Research article

Use of oyster mushrooms (*Pleurotus ostreatus*) for increased circularity and valorization of rapeseed residues

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ABSTRACT

In Europe, rapeseed is a common oilseed crop, resulting in the production of 20 million tons of rapeseed press cake yearly. This press cake can be further upcycled and a protein fraction can be extracted for food purposes, leaving de-proteinized fiber-rich residues. This study examined the use of these residues in the production of oyster mushrooms (*Pleurotus ostreatus*) and of the spent substrate as feed, since mushroom cultivation may improve the feed properties of substrate. In terms of mushroom production, the addition of rapeseed press residues was beneficial, giving significantly higher biological efficiency (BE = 93.1 ± 11.0%) compared with the control, sugar beet pulp substrate (70.0 ± 6.6%). This increase in productivity can most likely be explained by higher energy content in the substrate supplemented with lipid-rich rapeseed residues. Despite differences in BE between the substrates, high similarity was observed in lipid composition of the fruiting bodies (lipid profile dominated by linoleic acid (18:2), palmitic acid (16:0), and oleic acid (18:1)), and in protein and moisture content. After mushroom harvest, approximately 70% of the initial dry weight of both substrates remained as a possible feed source. Both substrates had significantly lower levels of carbohydrates and unchanged neutral detergent fiber content after mushroom harvest, and both gave lower *in vitro* digestibility, total gas production, and methane production. However, protein concentration differed between the substrates, with the highest concentration (15.8% of dry weight) found in spent substrate containing rapeseed press residues. The result of the present study suggests that the de-proteinized rapeseed press residue is a resource well-suited for use in the production of mushrooms and feed.

1. Introduction

Transition to a circular economy means that biomass previously considered as waste is valorized and recycled. Fungi have an important role to play in this transition, through their capacity to transform organic matter (Meyer et al., 2020). Fruiting bodies of edible mushrooms have been cultivated for centuries, in a production process based on solid-state fermentation where low-value agricultural waste is transformed into food. The specific composition of the substrate used in fermentation differs depending on the requirements of the fungal species cultivated, but it is generally based on various plant residues in different stages of degradation (Stamets, 2000).

Commonly cultivated mushrooms that are well known to consumers include white button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* spp.), and shiitake mushroom (*Lentinula edodes*). The latter two genera include primary decomposers that can grow directly on plant material rich in lignin and cellulose (Grimm and Wösten, 2018). In commercial production of these mushrooms, the substrate is often based on straw or sawdust (Sánchez, 2010). In order to decrease the carbon/nitrogen (C/N) ratio of the substrate, which can increase fruiting body production to some extent (Carrasco et al., 2018), wheat bran is also commonly added to the substrate. Another additive to the substrate, included with the main aim of balancing pH, is calcium carbonate. However, oyster mushrooms can achieve rapid growth and have the ability to produce fruiting bodies on a wide array of substrates, including waste products such as paper scraps and spent coffee grounds

([Fernandes et al., 2015](); [Alsanad et al., 2021]()). Thus, for this versatile and fast-growing genus, a broad range of lignocellulosic waste can be considered for the production of fruiting bodies.

Vegetable oils are produced globally and used for different purposes, e.g., as edible oils, for biofuel production, and in personal care products. Thus, residue of oilseed crops is abundantly available and therefore of interest to explore for the purpose of mushroom production. A number of different oilseed crops are grown world-wide and, after oil extraction from the seeds, a protein-rich press cake is obtained ([Ancuta and Sonia, 2020]()). This press cake typically contains around 30% fiber and 25–30% protein of high quality, comparable to soy protein ([Aider and Barbana, 2011]()). In Europe, rapeseed is a very common oilseed crop, with 10.8 million tons of rapeseed oil produced in 2020 ([FAOSTAT, 2023]()). This resulted in 20 million tons of rapeseed press cake, making it important to develop resource-efficient ways of handling this residue. Rapeseed press cake has an established use as animal feed, but its use in food applications is limited due to anti-nutritional factors such as phytic acid and glucosinolate. In order to upcycle the protein fraction in rapeseed press cake for food purposes, extraction can be performed to concentrate the protein and at the same time reduce the content of anti-nutrients. This protein upcycling process leaves de-proteinized fiber-rich residues. Since the protein content in these residues is typically low (around 20% protein on a dry weight (dwt) basis) and the fiber content is high, they are of little value as an animal feedstuff ([Ahlström et al., 2022]()).

A likely use of the de-proteinized rapeseed press residues is as low-value feedstock in composting and biogas processes. The use of this residue in mushroom production, as highlighted in the present study, has not previously been explored but would enable its valorization. Especially as spent mushroom substrate currently receives considerable attention for its potential in a wide range of applications including as a feed source ([Leong et al., 2022]()). The mushroom substrate is modified during fungal cultivation, due to degradation of the substrate in parallel with enrichment with fungal mycelium, which may improve the feed properties. The concept explored in the present study provides an opportunity to develop a completely circular process where rapeseed is utilized for the production of oil, proteins, and mushrooms, and finally used as feed.

Apart from enabling circular production, as described above, and acting as a tasty foodstuff with an interesting texture, edible mushrooms are usually a sustainable protein source containing all essential amino acids ([Ahlborn et al., 2019]()). Their lipid content and quality have received less attention, most probably because the lipid content is low, 1–4% dwt in most cases ([Cateni et al., 2022]()). Therefore, this study explored the use of de-proteinized rapeseed press residues for mushroom production, the feed potential of the substrate remaining after harvest, and the impact of substrate amendment with rapeseed press residues on the lipid profile in fruiting bodies of oyster mushrooms.

2. Material and methods

2.1. Fungal strain and mushroom substrate

Spawn of oyster mushroom (*Pleurotus ostreatus* M2191) obtained from Mycelia BVBA, Belgium, was used in the experiments. De-proteinized rapeseed press residues usually have a dense, compact structure, so in order to create a substrate that allowed gas exchange during fungal growth, sugar beet pulp (Betfor®, Nordic Sugar AB) was used as a base ingredient. The control substrate (substrate A) was composed of 100% sugar beet pulp, while the test substrate (substrate B) was composed of 66% sugar beet pulp and 34% de-proteinized rapeseed press residues (dwt/dwt). Industrially cold-pressed rapeseed press cake was obtained as a kind gift from Gunnarshögs Jordbruks AB (Hammenhög, Sweden). Extraction of proteins at pilot scale was performed as described in [Ahlström et al. (2022)](). In brief, 2 kg rapeseed press cake was dispersed in 18 kg water (1820 g dry solids) and the pH was increased to 10.5 with NaOH. After separation using a decanter, a liquid phase containing most of the proteins was obtained, together with a spent solids fraction (i.e., de-proteinized rapeseed press residues) of 3.1 kg (723 g dry solids). The pH of the sugar beet pulp was low (4.5), and substrate A was therefore amended with 2% calcium carbonate (dwt/dwt), which increased the pH to 6.4. The pH of the de-proteinized rapeseed press residues was 9.8, due to the alkaline conditions used for protein extraction, and when mixed with sugar beet pulp a pH of 6.9 was obtained in substrate B.

2.2. Mushroom production

Distilled water was added to the substrates to reach a moisture content of 65%. The substrates were packed in ventilated boxes suitable for mushroom production (Sac O2, Nevele, Belgium), with a total weight of 0.6 kg substrate (wet weight) per box corresponding to a dwt of 210 g of substrate per box. Three boxes were used for each treatment and the whole experiment was repeated. The boxes were pasteurized at 65 °C for 8 h and spawn of *P. ostreatus* was added in a concentration of 10% dwt to each box when the substrate had cooled down. The boxes were then incubated at 22 °C with the lids closed for 15 days, by which time the substrate was densely colonized with mycelium. The closed boxes were incubated at 4 °C in a refrigerator for a further three days to induce fructification, followed by removal of the lids and incubation in a climate chamber at 22–24 °C and 85% relative humidity until harvest of the first flush of fruiting bodies. The fruiting bodies were harvested five days after the emergence of the pins. After harvest of the first flush of mushrooms, the substrate was lyophilized and analyzed as described below.

2.3. Analysis

2.3.1. Substrate chemical composition

Total N and C were analyzed in lyophilized and milled samples of the substrates using a Vario Max CN. Proximate analysis of ash, total protein, neutral detergent fiber (NDF), water-soluble carbohydrates (WSC), and neutral lipids was performed on the substrates before and after mushroom production, according to [Ivarsson et al. (2021)](). Organic matter (OM) concentration was calculated as 1000 minus ash content.

2.3.2. Substrate in vitro evaluation

Two rumen-fistulated Nordic Red Breed dairy cows in late lactation fed *ad libitum* on a total mixed ration of grass silage and concentrate (60:40% on dry matter (DM) basis) were used as donors of rumen fluid inoculant for *in vitro* incubation of the spent substrates. Rumen fluid was collected and transported to the laboratory as described by [Chagas et al. (2019)](). Equal amounts of rumen fluid from each cow were immediately blended, strained through four layers of cheesecloth, and added to a buffered mineral solution (1:4 ratio) including Peptone™ (pancreatic digested casein; Merck, Darmstadt, Germany), according to [Menke and Steingass (1988)](). The buffered rumen fluid was kept at 39 °C under constant stirring and CO_2 flushing during the start-up of incubation.

For the *in vitro* incubation, substrate samples were weighed (±1000 mg) into serum bottles flushed with CO_2, and 60 mL of the previously prepared buffered rumen fluid were added. All bottles were placed in a water bath and gently and continuously agitated at 39 °C for 48 h. The substrates, before and after mushroom harvest, were each replicated in six bottles and randomly allocated to the *in vitro* incubation, which also included triplicate bottles with blanks (i.e., containing 60 mL of buffered rumen fluid but no substrate).

2.3.2.1. Gas production. An *in vitro* gas production experiment was conducted using a fully automated gas production technique described by [Cone et al. (1996)](), in which total gas volume is automatically recorded at 0.2-h intervals. Methane (CH_4) production *in vitro* was measured according to [Ramin and Huhtanen (2012)]() on gas samples

withdrawn (0.2 mL) at the end of the incubation (48 h) from each bottle. The concentration of CH_4 was determined using a gas chromatograph (GC) (Thermo Scientific, Trace 1300, USA) equipped with a thermal conductivity detector. Mean blank gas production within the run was subtracted from sample gas production. *In vitro* CH_4 production was calculated as described by Ramin and Huhtanen (2012) as:

$$CH_4 = 265 \times CH_4 \text{ concentration} + \text{Total gas production} \times CH_4 \text{ concentration} \times 0.55 \quad (1)$$

where total gas production is in mL g^{-1} sample, 265 is the total head-space volume (mL), and 0.55 is the ratio of CH_4 emissions in the outflow gas from the *in vitro* system.

2.3.2.2. In vitro true dry matter digestibility. *In vitro* true dry matter digestibility (TDMD) was determined for all samples by analyzing NDF concentrations in the residues using 07-11/5 Sefar Petex (Sefar AG, Heiden, Switzerland) *in situ* bags according to Krizsan et al. (2015). It was calculated as:

$$TDMD \left(g \ kg^{-1}\right) = \text{Incubated dry matter (DM; g)} - \text{NDF residue corrected for blank (g) } 1000 \ \times \ \text{Incubated DM (g)}/1000 \ \times \ \text{Incubated DM (g)} \quad (2)$$

2.3.3. Mushroom production

The amount of mushrooms (fresh weight and dwt) produced in the first flush was determined. The dry weight was recorded after lyophilization. Mushroom production (fresh weight) was related to the amount of substrate (dwt), in order to determine the biological efficiency (BE) of the substrate, calculated as:

$$BE = (\text{Mushroom (fresh weight)} / \text{Substrate (dwt)}) \text{ x } 100 \quad (3)$$

Total protein content in the fruiting bodies was analyzed by the Dumas method (Bellomonte et al., 1987), using a Vario Max CN and a conversion factor of 4.38 for total N (Barros et al., 2008).

2.3.4. Fatty acid methyl ester (FAME) content analysis

Fatty acid methyl ester (FAME) analysis was performed on lyophilized samples of de-proteinized rapeseed press residues, the substrates, and the fruiting bodies obtained. Approximately 50 mg of the sample was weighed into a glass tube and 2 mL of 2% H_2SO_4 in water-free methanol was added, together with a known amount of the internal standard 17:0-Me. Lipids were converted to FAME by heating in capped tubes at 90 °C for 60 min. The methyl esters were extracted into heptane by adding 2 mL of water and 0.75 mL of heptane and analyzed on a CP-wax 58 (FFAP-CB) column in an Agilent 8860 gas chromatography system. The injection temperature and detector temperature was 240 °C and 270 °C, respectively. The initial temperature was set at 150 °C and the temperature was raised at a rate of 4 °C/min up to 210 °C, followed by an increase of 10 °C/min until 250 °C, and finally held at 250 °C for 5 min.

Identification of FAME was performed by comparing retention times with those of authentic standards. The amount of FAME was calculated from the added internal standard (17:0-Me).

2.3.5. Analysis of glucosinolates

The concentration of glucosinolates in lyophilized de-proteinized rapeseed press residues was analyzed as described by Maldini et al. (2012).

2.4. Statistics

All experiments were set up with three replicates in each treatment and repeated once. Statistical analyses were carried out using Minitab version 2018 and data were tested for significant differences ($p < 0.05$) using ANOVA and Tukey's post-hoc test and *t*-test. Values presented are mean ± standard deviation (std).

3. Results and discussion

3.1. Production of fruiting bodies

The interval from inoculation of the substrate with spawn to harvest of the fruiting bodies was 36–38 days for substrate A and 33–34 days for substrate B. This is slightly longer than the commonly reported period of 21–28 days for oyster mushrooms in commercial production (Sánchez, 2010). The total yield of fruiting bodies was 147.0 ± 13.8 g/box for substrate A and 197.2 ± 23.0 g/box for substrate B. Thus, the biological efficiency (BE) of the substrates, describing conversion of substrate into fruiting bodies, was significantly higher for substrate B (93.1 ± 11.0%) compared with substrate A (70.0 ± 6.6%). For oyster mushrooms, which are known to be highly productive, BE values may exceed 100% (Stamets, 2000). The values obtained in the present study were based on the first flush only and substrate B in particular can be considered of interest for use in commercial production.

Selection of a suitable substrate for cultivation is essential in mushroom production. The substrate must provide an appropriate physical structure allowing gas exchange, and uptake of moisture, nutrients, and energy. In studies on mushroom substrate, commonly reported critical factors are pH and concentration of the macronutrients C and N, which are used to determine the C/N ratio (Carrasco et al., 2018). The energy content of mushroom substrate in relation to its BE is a factor that is rarely discussed, but our finding of higher BE for the substrate amended with lipid-rich residues suggests that this is a factor which should be considered when optimizing substrate. However, previous studies have reported that lipid-rich residues may contain compounds detrimental to fungal growth, an issue that needs to be considered. This was demonstrated by Krudpodorova and Barshteyn (2015), who examined mycelial growth in response to inclusion of oil press cake in liquid medium (submerged fermentation) and found that hyphal growth varied widely depending on the oilseed crop. In other studies, substrates with inclusion of olive press cake have been found to be less favorable for fruiting body production (Gregori and Pohleven, 2014; Koutrotsios et al., 2014). Rapeseed meal is reported to support efficient hyphal growth of *P. ostreatus* (Żuchowski et al., 2013), as also observed in a recent study (Heidari et al., 2022). However, both those studies focused on ways to decrease anti-nutritional compounds in solid-state fermentation with *P. ostreatus*, and did not consider fruiting body production. Their findings, taken together with the results obtained in the present study, suggest that residues based on rapeseed seems well-suited for cultivation of *P. ostreatus*.

3.2. Nutritional composition of fruiting bodies

Despite the difference in BE between substrates A and B, high similarity was observed in composition of the fruiting bodies. The protein concentration of the fruiting bodies was 20.5 ± 1.8% dwt for substrate A and 21.8 ± 2.2% dwt for substrate B, while the moisture content of the fruiting bodies ranged between 84 and 85% in both cases. Thus, both

protein and moisture concentration were well in line with previously reported values of approximately 85–95% of moisture and protein levels ranging between 20 and 25% of dwt (Lesa et al., 2022; Kalac, 2013). The total amount of fatty acids in the fruiting bodies was $3.3 \pm 0.5\%$ dwt for substrate A and $3.6 \pm 0.4\%$ dwt for substrate B. A total fatty acid concentration of 0.5–5% has been reported previously for *P. ostreatus* (Lesa et al., 2022).

Variations in the nutritional composition of fruiting bodies produced on different substrates are commonly observed (Koutrotsios et al., 2022; Guo et al., 2022; Carrasco et al., 2018). Variations in protein content are often explained by the N content of the substrate, with an increase in C/N ratio reported to be correlated to a decrease in protein concentration (Hoa et al., 2015). For the substrates used in the present study, the C/N ratio differed significantly (30.3 ± 0.9 in substrate A and 20.8 ± 1.2 in substrate B). As mentioned, oyster mushrooms have the capability to grow and produce fruiting bodies on a wide array of substrates, with a suitable C/N ratio reported to be within a wide range (32-150) (Chang and Miles, 2004). Despite the difference in C/N ratio between the substrates used in the present study, N availability can be considered high in both substrates, which may explain the similarity in the composition of the fruiting bodies. In addition, it should be pointed out that the correlation between N content in the substrate and protein content in the fruiting bodies is not straightforward since, as discussed by Koutrotsios et al. (2014), other factors such as the quality of carbohydrates in the substrate may also have an impact. The substrates used in the present study had similarities in this regard, as both were based on sugar beet pulp, which may be an additional explanation for the similarity in nutritional composition. Disparities in lipid concentration in *P. ostreatus* fruiting bodies observed in different studies may be partly explained by differences between strains. However, variations in lipid content of fruiting bodies produced by the same strain of *P. ostreatus* in response to growth on different substrates have also been reported (Alsanad et al., 2021; Ogwok et al., 2017; Koutrotsios et al., 2014). In contrast, in the present study, there were no significant differences between the substrates in total fatty acid content in the fruiting bodies. A likely explanation is that total fatty acid content was influenced by the same factors that affected the protein concentration (high N availability and similar carbohydrate composition).

The dominant fatty acid in the fruiting bodies was linoleic acid (18:2), corresponding to more than 75% of total fatty acids in fruiting bodies grown on both substrates (Table 1). Palmitic acid (16:0) and oleic acid (18:1) were also detected in substantial amounts. These observations are well in line with previously reported values for the fatty acid composition of fruiting bodies (both cultivated and wild) of *P. ostreatus* (Cateni et al., 2022; Fogarasi et al., 2018; Koutrotsios et al., 2017; Kalac,

2013). Overall, the fatty acid composition of the fruiting bodies produced on the different substrates (A, B) in the present study was very similar, with the only difference observed being a slightly higher concentration of oleic acid in fruiting bodies produced on substrate B. Other studies have also reported minor differences in fatty acid composition in response to different substrates (Alsanad et al., 2021; Ogwok et al., 2017; Koutrotsios et al., 2014) and the result of the present study further supports this finding (Table 1). In this regard, it should be noted that Koutrotsios et al. (2017) observed that fatty acid composition showed less variation in different strains of *P. ostreatus* than other common production parameters, such as earliness and yield. Thus, it seems reasonable to suggest that a fairly stable fatty acid composition, dominated by linoleic acid and also including palmitic acid and oleic acid, in fruiting bodies of *P. ostreatus* can be expected, irrespective of strain and substrate used.

3.3. Impact of mushroom production on substrates

Using product ingredients that are not edible for humans, such as agro-industrial waste, in animal feed is necessary and unavoidable for sustainable livestock production and therefore the mushroom substrates were evaluated from a feed perspective (Table 2). Adding de-proteinized rapeseed press residues to the sugar beet pulp substrate significantly increased the concentration of neutral lipids and total protein, while decreasing the concentration of carbohydrates (Table 2). The total fatty acid concentration was 10-fold higher in substrate B compared with substrate A and the fatty acid composition of the substrates also varied, as shown in Table 1. The de-proteinized rapeseed press residues had a total fatty acid content of $18.3 \pm 0.8\%$ dwt and the dominant lipids were oleic acid (18:1), linoleic acid (18:2), and alpha linolenic acid (18:3) (Fig. 1), which is in line with the fatty acid composition of cold-pressed rapeseed oil. The fatty acid composition in substrate B agreed well with the profile of de-proteinized rapeseed press residues (Figs. 1 and 2), demonstrating that the pasteurization performed on the substrates before mushroom cultivation did not affect the fatty acid composition.

The reduction in mass (dwt) after mushroom harvest, due to fungal

Table 1
Proportions of major fatty acids (% of total fatty acid content) in substrate A (100% sugar beet pulp) and substrate B (66% sugar beet pulp plus 34% de-proteinized rapeseed press residues), and in the fruiting bodies produced by oyster mushroom (*Pleurotus ostreatus*) grown on these substrates. Relative proportion of total fatty acids (Σ FAME), % dwt is also presented. Mean ± std, n = 3.

Fatty acid	Mushroom substrate		Fruiting bodies	
	Substrate A	Substrate B	Substrate A	Substrate B
16:0	$34.1 \pm 0.2a$[a]	$8.4 \pm 0.9b$	$11.0 \pm 0.3a$	$10.8 \pm 0.2a$
18:0	$0.2 \pm 0.3b$	$1.2 \pm 0.1a$	$0.5 \pm 0.4a$	$0.5 \pm 0.4a$
18:1	$17.0 \pm 0.7b$	$52.9 \pm 3.0a$	$7.6 \pm 0.8b$	$9.4 \pm 0.4a$
18:2	$40.1 \pm 0.7a$	$28.6 \pm 1.6b$	$78.3 \pm 1.5a$	$76.7 \pm 0.8a$
18:3	$4.9 \pm 0.4b$	$10.7 \pm 0.3a$	0.0	0.0
20:0	$1.5 \pm 0.4a$	$0.4 \pm 0.03a$	$2.7 \pm 0.4a$	$2.4 \pm 0.1a$
20:1	$1.1 \pm 0.1a$	$0.8 \pm 0.07b$	0.0	0.0
22:0	$0.9 \pm 0.06a$	$0.2 \pm 0.0b$	0.0	0.0
22:1	$0.2 \pm 0.4a$	0a	0.0	0.0
Σ FAME	0.7 ± 0.05	6.9 ± 0.9	3.3 ± 0.5	3.6 ± 0.4

[a] Values within rows followed by different letters are significantly different (p ≤ 0.05). Mushroom substrates and fruiting bodies were compared separately.

Table 2
Impact of *Pleurotus ostreatus* production on the chemical composition (g kg^{-1} dry weight (dwt)), true dry matter digestibility (TDMD, g kg^{-1} dry matter), and 48-h *in vitro* total gas and methane production (mL g^{-1} dry matter) of substrate based on sugar beet pulp only (substrate A) and 66% sugar beet pulp plus 34% de-proteinized rapeseed press residues (substrate B). The substrates were analyzed before inoculation (initial value) and after mushroom harvest (spent mushroom substrate, SMS). Mean ± std, n = 3.

	Substrate A		Substrate B	
	Initial value	SMS	Initial value	SMS
Ash	$75.6 \pm 9.2c$[a]	$193.4 \pm 11.6a$	$75.9 \pm 2.2c$	$141.9 \pm 13.7b$
Organic matter	$924 \pm 9.2a$[a]	$807 \pm 11.6c$	$924 \pm 2.2a$	$858 \pm 13.7b$
Neutral lipids	$2.9 \pm 0.02c$	$2.5 \pm 0.06c$	$51.0 \pm 1.4a$	$7.4 \pm 0.2b$
Total protein	$89.7 \pm 0.7c$	$93.8 \pm 6.5c$	$136.3 \pm 2.3b$	$157.7 \pm 10.7a$
Neutral detergent fiber	$335.3 \pm 8.8b$	$331.6 \pm 9.3b$	$342.0 \pm 6.0 ab$	$359.9 \pm 7.9a$
Water-soluble carbohydrates	$138.7 \pm 3.5a$	$28.5 \pm 1.7c$	$81.2 \pm 0.2b$	$14.9 \pm 1.3d$
TDMD	$956.0 \pm 5.1a$	$877.0 \pm 7.6b$	$861.7 \pm 14.6b$	$732.1 \pm 3.7c$
Total gas	$348.7 \pm 24.4a$	$296.4 \pm 40.2 ab$	$270.0 \pm 10.6bc$	$220.2 \pm 67.4c$
Methane	$62.7 \pm 3.8a$	$50.6 \pm 10.1b$	$45.7 \pm 2.5b$	$35.4 \pm 3.5c$

[a] Values within rows followed by different letters are significantly different (p ≤ 0.05).
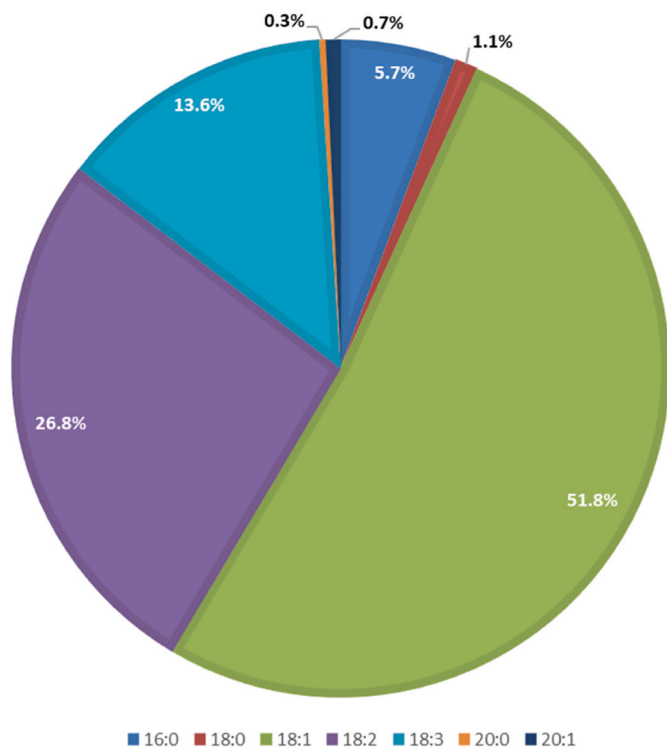
Fig. 1. Fatty acid composition (%, dry weight basis) of the de-proteinized rapeseed press residues used as mushroom substrate together with sugar beet pulp. Mean of three replicates, standard deviations were <10% of the mean.
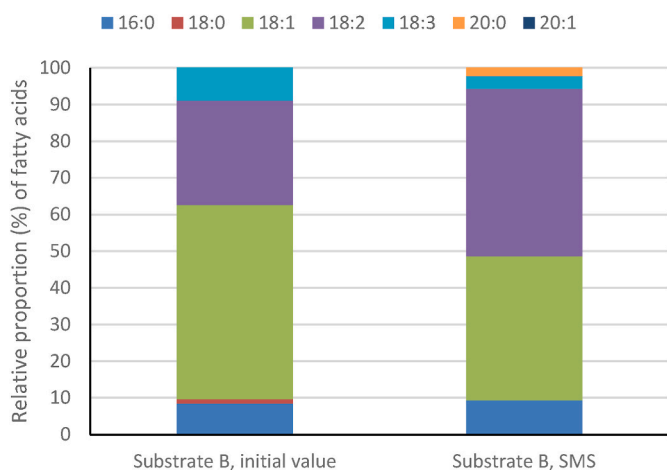

Fig. 2. Fatty acid profile (%, dry weight basis) of mushroom substrate comprising 66% sugar beet pulp and 34% de-proteinized rapeseed press residues (substrate B) before (initial value) and after mushroom cultivation (spent mushroom substrate, SMS). Mean of three replicates, standard deviation was <10% of the mean.

metabolism during growth, was 30.2 ± 1.8% dwt for substrate A and 30.5 ± 1.7% dwt for substrate B. Thus, approximately 70% of initial dry weight of both substrates remained as a possible feedstuff after harvest of the first flush of fruiting bodies. Both substrates had significantly lower levels of carbohydrates, unchanged NDF content, and higher levels of ash after harvest of the fruiting bodies (Table 2). The decrease in carbohydrates, but not NDF, demonstrates that the fungus preferably used more easily available carbohydrates as an energy source. Prolonging the cultivation process and harvesting a second flush of fruiting bodies would probably increase the degradation of NDF in the substrates, but would also have resulted in a larger reduction in mass, with

less substrate remaining as a potential feed ingredient. Increased ash level is commonly observed in spent mushroom substrate (Ivarsson et al., 2021; Koutrotsios et al., 2014), as the mass reduction is mainly due to degradation of organic carbon, explaining the organic matter depletion (Table 2).

For substrate A, the concentrations of neutral lipids and protein were similar before and after mushroom cultivation. For substrate B, the protein level was significantly increased, while the neutral lipid content decreased considerably (Table 2). The total fatty acid content in substrate B declined from an initial concentration of 6.9 ± 0.9% dwt to 1.5 ± 0.02% dwt after harvest. A detailed comparison revealed some minor variations in fatty acid profile in the substrate before and after fungus production, e.g., the relative content of linoleic acid (18:2) was slightly increased, suggesting that the lipolytic enzymes produced by *P. ostreatus* preferably degraded oleic acid (18:1) (Fig. 2). As the concentration of lipids was low in substrate B after harvest, this variation is not of great significance for its feed qualities. From a feed perspective, the increase in protein concentration is of greater interest and can be explained by several factors, including mass reduction and the occurrence of protein-rich fungal biomass in the spent substrate. However, it should also be pointed out that protein levels were calculated based on total N levels and the commonly used conversion factor of 6.25. As fungal biomass is rich in non-protein N, such as chitin (Barros et al., 2008), a detailed study of amino acid composition is needed to clearly determine the impact of mushroom cultivation on protein content and quality of the substrate.

Sugar beet pulp is a commonly used and popular feedstuff that also has a use as a mushroom substrate (Carrasco et al., 2018). Rapeseed press cake also has a use as animal feed, but the residues remaining after protein extraction are of less relevance in this regard due to lower protein levels, alkaline pH, and potential accumulation of anti-nutritional factors. The de-proteinized rapeseed press residues used in this study comprised the spent solids fraction after the first separation and were enriched in substances with low water solubility owing to the process conditions. No glucosinolates were detected in the residues, which was an expected finding since glucosinolates are water-soluble molecules and can be expected to be enriched in the aqueous phase in the protein extraction process. Another anti-nutrient of concern in rapeseed residues is phytate, a potent inhibitor of mineral absorption in the gastrointestinal tract, which in high concentrations can cause mineral deficiency (Mayer Labba et al., 2022). Phytate concentration was not analyzed in the present study but a previous study on wheat bran, which is well-known for its high content of phytate, found that *Pleurotus* spp. have the capacity for degradation of this anti-nutritional factor (Wanzenböck et al., 2017). In this context, it should be pointed out that parallel with the degradation, an uptake of certain organic compounds, e.g. caffeine, has been observed in fruiting bodies of oyster mushrooms (Chai et al., 2021). Thus, also the risk of accumulation of anti-nutritional factors in the fruiting bodies needs to be considered. For phytate specifically, this risk was evaluated by da Luz et al. (2013) and the compound could not be detected in the fruiting bodies despite a high concentration of phytate in the substrate.

Many factors should be considered when using an ingredient as a feedstuff, but especially its nutritional composition, digestibility (Ayaşan et al., 2020), and the target animal species. Regarding the proximate composition of the spent mushroom substrate (Table 2), the NDF content is a limitation for its use in non-ruminants diets, since dietary fiber content should not exceed e.g., 20–25% for pigs and 10–15% for poultry. On the other hand, the NDF content is acceptable for ruminant diets, where the protein concentration could be an advantage. In addition, mushroom cultivation is an aerobic solid-state fermentation process performed at room temperature with near-neutral pH. Thus, from a feed perspective the hygienic quality and the risk of establishment of pathogens such as *Salmonella* spp. is a factor that needs to be considered. In a challenge test, Ivarsson et al. (2021) observed an initial increase of the inoculated *Salmonella* strain (*Salmonella enterica* serovar Typhimurium)

in mushroom substrate followed by a decrease in its concentration during the spawn run. However, the concentrations of the inoculated food-borne pathogens did not decline to sanitary levels. High hygiene standards are needed during the mushroom cultivation process, possibly combined with heat treatment of the spent mushroom substrate, to ensure its hygienic quality.

In *in vitro* evaluations, the digestibility decreased when rapeseed press residue was added to the sugar beet pulp substrate (Table 2). This may be related to the high concentration of indigestible components, but further studies are needed to clarify this. Substrate A had a slightly lower organic matter content than substrate B after mushroom harvest, however, it had almost 50% more soluble carbohydrates, which can be ~98% digested according to Van Soest (1967), explaining the results obtained. Total gas production and CH_4 production were both lower for both substrates after harvesting the mushrooms (Table 2), which is in line with the decline in digestibility. Ramin and Huhtanen (2013) found that organic matter digestibility and CH_4 production are highly negatively correlated, which was also found to be the case in the present study. In addition, neutral lipid content was found to be much higher in substrate B compared with substrate A (initial value) and fat content is another factor affecting CH_4 emissions, with negative effects on digestibility and CH_4 production (Ramin and Huhtanen, 2013). There are three mechanisms involved in the inhibitory effect of fat on CH_4 production. First, biohydrogenation of unsaturated fatty acids utilizes hydrogen, making it unavailable for CH_4 production (Ramin and Huhtanen, 2013). Second, a higher fat concentration can decrease the relative supply of fermentable substrates, rather than having a direct effect on methanogenesis (Johnson and Johnson, 1995). Third, dietary fat concentration influences rumen fermentation by increasing the proportion of propionate at the expense of acetate or butyrate, and causes inhibition of rumen protozoa, depressing fiber digestion (McAllister et al., 1996).

4. Conclusion

De-proteinized rapeseed press residues were included in the mushroom substrate (sugar beet pulp) and used for production of oyster mushrooms. The inclusion gave a clear benefit in terms of mushroom production, with a significantly higher yield of fruiting bodies compared with the control substrate. The nutritional composition of the fruiting bodies was not affected by the addition of rapeseed press residues. The results indicated that both mushroom production and the inclusion of rapeseed press residues improved the spent substrate from a feed perspective. The highest level of total protein was observed in the press residues-supplemented substrate after harvest. Considering the alternative use for this residue, e.g., as a low-value feedstock in composting and biogas processes, it seems reasonable to explore de-proteinized rapeseed press residues further for use in the production of mushrooms and feed.

Credit author statement

K. Östbring: Conceptualization, Investigation, Resources, Writing – review and editing I Lager: Methodology, Investigation, Formal analysis, Writing – review and editing J. C. C. Chagas: Investigation, Writing – review and editing M. Ramin: Investigation, Writing – review and editing M. Hultberg: Conceptualization, Funding acquisition, Formal analysis, Investigation, Writing - original draft.

Ethical approval

Handling of the animals used as inoculum donors for the *in vitro* incubation test was approved by the Swedish Ethics Committee on Animal Research (Dnr A 6-21), represented by the Court of Appeal for Northern Norrland in Umeå.

Informed consent

All authors have the authority to publish this material and have agreed to its submission.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

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