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Research Article

Proximate analysis of cold-press oil cakes after biological treatment with *Trametes versicolor* and *Humicola grisea*

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In order to increase the current knowledge on cold-press oil cakes composition, the present study aims to determine the chemical composition of oil cakes from hull-less pumpkin (*Cucurbita pepo* L.), flax (*Linum usitatissimum* L.), and hemp (*Canabis sativa* L.) before and after the biological treatment with *Trametes versicolor* and *Humicola grisea* using fungal-based solid-state technology. After 10 days of treatment, the content of ash, total nitrogen, total proteins, and total organic carbon increased in all the three oil cakes, while the content of ether extracts decreased. After treatment, the concentration of soluble carbohydrates decreased in pumpkin and hemp seed oil cakes, whereas it increased in flaxseed oil cake. During treatment with *T. versicolor*, the content of fructose significantly increased in hull-less pumpkin seed oil cake. Fiber content decreased in pumpkin and flaxseed oil cakes after treatment with both of the fungi, whereas it increased in flaxseed oil cake.

Keywords: Cold-pressed oil cake / *Humicola grisea* / Proximate analysis / *Trametes versicolor* / Waste/by-product

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1 Introduction

The waste originating from the food industry is usually burned or used as fertilizer [1–3] and animal feed [4–8]. A few decades ago, a certain imbalance between the generation of food bio-waste and the number of livestock was perceived. The accumulation of food industry waste follows the growth of the food industry, but the demand for feed decreases due to the decline in livestock farms [9]. The awareness about the growth of environmental load due to waste or by-products originating from the food industry increases the tendency toward the search for new methods of its exploitation.

The waste/by-products that remain after oil extraction from seeds is called meal or cake. Although oil cakes do not have great commercial significance, due to their high nutritional value, they have immense possibilities for use in food production [10] or in a variety of biotechnological processes [11]. According to Cozea et al. [12], there are two types of oil cakes, inedible and edible. Inedible oil cakes are commonly used as organic fertilizers due to high N, P, and K contents. Edible oil cakes have higher nutritional value (protein content from 15 to 50%) and are preferable for

use as protein supplements and as flour. They can also be used in human nutrition when combined with wheat or other flours [13, 14]. The flour made from edible oil cakes can be used for the production of gluten-free products, which are known to have low contents of proteins, vitamins, and minerals, in order to enhance their nutritional value [15, 16]. Generally, oil cakes are used as food supplements [17–20]. It is reported that they have a positive effect on health [21] and are also known to reduce the risk of some diseases [12, 14, 22, 23].

Besides being used as food supplements, oil cakes can be utilized as substrates for the production of enzymes such as lipase [24] and cellulase [25], or in the production of antibiotics, mushrooms, vitamins, antioxidants, amino acids, organic acids, single-cell proteins, and biologically active secondary metabolites, as reviewed by Ramachandran et al. [11]. Their possible use for the production of industrial bio-products, including biogas and bio-oil, is also presented in the review by Ramachandran et al. [11].

One of the possible methods for the utilization of different types of agro-, food, or wood industry wastes for the production of value-added products or biofuels is solid-state fermentation with white-rot fungi [26].

The objective of this study was to determine the chemical composition of the three oil cakes obtained after cold-pressed oil production from hull-less pumpkin (*Cucurbita pepo* L.), flax (*Linum usitatissimum* L.), and hemp (*Canabis sativa* L.), before and after biological treatment with two different

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Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; NDF, neutral detergent fiber; TOC, total organic carbon

microorganisms; *Trametes versicolor* and *Humicola grisea*. The study aims to identify the possible pathways for the utilization of hull-less pumpkin, flax, and hemp oil cakes, before and after biological treatment. *T. versicolor* is known to be a good producer of lignolytic enzymes such as laccase [27] and was chosen for this study due to our previous experience with its solid-state cultivation on corn silage [28,29] and brewery spent grains [30]. On the other hand, there is scarce literature data on the use of *H. grisea* in solid-state fermentation. *Humicola* is a well-known genus of thermophilic fungi from the soil. Some species are known as good producers of cellulolytic enzymes [31]. During submerged cultivation, *H. grisea* was used for the production of chitinase [32], feruloyl esterase, and xylanase [33].

2 Materials and methods

2.1 Materials

Hull-less pumpkin (*C. pepo* L.), flax (*L. usitatissimum* L.), and hemp (*C. sativa* L.) oil cakes were obtained from the local company (SME Lazić, Vraneševci, Croatia) that uses cold-pressed technology based on screw presses for the production of oil from seeds. The oil cakes used in all experiments were from the same batch of oil production.

All the chemicals used for analysis were of the finest analytical grade: sodium dihydrogen phosphate, disodium hydrogen phosphate, isoamyl alcohol, and phenol were purchased from Kemika Zagreb, Croatia; sulfuric acid, sodium sulfate, copper sulfate, sodium hydroxide, methylene red, N-octanol, sodium sulfite, neutral detergent reagent, acid detergent reagent, acetone, sulfuric acid, phosphoric acid, D(+)-glucose, D(-)-fructose, D(+)-sucrose, D(+) maltose, maltotriose, vanillic acid, acetonitrile, and pN-phenol were purchased from Sigma Aldrich, Austria; hydrochloric acid and petrol ether were purchased from Carlo Erba Reagents, Rodano, Italy; pNP-palmitate (Alfa Aesar, Germany); tris(hydroxymethyl)aminomethane (Acros Organics, USD); 2-propanol (Alkaloid Skopje, Macedonia); chloroform (Carlo Erba, France).

2.2 Analytical methods

Dry matter and ash contents were determined according to the standard procedures of the Association of Official Analytical Chemists [34]. Total proteins were determined by the Kjeldahl method (pumpkin N $\times$ 5.3; flax N $\times$ 6.25; hemp N $\times$ 5.39; [35]). Soluble carbohydrates content was determined using the phenol-sulfuric acid method, while ether extract content was determined by the Soxhlet method [34]. Simple sugars were determined in the water extracts of oil cakes. Extraction was conducted with distilled water at 30°C during 30 minutes and extracts were analyzed by the gradient UHPLC method (Nexera XR UHPLC, Shimadzu, Japan; column: InertSustain NH₂; 5 μ m; 4.6 $\times$ 250 mm; mobile phase: acetonitrile:water 75:25; analysis time: 20 min; oven temp.: 40°C; mobile phase flow: 1 mL/min; sample volume: 10 μ L; detector: RID-20A). Total organic carbon (TOC) content was determined using the TOC Analyzer SSM 5000A (TOC LCPH/CPN, Shimadzu, Japan). Neutral detergent fiber

(NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed using a Velp Scientifica Fiber Analyzer FIVE Series (VELP Scientifica, Usmate, Italy) according to the Van Soest method [36].

All measurements were made in triplicates, and the results are expressed as mean values with SD.

2.3 Lipase assay

Lipase activity was determined by the end-point test, using pNP-palmitate as a substrate according to the slightly modified procedure of Palacios et al. [37]. The reaction mixture consisting 3.9 mL of 50 mM Tris-HCl buffer (pH 8) with gum arabic (1 g/L) and 1 mM pNP-palmitate was pre-warmed to 40°C whereupon crude enzyme extract was added ($V = 0.1$ mL). After 60 min of incubation ($T = 40^\circ\text{C}$) the reaction was stopped by the Marmur solution ($V = 1.5$ mL) and centrifuged (Hermle Z 326 K, Germany) at 15 000 rcf for 10 min at 4°C. Absorbance was measured spectrophotometrically (ThermoSpectronic, Helios Gamma, Germany) at 410 nm, and the amount of released pN-phenol was calculated using the molar extinction coefficient 0.2987 mL/mM cm determined from the calibration curve. One unit (U) is defined as the amount of lipase that degrades 1 mM of pNP-palmitate for 1 min.

The crude enzymatic extract was prepared by extraction of an oil cake (1 g) with a phosphate buffer (5 mL of 0.1 M, pH 7) at 4°C for 30 min and then centrifugation at 15 000 rcf for 10 min at 4°C.

2.4 Experimental set-up

Biological treatments of oil cakes with *T. versicolor* and *H. grisea* were carried out in laboratory jars ($V = 650$ mL). 50 grams of oil cake was mixed with water to obtain 60% of moisture, autoclaved (121°C/20 min), and cooled overnight. The substrate in each jar was inoculated with 5 mycelial discs (6 mm in diameter) and incubated at 27°C ($t = 10$ days) in an incubator with the air fan set to 10% (KB 115, BINDER GmbH, Germany). All experiments were performed in triplicate. For the purpose of measurements of chemical composition, samples were withdrawn from each jar in aseptic conditions and sterilized in an autoclave (121°C, 20 min).

2.5 Statistical analysis

Statistical analysis was performed using the TIBCO Software Inc. Statistica (data analysis software system), version 13, at the level of significance of $p < 0.05$. Differences in the chemical composition of untreated cakes, cakes before and after treatment, as well as cakes treated with *T. versicolor* and *H. grisea* were analyzed for significance using the analysis of variance (ANOVA). When ANOVA indicated significant differences among the mean values, post hoc analysis by Duncan's multiple-range test was performed.

Table 1. Proximate analysis of untreated cold-press oil cakes of hull-less pumpkin, hemp, and flax (means in the same row followed by different letters are significantly different, $p < 0.05$)

Parameters of analysis	Hull-less pumpkin oil cake	Hemp oil cake	Flax oil cake
Ash content, [%, db]	7.82 ± 0.00 ^a	7.48 ± 0.00 ^b	5.30 ± 0.00 ^c
Total proteins, [%, db]	38.27 ± 0.40 ^a	24.77 ± 0.41 ^b	32.83 ± 1.09 ^c
TOC, [%, db]	73.49 ± 0.76 ^a	66.74 ± 0.04 ^b	67.51 ± 0.86 ^b
Soluble carbohydrates, [%, db]	2.74 ± 0.40 ^a	1.84 ± 0.16 ^b	2.20 ± 0.36 ^b
Ether extracts, [%, db]	36.22 ± 0.58 ^a	12.34 ± 0.00 ^b	21.40 ± 0.38 ^c
NDF, [%, db]	11.81 ± 1.43 ^a	53.43 ± 1.21 ^b	18.83 ± 1.87 ^c
ADF, [%, db]	5.12 ± 1.77 ^a	39.19 ± 0.19 ^b	14.22 ± 0.30 ^c
ADL, [%, db]	0.74 ± 0.07 ^a	16.69 ± 0.35 ^b	6.02 ± 0.35 ^c

3 Results and discussion

3.1 Chemical analysis of the oil cakes before biological treatment

Dry matter, ash, total nitrogen, total proteins, TOC, soluble carbohydrates, simple sugars, ether extract, and fiber contents were measured before and after biological treatments. The proximate analysis of hull-less pumpkin, hemp, and flaxseed oil cakes before biological treatment with *T. versicolor* and *H. grisea* is presented in Table 1. The results are expressed as percentage of dry matter with SD. Dry matter content of the oil cakes before biological treatment was 93.07% (hull-less pumpkin seed oil cake), 92.92% (hemp seed oil cake), and 92.09% (flaxseed oil cake).

The results of total protein content of hull-less pumpkin oil cake (38.27 ± 0.40% db) are consistent with the literature data, where it was reported to be in the range from 45.6 to 59.8% db [14, 17, 25, 38]. The only lower value (29.3% db) found in literature was reported by Cozea et al. [12]. The total proteins determined in flaxseed oil cake (32.83 ± 1.09%) are almost equal as reported by Bochkarev et al. (32.6% db) [14]. Other values found in literature were lower [12, 23]. Regarding hemp seed oil cake, all values reported in the literature [39–41] were higher than the value determined in this paper (24.77 ± 0.41% db), except for those published by Cozea et al. [12], where the content of total proteins was 24.86%. The amounts of total proteins within untreated oil cakes are statistically significantly different (Table 1).

The results of ether extract content, however, slightly differ from those found in the literature. Ether extract content in hemp seed oil cake (12.34% db) differs to some extent from the results found in the literature (5.18–11.9% db) [12, 39–41]. The content of ether extract determined in hull-less pumpkin seed oil cake (36.22% db) considerably differs from the values reported by Bochkarev [14] (7 to 19.4% db) and Zdunczyk et al. (12.5% db) [38]. The content of ether extract in flaxseed oil cake (21.40%) is within the range found in literature, which varies from 6.83 [12] to 29.37% [23]. The observed discrepancies with literature data can be explained considering the different technology of oil production applied in the research. Pumpkin oil cake analyzed in the study by Zdunczyk [38] and Bochkarev [14] was produced using the technology of heated pressing. In our study, the cold pressing technology was applied. The process of oil production at higher temperatures (80–100°C) is suitable for the extraction of higher amounts of oil in comparison with

the processes performed at lower temperatures, e.g. when the temperature of the oil after pressing does not exceed 50°C. The statistically significant difference in the ether extract between untreated cakes is visible from the data presented in Table 1.

The literature data on fiber contents in oil cakes is scarce and mostly related to hemp seed oil cake. It is reported that NDF and ADF values are in the range from 19.0 to 41.5% db, and from 12.4 to 32.0% db, respectively [39]. In our study, it was found that hemp seed oil cake is comprised of 53.43% db of NDF and 39.19% db of ADF. The only data found in literature regarding fiber content (NDF content of 10.51% db) in hull-less pumpkin oil cake is that reported by Zdunczyk [38]. This result is similar to the results obtained in the present study (11.81% db). Comparing the amount of fibers (NDF, ADF, and ADL) in all the three oil cakes before biological treatment with microorganisms (Table 1), a statistically significant difference can be observed.

Hull-less pumpkin, hemp, and flaxseed oil cakes before biological treatment statistically differ in each analyzed parameter (statistical significance, $p < 0.05$), apart from TOC and soluble sugars. Concerning soluble sugars and TOC, the hull-less pumpkin seed oil cake is statistically different from hemp and flaxseed oil cakes, while hemp and flaxseed oil cakes are not mutually different (Table 1).

3.2 Chemical analysis of the oil cakes after biological treatment

After 10 days of biological treatment with *T. versicolor* and *H. grisea*, all the three oil cakes had statistically significant changes of the examined parameters, with the exception of TOC (Table 2, 3 and 4). This is the consequence of the heterogeneous chemical composition of the substrates, adsorption of mycelia to the substrates, and the formation and activities of the variety of enzymes which are affected by the nature of the carbohydrates available for the microorganism's growth.

After the treatment with *T. versicolor*, the amount of ash and protein generally increased in all the three oil cakes, where the highest increase of ash (40%) and protein (53%) was recorded in flaxseed oil cake (Table 4). A significant decrease in ether extract occurred with the highest decrease (84%) recorded in the samples of flaxseed oil cake. It is interesting to observe that total soluble carbohydrates in flaxseed oil cake increased by 509%. However, their concentrations in the samples of hull-less pumpkin seed (Table 2) and hemp seed oil cakes (Table 3) decreased by

Table 2. Proximate analysis of cold-press oil cakes of hull-less pumpkin before and after biological treatment with *T. versicolor* and *H. grisea* (means in the same row followed by different letters are significantly different, $p < 0.05$)

Parameters of analysis	Hull-less pumpkin oil cake before biotreatment	Hull-less pumpkin oil cake after biotreatment with	
		<i>Trametes versicolor</i>	<i>Humicola grisea</i>
Ash content, [%, db]	7.82 ± 0.00 ^a	8.59 ± 0.41 ^b	8.64 ± 0.05 ^b
Total proteins, [%, db]	38.27 ± 0.40 ^a	40.58 ± 0.22 ^b	41.91 ± 0.25 ^c
TOC, [%, db]	73.49 ± 0.76 ^a	73.86 ± 6.11 ^a	74.70 ± 0.61 ^a
Soluble carbohydrates, [%, db]	2.74 ± 0.40 ^a	1.10 ± 0.17 ^b	1.39 ± 0.24 ^b
Ether extracts, [%, db]	36.22 ± 0.58 ^a	16.28 ± 1.10 ^b	31.35 ± 0.57 ^c
NDF, [%, db]	11.81 ± 1.43 ^a	3.34 ± 0.19 ^b	10.04 ± 0.16 ^c
ADF, [%, db]	5.12 ± 1.77 ^a	1.25 ± 0.03 ^b	4.98 ± 0.15 ^a
ADL, [%, db]	0.74 ± 0.07 ^a	0.15 ± 0.01 ^b	1.42 ± 0.17 ^c

Table 3. Proximate analysis of cold-press oil cakes of hemp before and after biological treatment with *T. versicolor* and *H. grisea* (means in the same row followed by different letters are significantly different, $p < 0.05$)

Parameters of analysis	Hemp oil cake before biotreatment	Hemp oil cake after biotreatment with	
		<i>Trametes versicolor</i>	<i>Humicola grisea</i>
Ash content, [%, db]	7.48 ± 0.00 ^a	7.97 ± 0.12 ^b	8.19 ± 0.27 ^b
Total proteins, [%, db]	24.77 ± 0.41 ^a	28.63 ± 0.24 ^b	27.95 ± 0.23 ^c
TOC, [%, db]	66.74 ± 0.04 ^a	67.99 ± 4.14 ^a	67.70 ± 0.36 ^a
Soluble carbohydrates, [%, db]	1.84 ± 0.16 ^a	0.71 ± 0.16 ^b	0.89 ± 0.10 ^b
Ether extracts, [%, db]	12.34 ± 0.00 ^a	2.44 ± 1.06 ^b	11.80 ± 0.10 ^a
NDF, [%, db]	53.43 ± 1.21 ^a	63.30 ± 0.52 ^b	61.05 ± 0.31 ^c
ADF, [%, db]	39.19 ± 0.19 ^a	47.03 ± 0.62 ^b	43.13 ± 0.72 ^c
ADL, [%, db]	16.69 ± 0.35 ^a	19.05 ± 0.13 ^b	17.10 ± 0.44 ^a

Table 4. Proximate analysis of cold-press oil cakes of flax before and after biological treatment with *T. versicolor* and *H. grisea* (means in the same row followed by different letters are significantly different, $p < 0.05$)

Parameters of analysis	Flax oil cake before biotreatment	Flax oil cake after biotreatment with	
		<i>Trametes versicolor</i>	<i>Humicola grisea</i>
Ash content, [%, db]	5.30 ± 0.00 ^a	7.43 ± 0.71 ^b	5.38 ± 0.01 ^a
Total proteins, [%, db]	32.83 ± 1.09 ^a	50.05 ± 0.33 ^b	43.59 ± 0.27 ^c
TOC, [%, db]	67.51 ± 0.86 ^a	70.73 ± 4.80 ^a	71.27 ± 2.12 ^a
Soluble carbohydrates, [%, db]	2.20 ± 0.36 ^a	13.34 ± 3.12 ^b	10.09 ± 0.50 ^c
Ether extracts, [%, db]	21.40 ± 0.38 ^a	3.43 ± 0.98 ^b	15.06 ± 0.06 ^c
NDF, [%, db]	18.83 ± 1.87 ^a	16.42 ± 0.13 ^b	16.79 ± 0.18 ^b
ADF, [%, db]	14.22 ± 0.30 ^a	11.04 ± 0.16 ^b	12.53 ± 0.22 ^c
ADL, [%, db]	6.02 ± 0.35 ^a	4.15 ± 0.26 ^b	4.40 ± 0.14 ^b

approx. 60%. The increase of soluble carbohydrates in flaxseed oil cake is the consequence of the decrease of insoluble fibers, due to the activity of lignolytic enzymes produced by *T. versicolor* [29]. This was also the case with the other cakes treated with *T. versicolor*. The highest drop of insoluble fibers was recorded in hull-less pumpkin oil cake (NDF for 71%, ADF for 74%, and ADL for 80%), but in comparison to flaxseed oil cake, hull-less pumpkin oil cake before the treatment had a four-fold lower content of total fibers. A statistically significant increase of all fibers (NDF, ADF, and ADL) in hemp seed oil cake occurred after the treatments with both microorganisms, with the exception of an ADL increase of 2% in the case of the treatment with *H. grisea*, which did not show as statistically significant.

Similar results were obtained in our previous research, when *T. versicolor* was cultivated on corn silage [29]. In those experiments, the amount of ash, free fats, and protein content increased, while carbon to nitrogen ratio decreased. Some other materials were also used as substrates (sugar beet pulp, brewers' spent grains) for the cultivation of *T. versicolor* by our group, with the aim of producing laccase and manganese-peroxidase, or polyphenolic compounds [41,43].

In experiments performed with *H. grisea*, the share of total proteins and soluble carbohydrates was significantly different in all the three cakes before and after the treatment. Whilst the share of protein generally increased, the largest increase of 33% was found in flaxseed oil cake. Hemp and flax

Figure 1. Content of simple sugars in hull-less pumpkin, hemp and flax seed oil cakes during 10 days of biotreatment with *T. versicolor*.

Figure 2. Content of simple sugars in hull-less pumpkin, hemp and flax seed oil cakes during 10 days of biotreatment with *H. grisea*.

Table 5. Activity of lipase extracted from the oil cakes during the biological treatment with *T. versicolor* and *H. grisea*

Incubation time (days)	<i>T. versicolor</i>			<i>H. grisea</i>		
	Hull-less pumpkin oil cake	Hemp seed oil cake	Flax seed oil cake	Hull-less pumpkin oil cake	Hemp seed oil cake	Flax seed oil cake
	Lipase activity [U/g ds]					
3	0	0	0	0.82 ± 0.04	1.67 ± 0.11	0.29 ± 0.00
7	2.68 ± 0.13	4.35 ± 0.22	1.97 ± 0.11	0.62 ± 0.00	0.50 ± 0.03	0.54 ± 0.03
10	1.75 ± 0.09	1.92 ± 0.10	1.92 ± 0.13	0.56 ± 0.03	1.34 ± 0.07	0.92 ± 0.05

seed oil cakes did not show statistically significant differences in ash content, ether extract, and ADL content before and after the bio-treatment, respectively. There were no statistically significant differences in ADF content before and after the biotreatment with *H. grisea* for hull-less pumpkin seed oil cake. Unlike in hull-less and flaxseed oil cake, in hemp seed oil cake a statistically significant increase in total proteins did not result in the decrease of fibers. Similar to the experiments performed with *T. versicolor*, in experiments performed with *H. grisea*, the increase of soluble carbohydrates occurred after the

flaxseed oil cake treatment (369%), while approx. 50% of the decrease occurred for hull-less pumpkin seed and hemp seed oil cakes.

The carbohydrates can influence microbial growth rate and hence, indirectly, modulate the activity of enzymes with growth rate-related activities. They can also influence enzyme activities through induction and catabolite repression [44].

In order to better understand the utilization of soluble carbohydrates by both organisms during growth on different oil cakes, the measurements of simple sugars were done and the results are

presented in Figures 1 and 2. The amount of sucrose before the bio-treatment ranged from 17.59 mg/g db for hull-less pumpkin oil cake to 20.74 mg/g db for hemp oil cake. During the bio-treatment, the sucrose content decreased continuously, and after 10 days of cultivation, the amounts of sucrose were 3.73 mg/g db and 0.29 mg/g db for the hull-less pumpkin oil cake in case of the bio-treatment with *T. versicolor* and *H. grisea*, respectively. At the end of the bio-treatment of hemp oil cake with *T. versicolor* (after 10 days), the amount of sucrose was 1.56 mg/g db, and after the *H. grisea* treatment it was 2.57 mg/g db. In the first two days of flaxseed oil cake bio-treatment, the sucrose was not recorded. On the third day, the amount of sucrose was 17.12 mg/g db, and by the 10th day of cultivation the value decreased down to 4.02 mg/g db when the oil cake was bio-treated with *T. versicolor*, and to 1.97 mg/g db in the case of bio-treatment with *H. grisea*. During the bio-treatment with *H. grisea*, all the three oil cakes had no significant changes in the content of simple sugars (Figure 2). A significant change was recorded (Figure 1) in fructose content in the hull-less pumpkin oil cake bio-treated with *T. versicolor*. The fructose content was increasing continuously as fermentation took place. The fructose content, during the bio-treatment with *T. versicolor*, increased from 0.48 up to 20.9 mg/g db. This is in agreement with our previous study, where it was shown that *T. versicolor* has higher affinity towards glucose than fructose [43, 45].

Comparison of the results obtained by the statistical analysis of a single oil cake, with regard to the bio-treatment with two different fungi, shows that there is no statistically significant difference between the hull-less pumpkin seed and hemp seed oil cakes in the content of ash, TOC, and soluble carbohydrates. For flaxseed oil cake, there are no statistically significant differences, regardless of the applied fungi for the bio-treatment, in the content of NDE, ADL, and TOC.

The results show that the biological treatment with *T. versicolor* gave a more statistically significant difference in the chemical composition of studied oil cakes, in comparison to the bio-treatment with *H. grisea*, with regard to the content of total proteins, ether extract, and soluble carbohydrates.

The statistically significant decrease in fat content in all the three oil cakes after treatment with both microorganisms could be attributed not only to the use of fat as a source of nutrients for growth and development of fungi, but also to the results of extracellular lipase activity produced by *T. versicolor* and *H. grisea*. In order to confirm this hypothesis, the lipase activity in extracts obtained after 3, 7, and 10 days of oil cake bio-treatment with fungi was measured. Based on the obtained results (Table 5), it is apparent that *T. versicolor* and *H. grisea* did not produce an appreciable amount of lipase. The highest lipase activity of 4.35 U/g of dry fermented substrate was obtained on the 7th day, when *T. versicolor* was cultivated on hemp seed oil cake. In comparison with literature data, where different oil cakes were used for the cultivation of fungi from genera *Aspergillus* [46–48] (*Aspergillus malleus*, canola seed oil cake, 684.02 U/g ds; *Aspergillus ibericus*, palm kernel, and sesame oil cake, 460 U/g; *Aspergillus niger*, palm oil cake, 15.41 U/mL) or *Rhizopus* [49] (*Rhizomucor miehei*, babassu oil cake 43 U/g), the activities obtained in this study are low.

4 Concluding remarks

Oil cakes of hull-less pumpkin seed, flaxseed, and hemp seeds are highly valuable waste/by-products from the food industry that can be used as fertilizers, animal feed, human food supplements, or as substrates for enzyme production. Based on the results of the biological treatment of oil cakes with *T. versicolor* and *H. grisea*, it can be concluded that the application of fungal-based solid-state treatment of oil cakes can enhance their nutritional value, especially in the field of production of protein-enriched feed.

Practical application

The results of this research contribute to the knowledge on the application of the bioprocess (fungal-based solid-state treatment) for the development of nutritionally enriched products from the waste material obtained after oil production. Additionally, based on the results of the chemical composition of the three different oil cakes before the treatment, it can be suggested that oil cakes could be used for the production of enzyme lipase, using microorganisms which are known as good lipase-producing microorganisms.

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