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Evaluation of polyphenol content and lipoxygenase activity in selected oil cakes in terms of their valorization

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Abstract: Oil cakes are pomace that is a by-product of pressing oil from oilseeds. Due to the content of bioactive ingredients, there is more and more talk about their valorization through use in human and animal nutrition as well as microbiological processes aimed at the biosynthesis of biosurfactants, enzymes and fragrance compounds. The aim of this research was a qualitative assessment of selected oil cakes (linseed, rapeseed, hemp, safflower and camelina oil cakes))in the context of their potential use in microbiological cultures aimed at the biosynthesis of green note aroma compounds. The scope of the research included the analysis of the fatty acid composition of the lipid fraction of oil cakes as well as the determination of lipoxygenase activity and the polyphenol content in the extracts of these raw materials. Chromatographic analysis of the lipid fraction showed that hemp and safflower cakes had the highest content of polyunsaturated fatty acids. PUFAs constitute 67.18 ± 2.2% and 73.72 ± 1.8% in their extracts, respectively. Hemp cake extracts were also characterized by the highest lipoxygenase activity - 76.4–1.2 U/mL with a low content of phenolic compounds at the level of 0.116 ± 0.005 mg GAE/mL of extract.

Keywords: oil cakes; lipoxygenase; waste valorization; green note aroma compounds

1. Introduction

Oil cakes are the principal by-products obtained from pressing oil from oilseeds. Oil cakes can be classified into two categories - edible and inedible. Edible oil cakes among others (soybean, peanuts, rapeseed, sunflower, coconut, cotton seeds, safflower, linseed) have high nutritional value due to the content of lipids, proteins, carbohydrates and minerals, so they can be used as additives to products intended for human and animal consumption [1,2]. Inedible oil cakes, due to the presence of toxic compounds (castor, neem, mauha, karanja) can be used in the form of fertilizers [3]. Due to the content of bioactive compounds, cakes can also be a good component of microbiological media in the production of enzymes, antibiotics, biosurfactants [4].

The increasing emphasis on reducing the costs of industrial processes and increasing the added value of agro-industrial residues prompted us to undertake research on the possibility of using oil cakes in the biosynthesis of fragrance compounds from the so-called green note aroma compound note via *Yarrowia lipolytica* yeast. Green note compounds are substances responsible for the aroma of foliage, freshly cut grass, cucumber, apples, and other fruits. They comprise the short-chain C_6-C_9 aldehydes: hexanal, hexenal, nonenal, nonadienal as well as the alcohols hexanol and hexenol [5]. The green note biosynthetic pathway in plants is well understood, and the responsible enzymes as well as their corresponding genes have been identified [5,6]. The most important enzyme of the green note pathway is a lipoxygenase (LOXs, EC 1.13.11). Lipooxygenase catalyzes of

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hydroperoxidation of the polyunsaturated fatty acids: linoleic or linolenic acid yielding the corresponding 9- or 13-hydroperoxy fatty acid. The hydroperoxyl fatty acids can be metabolized by a number of different enzymes (e.g. hydroperoxide lyase) of the oxylipin pathway in plants into volatile compounds [7,8].

The aim of this study was to evaluate selected oil cakes (linseed, rapeseed, hemp, safflower and camelina) in terms of their potential use as a source of lipids in the biosynthesis of green note aroma compounds by *Yarrowia lipolytica* yeast. The composition of fatty acids in individual oil cakes was verified to determine which of them is the richest source of linoleic or linolenic acids. In addition, the activity of lipoxygenase in the oil cake extracts was also determined to know to what extent the yeast enzymatic apparatus can be enhanced with the activity of enzymes derived from the oil cake.

2. Materials and Methods

2.1. Materials

The materials consisted of 5 types of oil cakes, obtained after pressing oil from hemp seeds, rapeseed, safflower, camelina and flax, using the cold method at a temperature not exceeding 40°C. The oil cakes were obtained from the Oleovital Company (Grajów, Poland). Chemicals were purchased from Avantor Performance Matrials Poland S.A. (Gliwice, Poland).

2.2. Soxhlet Extraction

In order to extract fat, 15 g of oil cakes were weighed, crushed in a mortar, and then the crushed raw material was transferred to a Soxhlet apparatus. Extraction was carried out for approximately 1 hour (10 extraction cycles) with a portion of 150 ml of dichloromethane. The extract was dried with $MgSO_4$. The solvent was evaporated using a rotaevaporator (Bűchi B-490 Heating Bath) which was operated with a vacuum pump. The extraction from individual oil cakes was performed in two repetitions.

2.3. Chromatographic Analysis

The samples of extracted oil cakes were converted to fatty acid methyl esters in accordance with the PN-EN ISO 12966-2: 2017 standard [9]. The analysis was carried out in a YL6100 gas chromatograph, equipped with a flame-ionization detector and a BPX-70 capillary column (60 m x 0.25 mm x 0.25 μm) with N_2 as a carrier gas at a linear flow rate of 1.1 mL/min. The split injector temperature was set to 225°C and that of the FID detector to 250°C. The oven temperature was programmed to increase from 70°C to 160°C at a rate of 15°C/min, then an increment of 1.1°C/min to 200°C and another increment of 30°C/min to reach 225°C. The temperature was kept at this temperature for 1 minute.

2.4. Lipoxygenase Extraction and Enzymatic Assay

Lipoxygenase was extracted from around 0,5g of each type of oil cake by adding 10 ml of one of 4 different pH levels buffers (pH=2, pH=4, pH=7, pH=9). The extracts were centrifuged at 4500 rpm and filtrated on Büchner funnel. Enzymatic activity was measured spectrophotometrically (Rayleigh UV-1601 spectrophotometer), at a wavelength of 234 nm, using linoleic acid emulsion.

A linoleic acid emulsion was prepared consisting: 20 μL of linoleic acid, 200 μL of Tween 20 and 4,78 mL of pH 7 buffer by mechanical agitation (Ultra-Turrax, 10,000 rpm). Samples for spectrophotometry were prepared by combining 1,98 mL of pH 7 buffer, 1 mL extracted liquid and 20 μL of linoleic acid emulsion.

2.5. Determination of Total Phenolic Content

Methanol extracts of oil cakes were prepared by *n*-hexane/methanol extraction. Total phenolic contents in the obtained methanolic extracts were determined using the Folin–

Ciocalteu method according to Rybak et al. [10]. The content of phenolic compounds was calculated as gallic acid equivalents (mg GAE/mL of extract from oil cakes).

2.6. Statistical Analysis

Statistical analyses were performed with Statistica 13.3 software. One-way analysis of variance (ANOVA) and Tukey's test were used to determine significant differences among means ($p < 0.05$).

3. Results and Discussion

In the first stage of the experiment, fatty acids were analyzed, determining the percentage of individual acids in the lipid fraction of selected oil cakes. The results are presented in Figure 1.

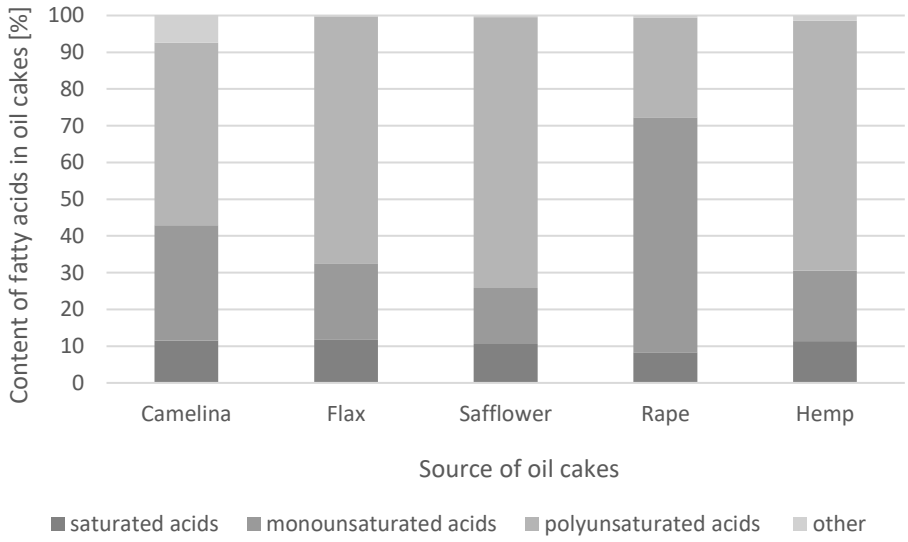

Figure 1. Percentage of monounsaturated, polyunsaturated and saturated acids in the lipid fraction extracted from oil cakes.

The collected data indicate that individual cakes differ in the composition of the lipid fraction. The fat fraction of rapeseed oil contains over 63% (63.95%) of monounsaturated fatty acids, while polyunsaturated fatty acids predominate in the remaining oil cakes analyzed. Flax, hemp and safflower oil cakes deserve special attention in terms of polyunsaturated fatty acids (PUFA) content. PUFA acids constitute from 67.18 ± 2.2% to 73.72 ± 1.8% in their extracts, respectively. In the context of the synthesis of green note aroma compounds, the presence of linoleic and linolenic acids (a precursors of green fragrances) is important. The highest concentration of linoleic acids, 44.77 ± 0.9%, occurs in the lipid fraction of hemp cake. In the other two raw materials mentioned above, the concentration of this acid ranges from 42.71 ± 1.1% to 43.07 ± 0.3%. Linolenic acid was present at a concentration of 23 - 31% in hemp and safflower cakes, respectively.

The key enzyme in the bioconversion of PUFA acids to green aroma compounds is lipoxygenase, therefore in the next stage of the study its activity was determined (Figure 2).

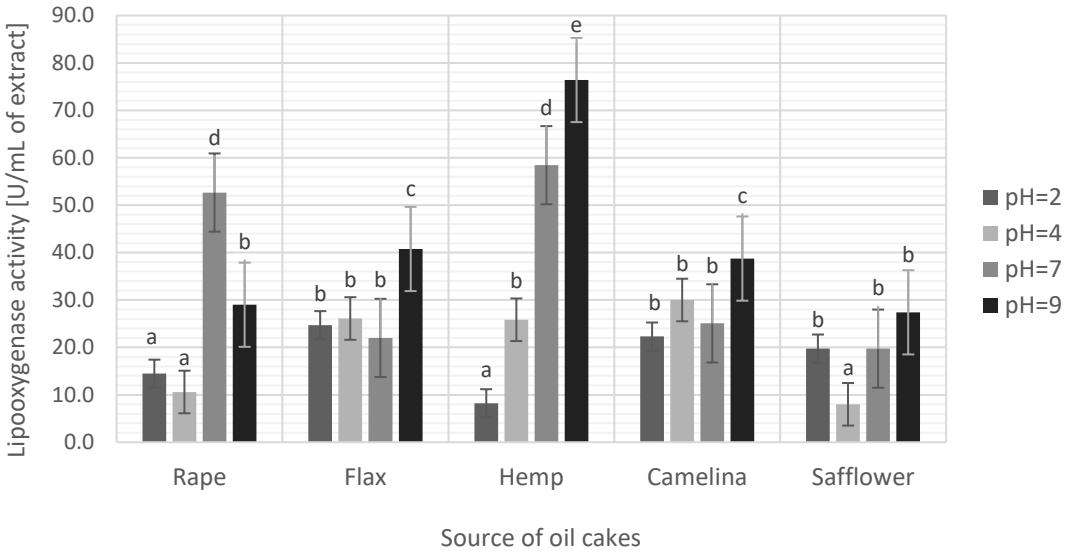

Figure 2. Lipoxygenase activity in the extracts of selected oil cakes. Mean values followed by the same letter are not significantly different according to HSD Tukey ($p \leq 0.05$).

In order to obtain an appropriately stable and active enzyme extract, the pH of the extraction environment was optimized. The assay used buffers with a pH range of 2-9. The differentiation of the extraction environment was dictated by the wide pH optimum of lipoxygenase activity. Literature data indicate the activity of these enzymes in the optimal pH range of 5-9 [11]. Analyzing Figure 2, one can see the influence of the extraction environment on the achieved enzyme activity. The highest lipoxygenase activity was observed in an alkaline environment, at pH 9, regardless of the type of oil cakes. Among the five tested raw materials, hemp extract had the highest lipoxygenase activity - 76.4 ± 1.2 U/mL. This value was approximately twice as high compared to other types of oil cakes.

Many scientists point out that the activity of lipoxygenase may be inhibited or disrupted by the presence of phenolic compounds in the reaction environment. Examples include studies indicating the inhibition of LOX in soybeans by polyphenols [12] or the inhibition of LOX activity by antioxidant compounds contained in olive extract [13].

To assess the inhibitory effect of phenolic compounds, in the next stage of the research, polyphenols were determined in the extracts of individual cakes using the Folin-Ciocolteu method. The results of this analysis are presented in Table 1, giving the polyphenol content expressed as equivalent of gallic acid.

Table 1. Total phenolic content in selected oil cakes.

	Source of oil cakes				
	Rape	**Flax**	**Hemp**	**Camelina**	**Safflower**
Total phenolic content [mg GAE/mL of extract from oil cakes]	$0.104^{a} \pm 0.025$	$0.198^{c} \pm 0.076$	$0.116^{a} \pm 0.005$	$0.137^{b} \pm 0.014$	$0.130^{b} \pm 0.010$

*Mean values followed by the same letter are not significantly different according to HSD Tukey ($p \leq 0.05$).

Flax oil cakes were characterized by the highest total phenolic content - 0.198 ± 0.076 mg GAE/mL, followed by camelina (0.137 ± 0.014 mg GAE/mL) and safflower oil cakes (0.130 ± 0.010 mg GAE/mL). The lowest concentration of phenolic compounds was found in rapeseed and hemp cakes. In the case of these raw materials, the dependence of the maximum LOX activity on the low phenolic compounds content is clearly visible.

4. Conclusion

This article presents promising research results on the valorization of oil cakes in the context of the biosynthesis of green note aroma compounds. The fatty acid composition of the lipid fraction extracted from the oil cakes indicates a high content of polyunsaturated acids, which are a substrate of bioconversion. Among the materials tested, hemp cakes attract particular attention. They are characterized by the highest content of linoleic and linolenic acid, and the extract obtained from them shows high lipooxygenase activity with a low concentration of phenolic compounds. High oxidative activity combined with the catalytic activity of microorganisms may give promising results in the context of efficient biosynthesis of green fragrance compounds. Therefore, it is worth undertaking further research involving hemp seed waste, striving to implement the assumptions of effective waste utilization and the successful implementation of the zero waste and a circular economy concepts. Currently, the valorization of waste in the perception of a circular economy is a global challenge.

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