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An Environmentally-Friendly Approach for the Release of Essential Fatty Acids from Cereal By-products Using Cellulose-Degrading Enzymes

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Abstract: The main intention of the present work was to investigate the ability of cellulose-degrading enzymes (C-DE) to release fatty acids (FAs) from complex matrices of cereal by-products during enzymatic hydrolysis (EH). For this purpose, three types of cereal bran (CB), i.e., wheat, rye, and oat were used as a lignocellulose substrate for three commercially available hydrolytic enzymes, i.e., Viscozyme L, Viscoferm, and Celluclast 1.5 L. The yield and composition of FFAs after EH was assessed and confronted with the yield obtained after either conventional Soxhlet extraction or alkaline-assisted hydrolysis (A-AH) with 10% KOH in 80% MeOH and subsequent liquid-liquid extraction. The experimental results demonstrated that up to 6.3% and 43.7% higher total FAs yield can be achieved within EH of rye bran using Celluclast 1.5 L than by A-AH and Soxhlet extraction, respectively. However, the application of Viscoferm for EH of wheat bran ensured up to 7.7% and 13.4% higher total FAs yield than A-AH and Soxhlet extraction, respectively. The concentration of essential linolenic acid (C18:3) in lipids extracted after EH of rye bran with Celluclast 1.5 L was up to 24.4% and 57.0% higher than in lipids recovered by A-AH and Soxhlet extraction, respectively. In turn, the highest content of linolenic in wheat bran lipids was observed after EH with Viscoferm and Viscozyme L, ensuring 17.0 and 13.6% higher yield than after A-AH, respectively. SEM analysis confirmed substantial degradation of CB matrix promoted by the ability of C-DE to act specifically on 1,4-β-D-glycosidic bonds in cellulose and on 1,2-α-,1,3-α-, and 1,5-α-L- arabinofuranoside and 1,4-β-D-xylosidic bonds in arabinoxylans, arabinans, and other arabinose-containing hemicelluloses. Structural alteration in cells integrity greatly contributed to the release of bound FAs and their better transfer into the extraction solvent. It has been shown that the proposed process of EH can be used for the efficient release of FAs from the CB matrix more sustainably and with a safer profile, thereby representing the further sustainable production of FAs for certain purposes.

Keywords: biorefining; by-products; enzymatic hydrolysis; essential fatty acids; green-extraction; lipids; sustainability

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1. Introduction

Technologies presently utilized for pretreatment of raw materials, i.e., pasteurization, sterilization, cooling, freezing, refining, defatting, deproteinization, debranning, etc., generally aimed at ensuring the safety of the end products result in substantial removal of valuable nutrients along with loss of functionality [1]. Cereal bran (CB) could be mentioned as one of the examples that despite the relative abundance of dietary fiber, are sorted out along the grain milling process and later disposed of as waste or utilized rather inefficiently [2]. Underestimation of CB as a food ingredient is conditioned by several drawbacks that are manifested in the form of negative influence on technological process and impairment of sensory quality of the end product by providing bitterness [3], reducing loaf volume, and contributing to textural changes, e.g., porosity, elasticity [4]. The reports of Jefremova et al. [5] and Aravind et al. [6] demonstrated that along with the increase of dietary fiber in the prepared products, the inclusion of wheat bran in the formulation of more than 7% and 30%, resulted in physical-chemical and sensory quality loss of bread and pasta, respectively. The adverse effect of CB was highlighted by Lebesi and Constantina [7], noting an increase in crumb firmness and darkening of cupcakes made with the addition of 10-30% leading also to lower sensory scores compared to control cupcakes. These observations have further been reinforced by Grigor et al. [8], conducting a meta-analysis of sensory acceptance of fiber-enriched cereal foods. It was revealed that adding as little as 2 g of fiber to the formulation resulted in moderate-large reductions of overall acceptability, flavor, and appearance in most elaborated products.

Due to palatability, refined food products rather than whole-grain products rich in dietary fiber are more appreciated by consumers [9]. There is a body of clinically-proven health benefits, however, supporting that whole-grain diets promote reductions in body weight, fat loss, systolic blood pressure, and LDL cholesterol level [10–14]. Given the above, new strategies aimed to process CB are needed to be elaborated that would allow attracting consumers with innovative food products enriched with nutrient back that were lost during processing, while not affecting the sensory quality of it. [15].

There have hitherto developed various pretreatment strategies aimed at valorization of CB to food and pharmaceutical applications, the vast majority of which, however, imply chemical modifications of plant cell walls by using potentially harmful to operators and the environment conditionally toxic solvents and catalysts [16,17]. Besides, physical and physicochemical pretreatments demonstrated such as mechanical extrusion [18] and steam explosion [19] are material-costly technologies requiring high initial investments [20]. Contrary to these methods, biorefining of lignocellulosic waste material due to its simplicity in operational and process conditions has been receiving tremendous interest amongst researchers as a potential strategy suitable for the enhancement of physical and physical-chemical features as well as production of biomolecules and chemicals [21,22]. Biorefining by enzymatic hydrolysis (EH) is an environmentally-friendly alternative that could ensure sustainability and circularity through the utilization of renewable raw materials and food-grade enzymes for obtaining high-added-value products and energy from biomass [23].

Owing to the relative abundance of phenolic compounds, which in CB are present mainly in non-extractable forms [24,25], the effectiveness of EH to release ferulic acid has repeatedly been proved [26,27]. Besides, the availability of dietary fiber, i.e., cellulose and hemicellulose [28,29] makes this material suitable for the production of xylooligosaccharides [30] and

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fermentable sugars, the compounds which are in demand for making functional food products and bioethanol, respectively [31,32]. The ability of commercial enzymes mixture and an *Escherichia coli* JM109 to produce biovanilin from wheat-bran-derived ferulic acid was demonstrated by Gioia et al. [33]. Furthermore, the single-step optimized process of EH utilizing filamentous fungi *Rhizopus oryzae* wild1.22 ensured the yield of fumaric acid up to 20.2 g L^{-1} from acid pretreated wheat bran hydrolysates [34].

The EH as a type of CB pretreatment has been well-studied, demonstrating the capability of hydrolytic enzymes to modify plant cell walls with the simultaneous release of valuable hydrophilic compounds. Less explored, however, is the influence of hydrolytic enzymes on the yield and chemical profile of lipophilic fraction (FAs) obtained upon direct hydrolysis of CB, in particular wheat, rye, and oat. EH as a novel plant pretreatment approach for the extraction/ release of FAs has been shown in a few reports by [35–37], where the effectiveness of hydrolytic enzymes in altering cell-wall structure and release of FAs was confirmed by observing a higher yield and qualitative profile of oils than from untreated matrices. Given that the majority of FAs (mainly in the form of triacylglycerols) in grain kernels are located in aleurone, and starchy endosperm fractions [38–40], it is hypothesized that the designed process of EH can be used for the efficient release of FAs from bran matrix in a more sustainable way and safer profile, thereby could represent the further sustainable production of FAs for certain purposes.

The main intention of the present study was to study the ability of cellulose-degrading enzymes (C-DE) to release fatty acids (FFAs) from complex matrices of cereal by-products during enzymatic hydrolysis (EH).

2. Materials and Methods

2.1. Plant Material

Three types of commercial food-grade CB samples were gathered from a local supplier the Ltd. "Voldemars", separated as wheat (*Triticum aestívum* L.), rye (*Secale cereále* L.), and oat (*Avena sativa* L). According to morphological assessment, such hydrological layers as inner pericarp (tube cells, cross cells), outer pericarp, aleurone and hyaline layers with attached starch granules, seed coat (testa). The proximate composition of the CB samples is shown in Table 1.

Table 1. Nutritional composition of cereal bran by-products derived from rye, wheat, and oat grains, g 100 g^{-1} DW.

	Major Nutrients Profile, g 100 g^{-1} DW					
Type of Bran	**Moisture, %**	**Crude carbohydrates**	**Starch**	**Crude Lipids**	**Crude Proteins**	**Dietary Fiber**
Wheat	11.9± 0.2 ^{a}	20.3 ± 0.4 ^{c}	8.7 ± 0.0 ^{c}	4.5 ± 0.1 ^{b}	16.2 ± 0.4 ^{a}	46.5 ± 2.1 ^{a}
Rye	11.7± 0.2 ^{a}	30.9 ± 0.5 ^{b}	18.6 ± 0.0 ^{b}	3.8 ± 0.1 ^{c}	16.9± 0.5 ^{a}	36.0 ± 1.9 ^{b}
Oat	12.4± 0.3 ^{a}	50.0 ± 0.9 ^{a}	47.6 ± 0.9 ^{a}	6.7 ± 0.5 ^{a}	14.0 ± 0.7 ^{b}	14.0 ± 1.7 ^{c}

Note: Values are means ± SD values of triplicates (n = 3). Means within the same column with different superscript letters (a, b, and c) are significantly different at $p < 0.05$; DW – dry weight; CH – carbohydrates; DF – dietary fiber.

2.2. Plant Material Preparation for Alkaline and Enzymatic Hydrolysis and Analysis of Hydroxycinnamates

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Each CB sample before EH was ground to reach Ø 0.5 mm particle size using the water-cooled "KN 295 Knifetec™" rotor mill (FOSS, Hilleroed, Denmark). Inactivation of native microorganisms and enzymes was done by mixing CB samples with double distilled water (DDW) at a ratio of 1:10 *w*/*v* in 50 mL reagent bottles with screw caps (VWR™, International, GmbH, Darmstadt, Germany) with the following subjecting to autoclaving using a digital autoclave with counter-pressure "Raypa, AES 110" (Barcelona, Spain) for 10 min at 121 ± 1 °C temperature and counter pressure 2.0 Pa. After thermal conditioning, the liquid fraction was decanted while solids were freeze-dried using a "Christ Alpha 1-2 LDplus" freeze-drying system (Osterode near Harz, Germany) at −51 ± 1 °C under a vacuum of 0.070–0.080 mBar for 72 h. Dried solids were packed in polypropylene zip-lock silver bags (high-density polyethylene polymer, density 3 mm, Impak Co., Los Angeles, CA, USA) (200 g in each) and stored at a temperature of −18 ± 1 °C until further analysis and use for a maximum of two wk. Moisture content was analyzed gravimetrically as proposed by Ruiz [41].

2.3. Chemicals and Reagents

A standard solution containing a mixture of C_4-C_{24} fatty acid methyl esters (FAMEs) with purity ≥99.0% was acquired from Sigma-Aldrich Chemie Ltd., (St. Louis, MO, USA). Sodium hydroxide (NaOH), potassium hydroxide (KOH), citric acid ($C_6H_8O_7$), sodium citrate dihydrate ($C_6H_5Na_3O_7{\cdot}2H_2O$), phenolphthalein ($C_{20}H_{14}O_4$), 0.5M trimethylphenylammonium hydroxide solution $(CH_3)_3N(OH)C_6H_5$ (TMPAH) in methanol (MeOH) for GC derivatization were of reagent grade gathered from Sigma-Aldrich Chemie Ltd. HPLC grade MeOH, pyridine (C_5H_5N), and boron trifluoride–methanol solution (BF_3/MeOH) were purchased from Sigma-Aldrich Chemie Ltd. Petroleum ether (puriss p.a., ≥99.9%, boiling point 40–60 °C) and diethyl ether ($(C_2H_5)_2O$)) (puriss p.a., ≥99.5%) were obtained from Chempur (Piekary Śląskie, Silesia, Poland). The ultrapure water was produced using the reverse osmosis PureLab Flex Elga water purification system (Veolia Water Technologies, Paris, France).

2.4. Enzymes

Commercially available food-grade C-DE preparations have been provided in kind by the company Novozymes® (Bagsvaerd, Denmark) for laboratory purposes. Since each enzyme preparation represents a mixture of diverse cellulolytic and xylanolytic enzymes, in this work they were used individually rather than as a mixture. A list of enzymes used is depicted in Table 2.

Table 2. The list of commercial cellulose-degrading enzymes was applied in this study.

Commercial Enzyme	Declared Activity	Enzyme Activity	Source	EC number
Viscozyme® L	100 FBG g^{-1}	Endo-1,3-(1,4)-β-D-glucanase, Endo-1,4-β-xylanase, Non-reducing end α-L-arabinofuranosidase	*Aspergillus aculeatus*	3.2.1.8 3.2.1.55 3.2.1.4
Viscoferm®	222 FBG g^{-1}	Endo-1,4-β-xylanase, Endo-1,3-(1,4)-β-D-glucanase	*Aspergillus* spp.	3.2.1.8 3.2.1.4
Celluclast® 1.5 L	700 EGU g^{-1}	Endo-1,3-(1,4)-β-D-glucanase	*Trichoderma reesei*	3.2.1.4

Note: EC – enzyme commission; EGU – endoglucanase units; FBG—fungal β-glucanase units.

2.5. Soxhlet Extraction

Soxhlet extraction was done using the method of Abdolshahi et al. [42] with slight modification. Triplicate samples about of 10 g of freeze-dried and finely ground CB were accurately weighted in extraction cellulose cotton thimbles (Whatman single thickness, 25 × 100 mm) (VWR™, International, GmbH, Darmstadt, Germany). Further, the thimbles were placed inside the extraction chambers and subjected to Soxhlet extraction using the system B-816 (BÜCHI Labortechnik AG, Flawil, Switzerland), which is fully automated. The extraction of the lipophilic fraction was done for 6 h using petroleum ether as the extraction solvent. Sufficient heat (heating plate temperature 195 °C) was used to give about 10 cycles of solvent per h. In the final preparatory stage, collected lipophilic fraction was dried for 30 min to release the solvent from the extraction beakers. To determine the yield of crude lipids, the collected samples were placed in a desiccator to cool and then weighted. Afterward, the collected dry lipids were re-dissolved in 3 mL of petroleum ether, filtered through a polytetrafluoroethylene hydrophobic (PTFE) membrane filter with a pore size of 0.45 µm (VWR™, International, GmbH.). The obtained filtrates were quantitatively transferred to 20 mL scintillation glass vials (Kimble® DWK Life Sciences, Millville, NJ, USA) and hereafter flushed with N_2 for 10 min to complete dryness. Dry residues were stored at a temperature of −18 ± 1 °C until further analysis and use, a maximum of five wk.

2.6. Preparation of the Lipid Fraction by Enzyme-Assisted Hydrolysis of Wheat, Rye, and Oat Bran with Subsequent Liquid-Liquid Extraction

EH of CB samples utilizing biocatalysts was performed in a water bath "SW23" with a capacity of 20.0 L and a horizontal shaking, and thermostatic and temporal control system (Julabo®, Saalbach-Hinterglemm, Germany). The optimal conditions for each enzyme were chosen individually based on Novozymes® guidance and following the protocol described by Juhnevica-Radenkova et al. [26]. The EH of N-SPs, i.e., cellulose and hemicellulose was accomplished using three commercially available multi-enzyme complexes, i.e., Viscozyme L, Viscoferm, or Celluclast 1.5 L. For this purpose, 30 mL 0.5 M sodium citrate buffer with the pH 4.6 containing 6 FBG mL^{-1} of endo-1,4-β-xylanase (Viscozyme L or Viscoferm), or 10 EGU mL^{-1} of endo-1,4-β-D-glucanase (Celluclast 1.5 L) was added to 3 g of each CB sample. The mixture was then vortexed for 2 min using the "ZX3" vortex mixer (Velp® Scientifica, Usmate Velate, Italy) and incubated in a water bath at 44 ± 1 °C and 100 rpm. After 48 h of EH to terminate the reaction, the obtained hydrolysates were subjected to thermal processing for 10 min at 99 ± 1 °C. The extraction of the lipophilic fraction was accomplished by liquid-liquid phase separation using petroleum ether as a solvent. Cooled down to ambient temperature (22 ± 1 °C) hydrolysates were quantitatively transferred to Falcon 50 mL conical centrifuge tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany). Afterward, 10 mL of petroleum ether was added to each tube, followed by vortex-mixing for 1 min. Separation of the layers was done by centrifugation at 4500 rpm (3169× *g*) for 10 min in a "Sigma, 2-16KC" centrifuge (Osterode near Harz, Germany). The top petroleum ether layer was separated and collected. The extraction procedure was repeated three times. The resulting lipophilic fraction (30 mL) was further evaporated using a "Laborota 4002" rotary evaporator (Heidolph, Swabia, Germany) at 65 °C and the dry fraction was then re-dissolved in 3 mL of petroleum ether, filtered through a PTFE membrane filter with a pore size 0.45 µm. The filtrates were quantitatively transferred to 20 mL scintillation glass vials and then subjected to drying under a gentle stream of N_2 to

complete dryness. Dry residues were kept at a temperature of −18 ± 1 °C until further analysis and use, a maximum of five wk.

2.7. Preparation of the Lipid Fraction by Alkaline-Assisted Hydrolysis of Wheat, Rye, and Oat Bran with Subsequent Liquid-Liquid Extraction

For the destruction purposes of the CB matrix and the release of bound forms of FAs, the 10% (*w*/*v*) KOH dissolved in 80% MeOH (*v*/*v* MeOH) was used. In the supremacy of MeOH, this approach allows the process of hydrolysis and release of FAs to be done more efficiently. Triplicate samples of 3.0 ± 0.1 g of ground CB were weighed in 50 mL reagent bottles with screw caps. For the hydrolysis 30 mL of prepared methanolic KOH was added to each CB sample and the mixture was subjected to incubation in a water bath "TW8" (Julabo®, Saalbach-Hinterglemm, Germany) at 65 °C for 3 h. After hydrolysis, the release of FAs from the salt form was done by shifting the pH of the medium from alkaline to acidic by adding 6 M HCl until the pH was 2.0 (10 mL). The extraction of lipophilic fraction was done using the liquid-liquid phase separation as described above (please refer to Section 2.6).

2.8. Preparation of Fatty Acids for GC/MS Analysis Using 0.5M Trimethylphenylammonium Hydroxide Solution (TMPAH)

The 0.5M trimethylphenylammonium hydroxide solution (TMPAH) in methanol (MeOH) reagent was utilized as a methylation agent. The methylation of polyfunctional groups to obtain volatile FAMEs derivatives was performed according to the protocol provided by Radenkovs et al. [21] with modifications. Briefly, 0.5 µL of lipid fraction was mixed with 10 µL of 1% phenolphthalein indicator ($C_{20}H_{14}O_4$:EtOH ratio 1:99 *w*/*v*), 70 µL of 0.5M TMPAH reagent and 919.5 µL of methanol:diethyl ether (MeOH:Et_2O ratio 50:50 *v*/*v*) in 2 mL chromatographic vial. The resulting permanent pink mixture was vortex-mixing for 1 min and kept in a GC oven at 60 °C for 30 min. Further, eliminating the mixture cooling step, the sample was injected into the heated injection port of the chromatograph, where the TMPAH salts were subjected to pyrolysis with subsequent transformation to their respective methyl esters.

2.9. The GC Conditions for FAMEs Analysis

The analysis of FAMEs was carried out on a "Clarus 600" system PerkinElmer, Inc. (Waltham, MA, USA) equipped with a quadrupole analyzer "Clarus 600 C" mass-selective detector (Waltham, MA, USA). The chromatographic separation of FAMEs was done using "Trace™ TR-FAME" (Thermo Fisher Scientific, Waltham, MA USA) column with a cyanopropylphenyl-based stationary phase (50 m × 0.22 mm, sorbent thickness – 0.25 µm) specifically designed for the separation of *cis*- and *trans*- isomers of FAMEs (Figure 1). The injector temperature was set to 280 °C; automatic injection using an autosampler, injection volume 0.5 µL; split ratio 4:1. The initial oven temperature was maintained at 70 °C for 2 min, then raised to 150 °C (rate of 20 °C min^{-1}), then increased to 250 °C (rate of 4 °C min^{-1}). Helium (ultra-high purity 5.0 grade – 99.9%) was used as a carrier gas at the constant flow rate of 1.0 min. The total separation time was 31.00 min. The analysis was performed in triplicate.

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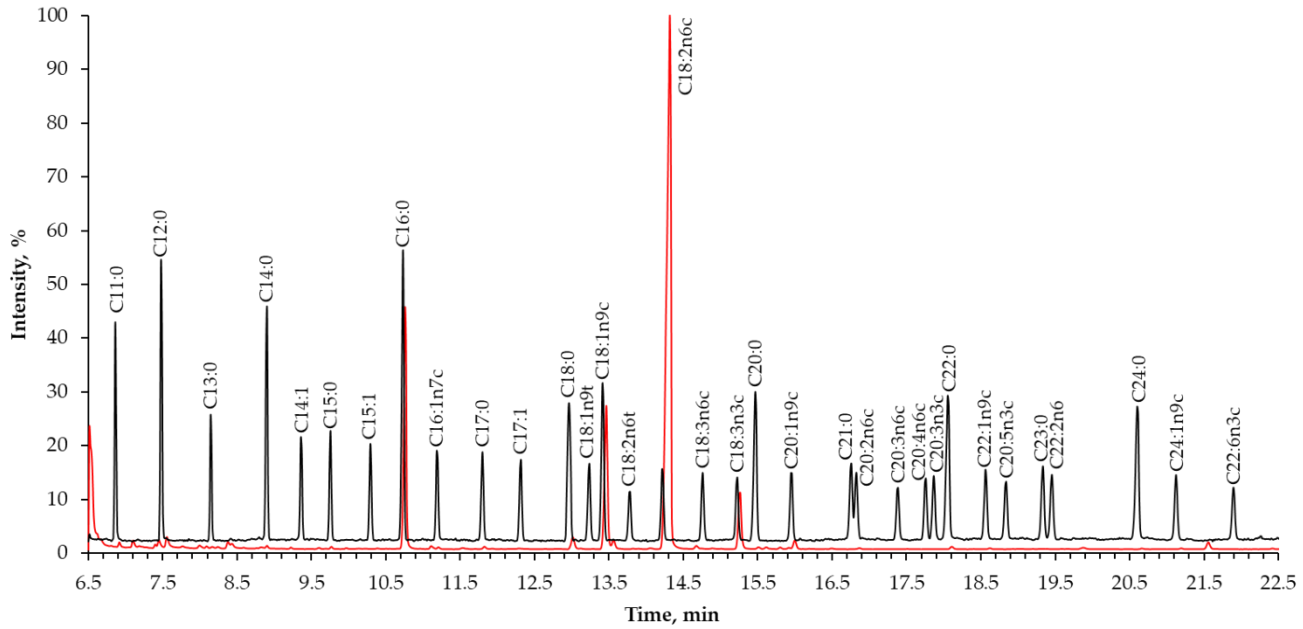

Figure 1. Representative chromatographic separation of C_4-C_{24} fatty acid methyl ester standards (black line) and FAME of lipids extracted after enzyme-assisted hydrolysis of rye bran using cellulose-degrading enzyme Celluclast 1.5 L for 48 h (red line). Samples injection volume 1.0 uL (0.5 ug mL^{-1}).

2.10. The MS Conditions for FAMEs Detection

Detector mode: Electron impact ionization was at 70 eV; ion source temperature: 230 °C; inlet temperature was 250 °C; capture time starting from 6.5 min (1.7 scan s^{-1}); ion multiplier: 240 V; and ion m/z interval: 41–500 atom mass units (AMU) for FAMEs.

2.11. The HPLC-RID Conditions for Carbohydrates Analysis

Quantitative analysis of mono- and disaccharides in hydrolysates after EH was accomplished on a Waters Alliance HPLC system (model No. e2695) coupled to a 2414 RI detector and a 2998 column heater (Waters Corporation, Milford, MA, USA) following the methodology described by Juhnevica-Radenkova et al. [26].

2.12. Scanning Electron Microscopy (SEM)

The morphology of untreated control and EH CB was analyzed by SEM using a Tescan Mira/LMU scanning electron microscope (Brno-Kohoutovice, Czech Republic) according to the method proposed by Juhnevica-Radenkova et al. [26].

2.13. Statistical Analysis

The results obtained are shown as means ± standard deviation of the mean from three replicates (n = 3). A p-value of <0.05 was used to denote significant differences between mean values determined using one-way analysis of variance (ANOVA) and Duncan's multiple range test performed using IBM® SPSS® Statistics version 20.0 (SPSS Inc., Chicago, IL, USA).

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3. Results and Discussion

3.1. Structural Changes in Wheat, Rye, and Oat Bran Morphology Induced by Cellulose-Degrading Enzymes

The assessment of morphology and microstructure of native CB samples revealed uniform homogeneity of the epidermal layer with no obvious signs of cracking or fractures (Figure 2A). Such histological layers as outer and inner pericarp (outer fraction), cross and tube cells (intermediate fraction) with tight adherence to the walls of the aleurone layer (inner fraction) were distinguished by performing SEM analysis (Figure 2B). Two fractions of starch granules were observed in the starchy endosperm of CB samples (Figure 2C). Spherical in shape starch granules with a size smaller than 10 μm taking prevalence over disk-shaped granules with size 15–35 μm, making a strong carcass and holding the integrity of the CB matrix.

However, further analysis revealed clear and extensive decomposition of CB non-starch polysaccharides caused by EH of CB for 48 h with multi-enzyme complex Viscozyme L. The EH resulted in the partial fracture of the epidermal layer of CB and the opening cross and tube cell (helix) microfibers can be seen in Figure 2E. Performing EH, visible void spaces in testa (seed coat) and nucellar tissues with an approximate holes size of 20–30 μm were revealed which are depicted in Figure 2F. Alike epidermal layer degradation and disruption of the cell integrity was observed in rye bran samples subjected to 48 h EH with Viscozyme L, indicating an equal hydrolytic performance of enzyme selected (Figure 2H). A similar observation has been made by Zhang et al. [22], noting the ability of xylanase and cellulase enzymes to modify wheat bran structure during complex EH and as a consequence better physicochemical and functional properties. The observed alteration in CB microstructure is associated foremost with the ability of cellulolytic and xylanolytic enzymes to act specifically on 1,4-β-D-glycosidic bonds in cellulose and on 1,2-*α*-,1,3-*α*-, and 1,5-*α*-L- arabinofuranoside and 1,4-β-D-xylosidic bonds in arabinoxylans, arabinans, and other arabinose-containing hemicelluloses, releasing relatively shorter polysaccharides or oligosaccharides composed of glucose, xylose, or arabinose [26].

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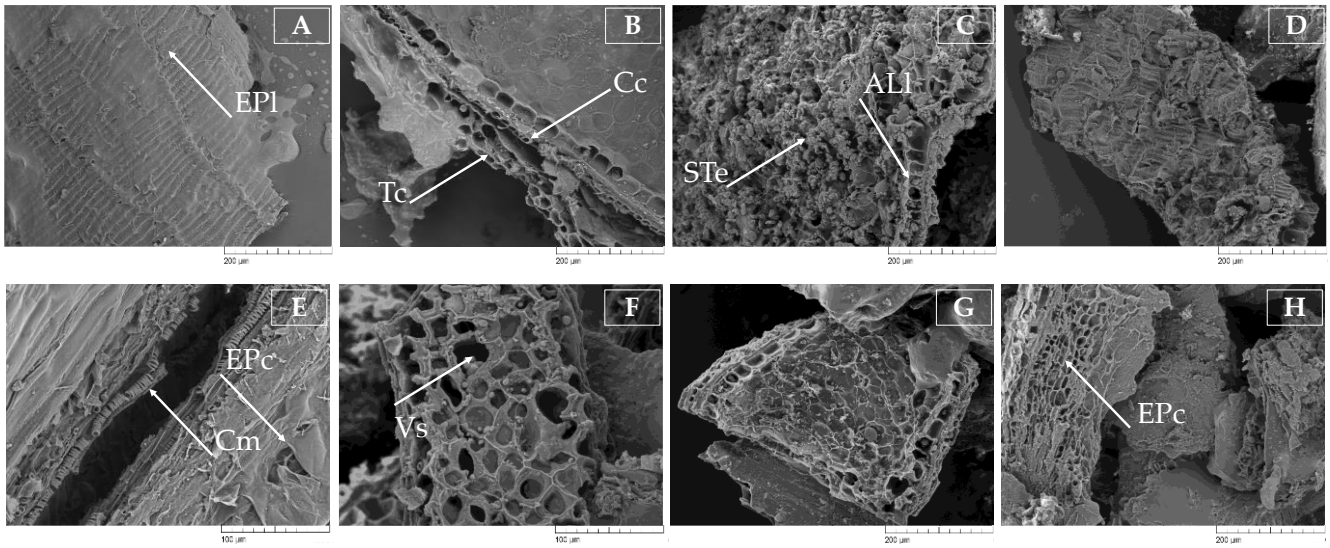

Figure 2. Representative SEM micrographs of untreated wheat (A,B) and rye (C,D) and enzymatically hydrolyzed wheat (E,F) and rye (G,H) cereal bran samples accomplished by cellulose-degrading commercial multi-enzyme complex Viscozyme L for 48 h. Note: EPl–epidermal layer; Tc–tube cells; Cc–cross cells; STe–starchy endosperm; ALl–aleurone layer; Cm–cellulose microfibers; EPc–epidermal layer cracking; Vs–void spaces in testa and nucellar tissues.

Given that the majority of FAs are distributed across the aleurone layer and starchy endosperm fraction, the action of C-DE would affect the release of subcellular organelles called oil bodies and thereby promote the mass transfer of FAs into the extraction solvent. This statement could be reinforced by an observation made by Kaseke et. al [34], revealing the ability of a hydrolytic enzyme cocktail, composed of Pectinex Ultra SPL, Flavourzyme 100 L, and cellulase in equal proportions to enhance the yield of oil, carotenoids, and phenolic compounds from EH pomegranate seeds.

3.2. Release of Mono- and Disaccharides from Wheat, Rye, and Oat Bran Using Enzyme-Assisted Hydrolysis

Based on the datasheets ensured by Novozymes® guidance, the enzyme preparations selected for EH of CB are multi-enzyme complexes consisting of such hydrolases as cellulase, xylanase, arabinofuranosidase that under favorable conditions promote the hydrolysis of cellulose, hemicellulose, and β-glucans present in CB. Simultaneous release of glucose, arabinose, xylose, maltose, and galactose can be achieved due to the breakdown of glycosidic bonds in these non-starch polysaccharides [43,44]. Therefore, the efficiency of EH in this study was assessed by determining the content of sugar monomers and dimers individually in CB that underwent hydrolysis with three C-DE for 48 h (Table 3). The results showed that glucose, xylose, arabinose, and fructose are the main end-products released after EH of wheat, rye, and oat bran samples. The number of sugars released was found to be the type of bran and hydrolytic enzyme-dependent. The prevalence of glucose over other sugars was observed in all CB hydrolysates. The glucose concentration fluctuated in the range of 7.9–47.6 g per 1000 mL^{-1} of bran hydrolysates, with oat bran having the highest content while wheat bran the lowest. A considerable higher amount of glucose in oat bran hydrolysates is due to the presence of β-glucans, which alongside starch and cellulose ensures the release of glucose

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monomers by endo-1,3-(1,4)-β-D-glucanase. Viscozyme L displayed superior hydrolytic performance since the yield of glucose monomers from wheat, rye, and oat bran were 58.4–126.6%, 163.4–58.4%, and 158.7–195.6% higher than that released after EH with Celluclast 1.5 L and Viscoferm, respectively. Higher glucose yield is conditioned by the composition and activity of hydrolytic enzymes presented in Viscozyme L preparation, which altogether promoted solubilization of cellulose and β-glucans and the rise of sugar monomers and dimers. This observation has been already confirmed in previous studies by Radenkovs et al. [21] and Bautista-Expósito et al. [45] working on CB hydrolysis with hydrolytic enzymes. In addition, the advantage of Viscozyme L over Celluclast 1.5 L was highlighted by Gama et al. [46], pointing to an increase in glucose level in the hydrolysates of apple pomace underwent EH.

The presence of xylose and arabinose monomers (except for oat after EH with Celluclast 1.5 L) was confirmed in all bran hydrolysates, the range of which fluctuated from 0.8 to 8.7 g 1000 mL^{-1} and from 0.8 to 6.2 g 1000 mL^{-1}. However, the ambiguity of the results obtained should be noted, since the highest yield of xylose in all bran hydrolysates was observed after EH with Celluclast 1.5 L, the enzyme which based on our knowledge have no xylanolytic activity. The highest yield of arabinose was observed in rye bran hydrolysates after EH with Viscozyme L, while no significant differences ($p < 0.05$) were found between arabinose values in wheat and oat bran hydrolysates and enzymes used.

Assessment of the total content of sugars released demonstrated that 1000 mL^{-1} wheat, rye, and oat bran hydrolysates contain up to 28.6 g, 48.6 g, and 52.9 g of sugars, respectively. In general, the highest content of total sugar was observed after performing EH with Viscozyme L, except for wheat bran, where no significant ($p < 0.05$) difference between Viscozyme L and Celluclast 1.5 L was revealed. Oat bran hydrolysates represented the highest total sugars content that was up to 84.9% and 8.8% higher in comparison to wheat and rye bran hydrolysates, respectively. A relatively high content of sugars is associated with a higher amount of starch in oat bran rather than with cellulolytic and xylanolytic activities of enzymes. Thermal processing that has been applied to terminate catalytic reactions after EH presumably contributed to the partial degradation of starch polysaccharide to its glucose monomer [47].

The concept of the Green Deal proposed by the EC could be supported by taking advantage of the ability of hydrolytic enzymes to release fermentable sugars more sustainably. Their further exploitation can be done by the production of bioethanol which would foster the use of grain by-products more efficiently and contribute to a circular economy [19,48].

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Table 3. The release of mono- and disaccharides after 48 h enzymatic hydrolysis of cereal bran samples using three multi-enzyme complexes, i.e., Viscozyme L, Viscoferm, and Celluclast 1.5 L (g 1000 mL^{-1} bran hydrolysate).

	Wheat bran				Rye bran				Oat bran			
Carbohydrate	**Control**	**Vzym**	**Vferm**	**Cell**	**Control**	**Vzym**	**Vferm**	**Cell**	**Control**	**Vzym**	**Vferm**	**Cell**
Xyl	0.4± 0.0 ^{d}	4.6 ± 0.0 ^{c}	6.1 ± 0.2 ^{b}	8.7 ± 0.1 ^{a}	0.2± 0.0 ^{d}	5.9 ± 0.2 ^{c}	7.8 ± 0.3 ^{b}	8.4 ± 0.1 ^{a}	n.d.	0.8 ± 0.0 ^{b}	1.2 ± 0.0 ^{b}	2.1 ± 0.0 ^{a}
Ara	0.5± 0.0 ^{b}	2.7 ± 0.0 ^{a}	3.2 ± 0.0 ^{a}	2.8 ± 0.0 ^{a}	0.6± 0.0 ^{d}	6.2 ± 0.2 ^{a}	4.9 ± 0.1 ^{b}	1.5 ± 0.0 ^{c}	n.d.	0.8 ± 0.0 ^{a}	0.9 ± 0.0 ^{a}	n.d.
Fru	n.d.	2.1 ± 0.0 ^{a}	0.7 ± 0.0 ^{b}	0.4 ± 0.0 ^{b}	0.2± 0.0 ^{c}	1.7 ± 0.0 ^{a}	0.8 ± 0.0 ^{b}	0.5 ± 0.0 ^{b}	n.d.	1.6 ± 0.1 ^{a}	0.3 ± 0.0 ^{b}	n.d.
Glu	n.d.	17.9 ± 0.2 ^{a}	7.9 ± 0.1 ^{c}	11.3 ± 0.3 ^{b}	n.d.	32.4 ± 0.4 ^{a}	12.3 ± 0.2 ^{c}	15.9 ± 0.3 ^{b}	n.d.	47.6 ± 1.3 ^{a}	16.1 ± 0.4 ^{c}	18.4 ± 0.5 ^{b}
Suc	1.9 ± 0.1	n.d.	n.d.	n.d.	2.9 ± 0.1	n.d.	n.d.	n.d.	0.9 ± 0.1	n.d.	n.d.	n.d.
Mal	0.3 ± 0.0 ^{c}	0.9 ± 0.0 ^{b}	0.9 ± 0.0 ^{b}	3.1 ± 0.0 ^{a}	0.2 ± 0.0 ^{c}	1.9 ± 0.0 ^{b}	0.7 ± 0.0 ^{c}	2.6 ± 0.0 ^{a}	n.d.	1.9 ± 0.0 ^{a}	1.1 ± 0.0 ^{b}	1.8 ± 0.0 ^{ab}
Tot	3.1 ± 0.1 ^{c}	28.2 ± 0.2 ^{a}	18.8 ± 0.3 ^{b}	28.6 ± 0.4 ^{a}	4.1 ± 0.1 ^{d}	48.6 ± 0.6 ^{a}	26.5 ± 0.6 ^{c}	28.9 ± 0.4 ^{b}	0.9 ± 0.1 ^{c}	52.9 ± 1.4 ^{a}	19.7 ± 0.4 ^{c}	22.9 ± 0.5 ^{b}

Note: Values are means ± SD of triplicates (n = 3). Xyl–xylose; Ara–arabinose; Fru–fructose; Glu–glucose; Suc–sucrose; Mal–maltose; Tot–total sugars content, Vzym–commercial multi-enzyme preparation Viscozyme L; Vferm–commercial multi-enzyme preparation Viscoferm; Cell–commercial enzyme preparation Celluclast 1.5 L; n.d.–not detected. The concentration of sugars in control CB samples is expressed as g 100 g^{-1} on a dry weight basis. Means within the same carbohydrate and bran type with different superscript letters (^{a,b,c,d}) are significantly different at $p < 0.05$.

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3.3. Lipids Yield from Wheat, Rye, and Oat Bran

Total lipids in tested CB yielded applying liquid-liquid extraction following A-AH varied in the range of 4.0–7.5%, with rye bran having the lowest amount and oat bran the highest (Figure 3). The recovered amount of lipids was in line with those reported by [49]. Amount of lipids recovered after A-AH was 2.6%, 2.2%, and 2.1% higher than that of Soxhlet extraction. The yield of lipids obtained using EH with Viscozyme L delivered 1.4–4.9%, which is 0.4–1.5% lower than recovered using Soxhlet type apparatus. Similar to Viscozyme L, the yield of lipids with EH of CB by Viscoferm was reached 1.8–5.1%.

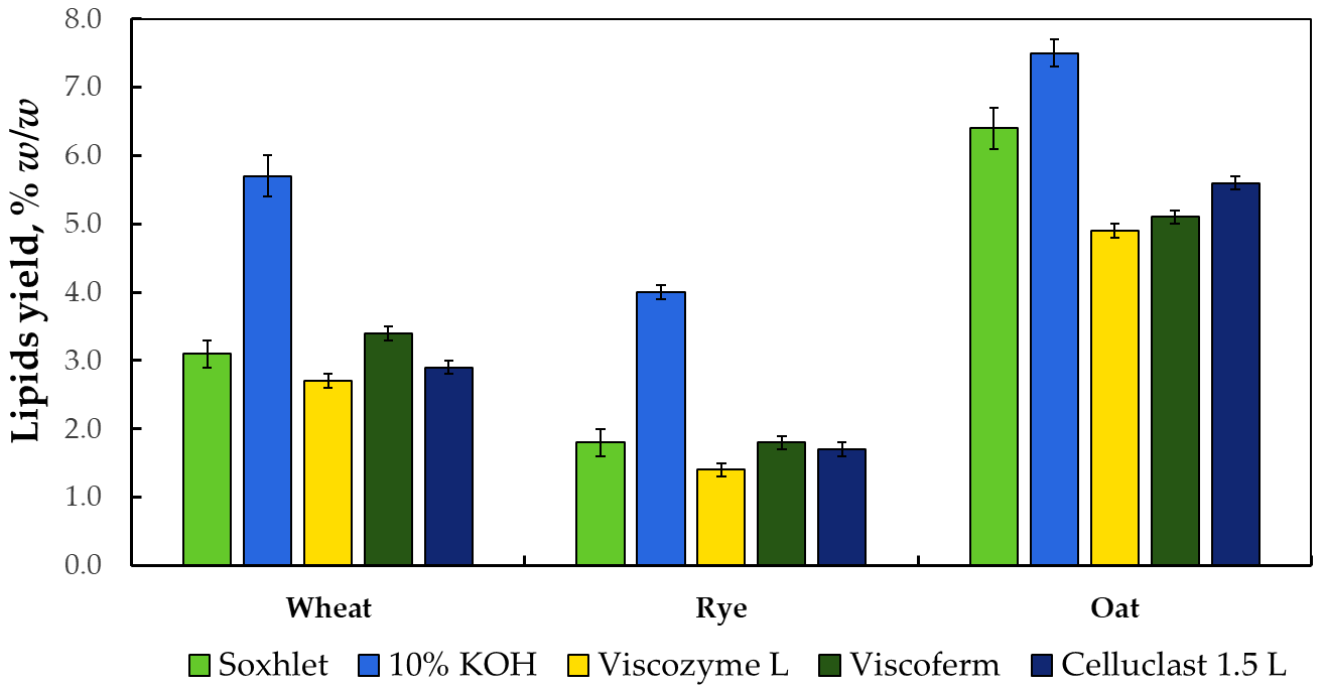

Figure 3. The content of lipids (% *w*/*w*) recovered from wheat, rye, and oat bran samples. Note: Values are means ± SD of triplicates (n = 3).

Although the amount of lipids extracted was 0.0–1.3% lower in comparison with the Soxhlet system. The advantage of Celluclast 1.5 L was confirmed by collecting a rather equal amount of lipids upon EH. The yield of lipids was in the range of 1.7 to 5.6%, which is 0.2–0.8% less than collected during Soxhlet extraction.

3.4. Effect of Alkaline- and Enzyme-Assisted Hydrolysis on Recovery of Lipids from Wheat, Rye, and Oat Bran

The composition of FAs in lipids recovered from wheat, rye, and oat bran is depicted in Tables 4–6. In total 18 FA were identified and quantified, among which the dominance of linoleic acid (C18:2) from 37.6 to 60.9%, followed by palmitic acid (C16:0) from 17.6 to 20.3%, oleic acid (C18:1) from 11.7 to 40.2%, linolenic acid (C18:3) from 0.8 to 4.9%, and stearic acid (C18:0) from 1.1 to 1.9% was found. The results are consistent with those of Narducci et al. [50], indicating a fairly similar descending order of FA content recovered from durum wheat grains.

Among saturated FAs (SFA), the prevalence of palmitic acid was observed in all bran samples and pre-treatment types applied. However, the highest concentration was revealed in oat, followed by wheat and rye bran lipids, corresponding to 137.0–150.5 mg g^{-1}, 127.0–143.9

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mg g^{-1}, and 90.2–125.5 mg g^{-1}, respectively. Up to 13.3% and 12.5% better palmitic acid release from wheat bran was achieved by subjecting the bran samples to EH with Viscozyme L and Viscoferm compared to Soxhlet type extraction, respectively. While a considerably higher yield of palmitic acid was recovered from rye bran samples after A-AH and EH with Celluclast 1.5 L, the values corresponded to an increase of 39.1% and 34.7% compared with Soxhlet extraction, respectively. However, neither EH nor A-AH did not have a tangible effect on the release of palmitic acid from the oat bran sample.

The concentration of oleic acid, the representative of monounsaturated FAs (MUFA) in wheat, rye, and oat bran lipids varied in the range of 104.5–121.3 mg g^{-1}, 64.2–87.2 mg g^{-1}, and 266.6–343.9 mg g^{-1}, respectively. Though, the results were inconsistent considering the yield of oleic acid as a function of enzyme and bran type. As seen, multi-enzyme complex Viscozyme L in the case of wheat bran samples demonstrated better hydrolytic performance since up to 16.1% and 14.3% higher yield of oleic acid was recovered than with Soxhlet extraction and A-AH hydrolysis, respectively. The superior hydrolysis efficiency and release of oleic acid was noticed after EH of rye bran with Celluclast 1.5 L. Up to 35.7% and 14.0% higher yield was reached compared with Soxhlet extraction and A-AH hydrolysis, respectively. It is worth noting that despite the highest sugar content being observed in rye bran hydrolysates (Table 3) obtained after EH with Viscozyme L, the release of oleic acid was using this enzyme is negligible. Considering this data become attainable to conclude that the release of oleic acid from rye bran matrix is rather associated with glycoside hydrolase activity (β-glucanase) that was the most intense in Celluclast 1.5 L than that of Viscozyme L and Viscoferm since the last two enzymes to a lesser extent were contributed to the release of oleic acid. A similar observation has been made by Radenkovs et al. [21], emphasizing the superiority of Celluclast 1.5 L in a more substantial performance of releasing polyunsaturated FAs over other hydrolytic enzymes. The obtained results indicate that rather a high side activity of triacylglycerol ester hydrolases likely present in Celluclast 1.5 L along with cellulolytic activity resulted in higher recovery of oleic acid through synergistic action. It is worth noting, that the content of oleic acid in lipids recovered from oat bran samples using either A-AH or EH was found to be 3.3–21.3% lower compared with lipids recovered with Soxhlet type extraction.

Among PUFA, the linoleic acid in CB lipids was found to be dominant, making the biggest contribution to the total content of PUFA in CB lipids. The concentration of linoleic acid varied in the range of 272.0–421.4 mg g^{-1}, with wheat and rye bran having the highest concentration and oat bran having the lowest. Obtained results are in line with data from [51], highlighting the supremacy of wheat lipids over barley, rice, sorghum, and oats. The highest linoleic acid recovery from CBs was achieved using EH with Celluclast 1.5 L, followed by Viscoferm, and Viscozyme L. Up to 49.1% and 10.7% higher release of linoleic acid was achieved after EH of rye bran with Celluclast 1.5 L compared with Soxhlet extraction and A-AH, respectively. However, the better recovery of linoleic acid from wheat bran was succeeded utilizing either Viscoferm or Viscozyme L, where up to 14.3% and 11.7% increase in the yield was observed compared with Soxhlet extraction, respectively.

The content of linolenic acid in lipids extracted from bran samples was found in the range of 6.1–33.9 mg g^{-1}, with rye bran lipids having the highest content and oat bran the lowest. Similar to oleic acid, the content of linolenic acid in rye bran lipids obtained after EH with Celluclast 1.5 L up to 57.0% and 24.4% higher than in lipids recovered with Soxhlet apparatus and after A-AH, respectively. The abundance of linolenic acid in wheat bran lipids was confirmed by getting up 17.0% and 13.6% higher content after EH with Viscoferm and Viscozyme L than A-AH did, respectively. The effectiveness of Viscozyme L over conventional

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solvent extraction has been highlighted by Díaz-Suárez et al. [52], pointing out on safer profile of recovered oil from castor seeds (*Ricinus communis*) seeds with an equal profile of FAs.

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Table 4. Fatty acid composition of wheat bran lipids according to the pre-treatment method, mg g^{-1} lipid.

Fatty acid	Wheat									
	Soxhlet		10% KOH		Vzym		Vferm		Cell	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Myristate C14:0	1.0	0.0	0.8	0.1	1.5	0.0	1.8	0.2	0.9	0.0
Pentadecanoate C15:0	0.8	0.0	1.0	0.1	0.8	0.0	0.8	0.0	0.7	0.0
Palmitate C16:0	127.0	1.9	132.3	3.1	143.9	1.2	142.9	1.9	126.4	2.0
Heptadecanoate C17:0	1.1	0.2	1.4	0.1	1.4	0.0	1.4	0.0	1.1	0.0
Stearate C18:0	8.5	0.3	8.6	0.3	9.6	0.1	9.7	0.0	8.5	0.0
Oleate C18:1 (c9)	104.5	1.1	106.2	2.5	121.3	2.3	117.2	0.9	104.8	1.9
Vaccenate C18:1 (t11)	2.6	0.4	2.3	0.0	2.8	0.2	3.0	0.0	2.0	0.1
Linoleate C18:2 (c9,c12)	365.8	0.0	379.9	7.3	408.6	6.1	417.9	4.2	377.6	10.8
Linolenate C18:3 (c9,c12,c15)	22.0	0.9	21.9	0.4	24.9	0.6	25.7	0.5	23.6	0.8
Arachidate C20:0	2.1	0.0	1.6	0.1	1.2	0.2	1.1	0.2	0.9	0.0
CLA linoleate C18:2 (c9,t11)	0.4	0.0	2.7	0.1	0.8	0.1	1.1	0.0	0.6	0.0
CLA linoleate C18:2 (t10,c12)	0.4	0.1	1.9	0.0	0.5	0.0	0.8	0.0	0.4	0.0
11-eicosenoate C20:1 (c11)	3.3	0.3	3.1	0.1	3.7	0.0	3.7	0.1	3.3	0.1
Eicosadienoate C20:2 (c11,c14)	BLQ	–	0.5	0.1	0.5	0.1	0.6	0.1	0.5	0.0
Behenate C22:0	3.1	0.7	3.3	0.2	2.2	0.2	2.0	0.1	1.7	0.1
Erucate C22:1 (c9,c11)	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
Tricosanoate C23:0	1.2	0.2	10.1	1.2	1.0	0.2	0.5	0.0	0.3	0.1
Tetracosanoate C24:0	0.8	0.2	1.1	0.1	0.8	0.1	0.6	0.0	1.3	0.1
SFA	145.6	3.6	160.3	5.2	162.5	2.1	160.8	2.5	141.7	2.4
MUFA	110.6	1.8	111.7	2.6	127.9	2.5	124.0	1.0	110.3	2.1
PUFA	388.6	1.0	406.9	7.9	435.4	6.9	446.1	4.9	402.7	11.7
$\sum_{MUFA + PUFA}$	499.2	2.9	518.6	10.5	563.3	9.4	570.1	5.9	513.0	13.7
$\sum_{SFA + MUFA + PUFA}$	644.8	6.5	678.9	15.7	725.8	11.5	730.9	8.4	654.7	16.1
CLA	0.8	0.1	4.6	0.1	1.4	0.1	1.9	0.0	1.0	0.0

Note: Values are means ± SD of triplicates (n = 3). SFA–saturated fatty acids; MUFA–monounsaturated fatty acids; PUFA–polyunsaturated fatty acids; CLA–conjugated linoleic acid; Vzym–commercial multi-enzyme preparation Viscozyme L; Vferm–commercial multi-enzyme preparation Viscoferm; Cell–commercial enzyme preparation Celluclast 1.5 L; L BLQ–below limit of quantification.

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The total content of FAs in bran-derived lipids is hydrolysis method dependent which varied in the range of 481.3–860.6 mg g^{-1} (Tables 4–6). Without reference to the CB pretreatment methods, the superiority of oat bran in the content of total FAs, including MUFA and PUFA over wheat and rye bran was revealed. The results are consistent with data obtained in an earlier study [53]. A relatively higher amount of FAs in oat bran than in other CB is explained by the presence of starchy endosperm, which is known to be a source of lipids [54]. The advantage of Celluclast L over other enzymes in the release of FAs from rye bran was established in this study. The experimental results demonstrated that up to 6.3% and 43.7% higher yield of total FAs can be achieved using EH with Celluclast 1.5 L than after A-AH and with Soxhlet extraction, respectively. However, in the case of wheat bran, the highest FAs yield was obtained after EH with Viscoferm. Up to 7.7% and 13.4% higher than after A-AH and with Soxhlet total FAs yield was ensured, respectively. In general, higher glycoside hydrolase activity (β-glucanase) declared for Celluclast 1.5 L led to a substantial breakdown of glycosidic bonds between starch and non-starch polysaccharides and lipids, which presumably promoted the release of FAs. Besides, superior xylanolytic than the cellulolytic activity of Viscozyme L and Viscoferm was also confirmed by [55], thereby explaining relatively less effectiveness in the release of glycolipids.

The extractability of FAs from the oat bran matrix using EH was observed to be the lowest since up to 3.8–17.6% lower yield of FAs was obtained than with Soxhlet. The EH of oat bran with Celluclast 1.5 L made the biggest contribution to the release of FAs, while Viscoferm had the lowest. Besides, no substantial release of FAs has been achieved even after the application of A-AH. It is assumed that a significantly lower content of FAs in oat bran lipids can be associated with its autoxidation reaction in the presence of oxygen and lack of natural antioxidants such as hydroxycinnamic and hydroxybenzoic acid derivatives abundantly present in wheat and rye bran hydrolysates [21]

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Table 5. Fatty acid composition of rye bran lipids according to the pre-treatment method, mg g^{-1} lipid.

Fatty acid	Rye									
	Soxhlet		10% KOH		Vzym		Vferm		Cell	
	Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV
Myristate C14:0	1.3	0.0	0.9	0.0	1.4	0.1	0.9	0.0	1.0	0.1
Pentadecanoate C15:0	0.8	0.0	1.2	0.0	0.8	0.1	0.9	0.1	0.8	0.1
Palmitate C16:0	90.2	1.5	125.5	1.3	117.1	0.6	114.0	2.0	121.5	3.3
Heptadecanoate C17:0	1.2	0.1	1.5	0.1	1.3	0.0	1.4	0.0	1.1	0.0
Stearate C18:0	6.9	0.2	7.2	0.2	6.8	0.3	6.6	0.0	7.5	0.4
Oleate C18:1 (c9)	64.2	1.4	76.5	1.1	71.2	0.1	72.8	0.5	87.2	3.3
Vaccenate C18:1 (t11)	1.7	0.0	2.3	0.1	2.0	0.2	2.0	0.3	2.3	0.2
Linoleate C18:2 (c9,c12)	282.7	6.4	380.6	9.1	341.4	4.6	357.1	5.5	421.4	8.8
Linolenate C18:3 (c9,c12,c15)	21.6	0.5	27.2	0.7	25.0	0.2	26.5	0.6	33.9	0.9
Arachidate C20:0	1.4	0.1	1.7	0.1	0.7	0.0	0.8	0.1	0.8	0.1
CLA linoleate C18:2 (c9,t11)	0.1	0.0	2.1	0.1	0.6	0.0	1.7	0.1	0.9	0.1
CLA linoleate C18:2 (t10,c12)	0.2	0.0	1.6	0.0	0.5	0.0	1.1	0.0	0.5	0.0
11-eicosenoate C20:1 (c11)	3.9	0.3	4.3	0.1	4.0	0.1	4.1	0.2	5.2	0.1
Eicosadienoate C20:2 (c11,c14)	BLQ	–	0.8	0.1	0.5	0.1	0.8	0.0	0.8	0.0
Behenate C22:0	2.7	0.1	2.9	0.1	2.0	0.1	1.8	0.0	1.6	0.1
Erucate C22:1 (c9,c11)	0.4	0.0	0.4	0.0	0.3	0.0	0.4	0.0	0.5	0.0
Tricosanoate C23:0	0.9	0.1	12.9	1.1	0.7	0.0	0.4	0.0	0.3	0.0
Tetracosanoate C24:0	1.1	0.1	1.1	0.1	0.7	0.1	0.6	0.1	4.6	0.0
SFA	106.4	2.2	154.9	3.1	131.6	1.2	127.2	2.3	139.2	4.1
MUFA	70.3	1.8	83.5	1.4	77.6	0.4	79.3	1.0	95.2	3.6
PUFA	304.6	6.9	412.3	9.9	368.1	4.9	387.2	6.2	457.3	9.9
$\sum_{MUFA + PUFA}$	374.9	8.6	495.8	0.0	445.7	5.4	466.5	7.2	552.5	13.5
$\sum_{SFA + MUFA + PUFA}$	481.3	10.9	650.7	14.3	577.3	6.5	593.7	9.5	691.7	17.6
CLA	0.3	0.0	3.7	0.1	1.2	0.0	2.9	0.1	1.3	0.1

Note: *Please see Table 4.*

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Table 6. Fatty acid composition of oat bran lipids according to the pre-treatment method, mg g^{-1} lipid.

Fatty acid	Oat									
	Soxhlet		10% KOH		Vzym		Vferm		Cell	
	Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV
Myristate C14:0	2.6	0.0	1.8	0.0	2.3	0.1	2.1	0.1	1.4	0.1
Pentadecanoate C15:0	BLQ	–	0.2	0.0	BLQ	–	BLQ	–	BLQ	–
Palmitate C16:0	150.5	0.1	149.0	0.7	144.4	0.4	137.0	2.4	143.1	0.5
Heptadecanoate C17:0	1.0	0.0	1.0	0.0	0.7	0.0	1.0	0.1	1.0	0.0
Stearate C18:0	16.6	0.2	14.4	0.2	14.0	0.2	12.4	0.2	15.8	0.1
Oleate C18:1 (c9)	343.9	4.8	300.0	1.0	270.6	1.8	266.6	1.3	332.6	0.8
Vaccenate C18:1 (t11)	4.2	0.1	4.7	0.1	3.7	0.0	2.4	0.3	3.2	0.4
Linoleate C18:2 (c9,c12)	323.7	4.5	321.4	2.9	272.9	1.0	272.0	1.3	314.1	3.0
Linolenate C18:3 (c9,c12,c15)	7.7	0.2	7.4	0.2	6.1	0.0	6.2	0.2	7.3	0.0
Arachidate C20:0	0.9	0.8	0.7	0.1	0.5	0.0	0.7	0.1	0.7	0.1
CLA linoleate C18:2 (c9,t11)	0.4	0.0	1.7	0.1	0.3	0.0	0.9	0.0	0.3	0.0
CLA linoleate C18:2 (t10,c12)	0.1	0.0	1.1	0.1	0.3	0.0	0.5	0.0	0.2	0.0
11-eicosenoate C20:1 (c11)	5.7	0.1	4.7	0.0	3.9	0.0	4.4	0.1	5.7	0.0
Eicosadienoate C20:2 (c11,c14)	BLQ	–	BLQ	–	BLQ	–	0.1	0.0	0.1	0.0
Behenate C22:0	1.7	0.2	1.3	1.7	1.6	0.1	1.3	0.1	1.3	0.0
Erucate C22:1 (c9,c11)	0.2	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
Tricosanoate C23:0	1.0	0.1	1.6	0.7	0.7	0.2	0.7	0.0	0.6	0.0
Tetracosanoate C24:0	0.5	0.0	0.4	0.0	0.4	0.0	0.3	0.0	0.4	0.0
SFA	174.8	1.4	170.5	3.7	164.6	1.0	155.5	2.9	164.3	0.9
MUFA	353.9	5.1	309.4	1.1	278.4	1.9	273.5	1.7	341.7	1.2
PUFA	331.8	4.7	331.7	3.3	279.6	1.1	279.7	1.6	322.0	3.1
$\sum_{MUFA + PUFA}$	685.7	9.8	641.1	4.4	558.0	3.0	553.2	3.3	663.6	4.3
$\sum_{SFA + MUFA + PUFA}$	860.6	11.2	811.6	8.1	722.6	4.0	708.7	6.2	828.0	5.2
CLA	0.4	0.0	2.8	0.2	0.6	0.0	1.3	0.1	0.4	0.0

Note: *Please see Table 4.*

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4. Conclusions

The present study was undertaken to clarify the ability of C-DE to release FAs from complex matrices of CBs during EH. For this purpose, three commercially available hydrolytic enzymes with cellulolytic, xylanolytic, and β-glucanolytic activities were tested and the cell-wall components' degradation products were selectively determined. Among hydrolytic enzymes selected, the superiority of Viscozyme L was revealed since the highest total sugars content in the rye and oat bran hydrolysates was observed after performing EH for 48 h. While no significant ($p < 0.05$) difference in the content of total sugars between Viscozyme L and Celluclast 1.5 L was observed in wheat bran hydrolysates. Oat bran hydrolysates contained the highest amount of total sugars and this amount was 87.6% and 8.9% higher than in wheat and rye bran hydrolysates, respectively. Relatively high sugar content is associated with a higher amount of starch in oat bran rather than with cellulolytic and xylanolytic activities of enzymes. Thermal processing used to terminate the catalytic reactions after EH presumably contributed to the partial degradation of starch polysaccharide to its glucose monomers. Structural alteration in cell walls integrity was confirmed by SEM analysis, and obvious signs of epidermal cracking or fractures were most evident after EH with Viscozyme L. In general, the highest content of total sugar was observed after performing EH with Viscozyme L, except for wheat bran, where no significant ($p < 0.05$) difference between Viscozyme L and Celluclast 1.5 L was revealed. The results showed that the action of C-DE promoted the release of subcellular organelles and the breakdown of glycosidic bonds between starch and non-starch polysaccharides and lipids in glycolipids, thus contributing to better transfer of FAs into the extraction solvent. Among the hydrolytic multi-enzymes tested, the advantage of Celluclast 1.5 L and Viscoferm was highlighted. Up to 6.3% higher content of total FAs in rye bran lipids was observed compared to A-AH. The application of Viscoferm ensured up to 7.7% higher content of FAs in wheat bran lipids than A-AH. The amount of oleic, linoleic acids, and linolenic in rye bran lipids extracted after EH with Celluclast 1.5 L was 14.0%, 10.7%, and 24.4% higher than in lipids after A-AH. A fairly lower but still relevant difference in the yield of oleic, linoleic acids, and linolenic between EH and A-AH lipids was observed after EH of wheat bran with Viscoferm, up to 10.4%, 10.0%, and 17.0% higher amount was achieved, respectively. In the context of waste-free technology, the established process of EH can find application within the food industry and successfully be used for the production of both FAs and fermentable sugars in a more sustainable way.

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