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Optimization of supercritical carbon dioxide extraction of rye bran using response surface methodology and evaluation of extract properties

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ABSTRACT

Rye bran, which is often discarded after flour milling, contains valuable compounds that may be used in the production of functional ingredients. In this study supercritical CO_2 extraction process of rye bran was optimized to obtain the highest extract yields by applying central composite design with three independent variables, pressure (25, 40, 55 MPa), temperature (30, 50, 70 °C) and dynamic extraction time (60, 90, 120 min). The importance of evaluated parameters could be arranged in the following order: temperature > pressure > dynamic extraction time. Calculated response surface model was found to be significant and enabled to select preferable extraction parameters: the highest extract yield (~2.5%) was obtained at 55 MPa, 70 °C, and 120 min. The interactions between different parameters were also evaluated. The dominant fatty acids in extracted oil were linoleic (61.09%), palmitic (13.74%), oleic (13.65%) and linolenic (6.37%); oxygen radical absorbance (ORAC) and DPPH• scavenging capacities of rye bran extract were 683.8 ± 45 and 62.28 ± 1.2 μM trolox equivalents/g, respectively, while total phenolic content was 14.62 ± 0.61 mg gallic acid equivalents/g.

1. Introduction

The bran is a hard part of a cereal grain, which is removed in the production of flour during milling process. Being rich in various bioactive compounds such as vitamins, phenolics, flavonoids, glucans and pigments the bran is used for various applications as a flour production by-product. For instance, bran products were reported as nutritional ingredients in functional foods possessing antioxidant and anticancer properties [1]. Wheat and rye are the major cereals for bread making; however, the composition of their grains is different; rye contains more fiber, less fat and storage protein, it is a good source of minerals and vitamins [2]. Rye bran is also a rich source of phenolic lipids, alkylresorcinols (1,3-dihydroxy-5-alkylbenzene homologs with an odd-numbered alkyl tail in the range of 15:0–25:0 carbon atoms), which are present at high concentration in the outer parts of wheat and rye grains [3,4].

Valuable compounds from cereal bran can be extracted with organic solvents by various conventional extraction procedures, which are usually time consuming and require large volumes of solvents, which are difficult to remove for extract purification [5–8]. Cleaner extracts may be obtained by using extraction techniques with pressurized liquids and/or supercritical fluids. Supercritical fluid extraction with carbon dioxide (SFE-CO_2) was applied for the extraction of rye bran and it was shown that extract yield was dependent on pressure and temperature [9,10]; however, due to amphiphilic character of alkylresorcinols pure CO_2 was not efficient. Two-step procedure was suggested, which consisted of removal on nonpolar lipids and further isolation of alkylresorcinol-rich fraction by adding 10% of co-solvent ethanol. Later it was advanced by supplementing with chromatographic purification procedure [11]. In the other study SFE-CO_2 was compared with ethyl acetate extraction, however no significant differences were found [12]. It was also suggested using intact kernels instead of milled ones because the former yielded the same amount of alkylresorcinols less diluted with other substances. Purified fractions were applied in bioactive emulsions protecting apples against *Penicillium expansum* [13].

Literature survey shows that the majority of studies on SFE-CO_2 of rye bran have been focused on the isolation of alkylresorcinol-rich fraction. However, optimization of SFE-CO_2 parameters for the isolation of CO_2-soluble fraction from rye bran has not been performed previously, while many reports demonstrated that

Abbreviations: CCD, central composite design; DW, dry weight basis; SFE-CO_2, supercritical carbon dioxide extraction; RSM, response surface methodology.

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application of response surface methodology (RSM) for the optimization of extraction parameters may substantially improve process effectiveness in terms of product yield [14,15]. Therefore, the aim of this study was to optimize SFE-CO_2 parameters for the isolation of the highest yields of non-polar rye bran fraction by using central composite design (CCD) with RSM and also to evaluate fatty acid composition and antioxidant capacity of extracts. It may be hypothesized that properly selected parameters may give substantially higher extract yields from rye bran.

2. Experimental

2.1. Materials

Rye brans, donated by AB "Kauno Grūdai" (Kaunas, Lithuania), were ground in a Retsch ZM 200 laboratory rotor mill (Miag, Braunscheweig, Germany) using different mesh size sieves for obtaining 3 fractions, <0.2 mm, <0.5 mm, and <1.0 mm. Carbon dioxide (99.9%) was obtained from AGA (Vilnius, Lithuania). Analytical grade solvent for extraction and HPLC grade solvents for chromatographic analysis were from Chempur (Piekary Śląskie, Poland). 6-Hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid (Trolox), [2,2-azobis(2-methyl-propionamidine) dihydro-chloride] (AAPH), Folin–Ciocalteu phenol reagent (2 M), 3,4,5-trihydroxybenzoic acid (gallic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH•) were from Sigma–Aldrich Chemie (Steinheim, Germany); fluorescein (FL) sodium salt from Fluka Analytical (Buchs, Switzerland). Randomly methylated β-cyclodextrin (RMCD) (Trappsol, pharmacy grade) was purchased from CTD Holdings, Inc. (High Springs, FL, USA).

2.2. Supercritical fluid extraction with CO_2 (SFE-CO_2)

SFE-CO_2 optimization experiments were performed in a laboratory extraction system Helix (Applied Separations, PA, USA) using 50 mL stainless steel extraction vessel filled with 10 g of ground rye bran. The flow chart diagram of the instrument used is presented elsewhere [14]. To avoid system clogging the sample was placed between two layers of coton wool. The volume of CO_2 was measured by a digital mass flow meter in standard liters per minute (SL/min) at a standard state ($P_{CO_2} = 100$ kPa,$T_{CO_2} = 20\,^{\circ}$C,$\rho_{CO_2} = 0.0018$ g/mL). The process consisted of static (10 min) and dynamic extraction steps. SFE-CO_2 at optimal conditions was upscaled in a pilot system (Applied Seperations, PA, USA) using 3650 g of bran flour and 10 L stainless steel extraction vessel. For exhaustive extraction in this system static and dynamic extraction time were prolonged to 30 min and 210 min, respectively. Collected extracts were kept at −22 °C temperature before analysis. Soxhlet extraction was performed in an automated extractor (Behr Labor-Technik, Düsseldorf, Germany) as a standard technique (AOAC) using hexane [16]. The solvent was removed in a rotary vacuum evaporator (Büchi, Flawil, Switzerland) at 42 °C and the residue was weighed by analytical balances. Extractions were replicated three times.

2.3. Oxygen radical absorbance capacity (ORAC) assay

Extracts obtained at optimal conditions in a pilot extraction system were analyzed using L-ORAC (oxygen radical absorbance capacity) assay [17]. Briefly, 10 mg of extract were dissolved in 1 mL of 7% RMCD solution in acetone/water (1/1, v/v) and diluted to a final concentration of 0.000033%. RMCD solution (7%) was used as a blank. The solutions of samples (25 μL) and fluorescein (150 μL, 14 μM) were placed in a 96 well black and opaque microplate with transparent flat-bottom, which was sealed and incubated at 37 °C for 15 min. Then AAPH solution as a peroxyl radical generator (26 μL, 240 mM) was added with a multichannel pipette and the microplate was inserted in a FLUOstar Omega fluorescent reader (BMG Labtech GmbH, Offenburg, Germany). The microplate was shaken prior to each reading, fluorescence measurements (excitation 485 nm and emission 510 nm) were recorded every 66 s, in total 120 cycles. At least three independent measurements were performed for each sample. Raw data were analyzed using software MARS (BMG Labtech GmbH, Offenburg, Germany). Antioxidant curves (fluorescence versus time) were normalized and the area under the fluorescence decay curve (AUC) was calculated as $AUC = 1 + \Sigma_{i=1}^{i=120} f_i/f_0$, were f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i. The final ORAC values were calculated by using a regression equation between the trolox concentration ant the net area under the curve (AUC). Trolox solutions in the concentration range of 0–250 μM were used for calibration and ORAC was expressed in trolox equivalent (TE) antioxidant capacity, μM TE/g of extract dry weight (EDW).

2.4. DPPH• scavenging assay

The assay [18] was performed in a 96-well microtiter plates using FLUOstar Omega fluorescent reader. The reaction mixture consisted of 7.5 μL of rye bran extract (1%) and 300 μL methanolic solution of DPPH• (6×10^{-5}M). The mixture was left to stand for 45 min in the dark and the reduction of DPPH• was determined by measuring the absorption at 515 nm. All measurements were performed in triplicate. Radical scavenging capacity (RSC) was determined from the calibration curve, which was drawn by using 50, 100, 125, 250, 500, 1000 μM/L concentration solutions of trolox and expressed in μM TE/g EDW.

2.5. Determination of total phenolic content (TPC)

Ten microliter of appropriate dilutions of the extracts or gallic acid solutions were oxidized with 190 μL Folin–Ciocalteau's reagent solution in deionized water (1:13) [19]. The reagents were mixed, allowed to stand for 3 min and then neutralized with 100 μL of 7% Na_2CO_3. The mixture was vortexed for 90 min and the absorbance was measured at 765 nm in a FLUOstar Omega reader. The TPC was calculated using gallic acid calibration curve and expressed in mg gallic acid equivalents per gram (mg GAE/g EDW).

2.6. Experimental design

Response surface methodology (RSM) using central composite design (CCD) [20] was applied for experimental design of rye bran SFE-CO_2. Three independent variables and their variation levels were chosen based on previously reported data: pressure (25–55 MPa), temperature (30–70 °C), and dynamic extraction time (60–120 min). It should be noted that the max level of pressure in our study was selected remarkably higher than the highest level (35 MPa), which was used in the previously performed studies with rye bran [9–12]. Francisco et al. [9] concluded that extract yield was proportional to pressure, when it is higher than 30 MPa. The number of experiments is defined by the formulae:

$$N = 2^f + 2f + c \quad (1)$$

where f is the number of factors and c is the number of center points. Complete design consisted of 20 experimental runs with 8 factorial points, 6 axial points and 6 center points. The multiple regression equation was used to fit the second-order polynomial equation based on the experimental data as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_{i+} \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j>1}^{3} \beta_{ij} X_i X_j \quad (2)$$