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ARTICLE

Determination of Antioxidant Capacity, Phenolic Acids, and Fatty Acid Composition of Rapeseed Varieties

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Three different analytical methods: ferric-reducing antioxidant power (FRAP), 2,2′-diphenyl-1-picrylhydrazyl (DPPH), and oxygen radical absorbance capacity (ORAC) were used for determination of antioxidant capacity of seven rapeseed varieties. Antioxidant capacity and levels of the total phenolic content, individual phenolic acids, fatty acid composition, and the selected physicochemical properties of the studied rapeseed cultivars were determined. Mean ORAC values for methanolic extracts of rapeseeds (4092−12989 mmol of Trolox/100 g) were significantly higher than FRAP and DPPH values (6218−7641 and 6238−7645 μmol of Trolox/100 g, respectively). Although FRAP and DPPH results were lower than ORAC values for all studied rapeseed varieties, there are linear and significant correlations between these three analytical methods (correlation coefficients ranged between 0.9124 and 0.9930, $p < 0.005$). Also, total phenolic compounds in rapeseeds correlated with antioxidant capacity (correlation coefficients ranged between 0.8708 and 0.9516, $p < 0.01$). Total phenolic acids determined by HPLC varied from 20.3 mg to 40.7 mg per 100 g of rapeseed flour, and the main phenolic acid is sinapic acid (17.4−36.4 mg/100 g). Fatty acid composition (SAFA = 7.2−8.6%, MUFA = 58.5−68.0%, PUFA = 24.7−33.9%) and the absence of *trans*-fatty acids indicate that the studied rapeseed varieties can be a source of unsaturated fatty acids and have a positive impact on human health.

KEYWORDS: Rapeseed varieties; antioxidant capacity; phenolic compounds; principal component analysis

INTRODUCTION

Rapeseed (*Brassica napus*) is the most important oil crop in the continental temperate regions and ranks second among oilseed crops produced worldwide. Cultivars strongly reduced in erucic acid and glucosinolates (00 quality) give one of the healthiest vegetable oils for human consumption. The development of oilseeds with altered lipid composition has been the subject of intensive research in recent years due to the industrial and nutritional importance of rapeseed oil (*1*−*3*). Fatty acid composition varied among varieties and changed according to environmental conditions (*4*). Rapeseeds and oil extracted from them contain low levels of saturated fatty acids (SAFA = 5−10%) and significant amounts of monounsaturated fatty acids (MUFA ranging from 44 to 75%), and some amounts of polyunsaturated fatty acids (PUFA = 22−35%) with a significant fraction of linolenic acid (9−13%) (*4*−*10*). This is an omega-3 PUFA that is acknowledged to provide protection from cardiovascular diseases by counteracting thrombosis (*11*).

Moreover, antioxidant compounds present in rapeseeds and the crude oil, including polyphenols, sterols, flavonoids, tocopherols etc., reveal an important function in prevention and treatment of some chronic diseases, such as heart, neurodegenerative, aging, cancer, and rheumatoid arthritis and exhibit antiradical activity (*12*−*21*). These compounds, as natural antioxidants, possess important biological and chemical properties such as antioxidant capacity, hydrogen peroxide production in the presence of certain metals, and the ability to scavenge active oxygen species and electrophiles, inhibit nitrosation reactions, and chelate metals (*22*).

Rapeseeds contain high amounts of phenolic compounds, which can be fractionated into free phenolic acids, soluble esters and glycosides of phenolic acids, and insoluble-bound phenolic compounds (*16*, *23*, *24*). The main free phenolic acid found in rapeseeds is sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid), although other phenolic acids (gallic, protocatechuic, syringic, chlorogenic, ferulic, vanillic, caffeic, *p*-coumaric, and *p*-hydroxybenzoic) were analyzed in rapeseeds as well (*12*−*20*). High performance liquid chromatography with diode array detector (HPLC-DAD) and UV detector (HPLC-UV) is the most common used method for determination of individual phenolic acids in methanolic and methanolic−water extracts from rapeseeds (*13*, *14*, *17*, *20*, *25*−*27*). Furthermore, liquid chromatography coupled with mass spectrometry (MS) was applied to determination of decarboxylated products of sinapine in rapeseeds (*27*).

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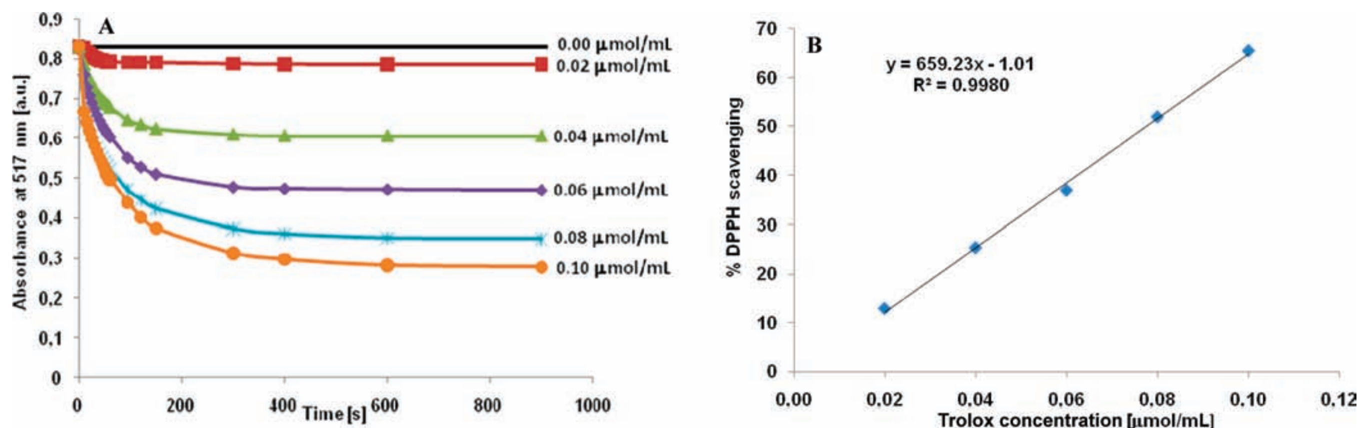

Figure 1. Kinetic profiles of DPPH free radicals scavenging by standard methanolic solutions of Trolox (**A**) and calibration curve for DPPH method (**B**).

Also, size exclusion high-performance liquid chromatography (SE-HPLC) (*28*) and gas−liquid chromatography (GLC) were used for determination of phenolic acids in rapeseed extracts (*16*, *24*). However, total phenolic content in rapeseeds was determined by spectrophotometric methods using the Folin-Denis (388−29600 mg/100 g) (*12*, *29*, *30*) and Folin-Ciocalteu (1505.5−2659.7 mg/100 g) (*14*, *20*) reagents.

To the best of our knowledge, only a few methods, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), β-carotene−linoleic acid, the reducing power and Trolox equivalent antioxidant capacity (TEAC), and electron spin resonance (ESR), have been used for determination of antioxidant capacity of six rapeseed and one canola varieties (*12*, *18*, *21*). However, there has been no reference to the determination of antioxidant capacity of rapeseed varieties by ferric-reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays.

Therefore, in this study, three different analytical methods, FRAP, DPPH, and ORAC, after some modifications, were employed for the determination of the total antioxidant capacity of seven winter rapeseed varieties. Moreover, total phenolic compounds, individual phenolic acids, fatty acid composition, and selected physicochemical properties (moisture and oil content in seeds) were analyzed, and possible correlations between these parameters and antioxidant capacity were studied.

MATERIALS AND METHODS

Plant Material and Chemicals. Seven commercial winter rapeseed varieties of *Brassica napus*, three pollinated: seed 1, seed 4, and seed 5 supplied by Monsanto Company (Lyon, France), hybrid variety - seed 2 and pollinated variety - seed 6 provided by Syngenta Seeds, Inc. (Warsaw, Poland), pollinated variety - seed 3 from KWS seed company (Einbeck, Germany) and pollinated variety - seed 7 (HR Strzelce, Poland) were stored in the dark at ambient temperature, until treatment and further analysis.

All reagents were of analytical or HPLC grade. 2,2′-Diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$, 95%), fluorescein disodium, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%), 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (AAPH, 97%), 2,4,6-tris-(2-pyridyl)-*s*-triazine (TPTZ, 99%), sinapic acid (98%), caffeic acid (98%), and ferulic acid (99%) were supplied by Sigma-Aldrich (Poznań, Poland). Gallic acid (98%) and *p*-coumaric acid (98%) were purchased from POCh (Gliwice, Poland). Methanol (99.8%) and acetic acid (99.7%) of HPLC grade were purchased from Chempur (Piekary Śląskie, Poland). Deionized water was used for the preparation of solutions.

Determination of Fatty Acid Composition. Fatty acid composition of rapeseed samples was determined according to the official method ISO 5508:1990 (*31*). Fatty acid methyl esters were prepared from the hexane extracted oils (ISO 5509:1990 (E)). Fatty acid analysis was carried out on a HP 5890 GC gas chromatograph with a flame ionization detector (FID) (Hewlett-Packard, Avondale, Pennsylvania, USA) and split/splitless injector. The column used was a 50 m × 0.22 mm i.d., 0.25 μm (SGE Pty. Ltd. Ringwood Victoria, Australia). The temperatures of injector and detector were adjusted to 250 °C, while oven temperature program was as follows: heating from 150 to 210 °C at 1.3 °C/min, hold at 210 °C for 5 min. The carrier gas was hydrogen at a flow rate of 0.6 mL/min.

Fatty acids were identified by comparing the gas chromatograph retention time of each peak with that of the authorized pure individual standard compounds, and they were quantified using the area under each fatty acid peak. A fatty acid standard was analyzed once a day to check the repeatability of the GC instrument. Triplicate measurements were performed.

Determination of Oil and Moisture Contents in Rapeseeds. Oil content was determined by solvent extraction according to AOAC methods (*32*). Moisture content was determined by vacuum drying at 60 °C to constant weight. All rapeseed samples were analyzed in triplicate.

Extraction Procedure. A portion (1.0−2.0 g) of grounded rapeseed sample and 15 mL of methanol−water (1:1 v/v) were transferred into a round-bottomed flask and shaken at room temperature for 60 min. Each sample was extracted in triplicate, and the residual rapeseed flour was separated by centrifugation (4500 rpm, 15 min). The pooled extracts were filtered and stored in a refrigerator prior to analysis.

Determination of Antioxidant Capacity. *FRAP Method.* Antioxidant capacity of the studied rapeseed cultivars was determined by the spectrophotometric FRAP method (*33*). In our procedure, freshly prepared FRAP reagent (2.5 mL of a 10 mmol/L TPTZ solution in 40 mmol/L HCl, 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 0.1 mol/L acetate buffer, pH 3.6) was incubated at 37 °C for 10 min. Then, 0.05 mL of rapeseeds extracts and 2 mL of FRAP reagent were transferred into a 10 mL volumetric flask and made up to volume with redistilled water. The obtained blue solutions were kept at room temperature for 20 min. The absorbance was measured at 593 nm against a reagent blank (2 mL of FRAP reagent made up to 10 mL with redistilled water) using a Helios α- spectrophotometer (Unicam, Cambridge, United Kingdom) in a 1-cm quartz cell.

DPPH Method. The modified DPPH method was used for determination of antioxidant capacity of rapeseed varieties. A methanolic solution (12.0 mg/L) of the radical DPPH$^{\bullet}$ was prepared daily and protected from light. Absorbance was recorded to check the stability of the radical throughout the time of analysis using a Helios α-spectrophotometer (Unicam, Cambridge, United Kingdom). The effect of methanolic Trolox standard solutions (0.02−0.10 μmol/mL) or methanolic rapeseed extracts on the DPPH$^{\bullet}$ absorbance was estimated, according to the following procedure: 0.5 mL of each Trolox solution (or extract) was added to 1.5 mL of methanol and 0.5 mL of DPPH$^{\bullet}$ methanolic solution (**Figure 1**).

Absorbance at 517 nm was recorded at different time intervals until the reaction reached an equilibrium. The initial absorbance was close to 0.830 in all cases. The blank reference cuvette contained methanol. All measurements were performed in 5-fold. The scavenging of DPPH was calculated as follows: %DPPH scavenging = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$, where $Abs_{control}$ = absorbance of DPPH radical + methanol; Abs_{sample} = absorbance of DPPH radical + standard (or seed extract). However, DPPH values expressed as micromoles of Trolox

equivalents per 100 g of seed samples were obtained from the following linear relationship: f(concentration of Trolox) = %DPPH for five Trolox standard solutions.

ORAC Method. The reaction mixture for the ORAC assay can be prepared in quartz cuvette as follows: 1.5 mL of 0.0816 μmol/L fluorescein disodium in 0.075 mol/L phosphate buffer (pH = 7.0), 0.25 mL of diluted methanolic rapeseed extract (0.01 mL into 250 mL volumetric flask), or 0.25 mL of Trolox standard solutions (0.008−0.048 μmol/mL) or blank (phosphate buffer). The mixture was kept 10 min at 37 °C in the dark, and the reaction was initiated by addition of 0.25 mL of 153 mmol/L AAPH. The fluorescence decay was measured at 37 °C every 1 min at 525 nm emission and 485 nm excitation, using a Hitachi F-7000 Fluorescence spectrophotometer (Hitachi Ltd., Tokyo, Japan). A calibration curve was generated using the net area under the curve (AUC) of fluorescein decay in the presence of five standard concentrations of Trolox (AUC_{Trolox}) minus AUC_{blank} for blank. $ORAC_{seed}$ values were obtained from the following linear relationship: f(concentration of Trolox) = ($AUC_{Trolox} - AUC_{blank}$). Data were expressed as milimoles of Trolox equivalents per 100 g of rapeseed samples.

Calibration curves were prepared using working solutions of Trolox in methanol between 0.002−0.018, 0.02−0.10, 0.008−0.048 μmol/mL for FRAP, DPPH, and ORAC methods, respectively. Five calibration curves were plotted using the least-squares method resulting in equations: $y = (42.36 \pm 0.28)x + (0.030 \pm 0.003)$, $R^2 = 0.9998$, $RSD_{slope} = 1.1\%$ for the FRAP method, $y = (659.23 \pm 17.14)x - (1.01 \pm 1.14)$, $R^2 = 0.9980$, $RSD_{slope} = 2.8\%$ for the DPPH method and $y = (9057.4 \pm 305.0)x + (230.2 \pm 9.5)$, $R^2 = 0.9955$, $RSD_{slope} = 3.2\%$ for the ORAC method. The calculated detection limits (2.98×10^{-4}, 4.93×10^{-3}, 3.38×10^{-3} μmol/mL for the FRAP, DPPH, and ORAC methods, respectively) and quantification limits (9.92×10^{-4}, 1.64×10^{-2}, 1.13×10^{-2} μmol/mL for FRAP, DPPH, and ORAC methods, respectively) for standard methanolic solutions of Trolox confirm the linear concentrations range for antioxidant capacity determinations of the investigated samples.

Determination of Phenolic Compounds in Rapeseed Varieties

HPLC Analysis. A Shimadzu HPLC system (Shimadzu, Kyoto, Japan) with a SPD-M20A diode-array detector (DAD) and SIL-20AC TH autosampler coupled with analytical software (LC Solution-Release 1.23SP1) were applied for determination of phenolic acids in rapeseed varieties. The column used was a 150 mm × 4.6 mm i.d., 5 μm, Discovery RP-C18, (Supelco, Bellefonte, PA). The column thermostat was set at 30 °C. The mobile phase was 2% acetic acid in water, pH 3.2 (A) and methanol (B) at a total flow rate of 1 mL/min. The gradient program of solvent A in B (v/v) was as follows: 0−25% B (11 min), 25−28.75% B (4 min), 28.75−36% B (10 min), 36−45% B (10 min), 45−65% B (3 min), 65% B−100% A (3 min), and finally 100% A (4 min). Eluates were detected at 254 nm (gallic acid), 295 nm (p−coumaric acid), and 325 nm (caffeic, ferulic, and sinapic acids). Peak identities were confirmed from retention data and by spiking of extracts with five standards. Calibration curves: $y = (54718 \pm 646)x + (508 \pm 450)$, $y = (30655 \pm 409)x + (3679 \pm 838)$, $y = (50023 \pm 431)x + (8169 \pm 7040)$, $y = (61893 \pm 636)x + (30754 \pm 3672)$, and $y = (20840 \pm 785)x + (2158 \pm 452)$ with determination coefficients of $R^2 = 0.9994$, 0.9991, 0.9997, 0.9994, and 0.9990 in the concentration range 0.0−25.0 mg/L of standard solutions of caffeic, p-coumaric, ferulic, sinapic, and gallic acids were obtained for the quantification of individual phenolic acids. The calculated values of the slope relative standard deviations (RSD = 2.3−2.5%, $n = 5$) indicate reasonable repeatability of the HPLC method. Moreover, limits of detection and quantification ranged between 0.02−0.26 mg/L and 0.07−0.87 mg/L, respectively, for HPLC analysis of all studied phenolic acids.

The rapeseed extracts were centrifuged, filtered through a 0.45 μm nylon filter, and analyzed directly by HPLC. After each run, the column was washed with 100% methanol and equilibrated to initial conditions for 15 min.

Determination of Total Phenolic Content (TPC). Total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu reagent, according to procedures described previously (*33*). Calibration curves were prepared for the working solutions of sinapic acid in the concentration range 1−10 μg/mL. Five calibration curves were plotted using the least-squares method resulting in equation $y = (0.072x \pm 0.001) + (0.040 \pm 0.004)$, $R^2 = 0.9993$, and $RSD_{slope} = 1.0\%$ ($n = 5$).

Statistical Analysis. The results of antioxidant capacity, total phenolic content, and individual phenolic acids in the studied rapeseed cultivars were determined (five portions of each extract analyzed within 1 day) by the FRAP, DPPH, ORAC, Folin-Ciocalteu, and HPLC methods, respectively. The obtained results were presented as mean (c) ± standard deviation (SD).

The reproducibility of the applied analytical methods was checked by five replicate determinations of antioxidant capacity, total phenolic content, amounts of individual phenolic acids in the same seed sample over a period of three days.

Moreover, the Pearson correlation test was used to determine the correlations between variables: antioxidant capacity results and total phenolic content in different rapeseed samples. Mean differences were considered significant at the $p < 0.05$ level. One-way ANOVA, followed by Duncan test, were performed to analyze the significant differences between data ($p < 0.05$).

Principal component analysis (PCA) was performed for the results of antioxidant capacity, total phenolic content, total phenolic acid, fatty acid composition, and physicochemical parameters of the studied rapeseed cultivars using the Statistica (Windows software package, version 8.0). PCA score plot was used to determine whether various rapeseed cultivars could be grouped into different classes.

RESULTS AND DISCUSSION

Composition Analysis of Rapeseed Varieties. The results of fatty acid composition and amounts of moisture and oil in the studied rapeseed samples are listed in **Table 1**.

It can be noted that the studied rapeseed varieties contain significantly different amounts of oil ranging between 40.5−46.9%, whereas the moisture content varied from 6.6 to 8.5%, with insignificant differences among seed 2 and seed 6, seed 3, seed 5 and seed 7 (Duncan test, $p > 0.05$). Fatty acid profiles are within the official ranges for rapeseed oils specified in the Codex Alimentarius (*34*); thus, the results obtained do not require any additional comments. It is noteworthy that all seven rapeseed varieties contain small amounts of saturated fatty acids (SAFA = 7.2−8.6%), whereas polyunsaturated fatty acids (PUFA) range between 24.7% (seed 6) and 33.9% (seed 3). The main fraction of fatty acids in rapeseed samples are monounsaturated fatty acids (MUFA = 58.5−68.0%) with predominant oleic acid (C 18:1), which varied from 56.8% (seed 3) to 66.6% (seed 6). Significant differences ($p < 0.05$) were found between amounts of poly- and monounsaturated fatty acids in all rapeseed varieties (except MUFA in seed 1 and seed 5), while the level of saturated fatty acids in seed 1, seed 2, seed 3, seed 4, and seed 6 did not differ significantly. The studied rapeseed samples do not contain *trans*-fatty acids, while a low level of erucic acid (0−0.2%) was determined. The highest erucic acid (C 22:1) content was shown for seed 1 and seed 4, with significant differences in relation to the other studied rapeseed cultivars (**Table 1**). In addition, the omega-6/omega-3 acid ratio (ω-6/ω-3) for all studied rapeseed varieties was fairly constant (1.7−2.4) and an insignificant difference for seed 1 and seed 6, seed 2 and seed 7, seed 3, seed 5 and seed 7 (Duncan test $p > 0.05$).

Literature studies indicate that the amounts of oleic (C 18:1), linoleic (C 18:2), and linolenic (C 18:3) acids ranged as follows: 10.7−72.0%, 12.0−29.0%, and 2.4−18.3%, respectively (*4*, *5*, *8*−*10*). Other authors (*6*−*8*) reported that the amounts of MUFA decrease if the content of erucic acid increases. The rapeseed varieties studied in this work contain small amounts of erucic acid (0−0.2%); thus, the content of MUFA is relatively high (58.5−68.0%).

The calculated values of RSD ranged between 0.0 to 4.5% indicating reasonable repeatability of the official GC method for determination of fatty acid composition.

Antioxidant Capacity of Rapeseed Varieties. The antioxidant capacities of the studied rapeseed varieties were determined by

Table 1. Physicochemical Properties and Fatty Acid Composition of the Studied Rapeseed Varieties

	rapeseed varieties						
	seed 1	seed 2	seed 3	seed 4	seed 5	seed 6	seed 7
Physicochemical Properties[a]							
moisture content [%]	8.5 ± 0.1 d	6.6 ± 0.1 a	7.6 ± 0.1 b	8.0 ± 0.1 c	7.4 ± 0.1 b	6.8 ± 0.2 a	7.4 ± 0.2 b
oil content [%]	43.6 ± 0.2 c	46.9 ± 0.1 g	40.5 ± 0.4 a	44.5 ± 0.0 d	42.6 ± 0.1 b	46.5 ± 0.2 f	45.6 ± 0.2 e
oil/dry mass [%]	47.7 ± 0.2 c	50.2 ± 0.2 g	43.8 ± 0.1 a	48.4 ± 0.2 d	46.0 ± 0.1 b	49.9 ± 0.2 f	49.2 ± 0.1 e
Fatty Acid Composition[a] [wt %]							
C 16:0	4.4 ± 0.2 a,b	4.6 ± 0.1 b	4.6 ± 0.0 b	4.5 ± 0.1 a,b	5.1 ± 0.1 c	4.3 ± 0.1 a	5.1 ± 0.1 c
C 16:1	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a
C 18:0	1.8 ± 0.0 b	1.6 ± 0.0 a	1.5 ± 0.0 a	2.0 ± 0.0 c	1.9 ± 0.0 b,c	1.9 ± 0.0 b,c	2.2 ± 0.1 d
C 18:1 *cis*	61.9 ± 0.3 b	66.0 ± 0.3 d	56.8 ± 0.2 a	63.5 ± 0.3 c	62.2 ± 0.1 b	66.6 ± 0.1 e	63.4 ± 0.2 c
C 18:1 *trans*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C 18:2 *cis*	19.0 ± 0.2 d	17.3 ± 0.1 b	23.9 ± 0.1 f	17.1 ± 0.1 b	19.6 ± 0.2 e	16.1 ± 0.1 a	18.4 ± 0.2 c
C 18:2 *trans*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C 18:3 *cis*	9.9 ± 0.1 d	7.8 ± 0.2 a	10.0 ± 0.0 d	10.0 ± 0.1 d	8.4 ± 0.1 b	8.6 ± 0.2 c	7.9 ± 0.0 a
C 18:3 *trans*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C 20:0	0.7 ± 0.0 a,b	0.6 ± 0.0 a	0.7 ± 0.0 a,b	0.7 ± 0.0 a,b	0.7 ± 0.0 a,b	0.7 ± 0.0 a,b	0.7 ± 0.0 b
C 20:1	1.5 ± 0.0 c	1.3 ± 0.0 b	1.4 ± 0.0 c	1.4 ± 0.0 c	1.2 ± 0.0 b	1.2 ± 0.0 b	1.1 ± 0.0 a
C 22:0	0.4 ± 0.0 a,b	0.3 ± 0.0 a	0.5 ± 0.0 b	0.4 ± 0.0 a,b	0.4 ± 0.0 a,b	0.4 ± 0.0 a,b	0.4 ± 0.0 a,b
C 22:1	0.2 ± 0.0 c	0.1 ± 0.0 b	0.1 ± 0.0 b	0.2 ± 0.0 c	0.1 ± 0.0 b	0 ± 0.0 a	0.1 ± 0.0 b
C 24:0	0.1 ± 0.0 a	0.1 ± 0.0 a	0.2 ± 0.0 b	0.1 ± 0.0 a,b	0.2 ± 0.0 b	0.1 ± 0.0 a,b	0.2 ± 0.0 b
SAFA	7.4 ± 0.2 a,b	7.2 ± 0.1 a	7.5 ± 0.1 b	7.7 ± 0.2 b	8.3 ± 0.1 c	7.4 ± 0.1 a,b	8.6 ± 0.2 d
MUFA	63.8 ± 0.1 b	67.6 ± 0.1 e	58.5 ± 0.1 a	65.4 ± 0.1 d	63.7 ± 0.4 b	68.0 ± 0.1 f	64.8 ± 0.1 c
PUFA	28.9 ± 0.1 f	25.1 ± 0.1 b	33.9 ± 0.1 g	27.1 ± 0.1 d	28.0 ± 0.1 e	24.7 ± 0.2 a	26.3 ± 0.0 c
omega-6/omega-3	1.9 ± 0.0 b	2.2 ± 0.0 c	2.4 ± 0.1 d	1.7 ± 0.0 a	2.3 ± 0.0 d	1.9 ± 0.0 b	2.3 ± 0.0 c,d

[a] Values are means ± standard deviation, $n = 3$. Different letters within the same row indicate significant differences (one-way ANOVA and Duncan test, $p < 0.05$). SAFA − saturated fatty acids; MUFA − monounsaturated fatty acids; PUFA − polyunsaturated fatty acids.

FRAP, DPPH, and ORAC methods and the results are presented in **Table 2**.

It is evident that the antioxidant capacity results for each of the rapeseed sample are different from one another. This variability among the rapeseeds can be explained by the influences of genetic and environmental factors, which would affect the level of antioxidants. The highest antioxidant capacity (FRAP = 7641 μmol of Trolox/100 g, DPPH = 7645 μmol of Trolox/100 g, and ORAC = 12989 mmol of Trolox/100 g) was in seed 1, whereas FRAP (6218 μmol of Trolox/100 g), DPPH (6238 μmol of Trolox/100 g) and ORAC (4092 mmol of Trolox/100 g) results were the lowest for seed 5 (**Table 2**). The significant differences in antioxidant capacity of two pollinated varieties, seed 4 and seed 5, determined by three analytical methods were found, whereas similar FRAP and DPPH values were found for seed 2 (hybrid variety) and seed 3 (pollinated variety), and two pollinated varieties, seed 1 and seed 7 (**Table 2**). Moreover, Duncan test indicated that the hybrid variety - seed 2 and pollinated variety - seed 6 from the same agricultural company did not differ significantly in DPPH and ORAC results. Also, insignificant differences for mean ORAC values were observed between seed 6 and seed 7. It is noteworthy that antioxidant capacities of rapeseed varieties determined by FRAP method (6218−7641 μmol of Trolox/100 g) were similar in comparison to the antioxidant capacities analyzed by the DPPH method (6238−7645 μmol of Trolox/100 g). However, FRAP and DPPH values were about 650 and 1700 times lower in comparison with ORAC results (4092−12989 mmol of Trolox/100 g) for the studied rapeseed varieties (**Table 2**). The ORAC results of rapeseed samples were quite different from the FRAP and DPPH results. This fact can be explained by the fact that FRAP and DPPH assays are a single electron-transfer based reactions, whereas the ORAC assay involves a hydrogen atom transfer reaction. Hence, the difference in the mechanism of ORAC, FRAP, and DPPH assays could have resulted in differences between these methods. Moreover, this difference between ORAC and FRAP and DPPH methods may indicate that phenols are not the only compounds with antioxidant capacity in the rapeseed extracts. Proteins and amino acids, which can be present in high concentrations in the studied seed extracts (*35*) produce considerable interference and overestimated ORAC results. Rapeseed and canola proteins have been reported to act as direct scavengers toward diverse free radicals or antioxidants (*21*, *36*, *37*). Therefore, a protein-rich samples presented high ORAC values (*38*). However, FRAP assay does not detect thiols because the reduction potential of thiols is generally lower than that of the Fe^{3+}/Fe^{2+} half-reaction. Only, the DPPH method was used for analysis of antioxidant capacity of two European deoiled rapeseed cultivars (Lion and Express) (*21*). The ED_{50} values for Lion rapeseed (3.70−4.08 mg/mL) were significantly lower than those for Express rapeseed. For comparison in the report of Matthäus (*18*), the water extract from rapeseed revealed a DPPH radical scavenging activity at a concentration 0.2 mg/mL. In addition, the antioxidant activity of crude tannins of canola (Cyclone) and rapeseed (Kolner, Ligaret,

Table 2. Antioxidant Capacities of Rapeseed Varieties Determined by FRAP, DPPH and ORAC Methods

rapeseed varieties	FRAP[a] [μmol of Trolox/100 g]	DPPH[a] [μmol of Trolox/100 g]	ORAC[a] [mmol of Trolox/100 g]
seed 1	7641 ± 111 e	7645 ± 88 e	12989 ± 219 f
seed 2	6949 ± 99 c	7105 ± 98 c,d	10659 ± 197 d
seed 3	6960 ± 122 c	7029 ± 78 c	7981 ± 159 c
seed 4	6718 ± 96 b	6805 ± 80 b	5112 ± 174 b
seed 5	6218 ± 95 a	6238 ± 84 a	4092 ± 102 a
seed 6	7180 ± 148 d	7198 ± 94 d	10809 ± 228 d,e
seed 7	7574 ± 102 e	7551 ± 78 e	11125 ± 186 e

[a] Values are means ± standard deviation, $n = 5$. Different letters within the same column indicate significant differences (one-way ANOVA and Duncan test, $p < 0.05$).

Table 3. Content of Free Phenolic Acids, Total Phenolic Acid, and Total Phenolic Content in the Studied Rapeseed Varieties

	content of phenolic acids in rapeseed samples[a] [mg/100 g]						
rapeseed varieties	gallic acid	caffeic acid	ferulic acid	sinapic acid	*p*-coumaric acid	TPA [mg/100 g]	TPC [mg SA/100 g]
seed 1	0.97 ± 0.02 a	0.03 ± 0.00 c	0.25 ± 0.01 b	20.6 ± 0.4 b	0.57 ± 0.00 a	22.4 ± 0.4 b	1711 ± 36 d
seed 2	1.18 ± 0.03 b	0.02 ± 0.00 b	0.31 ± 0.01 c	20.9 ± 0.0 c	0.83 ± 0.00 f	23.2 ± 0.1 c	1640 ± 39 c
seed 3	1.97 ± 0.01 e	0.07 ± 0.00 e	0.27 ± 0.01 b,c	17.4 ± 0.1 a	0.64 ± 0.01 c	20.3 ± 0.1 a	1643 ± 28 c
seed 4	1.80 ± 0.02 d	0.02 ± 0.00 b	1.96 ± 0.07 e	36.4 ± 0.4 e	0.62 ± 0.02 b	40.7 ± 0.4 f	1524 ± 36 b
seed 5	1.17 ± 0.03 b	0.04 ± 0.00 d	0.28 ± 0.01 b,c	20.9 ± 0.1 c	0.69 ± 0.02 d	23.1 ± 0.1 c	1378 ± 34 a
seed 6	1.63 ± 0.03 c	0.04 ± 0.00 d	0.45 ± 0.01 d	23.0 ± 0.1 d	0.92 ± 0.01 g	26.0 ± 0.2 e	1676 ± 40 c,d
seed 7	1.19 ± 0.03 b	0.01 ± 0.00 a	0.10 ± 0.00 a	23.2 ± 0.1 d	0.78 ± 0.00 e	25.3 ± 0.1 d	1821 ± 32 e

[a] Values are means ± standard deviation, $n = 5$. Different letters within the same column indicate significant differences (one-way ANOVA and Duncan test, $p < 0.05$). TPA - total phenolic acid. TPC - total phenolic content. SA - sinapic acid.

and Leo) hulls was determined by β-carotene-linoleate, DPPH, and reducing power assays (*12*), whereas FRAP and ORAC methods were not applied for antioxidant capacity determination of the rapeseed cultivars.

The within-day precision of FRAP, DPPH, and ORAC methods was tested by analyses of all rapeseed samples in five replicates. The values of RSD ranged between 1.4−2.1%, 1.0−1.3%, and 1.7−3.4%, respectively, indicating reasonable repeatability of the FRAP, DPPH, and ORAC determinations for the studied rapeseed extracts. The between-day precision of the proposed methods was evaluated by performing the determination within three days on all seed samples ($n = 5$), and the obtained results were satisfactory with RSD ranging between 1.8−2.9%, 2.0−2.9%, and 2.2−4.1% for FRAP, DPPH, and ORAC values: 6205−7649 μmol of Trolox/100 g, 6241−7654 μmol of Trolox/100 g, and 4101−12983 mmol of Trolox/100 g, respectively.

Determination of Five Individual Phenolic Acids by HPLC-DAD. The results of free phenolic acids in the studied rapeseed varieties analyzed by the HPLC-DAD method and total phenolic content determined by Folin-Ciocalteu method are listed in **Table 3**.

It is noteworthy that the amount of phenolic acids in the studied seed samples depends on the rapeseed variety. The main phenolic acid in all rapeseed varieties was sinapic acid (17.4−36.4 mg/100 g), while the content of caffeic acid was the lowest for all studied samples (0.01−0.07 mg/100 g). It can be noted that the level of sinapic acid in the discussed rapeseed samples was lower, when compared to results obtained by Kozlowska et al. (*16*) (41.3−51.6 mg/100 g for Polish rapeseed varieties), Cai and Arntfield (*14*) (34.0−49.0 mg/100 g for canola flour), and Krygier et al. (*24*) (73.2 mg/100 g for Candle cultivar and 80.1 mg/100 g for Tower cultivar), although similar to those reported by Siger et al. (*20*) (19.8−67.3 mg/100 g). However, the studied rapeseed varieties contain about 5 and 10 times higher amount of sinapic acid than the summer rapeseed cultivar, Yellow Sarson (3.5−3.7 mg/100 g) (*16*, *24*). Besides other phenolic acids, gallic, caffeic, ferulic, and *p*-coumaric occurred in considerably smaller quantities (**Table 3**). For comparison, the concentrations of caffeic, ferulic, and *p*-coumaric acids in different rapeseed varieties reported by other authors were in the same range between trace amounts and 1.8 mg/100 g, 0.5−6.8 mg/100 g, and trace amounts and 3.1 mg/100 g, respectively (*16*, *20*, *24*). The pollinated variety of seed 4 had the highest mean concentrations of sinapic and ferulic acids, and was significantly different from all other varieties. However, there were no significant differences in the amounts of these phenolic acids in seed 2 and seed 5. Also, similar sinapic acid content was determined in seed 6 and seed 7, while three pollinated rapeseed varieties: seed 1, seed 3, and seed 5 did not differ significantly in ferulic acid content. The same concentration of caffeic acid in hybrid variety - seed 2 and pollinated variety - seed 4 and in two pollinated rapeseed cultivars: seed 5 and seed 6 was found. In addition, the level of gallic acid in seed 2, seed 5, and seed 7 was not significantly different (Duncan test $p > 0.05$). In contrast, significant differences were observed for the amount of *p*-coumaric acid in all studied rapeseed varieties.

Total phenolic acid content calculated from HPLC data varied from 20.3 mg/100 g (seed 3) to 40.7 mg/100 g (seed 4), whereas total phenolic content determined by the Folin-Ciocalteu method ranged from 1378 mg of sinapic acid/100 g (seed 5) to 1821 mg sinapic acid/100 g (seed 7) of rapeseed flour. The obtained results of total phenolic content are similar to those reported by other authors (400−2659.7 mg of sinapic acid/100 g) (*14*, *18*, *20*). The total phenolic content in three rapeseed varieties: seed 2 (hybrid variety), seed 3 (pollinated variety), seed 6 (pollinated variety), and two pollinated cultivars: seed 1 and seed 6 revealed an insignificant differences (Duncan test $p > 0.05$), while amounts of total phenolics in other studied cultivars were statistically different (Duncan test $p < 0.05$). Moreover, the highest total phenolic acid content was for seed 4, with significant differences in relation to the other studied cultivars. Although total amount of phenolic acids differed insignificantly between seed 2 and seed 5.

The high variability in the amounts of the individual phenolic acids, total phenolic acid, and total phenolic content in rapeseed varieties has been widely reported and depends on several factors, such as genetic, agronomic, environmental, and extraction procedures (*14*, *18*, *20*, *23*).

The repeatability of the proposed HPLC and Folin-Ciocalteu methods was tested by analyses of all rapeseed samples in five replicates. The values of RSD were below 4.0% for individual phenolic acids and 2.5% for total phenolic content determination, indicating reasonable repeatability of the used methods (**Table 3**). Also, the reproducibility ($n = 5$ within 3 days) of these methods is satisfactory with RSD ranging between 0.3 and 5.3% for concentrations of gallic acid (1.02−2.08 mg/100 g), caffeic acid (0.01−0.08 mg/100 g), ferulic acid (0.09−2.03 mg/100 g), sinapic acid (17.8−36.1 mg/100 g), *p*-coumaric acid (0.60−1.01 mg/100 g), total phenolic acids (20.7−40.4 mg/100 g), and total phenolic content (1372−1824 mg of sinapic acid/100 g) in studied rapeseed cultivars.

Correlation Between Total Phenolic Content and Antioxidant Capacity of Rapeseed Varieties. Regression analysis was performed for correlations among FRAP, DPPH, ORAC, and TPC in the studied rapeseed varieties. Linear and significant correlations ($p < 0.001$ and $p < 0.05$) were found between three various methods used to determine the antioxidant potential and total phenolic content (correlation coefficients ranged between 0.8708 and 0.9930). The lowest correlation coefficient ($r = 0.8708$) was observed between the ORAC assay and TPC determined by the Folin-Ciocalteu method. This fact can be explained by the fact that phenols are not the only compounds with antioxidant potential in the studied rapeseed extracts. Also, lower correlation coefficients were calculated between the ORAC − FRAP ($r = 0.9124$) and ORAC − DPPH assays ($r = 0.9260$). The ORAC method takes into account the kinetic action of antioxidants,

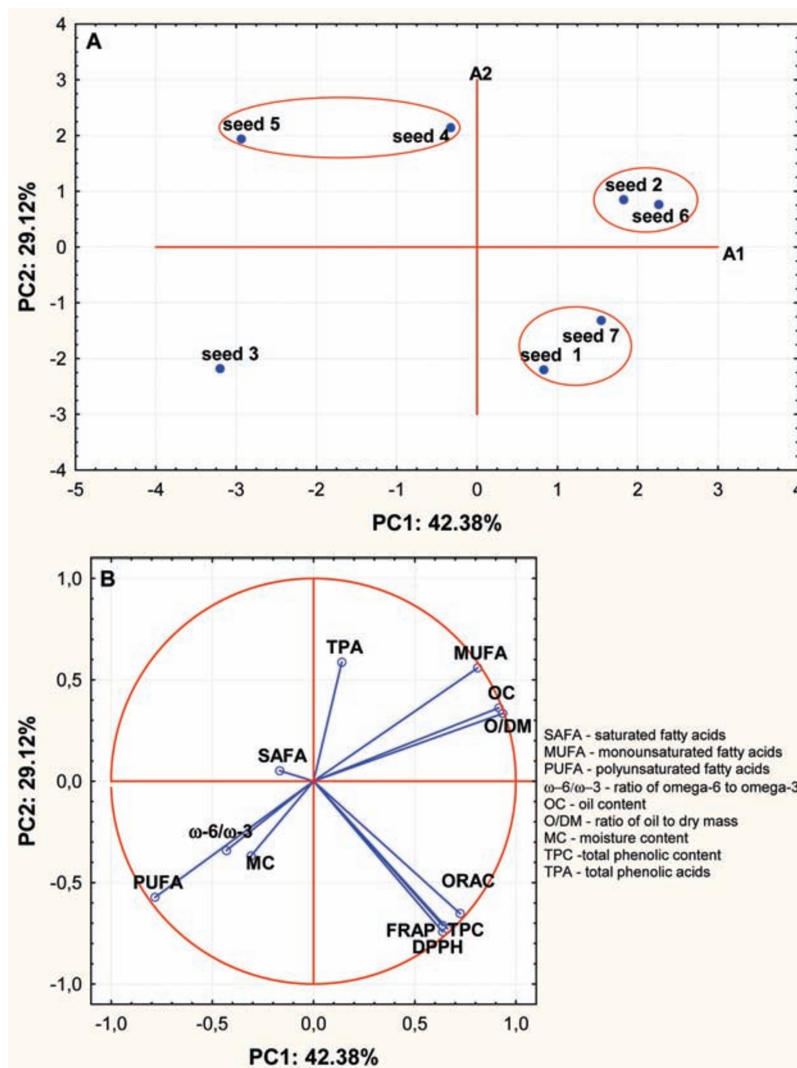

Figure 2. Score plot (**A**) and loading plot (**B**) of first two principal components (PC1 and PC2) for classification of different rapeseed varieties and describing the variation among the physicochemical properties of rapeseed samples.

which might explain the discrepancy between the results obtained with the ORAC assay and those obtained with the other assays. However, DPPH and FRAP values for all rapeseed varieties significantly correlated with TPC ($r = 0.9516$, $p = 0.00096$ and $r = 0.9468$, $p = 0.0012$). These results indicate a relationship between phenolic compound concentration in rapeseed extracts and their free radical scavenging and ferric reducing capacities. Therefore, the presence of phenolic compounds in the studied extracts contributes significantly to their antioxidant capacity.

For comparison, significant linear correlations (r ranged from -0.812 to 0.972) between total phenolics content and antioxidant activity of canola meal extracts analyzed by DPPH, TEAC, β-carotene−linoleic acid (linoleate) model and the reducing power methods were demonstrated by Hassas-Roudsari et al. (*35*). In these cases, a high phenolic content is an important factor in determining the antioxidant capacity of rapeseeds. Moreover, a similar correlation coefficient ($r = 0.966$, $p = 0.002$) for the relationship between the reducing power of Cyclone canola hull and total content of phenolics was reported by Amarowicz et al. (*12*). However, Yoshie-Stark et al. (*21*) and Matthäus (*18*) did not find a linear correlation between total phenolics and antioxidant activity of rapeseed cultivars determined by different analytical methods ($r = 0.0117$, 0.0092, and 0.0079 for DPPH, β-carotene bleaching, and electron spin resonance spectroscopy).

Principal Component Analysis. Principal component analysis (PCA) was applied to observe any possible clusters within analyzed rapeseed samples. The first two principal components took into account 71.50% (PC1 = 42.38% and PC2 = 29.12%, respectively), of the total variation. The scores of the first two principal components, for seven rapeseed varieties are presented in **Figure 2A**.

In the score plot rapeseeds with high antioxidant capacities, total phenolic content, and lower PUFA amount (seed 1, seed 2, seed 6, and seed 7) are located to the right, whereas seed samples (3, 4, and 5) with low FRAP, DPPH, ORAC, TPC values and higher PUFA are situated at the left in the diagram. The studied rapeseeds fell into three distinct groups, respectively. These groups generally have similar antioxidant capacities and total phenolic content. Moreover, seeds 2 and 6 showed similar contents of moisture and extracted oil, SAFA, MUFA, and PUFA. The rapeseed samples 4 and 5 with the lowest antioxidant capacities and TPC were located in the same quarter of the PCA graph. However, there is a large distance between samples 4 and 5. This fact can be explained by the fact that rapeseed sample 4 contains about two times higher amounts of total phenolic acids (40.7 mg/100 g) than other rapeseed varieties (**Table 3**). It is noteworthy that seed 3 with the lowest content of oil, phenolic acids, MUFA and the highest level of PUFA and ratio of omega-6/omega-3 was separated from three clusters (**Figure 2A**).

The relationships between the first two principal components and the studied variables were presented graphically by the loading plot (**Figure 2B**). The first principal component (PC1) was highly, positively contributed by the ratio of oil content to dry mass, oil content, monounsaturated fatty acids, antioxidant capacity determined by three different analytical methods and total phenolics in rapeseed varieties (PC1 = 0.934, 0.916, 0.811, 0.724, 0.662, 0.637, and 0.637 for O/DM, OC, MUFA, ORAC, DPPH, FRAP, and TPC, respectively) but negatively associated to polyunsaturated fatty acids (−0.783). However, the second principal component (PC2) was inversely correlated with FRAP (−0.742), DPPH (−0.728), TPC (−0.711), ORAC (−0.653), PUFA (−0.572), and positively related to total phenolic acid content and monounsaturated fatty acids (PC2 = 0.587 and 0.559 for TPA and MUFA, respectively). The statistical analysis for the data and results depicted in **Figure 2B** confirmed positive correlations between total phenolic content in the studied rapeseed varieties and their antioxidant capacities ($r = 0.8708-0.9516$, $p < 0.05$) and antioxidant capacities determined by different analytical methods ($r = 0.9124-0.9930$, $p < 0.05$). However, antioxidant capacities and total phenolic content (TPC) could not be associated with total phenolic acids (TPA) in rapeseeds (r ranged from −0.1942 to −0.4235, $p > 0.1$). In addition, oil content (OC) and ratio of oil to dry mass (O/DM) correlated significantly, positively with monounsaturated fatty acids ($r = 0.9522$ and 0.9472, $p < 0.005$), but negatively with polyunsaturated fatty acids ($r = -0.9438$ and −0.9385, $p < 0.005$) in all seeds. Also, a significant, negative correlation for MUFA and PUFA was observed ($r = -0.9875$, $p < 0.0001$). Thus, the higher PUFA in rapeseeds the lower MUFA, oil content, and ratio of O/DM. However, polyunsaturated fatty acids in the studied rapeseed varieties are insignificantly related to the omega-6/omega-3 acid ratio ($r = 0.4159$, $p = 0.353$).

The proposed FRAP, DPPH, and ORAC methods are relatively simple, precise, and convenient for the determination of antioxidant capacities of rapeseed varieties. All studied rapeseed varieties are rich in antioxidants. Although protein-rich rapeseed samples presented high ORAC values, proteins and amino acids could produce considerable interference in these antioxidant capacities determined by the ORAC method. It is noteworthy that there are linear and significant correlations between total phenolic content and antioxidant capacity of rapeseed varieties. Also, oil content and ratio of oil to dry mass correlated significantly, positively with the level of monounsaturated fatty acids, but negatively with the content of polyunsaturated fatty acids in all seeds. Fatty acid composition and the absence of *trans*-fatty acids indicate that the studied rapeseed varieties are good sources of unsaturated fatty acids and should present a positive impact on human health. In addition, a rapid and effective chromatographic procedure was applied to determine five individual phenolic acids in methanol−water extracts of rapeseed samples. The predominant phenolic acid in the studied rapeseeds is sinapic acid, whereas the others occur in small amounts. The proposed analytical methods can be usefully employed by the processing industry in assessing the antioxidant potential of rapeseed varieties.

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