

# Dehulling and microwave pretreatment effects on the physicochemical composition and antioxidant capacity of virgin rapeseed oil

Agnieszka Rękas<sup>1</sup> · Aleksander Siger<sup>2</sup> · Małgorzata Wroniak<sup>1</sup> · Iwona Ścibisz<sup>1</sup> · Dorota Derewiaka<sup>3</sup> · Andrzej Anders<sup>4</sup>

Revised: 28 December 2016 / Accepted: 2 January 2017 / Published online: 20 February 2017  
© Association of Food Scientists & Technologists (India) 2017

**Abstract** The effect of microwave heating (800 W) of whole and dehulled rapeseeds for 2 to 8 min was investigated in order to evaluate the impact of dehulling in conjunction with microwaving on the nutritional value, antioxidant activity and physicochemical properties of virgin rapeseed oil. Control oil produced from dehulled seeds (DRO) had higher amounts of bioactive compounds, such as tocopherols and phytosterols, lower content of pigments, and higher content of primary and secondary oxidation products compared to oil pressed from whole seeds (WRO). Oils pressed from seeds that had previously undergone thermal treatment demonstrated gradual increase of oxidative stability, radical scavenging activity, moreover microwave treatment to caused darkening of oil, assessed in terms of changes in L\*a\*b\* coordinates as well as browning index. Thermally-induced compositional changes were seen mainly in canolol, phytosterols, and carotenoids content, while only slight increase of tocopherols and phenolics was observed. The most pronounced

effect of microwave pretreatment was noted for canolol formation—for 8-min MV exposure canolol quantity was approximately 7- and 23-fold higher, in comparison with control WRO and DRO samples, respectively (increase from 61.39 to 456.04 µg/g, and from 13.39 to 320.44 µg/g).

**Keywords** Bioactive compounds · Dehulling · Microwave treatment · Physicochemical properties · Radical scavenging activity · Rapeseed oil

## Abbreviations

AHC	Agglomerative cluster analysis
DRO	Rapeseed oil pressed from dehulled seeds
HF	Hydrophilic fraction
IP	Induction period
LF	Lipophilic fraction
MV	Microwave
<i>p</i> -AnV	<i>p</i> -anisidine value
PC-8	Plastochromanol-8
PV	Peroxide value
RSC	Radical scavenging activity
WRO	Rapeseed oil pressed from whole seeds
α-T	Alpha tocopherol
β-T	Beta-tocopherol
γ-T	Gamma-tocopherol
δ-T	Delta-tocopherol

## Introduction

Rapeseed belongs to the dicotyledonous plants in which the embryo constitutes as much as 80% of the mature seed. Seeds of rape are complex structures that consist of three major components: (1) the embryo, composed of

✉ Agnieszka Rękas  
agnieszka\_rekas@sggw.pl

<sup>1</sup> Department of Food Technology, Faculty of Food Sciences, Warsaw University of Life Sciences, Nowoursynowska St. 159c, 02-787 Warsaw, Poland

<sup>2</sup> Department of Food Biochemistry and Analysis, Faculty of Food Sciences and Nutrition, Poznań University of Life Sciences, Wojska Polskiego St. 28, 60-637 Poznań, Poland

<sup>3</sup> Department of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Sciences, Warsaw University of Life Sciences, Nowoursynowska St. 159c, 02-787 Warsaw, Poland

<sup>4</sup> Department of Machines and Research Methodology, Faculty of Technical Sciences, University of Warmia and Mazury in Olsztyn, M. Oczapowskiego St. 11, 10-736 Olsztyn, Poland

cotyledons, hypocotyl, and radicle; (2) the endosperm; and (3) the seed coat (hull), which surrounds the embryo and the endosperm (Hu et al. 2013). Generally, rapeseeds contain high amounts of oil (38–50%) and proteins (36–44%), as well as crude fibers (10–15%). These major rapeseed constituents are non-uniformly distributed in different fractions of seed. The cotyledon contains high amounts of oil (50–56% of dry mass), proteins (28–44% of fat free dry mass), and low amounts of crude fiber (4–7% of fat free dry mass). On the other hand, the hulls consist mainly of crude fiber (40–48% of fat free dry mass), protein (up to 15% of fat free dry mass), and low amounts of oil (6–14% of dry mass). Besides fiber, the hulls consist of low molecular weight carbohydrates, polysaccharides, pectins, cellulose, lignin, as well as polyphenolics, glucosinolates and minerals (Rotkiewicz and Zadernowski 1997). Phenolic compounds (phenolic acids and tannins) are responsible for bitter and astringent after-taste, also they contribute to the dark colour of crude rapeseed oil. Moreover, residual amounts of glucosinolate degradation products, such as isothiocyanates, may contribute to the bitterness and pungency of rapeseed products (both oil and meal) (Naczek et al. 1998).

Extensive studies on the possibility of application of rapeseed dehulling were carried out in the late 70 and 80 s of the past century. At the time, developed dehulling techniques comprised: air-classification of the defatted meal, liquid cyclone fractionation after solvent extraction, cracking and air-classification of seeds before oil extraction. However, because of the necessity to apply solvents, which diminished the quality of oil and meal and generated additional production costs, these technologies haven't found practical application (Carré et al. 2015; Koubaa et al. 2016). Recently, for separation of hulls from rapeseeds, the mechanical methods are applied. Mechanical rapeseed dehulling may be accomplished either by crushing seeds directly on a hard surface or in the gap between two rotating rolls (Rotkiewicz and Zadernowski 1997; Anders 2003). Despite the fact that the technical feasibility of mechanical purification of the hulls appears to be very uncertain, it has many advantages. Dehulling of rapeseed prior to oil pressing allows to maintain the screw press temperature below 40 °C, while oil extraction from dehulled rapeseeds enables recovery of most of the oil from kernel (Carré et al. 2015). Oil produced from dehulled seeds has better sensory characteristics—milder taste and flavour, bright yellowish colour, and lower content of waxes (Koubaa et al. 2016).

First studies concerning rapeseed thermal pretreatment by microwaves prior to pressing were directed toward rapid heat inactivation of seed-specific enzymes, such as myrosinase, thioglucoside glucohydrolase, its effect on the sulfur content and colour of crude rapeseed oil

(Maheshwari et al. 1980). Recent studies demonstrate favourable effect of microwave pretreatment of rapeseeds on the formation of phenolic-type compound described as potent antioxidant and an alkylperoxyl radical scavenger, known as canolol (2,6-dimethoxy-4-vinylphenol) (Wakamatsu et al. 2005). As distinct from other phenolic compounds showing low solubility in non-polar media, canolol is less hydrophilic thus it has better solubility in lipophilic fraction of oil. As a result, canolol constitute a major phenolic compound found in rapeseed oil obtained from seeds heated prior to pressing (Koski et al. 2003).

To best of our knowledge, the effect of dehulling in conjunction with microwaving on the nutritional value, antioxidant activity and physicochemical properties of rapeseed oil has not been reported. Therefore, the present study was undertaken to investigate the effects of microwave pretreatment of whole and dehulled seeds on changes in the chemical composition (tocochromanols, phytosterols, phenolic compounds, carotenoids), oxidative stability parameters and antioxidant capacity of virgin rapeseed oils.

## Materials and methods

### Material

Seeds of winter type rapeseed Bojan, were provided by the Plant Breeding Strzelce Ltd. Co.—IHAR Group, Poland. Seeds were harvested at optimum maturity, and did not contain any impurities or broken seeds. They were stored in paper bags in atmospheric conditions at  $19 \pm 1$  °C. The moisture and oil content were 5.3, and 45.7%, respectively.

### Reagents

Analytical standards of tocopherols (>95%), HPLC-grade *n*-hexane, methanol, formic acid, orthophosphoric acid, and 1,4-dioxane were purchased from Calbiochem-Merck Biosciences (Darmstadt, Germany). Phenolic acid standards, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 5 $\alpha$ -cholestane (>97%) and Sylon BTZ were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### Dehulling

Dehulling of rapeseeds was performed using a disc sheller equipped with cylindrical blades, developed by Anders (2003). The height of the slot between the hulling disk and the top cover of the working space was 3 mm. The hulling disk at a diameter of 140 mm, equipped with blades inclined at 45 degrees to the flat surface of the disk. Dehulled rapeseeds were subjected to pneumatic separation

on a laboratory separator Petkus K-293 (PETKUS Technologie GmbH, Germany).

### Microwave pretreatment

Whole and dehulled rapeseeds were equilibrated at refrigerated temperature ( $4 \pm 2$  °C) in closed containers for 72 h to reach moisture content of 7.5% by spraying the seeds with a required amount of water. For each microwave (MV) pretreatment, 500 g of seeds were placed in glass beaker (16-cm diameter) inside the microwave (Model: NN-J155 W). Seeds were exposed to microwave irradiation for 2, 4, 6, and 8 min (2450 MHz, 800 W). Immediately after every heating run the temperature of the seeds was determined using handheld infrared thermometer (KC-180B, Tynaxtools, Poland). Whole and dehulled rapeseed sample without microwave radiation (0 min radiation time) was used a control sample. Each experiment was performed in triplicate for all variants of the microwave radiation. Following each heating run, seeds were allowed to cool to ambient temperature and thoroughly mixed to obtain a homogeneous sampling.

### Oil extraction by cold-pressing

Thermally treated seeds were then moisturised in a similar manner as seeds before microwaving to reach required moisture content of 8.5%. The oil was pressed with the use of screw press (Farmet, Czech Republic). During the pressing, the temperature was kept below 40 °C. After pressing oils were collected, subjected to natural sedimentation (3 days) under refrigeration conditions ( $4 \pm 2$  °C) and decanted.

### Determination of tocochromanols

Tocochromanols ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherol and PC-8) were determined according to the method described by Siger et al. (2015). In brief, a 200 mg oil sample was dissolved in 10 ml of *n*-hexane and transferred to vials for further analysis. Separation was performed using a Waters HPLC system (Waters, Milford, MA, USA) coupled with a FLD detector (Waters 474), a PDA detector (Waters 2998), and a LiChrosorb<sup>®</sup> Si-60 column (250 × 4.6 mm, 5  $\mu$ m, Merck Millipore, Darmstadt, Germany). The mobile phase was a mixture of *n*-hexane with 1,4-dioxane (96:4 *v/v*) with a flow rate 1.0 ml min<sup>-1</sup>. Quantification of tocochromanols was conducted using data from the FLD with excitation/emission wavelengths of 295/330 nm, respectively. The plastochochromanol-8 contents were assayed and calculated following method described by Siger et al. (2014).

### Determination of phytosterols

Phytosterols were determined following procedures described by the AOCS Official Method Ch 6–91 (1997). In brief, a 50-mg oil sample was saponified with 1 M methanolic KOH at room temperature for 18 h. The extraction of the unsaponifiable fraction was carried out with a mixture of *n*-hexane/methyl *tert*-butyl (1:1 *v/v*) followed by solvent evaporation to dryness under nitrogen. Dry residues were dissolved in 100  $\mu$ l pyridine and silylated with 400  $\mu$ l of Sylon BTZ. Derivatives of the sterols were separated on a gas chromatograph (Shimadzu, Japan) equipped with a FID detector, using DB-5MS capillary column (30 m × 0.25 mm × 0.25  $\mu$ m; Phenomenex Torrance, CA, USA). A 1  $\mu$ l of the sample was injected in splitless mode (setup: hydrogen carrier gas at 1.5 ml min<sup>-1</sup> flow rate, the detector temperature was set at 300 °C). The column temperature: 50 °C hold for 2 min, ramped to 230 °C at 15 °C min<sup>-1</sup>, ramped to 310 °C at 3 °C min<sup>-1</sup>, hold for 10 min. All sterols were quantified using a 5 $\alpha$ -cholestane as an internal standard. All sterols were quantified using a 5 $\alpha$ -cholestane as an internal standard. Phytosterols were identified based on a GC-MS<sup>n</sup> laboratory phytosterol spectra library, as well as the online NIST mass spectra library.

### Phenolic compounds analysis

A 250-mg of oil sample was dissolved in 5 ml of hexane and the mixture was stirred for 1 min with a vortex. A SPE column filled with diol (Supelco) was placed in a vacuum elution apparatus (Chromabond<sup>®</sup> System, Macherey–Nagel, Germany) and conditioned by consecutive addition of 5 ml of methanol and 5 ml of *n*-hexane. Then, the oil solution was applied to the column, and the solvent was pulled through, leaving the sample on the solid phase. The column was eluted with 5 ml of *n*-hexane and 5 ml *n*-hexane/ethyl acetate (90:10 *v/v*). The polar compounds retained were recovered with 5 ml of methanol.

Analysis was carried out using Shimadzu HPLC system (Shimadzu, Japan), equipped with a Luna C18(2) reversed-phase column (4.6 × 250 mm; 5  $\mu$ m, Phenomenex, Torrance, CA, USA), and a DAD detector. Gradient elution was used, combining solvent A (formic acid/H<sub>2</sub>O 900:100 *v/v*) and solvent B (methanol) as follows: 10% B (0–1 min), 20% B (10–22 min), 50% B (22–45 min), 70% B (45–55 min), 90% B (55–60 min), 10% B (60–65 min), 10% B (65–75 min). The flow rate was 1.0 ml min<sup>-1</sup>. The injection volume was 20  $\mu$ l, while the column temperature was maintained at 25 °C. The signal was monitored at 200–600 nm using a DAD detector (SPD-M20A, Shimadzu, Japan). Quantitative determination of phenolic

compounds was carried out by comparing the retention times and diode array spectral characteristics with the appropriate standards. HPLC/ESI/MS<sup>n</sup> analyses were performed to qualitatively identify polar phenolic compounds that differed in their retention times from the standards.

### Isolation and identification of canolol

Analysis was carried out using a Waters HPLC system (Waters, Milford, MA, USA) with a FLD detector (Waters 474), a PDA detector (Waters 2998), equipped with a Nova-Pack silica semi-preparative column (19 × 300 mm; 6 μm, Waters, USA). The oil (1 g) was dissolved in *n*-hexane, made up to 10 ml, and 200 μl were applied onto the column. The mobile phase consisted of *n*-hexane and 1,4-dioxane (96:4 v/v) and the flow rate was 3 ml min<sup>-1</sup>. The canolol containing fraction (RT = 43,106 min) was collected under nitrogen in a sealed round bottom flask. This procedure was repeated several times to obtain a high concentration of canolol. The solvent was evaporated under nitrogen and the isolated canolol was dissolved in *n*-hexane in a 10 ml volumetric flask. The concentration of canolol was evaluated spectrophotometrically according to its specific absorption coefficient: 29,000 (λ = 218 nm) and 13,000 (λ = 269 nm). (Aachary and Thiyam-Holländer 2013). Peak identity and homogeneity was verified using HPLC-MS<sup>n</sup>.

### Determination of colour development

Total carotenoid pigments, expressed as β-carotene, were assayed for oil samples diluted in cyclohexane at 445 nm (BSI 1977). The total chlorophyll pigments, expressed as pheophytin *a*, were determined according to the AOCS Method (1997) by measuring the absorbance of the oil against the air at 630, 670, and 710 nm.

The non-enzymatic browning index was assayed spectrophotometrically for oil samples diluted in chloroform at 420 nm (Yoshida et al. 1999).

The colour of the oils was determined by CIE L\*a\*b\* colour scales (CM-3600d spectrophotometer, Konica Minolta, Japan), whereas colour difference (ΔE) was calculated as  $\Delta E = [(L_0 - L)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2]^{1/2}$ , where L<sub>0</sub>, a<sub>0</sub><sup>\*</sup>, and b<sub>0</sub><sup>\*</sup> are the colour parameters of the control WRO and DRO oil samples.

### Radical scavenging activity (RSC)

To evaluate the antioxidant capacity of the oils, spectrophotometric analysis was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), following method described by Tuberoso et al. (2007). The antioxidant capacity of oil (TF) and both lipophilic (LF) and hydrophilic (HF) fractions was

determined. To separate the HF and LF, 500 μl of oil was mixed with 500 μl of methanol and centrifuged at 700 g for 10 min to allow the fractions to separate. HF was tested using 20 μl of extract added to 3 ml of methanolic DPPH (0.04 mM), while LF and TF assay was performed using DPPH dissolved in ethyl acetate. Spectrophotometric readings were carried out after a 1 h period of incubation with a Spectronic Helios β UV–Vis spectrophotometer at 517 nm using a 10-mm quartz cuvette. Results were expressed as a Trolox equivalent antioxidant capacity (TEAC, mmol/l), using a Trolox calibration curve in the range 0.02–4.00 mM.

### Oxidative stability

The peroxide value (PV), *p*-anisidine value (*p*-AnV), and specific UV extinctions (*K*<sub>232</sub> and *K*<sub>268</sub> values) were determined in accordance with ISO standard methods (3960:2005; 6885:2008, 3656:2011, respectively).

The oxidative stability of the oil samples was evaluated by Metrohm Rancimat apparatus model 743 (Herisau, Switzerland). In brief, oil samples were weighed (2.5 g) into the reaction vessel and heated to 120 °C under air flow of 20 L/h. The induction period (IP) was expressed in hours (h).

### Statistical analysis

Statistical analysis was carried out with Statistica v. 12 software (StatSoft, Inc., Tulsa, OK, USA). Significant differences between means were determined through Tukey's Multiple Range Tests. Correlations between variables studied were determined using *Pearson's* correlation coefficient (*r*). Additionally, data were subjected to agglomerative cluster analysis (AHC), applying the Ward's method with the Euclidean distance measure based on presence or absence of a particular component, using XLStat software (Addinsoft, Paris, France).

## Results and discussion

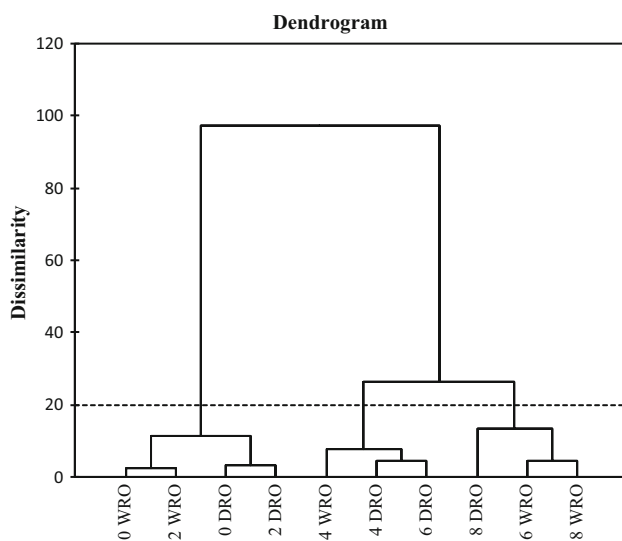
### Preliminary multivariate statistical analysis

The application of an agglomerative cluster analysis (AHC) allowed to classify oil samples on the basis of seeds type (whole/dehulled) and microwave time (0–8 min), which facilitated identification of similarities between analysed rapeseed oils. AHC was applied to data of variables, including: bioactive compounds (tocopherols, PC-8, phytosterols, phenolic compounds, carotenoids, chlorophylls), physicochemical parameters (PV, *p*-AnV, *K*<sub>232</sub>, *K*<sub>268</sub>, IP, browning index, CIE L\*a\*b\* coordinates), RSC

of whole oil and its lipophilic and hydrophilic fractions and two types of rapeseed oils (WRO and DRO) that had previously undergone microwave pretreatment under five different exposure times. The dendrogram of divided analysed oils into two major clusters (Fig. 1). Cluster 1 contained WROs and DROs pressed from control oils and 2-min MV treated seeds. These oils formed distinct cluster, linked to the other cluster at long distance, which indicates significant differences with respect to other oils. Cluster 2 contained two distinct subclusters: first combined DROs obtained from seeds after 4- and 6-min exposure, and WRO pressed from 4-min MV treated seeds, while second joined WROs and DROs pressed from 8-min MV treated seeds and WRO obtained from seeds MV treated for 6 min.

### Microwave pretreatment time and temperature effect on the compositional changes of oil

Quality of extracted oil dependent on the level of potency, time of exposure and temperature of microwave pretreatment (Azadmard-Damirchi et al. 2011). Application of thermal pretreatment of rapeseeds prior to pressing caused rupture of the cell membrane which facilitated extraction of phytochemicals, such phytosterols, tocopherols, and carotenoids into the crude oil during pressing. The effect of roasting temperature on the content of bioactive compounds in the oil have been well investigated (Vaidya and Choe 2011; Cai et al. 2013; Shrestha and De Meulenaer 2014; Siger et al. 2015), whereas literature data considering seeds temperature reached during microwave pretreatment



**Fig. 1** AHC dendrographic classification of rapeseed oils according to the type of seeds (whole/dehulled) and microwave time exposure. Explanatory notes: WRO, rapeseed oil pressed from whole seeds; DRO, rapeseed oil pressed from dehulled seeds; 0–8, microwave pretreatment time

was scarce, and relate mainly to changes in the content of canolol. Studies conducted by Mayengbam et al. (2013) explained formation of canolol in rapeseed as result of consecutive reactions initiated by thermally-induced breakdown of sinapoyl glucose and sinapine to sinapic acid followed by its decarboxylation to canolol. According to Spielmeyer et al. (2009), canolol is thermally unstable and at temperature above 160 °C, achieved after 7.5 min of MV exposure, undergoes degradation. Yang et al. (2014) reported canolol decrease following 7 min MV exposure; however no temperature measurements were carried out in line with seeds pretreatment. Additionally, the aspect of rapeseeds moisture content prior to MV pretreatment was omitted in both studies, which, as pointed out by Yang et al. (2013) and Wroniak et al. (2016), in addition to the period of MV exposure, exert an effect on the degree of quality changes occurring in seeds during heating. As can be seen from Table 3, the longer MV exposure time, caused heating of seeds to higher temperature. Moreover, hulls removal from the seeds was found to be of great importance. Even when two batches of seeds (dehulled and whole seeds) were prepared in the same manner prior to pressing (seeds were moisturised to reach the same moisture content), dehulled seeds had lower temperatures compared to whole seeds. Followings 2, 4, 6, and 8 min MV exposure whole seeds temperature was 56, 101, 125, and 144 °C. The respective temperatures reached by dehulled seeds were: 31, 46, 89, and 119 °C. This observation may be explained by considering differences in water distribution in moisturised seeds which determines the rate of heating up during thermal treatment. During microwave pretreatment tocopherols were found to be thermally-stable, most likely due to antioxidants, released during microwaving, which protected tocopherols from degradation under elevated temperatures (Dean et al. 2011). According to Matthäus (2012) canolol, formed during rapeseeds thermal pretreatment, acted as antioxidant protecting tocopherols from degradation during heating. The amount of phytosterols, plastochromanol-8, carotenoids and phenolic compounds, mainly canolol, increased gradually, reaching maximum concentration after 8 min of MV exposure, where seeds temperatures were the highest (Tables 1, 2, 3, 4).

### Effects of dehulling and microwave pretreatment on bioactive compounds

#### Tocochromanols

Tocopherols are the most abundant antioxidants in rapeseed oil, where  $\gamma$ - and  $\alpha$ -tocopherol are predominant,

**Table 1** Tocochromanols content in oils produced from microwave treated whole and dehulled rapeseed

Oil source	Microwave treatment time (min)	Tocopherols (mg/100g)					PC-8 (mg/100 g)
		$\alpha$ -T	$\beta$ -T	$\gamma$ -T	$\delta$ -T	Total	
Whole seeds	0	25.84 $\pm$ 0.15 <sup>a</sup>	0.08 $\pm$ 0.02 <sup>a</sup>	38.66 $\pm$ 0.17 <sup>a</sup>	0.61 $\pm$ 0.04 <sup>a</sup>	65.18 $\pm$ 0.21 <sup>a</sup>	2.42 $\pm$ 0.05 <sup>a</sup>
	2	25.87 $\pm$ 0.10 <sup>a</sup>	0.06 $\pm$ 0.01 <sup>a</sup>	39.80 $\pm$ 0.14 <sup>a</sup>	0.62 $\pm$ 0.05 <sup>a</sup>	66.53 $\pm$ 0.24 <sup>a</sup>	2.59 $\pm$ 0.05 <sup>a</sup>
	4	26.04 $\pm$ 0.08 <sup>b</sup>	0.06 $\pm$ 0.02 <sup>a</sup>	41.06 $\pm$ 0.12 <sup>b</sup>	0.63 $\pm$ 0.03 <sup>a</sup>	67.79 $\pm$ 0.19 <sup>b</sup>	3.82 $\pm$ 0.19 <sup>b</sup>
	6	25.61 $\pm$ 0.15 <sup>a</sup>	0.08 $\pm$ 0.03 <sup>a</sup>	39.98 $\pm$ 0.12 <sup>a</sup>	0.85 $\pm$ 0.07 <sup>b</sup>	66.53 $\pm$ 0.25 <sup>a</sup>	4.04 $\pm$ 0.14 <sup>b</sup>
	8	25.51 $\pm$ 0.12 <sup>a</sup>	0.03 $\pm$ 0.01 <sup>a</sup>	40.81 $\pm$ 0.17 <sup>b</sup>	0.56 $\pm$ 0.05 <sup>a</sup>	66.92 $\pm$ 0.23 <sup>a</sup>	4.19 $\pm$ 0.01 <sup>b</sup>
Dehulled seeds	0	27.46 $\pm$ 0.14 <sup>A</sup>	0.13 $\pm$ 0.06 <sup>A</sup>	41.23 $\pm$ 0.12 <sup>A</sup>	0.89 $\pm$ 0.05 <sup>A</sup>	69.71 $\pm$ 0.15 <sup>A</sup>	2.70 $\pm$ 0.05 <sup>A</sup>
	2	27.41 $\pm$ 0.12 <sup>A</sup>	0.13 $\pm$ 0.05 <sup>A</sup>	41.35 $\pm$ 0.10 <sup>A</sup>	0.88 $\pm$ 0.04 <sup>A</sup>	69.70 $\pm$ 0.19 <sup>A</sup>	2.99 $\pm$ 0.02 <sup>A</sup>
	4	27.26 $\pm$ 0.17 <sup>A</sup>	0.12 $\pm$ 0.04 <sup>A</sup>	41.49 $\pm$ 0.13 <sup>A</sup>	0.84 $\pm$ 0.04 <sup>A</sup>	69.72 $\pm$ 0.13 <sup>A</sup>	3.98 $\pm$ 0.07 <sup>B</sup>
	6	27.36 $\pm$ 0.08 <sup>A</sup>	0.14 $\pm$ 0.10 <sup>A</sup>	42.46 $\pm$ 0.24 <sup>B</sup>	0.84 $\pm$ 0.04 <sup>A</sup>	70.79 $\pm$ 0.39 <sup>B</sup>	4.69 $\pm$ 0.17 <sup>C</sup>
	8	26.63 $\pm$ 0.39 <sup>B</sup>	0.09 $\pm$ 0.01 <sup>A</sup>	42.60 $\pm$ 0.14 <sup>B</sup>	0.81 $\pm$ 0.02 <sup>A</sup>	70.13 $\pm$ 0.35 <sup>AB</sup>	4.44 $\pm$ 0.22 <sup>C</sup>

Mean values denoted by the same letter (a, b, A, B, ...) in the columns do not constitute statistically significant differences at  $p < 0.05$

usually with the proportion of 65 to 35%. The amounts of tocochromanols identified in the analysed oil samples are summarized in Table 1. Analysed oil contained mostly  $\gamma$ - and  $\alpha$ -T, and minor amounts of  $\delta$ - and  $\beta$ -T. In contrary to Yang et al. (2011), who reported higher level of tocopherols in rapeseed oil pressed from whole seeds than in oil from dehulled seeds (50.6 and 47.6 mg/100 g, respectively), in this current study control WRO contained lower level of tocopherols compared to control DRO (65.18 and 69.71 mg/100 g, respectively).

MV pretreatment of rapeseed significantly ( $p < 0.05$ ) influenced the concentration of tocochromanols in oils. Although changes in the contents of  $\alpha$ -T homologue were assigned as statistically significant, there appeared to be only slight alteration in their concentration (not exceeding 1%). On the other hand, the content of  $\gamma$ -,  $\delta$ -, and  $\beta$ -T first increased and then decreased depending on the period of microwave irradiation (Table 1). As Wijesundera et al. (2008) suggested, slight alterations in tocopherol levels in oils produced from roasted seeds may result from their co-elution in HPLC with another compounds formed during seeds roasting. Similarly to tocopherols content, control DRO contained higher level of plastochromanol-8 (PC-8) than control WRO (2.70 and 2.42 mg/100 g, respectively). MV pretreatment of rapeseed prior to pressing significantly ( $p < 0.05$ ) increased the content of PC-8 in the analysed oil samples (Table 1). The 8-min MV exposure resulted in an increase of PC-8 content of 73 and 63%, for WRO and DRO, compared to control oil sample. Similar results were obtained by Shrestha and De Meulenaer (2014) who noted significant increase of PC-8 after rapeseed roasting at 165 °C for 10 min (increase from 1.80 to 40.29 mg/100 g). On the other hand, Siger et al. (2015) observed gradual degradation of PC-8 during rapeseed roasting at 140 and 160 °C, while further

temperature elevation to 180 °C resulted in a slight increase of this compound. According to Shrestha and De Meulenaer (2014), increased extractability of PC-8 from seeds may result from thermally-induced disruption of internal structure of oilseeds during roasting.

### Phytosterols

The phytosterols content in the analysed oil samples is summarised in Table 2. The amount of total phytosterols in control WRO and DRO was 567.36 and 591.08 mg/100 g, respectively. Similarly to tocopherols composition, higher content of individual and total phytosterols was detected in oil samples from dehulled seeds.  $\beta$ -Sitosterol and campesterol were the dominant phytosterols found in the studied oils. As can be seen from Table 2 individual phytosterol contents were significantly affected by the MV pretreatment of rapeseed prior to pressing. Sterol composition varied among the studied oils attaining a maximum concentration of 625.09 and 658.73 mg/100 g, after 8-min exposure, in the case of phytosterols in WRO and DRO, respectively. Similar relationship between the MV exposure period and the content of individual phytosterols in WRO was observed—after 8-min exposure the amount of brassicasterol, campesterol and  $\beta$ -sitosterol increased by 14.45, 11.26, and 6.90%, respectively, compared to the control sample. Respective increase of brassicasterol, campesterol and  $\beta$ -sitosterol in DRO was as follows: 14.40, 8.90, 8.28%. Azadmard-Damirchi et al. (2010) and Yang et al. (2013) reported that the amount of phytosterols increased significantly depending on the MV pretreatment time. However, Yang et al. (2013) found an adverse correlation between seeds moisture and phytosterols content in rapeseed oil pressed from MV-treated seeds.

**Table 2** Phytosterols content in oils produced from microwave treated whole and dehulled rapeseed

Oil source	Microwave treatment time (min)	Phytosterol contents (mg/100 g)							Total
		Cholesterol	Brassicasterol	Campesterol	Stigmasterol	β-Sitosterol	Δ5-Avenasterol		
Whole seeds	0	1.77 ± 0.01 <sup>a</sup>	77.01 ± 0.09 <sup>a</sup>	175.29 ± 1.14 <sup>a</sup>	2.61 ± 0.03 <sup>a</sup>	293.07 ± 3.93 <sup>a</sup>	14.56 ± 0.04 <sup>a</sup>	567.36 ± 5.63 <sup>a</sup>	
	2	1.78 ± 0.01 <sup>a</sup>	77.39 ± 4.24 <sup>a</sup>	178.88 ± 1.04 <sup>a</sup>	2.65 ± 0.42 <sup>a</sup>	296.59 ± 3.93 <sup>a</sup>	15.19 ± 0.09 <sup>a</sup>	572.48 ± 5.63 <sup>a</sup>	
	4	1.77 ± 0.01 <sup>a</sup>	78.51 ± 3.58 <sup>a</sup>	185.66 ± 2.72 <sup>b</sup>	3.04 ± 0.28 <sup>b</sup>	302.17 ± 4.11 <sup>a</sup>	18.07 ± 3.59 <sup>b</sup>	589.22 ± 4.13 <sup>b</sup>	
	6	1.79 ± 0.02 <sup>a</sup>	85.05 ± 0.94 <sup>b</sup>	192.40 ± 3.86 <sup>c</sup>	3.32 ± 0.12 <sup>b</sup>	307.82 ± 2.61 <sup>b</sup>	19.00 ± 1.29 <sup>b</sup>	609.38 ± 3.54 <sup>c</sup>	
	8	1.80 ± 0.01 <sup>a</sup>	88.14 ± 1.16 <sup>b</sup>	195.03 ± 2.51 <sup>c</sup>	3.56 ± 0.07 <sup>b</sup>	313.30 ± 1.43 <sup>b</sup>	23.26 ± 1.63 <sup>c</sup>	625.09 ± 3.23 <sup>d</sup>	
Dehulled seeds	0	1.80 ± 0.01 <sup>A</sup>	81.96 ± 2.18 <sup>A</sup>	183.27 ± 2.05 <sup>A</sup>	2.77 ± 0.30 <sup>A</sup>	304.61 ± 3.92 <sup>A</sup>	16.67 ± 1.09 <sup>A</sup>	591.08 ± 4.68 <sup>A</sup>	
	2	1.80 ± 0.01 <sup>A</sup>	82.39 ± 4.24 <sup>A</sup>	189.88 ± 1.14 <sup>A</sup>	2.85 ± 0.42 <sup>A</sup>	309.59 ± 3.93 <sup>A</sup>	18.19 ± 2.09 <sup>B</sup>	604.70 ± 5.63 <sup>B</sup>	
	4	1.81 ± 0.01 <sup>A</sup>	85.55 ± 4.90 <sup>B</sup>	190.23 ± 2.42 <sup>A</sup>	2.89 ± 0.24 <sup>A</sup>	313.97 ± 1.20 <sup>A</sup>	17.85 ± 2.45 <sup>A</sup>	612.26 ± 5.39 <sup>B</sup>	
	6	1.80 ± 0.01 <sup>A</sup>	89.92 ± 3.21 <sup>B</sup>	197.52 ± 3.13 <sup>B</sup>	3.15 ± 0.07 <sup>B</sup>	320.08 ± 7.74 <sup>B</sup>	18.79 ± 1.38 <sup>B</sup>	631.26 ± 5.66 <sup>C</sup>	
	8	1.81 ± 0.02 <sup>A</sup>	93.76 ± 4.67 <sup>C</sup>	199.59 ± 5.41 <sup>B</sup>	3.29 ± 0.11 <sup>B</sup>	329.83 ± 4.32 <sup>B</sup>	20.45 ± 2.01 <sup>C</sup>	658.73 ± 3.74 <sup>D</sup>	

Mean values denoted by the same letter in the columns do not constitute statistically significant differences at  $p < 0.05$

### Phenolic compounds

Rapeseed contain the highest amount of phenolic compounds among other oilseeds. However, phenolics are non-uniformly distributed in different fractions of seed. Studies conducted by Krygier et al. (1982) revealed that rapeseed hulls contain no free phenolic acids, while esterified phenolic acids are the major phenolics found in cotyledons. Because of their hydrophilic nature, only small proportion of phenolic acids is transferred to the oil. The amount of phenolic compounds in cold-pressed rapeseed oil vary from 1.8 to even 4 µg/g (Koski et al. 2003). In our study the concentration of polar phenolic acids, namely: *trans*-sinapic acid and its methyl ester, sinapine, ferulic acid and *p*-coumaric acid was very low, and did not exceed 5 µg/g (Table 3). As distinct from phenolic acids showing low solubility in non-polar media, canolol is less hydrophilic than other phenolics, thus it has better solubility in lipophilic fraction of oil (Matthäus 2012), which is why the dominant polyphenol in the analysed oil samples was canolol (Table 3). Dehulling affected the content of phenolic compounds of the oils, the initial content of phenolic acids was higher in DRO, in contrary to canolol level, which was higher in WRO. The differences in phenolic levels can be explained by their distribution in seeds, where certain fractions of oilseeds contain more phenolic than others. Moreover, as Yang et al. (2011) suggested, the pressure form under the same cold-pressing condition are different between whole and dehulled seeds, which differentiate the transfer ratio of phenolics to the crude oil.

Differences in polarity and molecular structure and thus solubility in the oil is the main factor influencing transfer ratio of phenolic compounds into the crude oil—the higher number of hydroxyl groups (OH) is in a molecule, the more soluble in water it is, loss of polar hydroxyl groups and/or addition of methoxy groups (CH<sub>3</sub>) can decrease the polarity (Brewer 2011). Along with increasing MV pretreatment time only minor changes in the quantities of sinapine, sinapic acid methyl ester, ferulic acid and *p*-coumaric acid in the oil were found, in contrary to canolol, which was found to be the dominants phenolic compound in oil (Table 3). The amount of canolol detected in control WRO and DRO was 61.39 and 13.39 µg/g, respectively. The maximum canolol formation was achieved following 8-min MV exposure, canolol content increased to 456.04 and 320.44 µg/g.

### Effects of dehulling and microwave pretreatment on the colour development of oils

The effect of seeds dehulling and microwaving on the oils colour development is summarised in Table 4. The content of chlorophyll pigments in cold-pressed oil is of great

**Table 3** Phenolic compounds in oils produced from microwave treated whole and dehulled rapeseed

Oil source	Microwave treatment time (min)	Seeds temperature (°C)	Phenolic compounds (µg/g)					
			<i>trans</i> -Sinapic acid	Sinapic acid methyl ester	Ferulic acid	Sinapine	<i>p</i> -Coumaric acid	Canolol
Whole seeds	0	20	1.99 ± 0.002 <sup>a</sup>	0.00 ± 0.000 <sup>a</sup>	0.15 ± 0.000 <sup>a</sup>	0.25 ± 0.001 <sup>a</sup>	0.17 ± 0.001 <sup>a</sup>	61.39 ± 0.07 <sup>a</sup>
	2	56	2.27 ± 0.002 <sup>a</sup>	0.08 ± 0.000 <sup>a</sup>	0.16 ± 0.001 <sup>a</sup>	0.66 ± 0.001 <sup>b</sup>	0.17 ± 0.001 <sup>a</sup>	86.52 ± 0.10 <sup>b</sup>
	4	101	2.23 ± 0.001 <sup>a</sup>	0.08 ± 0.000 <sup>a</sup>	0.13 ± 0.001 <sup>a</sup>	0.94 ± 0.002 <sup>c</sup>	0.16 ± 0.000 <sup>a</sup>	136.84 ± 0.25 <sup>c</sup>
	6	125	2.43 ± 0.004 <sup>a</sup>	0.09 ± 0.000 <sup>a</sup>	0.14 ± 0.000 <sup>a</sup>	1.11 ± 0.002 <sup>c</sup>	0.19 ± 0.001 <sup>a</sup>	365.64 ± 0.37 <sup>d</sup>
	8	144	1.99 ± 0.002 <sup>a</sup>	0.00 ± 0.000 <sup>a</sup>	0.15 ± 0.000 <sup>a</sup>	0.25 ± 0.001 <sup>a</sup>	0.17 ± 0.001 <sup>a</sup>	456.04 ± 0.42 <sup>e</sup>
Dehulled seeds	0	20	3.11 ± 0.005 <sup>B</sup>	0.00 ± 0.000 <sup>A</sup>	0.26 ± 0.001 <sup>A</sup>	0.09 ± 0.001 <sup>A</sup>	0.34 ± 0.001 <sup>A</sup>	13.39 ± 0.04 <sup>A</sup>
	2	31	3.01 ± 0.004 <sup>B</sup>	0.00 ± 0.000 <sup>A</sup>	0.23 ± 0.001 <sup>A</sup>	0.09 ± 0.001 <sup>A</sup>	0.31 ± 0.001 <sup>A</sup>	24.78 ± 0.15 <sup>B</sup>
	4	46	2.07 ± 0.013 <sup>A</sup>	0.00 ± 0.000 <sup>A</sup>	0.20 ± 0.001 <sup>A</sup>	0.09 ± 0.000 <sup>A</sup>	0.29 ± 0.001 <sup>A</sup>	33.45 ± 0.04 <sup>C</sup>
	6	89	2.51 ± 0.005 <sup>A</sup>	0.02 ± 0.001 <sup>A</sup>	0.19 ± 0.001 <sup>A</sup>	0.01 ± 0.001 <sup>A</sup>	0.29 ± 0.001 <sup>A</sup>	141.22 ± 0.06 <sup>D</sup>
	8	119	3.07 ± 0.005 <sup>B</sup>	0.07 ± 0.001 <sup>A</sup>	0.25 ± 0.001 <sup>A</sup>	1.05 ± 0.001 <sup>B</sup>	0.22 ± 0.002 <sup>A</sup>	320.44 ± 0.12 <sup>E</sup>

Mean values denoted by the same letter in the columns do not constitute statistically significant differences at  $p < 0.05$

**Table 4** Colour development in oils produced from microwave treated whole and dehulled rapeseed

Oil source	Microwave treatment time (min)	Total chlorophylls (mg/kg)	Total carotenoids (mg/kg)	Browning index ( $\lambda = 420$ nm)	CIE L*a*b* coordinates <sup>a</sup>			$\Delta E$
					L*	a*	b*	
Whole seeds	0	1.58 ± 0.05 <sup>a</sup>	7.44 ± 0.06 <sup>a</sup>	0.17 ± 0.02 <sup>a</sup>	97.11 ± 0.02 <sup>b</sup>	-3.06 ± 0.01 <sup>a</sup>	27.82 ± 0.07 <sup>a</sup>	-
	2	2.56 ± 0.05 <sup>b</sup>	7.03 ± 0.08 <sup>a</sup>	0.26 ± 0.02 <sup>b</sup>	96.97 ± 0.01 <sup>b</sup>	-3.16 ± 0.01 <sup>a</sup>	30.05 ± 0.01 <sup>a</sup>	2.24 ± 0.03 <sup>a</sup>
	4	3.66 ± 0.14 <sup>c</sup>	11.32 ± 0.36 <sup>b</sup>	0.35 ± 0.01 <sup>b</sup>	95.18 ± 0.02 <sup>a</sup>	-3.80 ± 0.01 <sup>a</sup>	49.86 ± 0.04 <sup>c</sup>	22.14 ± 0.03 <sup>c</sup>
	6	4.56 ± 0.07 <sup>d</sup>	11.78 ± 0.16 <sup>b</sup>	0.46 ± 0.04 <sup>c</sup>	95.51 ± 0.01 <sup>a</sup>	-4.10 ± 0.01 <sup>b</sup>	45.28 ± 0.03 <sup>b</sup>	17.57 ± 0.02 <sup>b</sup>
	8	5.64 ± 0.26 <sup>e</sup>	12.17 ± 0.26 <sup>b</sup>	0.55 ± 0.01 <sup>c</sup>	95.15 ± 0.01 <sup>a</sup>	-4.23 ± 0.00 <sup>b</sup>	49.18 ± 0.01 <sup>c</sup>	21.49 ± 0.01 <sup>c</sup>
Dehulled seeds	0	0.96 ± 0.08 <sup>A</sup>	9.69 ± 0.07 <sup>A</sup>	0.13 ± 0.02 <sup>A</sup>	97.08 ± 0.04 <sup>B</sup>	-3.11 ± 0.01 <sup>A</sup>	29.88 ± 0.13 <sup>A</sup>	-
	2	1.99 ± 0.06 <sup>B</sup>	9.00 ± 0.38 <sup>A</sup>	0.21 ± 0.06 <sup>B</sup>	96.17 ± 0.01 <sup>B</sup>	-3.66 ± 0.01 <sup>A</sup>	33.43 ± 0.03 <sup>B</sup>	3.71 ± 0.02 <sup>A</sup>
	4	2.16 ± 0.13 <sup>C</sup>	13.49 ± 0.14 <sup>B</sup>	0.28 ± 0.02 <sup>B</sup>	95.36 ± 0.01 <sup>A</sup>	-4.15 ± 0.00 <sup>B</sup>	47.87 ± 0.04 <sup>C</sup>	18.10 ± 0.03 <sup>B</sup>
	6	2.89 ± 0.04 <sup>C</sup>	13.56 ± 0.10 <sup>B</sup>	0.32 ± 0.02 <sup>C</sup>	94.82 ± 0.01 <sup>A</sup>	-4.44 ± 0.01 <sup>B</sup>	52.63 ± 0.11 <sup>D</sup>	22.89 ± 0.06 <sup>D</sup>
	8	3.67 ± 0.04 <sup>D</sup>	13.84 ± 0.10 <sup>B</sup>	0.36 ± 0.03 <sup>C</sup>	95.40 ± 0.06 <sup>A</sup>	-4.53 ± 0.05 <sup>B</sup>	50.36 ± 0.30 <sup>D</sup>	20.59 ± 0.04 <sup>C</sup>

<sup>a</sup> L\* lightness of the sample (0 = black, 100 = white); a\* indicates redness by positive or greenness by negative; b\* indicates yellowness by positive or blueness by negative;  $\Delta E$  colour difference

Mean values denoted by the same letter in the columns do not constitute statistically significant differences at  $p < 0.05$

significance because of its potential participation in photosensitized oxidation and thereby contribution to reduced oxidative stability of oil. DROs contained nearly 2-fold lower level of pheophytin *a* compared to WROs, most likely due to removal of hulls, which impart a greenish colour to cold-pressed rapeseed oil (Maheshwari et al. 1980). Analysed control WRO and DRO contained a small amount of total chlorophylls (1.58 and 0.96 mg/kg, respectively), which were increased by microwave treatment, reaching the highest concentration after 8-min exposure (5.64 and 3.67 mg/kg, respectively). Carotenoid pigments represent a group of lipid soluble antioxidant components of oilseeds. The content of carotenoids was at the level of 9.69 and 7.44 mg/100 g, for control DRO and

WRO, respectively. Extending the time of the seeds' thermal treatment yielded to a significant increase of carotenoids. After 8-min MV seeds exposure, the respective increase in carotenoids quantity amounted to 42.8 and 63.6%. As suggested by Vaidya and Choe (2011), increased carotenoid content in oils produced from heated seeds may be partially explained by the fact that carotenoids bound to proteins and form thermostable carotenoid-protein complexes. Thermally induced process of destruction of the internal seed cell structure, including protein denaturation, increases the oil's accessibility to lipid-soluble carotenoids.

MV pretreatment of seeds resulted in darkening of oils, increased browning index and the calculated colour



**Table 5** Oxidative stability and DPPH radical scavenging activity<sup>†</sup> of oils produced from microwave treated whole and dehulled rapeseed

Oil source	Microwave treatment time (min)	Oxidative stability parameters*				DPPH Radical scavenging activity (mmol TEAC/l) <sup>†</sup>			
		PV (meq O <sub>2</sub> /kg)	<i>p</i> -AnV	<i>K</i> <sub>232</sub>	<i>K</i> <sub>268</sub>	IP (h)	HF	LF	TF
Whole seeds	0	0.87 ± 0.09 <sup>a</sup>	0.26 ± 0.03 <sup>a</sup>	0.88 ± 0.04 <sup>a</sup>	0.10 ± 0.03 <sup>a</sup>	4.27 ± 0.03 <sup>a</sup>	0.64 ± 0.00 <sup>a</sup>	1.20 ± 0.06 <sup>a</sup>	1.92 ± 0.00 <sup>a</sup>
	2	0.99 ± 0.06 <sup>a</sup>	0.31 ± 0.05 <sup>b</sup>	0.97 ± 0.04 <sup>a</sup>	0.13 ± 0.03 <sup>a</sup>	4.64 ± 0.09 <sup>a</sup>	1.13 ± 0.02 <sup>b</sup>	1.31 ± 0.00 <sup>b</sup>	2.37 ± 0.01 <sup>b</sup>
	4	1.18 ± 0.11 <sup>a</sup>	0.38 ± 0.04 <sup>b</sup>	1.33 ± 0.02 <sup>b</sup>	0.15 ± 0.12 <sup>a</sup>	4.83 ± 0.12 <sup>a</sup>	1.20 ± 0.00 <sup>c</sup>	1.34 ± 0.08 <sup>b</sup>	2.63 ± 0.01 <sup>c</sup>
	6	1.62 ± 0.05 <sup>b</sup>	0.48 ± 0.06 <sup>c</sup>	1.45 ± 0.06 <sup>b</sup>	0.22 ± 0.07 <sup>b</sup>	6.13 ± 0.07 <sup>b</sup>	2.02 ± 0.01 <sup>c</sup>	1.51 ± 0.02 <sup>c</sup>	3.68 ± 0.00 <sup>c</sup>
	8	1.96 ± 0.08 <sup>b</sup>	0.59 ± 0.09 <sup>c</sup>	2.06 ± 0.01 <sup>c</sup>	0.31 ± 0.07 <sup>c</sup>	6.82 ± 0.07 <sup>b</sup>	2.54 ± 0.05 <sup>d</sup>	1.61 ± 0.00 <sup>d</sup>	4.18 ± 0.00 <sup>d</sup>
Dehulled seeds	0	0.89 ± 0.14 <sup>A</sup>	0.31 ± 0.04 <sup>A</sup>	0.99 ± 0.05 <sup>A</sup>	0.14 ± 0.16 <sup>A</sup>	3.91 ± 0.16 <sup>A</sup>	0.51 ± 0.05 <sup>A</sup>	1.28 ± 0.01 <sup>A</sup>	1.81 ± 0.03 <sup>A</sup>
	2	1.40 ± 0.14 <sup>A</sup>	0.38 ± 0.07 <sup>A</sup>	1.20 ± 0.05 <sup>A</sup>	0.19 ± 0.16 <sup>A</sup>	4.19 ± 0.16 <sup>A</sup>	0.98 ± 0.04 <sup>B</sup>	1.31 ± 0.01 <sup>A</sup>	2.33 ± 0.05 <sup>B</sup>
	4	1.44 ± 0.19 <sup>A</sup>	0.49 ± 0.08 <sup>B</sup>	1.39 ± 0.02 <sup>B</sup>	0.28 ± 0.08 <sup>B</sup>	4.39 ± 0.08 <sup>AB</sup>	1.01 ± 0.06 <sup>B</sup>	1.39 ± 0.01 <sup>A</sup>	2.42 ± 0.06 <sup>B</sup>
	6	1.52 ± 0.13 <sup>A</sup>	0.58 ± 0.06 <sup>BC</sup>	1.56 ± 0.03 <sup>C</sup>	0.30 ± 0.38 <sup>B</sup>	4.93 ± 0.38 <sup>B</sup>	1.23 ± 0.06 <sup>C</sup>	1.64 ± 0.04 <sup>B</sup>	2.93 ± 0.06 <sup>C</sup>
	8	2.00 ± 0.22 <sup>B</sup>	0.67 ± 0.03 <sup>C</sup>	2.13 ± 0.08 <sup>D</sup>	0.38 ± 0.01 <sup>C</sup>	5.22 ± 0.01 <sup>B</sup>	1.89 ± 0.03 <sup>D</sup>	1.73 ± 0.05 <sup>B</sup>	3.69 ± 0.08 <sup>D</sup>

Mean values denoted by the same letter (a, b, A, B, ...) in the columns do not constitute statistically significant differences at *p* < 0.05

\* PV peroxide value; *p*-AnV *p*-anisidine value; *K* specific UV extinction at the indicated wavelength (nm); IP induction period (h) determined by Rancimat test at 120 °C

† Antioxidant activity of: hydrophilic fraction (HF); lipophilic fraction (LF); whole oil (TF)

difference (ΔE) value. The colour of oils gradually changed from light yellow to light brown, which was also observed earlier in oils produced from roasted (Cai et al. 2013). As expected, significant negative correlation between the amount of carotenoids and L\* and a\* colour parameters was found (*r* = −0.777 and −0.692, respectively), whereas strong positive correlation was noted for b\* parameter (*r* = 0.827). A similar correlation between the browning index and CIE L\*a\*b\* coordinates was found. Shrestha and De Meulenaer (2014) reported a significant increase (*p* < 0.05) of the browning index of oil may be due to formation of Maillard type browning reaction products. Furthermore, Durmaz and Gökmen (2010), reported that furfural derivatives were also responsible for darkening of oils produced from seeds that had previously undergone thermal pretreatment.

### Effects of dehulling and microwave pretreatment on antioxidant capacity

Table 5 shows the antioxidant capacity of WROs and DROs, expressed in TEAC for oil (TF), and both for their hydrophilic phase (HF) and the lipophilic fraction (LF). Antioxidant capacity of oils was assessed through DPPH assay, because it enables comparison of the data with the results published by other authors. Based on the results listed in Table 5, both dehulling and MV treatment significantly affected TEAC values (*p* < 0.05). The HF of control WRO and DRO sample showed radical scavenging activity (RSC) value of 0.64 and 0.51 mmol TEAC/l, respectively. The respective RSC value of LF was 1.20 and 1.28 mmol TEAC/l. DROs were characterised by higher RSC of the LF, most likely due to higher concentration of lipophilic-like antioxidants (tocopherols, phytosterols, carotenoids) compared to WROs (Tables 1, 2, 4). On the other hand, higher contribution of HF to RSC was found in WROs, which might be attributed to considerably higher content of hydrophilic antioxidants, especially canolol, than in DROs (Table 3). Along with seeds MV exposure elongation, a significant increase in the RSC of the HF occurred. After 8-min MV exposure, the HF of WRO and DRO showed RSC value of 2.54 and 1.89 mmol TEAC/l, respectively. Statistical analysis showed a significant effect of increased content of canolol and TEAC values of HF of oils. The *Pearson* correlation coefficient was *r* = 0.981 and *r* = 0.933, for WROs and DROs, respectively. A gradual increase of antioxidant capacity of LF along with heating time prolongation was also noted for both WROs and DROs, however, those changes were less pronounced, when compare to HF values. Following 8-min MV exposure, the RSC of the LF of WROs and DROs was 1.61 and 1.73, 1.89 mmol TEAC/l. Significant correlation between

antioxidant capacity of LF and the content of lipophilic antioxidants was found. The RSC of the LF was found to correlate best with the content of total phytosterols ( $r = 0.984$ , for WROs,  $r = 0.965$ , for DROs), total carotenoids ( $r = 0.788$ , for WROs,  $r = 0.850$ , for DROs), and total tocopherols ( $r = 0.451$ , for WROs,  $r = 0.766$ , for DROs).

The RSC value of whole oil was 1.92 and 1.89 mmol TEAC/l, for control WROs and DROs, respectively, and respective antioxidant capacity after 8-min exposure was 4.18 and 3.69 mmol TEAC/l. Similar scavenging activity of DPPH radicals of the oil from 7-min MV treated rapeseeds (3.92 mmol TEAC/l), was obtained by Zheng et al. (2014).

The RSC assayed for whole oil (TF) was higher than the calculated RSC based on arithmetic sum of lipophilic (LF) and hydrophilic (HF) fractions of the oil (Table 5), which may result from cooperative effects of antioxidants in oil. Espin et al. (2000) found, that synergism of phenolic compounds with one another and/or other components present in each fraction may contribute to the high antioxidant activity of rapeseed oil.

## Effects of dehulling and microwave pretreatment on physicochemical properties

### Oxidative stability parameters

The content of primary oxidation products measured as PV of the cold-pressed (control) WRO and DRO were in the range 0.87 and 0.89 meq  $O_2$ /kg. As can be seen from Table 5, heating altered the content of lipid oxidation products considerably ( $p < 0.05$ ); however, none of the oil samples reached upper limit of PV of 15 meq  $O_2$ /kg (CODEX STAN, 2013). Generally, PV is not good index for measurement of oxidation because hydroperoxides are thermally unstable and easily decompose to the more stable secondary oxidation products, such as aldehydes, carbonyls, dienes, trienes, ketones (Halvorsen and Blomhoff 2011). Extending the time of seeds' MV pretreatment time yielded a statistically significant ( $p < 0.05$ ) increase in the accumulation of aldehydes, assessed in terms of changes in *p*-AnV, additionally, a simultaneous increase of conjugated dienes and trienes level took place, resulting from the oxidation process of PUFAs.

Conductometric analysis of the total volatile compounds formed during lipid hydroperoxides degradation based on the results of the Rancimat test are given in Table 5. The induction period (IP) of the control DRO was slightly lower than that of the control WRO (3.91 and 4.27 h, respectively). These minor differences resulted most likely from various amounts of compounds with antioxidant properties. Wroniak

et al. (2013) reported no significant difference in the IP of rapeseed oil pressed from whole and dehulled seeds (IP for cold-pressed oil from *cv.* Monolit was 3.62 h, regardless of dehulling process). On the other hand, Yang et al. (2011) found that dehulling significantly influenced the oxidative stability of the oils, rapeseed oil pressed from dehulled seeds was more easily oxidized. According to Rotkiewicz and Zadernowski (1997) lower content of triglycerides, and higher concentration of mono- and diacylglycerols and free fatty acids is responsible for diminished oxidative stability of rapeseed oil pressed from dehulled seeds.

It was found that microwave time exposure exert a statistically significant effect on the oxidative stability of oils. A gradual increase in the oxidative stability along with increase in heating time occurred for both WROs and DROs, reaching the maximum after 8-min exposure (6.82 and 5.22 h, respectively). This observation concurred with previously published reports (Azadmard-Damirchi et al. 2010, Yang et al. 2013, Wroniak et al. 2016). These authors reported thermally-induced increase of oxidative stability of oils, the reason was probably due to improved extractability of compounds with antioxidant properties, such as tocopherols, canolol, and phytosterols. However, Shrestha and De Meulenaer (2014) pointed out that lipophilic Maillard type browning reaction products, formed in seeds as a results of thermal pretreatment, may also be responsible for enhanced oxidative stability of oil produced from seeds that had previously thermally pretreated. Additionally, phospholipids, released as a result of thermally-induced degradation of lipid bilayer may affect through sequestering of trace pro-oxidant metals, such as iron (Choe and Min 2006). In order to verify the correlation between the aforementioned compounds and oxidative stability of oil, the *Pearson* correlation coefficients were calculated. A positive significant correlation between IP and canolol content was found ( $r = 0.996$ , for WROs,  $r = 0.920$ , for DROs), followed by phytosterols content ( $r = 0.981$ , for WROs,  $r = 0.982$ , for DROs), and total carotenoids ( $r = 0.757$ , for WROs,  $r = 0.860$ , for DROs), and the content of tocopherols ( $r = 0.338$ , for WROs,  $r = 0.715$ , for DROs).

## Conclusion

Obtained results showed that dehulling of rapeseeds increased the concentration of lipophilic bioactive compounds, such as tocopherols, phytosterols and carotenoids in the oil. Rapeseed microwave pretreatment prior to pressing led to gradual increase of phytosterols, carotenoids and phenolic compounds, mainly canolol, while slight changes were observed in terms of tocopherols contents. Along with increased microwave pretreatment

time explicit changes in the oxidative stability and radical scavenging activity of oils were noted. Moreover, seeds thermal treatment accelerated the browning process of oils. Along with increased microwave pretreatment time explicit changes in the oxidative stability and radical scavenging capacity of oils were noted. As many authors suggested at least three factors, namely canolol and MRPs formation under elevated temperatures and phospholipids release from lipid bilayer are responsible for such effects.

#### Compliance with ethical standards

**Conflict of interest** The authors have declared no conflict of interest.

## References

- Aachary AA, Thiyam-Holländer U (2013) An update on characterization and bioactivities of sinapic acid derivatives. In: Thiyam-Holländer U, Eskin NAM, Matthäus B (eds) *Canola and rapeseed: production, processing, food quality, and nutrition*. Taylor & Francis, Boca Raton, pp 21–38
- Anders A (2003) Rapeseed coat removal using disks equipped with cylindrical blades. *Tech Sci* 6:65–72
- AOCS Official Method Cc 13d-55 (1997) Determination of chlorophyll related pigments in oil
- AOCS Official Method Ch 6-91 (1997) Determination of the composition of the sterol fraction of animal and vegetable oils and fats by TLC and capillary GLC. Champaign, IL
- Azadmard-Damirchi S, Habibi-Nodeh F, Hesari J, Nemati M, Achachlouei BF (2010) Effect of pretreatment with microwaves on oxidative stability and nutraceuticals content of oil from rapeseed. *Food Chem* 121:1211–1215
- Azadmard-Damirchi S, Alirezalu K, Achachlouei BF (2011) Microwave pretreatment of seeds to extract high quality vegetable oil. IIRSE: [waset.org/Publication/5056](http://waset.org/Publication/5056)
- Brewer MS (2011) Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Compr Rev Food Sci Food Saf* 10:221–247
- BSI 684-2.20 (1977) Methods of analysis of fats and fatty oils. Other methods. Determination of carotene in vegetable oils. British Standards Institution, London
- Cai L, Cao A, Aisikaer G, Ying T (2013) Influence of kernel roasting on bioactive components and oxidative stability of pine nut oil. *Eur J Lipid Sci Technol* 115:556–563
- Carré P, Quinsac A, Citeau M, Fine F (2015) A re-examination of the technical feasibility and economic viability of rapeseed dehulling. OCL: [10.1051/oc/2014044](https://doi.org/10.1051/oc/2014044)
- Choe E, Min DB (2006) Mechanisms and factors for edible oil oxidation. *Compr Rev Food Sci Food Saf* 5:s169–s186
- CODEX STAN (2013) Codex standard for named vegetable oils. FAO/WHO, Rome. (CODEX STAN 210-1999)
- Dean LL, Davis JP, Sanders TH (2011) Groundnut (Peanut) Oil. In: Gunstone F (ed) *Vegetable oils in food technology: composition, properties and uses*. Wiley, Chichester, pp 225–242
- Durmaz G, Gökmen V (2010) Determination of 5-hydroxymethyl-2-furfural and 2-furfural in oils as indicators of heat pre-treatment. *Food Chem* 123:912–916
- Espin JC, Soler-Rivas C, Wichers HJ (2000) Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. *J Agric Food Chem* 48:648–656
- Halvorsen B, Blomhoff R (2011) Determination of lipid oxidation products in vegetable oils and marine omega-3 supplements. *Food Nutr Res*. doi:[10.3402/fnr.v55i0.5792](https://doi.org/10.3402/fnr.v55i0.5792)
- Hu Z-Y, Hua W, Zhang L, Deng L-B, Liu G-H, Hao W-J (2013) Seed structure characteristics to form ultrahigh oil content in rapeseed. *PLoS ONE*. doi:[10.1371/journal.pone.0062099](https://doi.org/10.1371/journal.pone.0062099)
- ISO 3960 (2005) Animal and vegetable fats and oils. Determination of peroxide value. International Organization for Standardization, Geneva
- ISO 6885 (2008) Animal and vegetable fats and oils. Determination of anisidine value. International Organization for Standardization, Geneva
- ISO 3656 (2011) Animal and vegetable fats and oils. Determination of ultraviolet absorbance expressed as specific UV extinction. International Organization for Standardization, Geneva
- Koski A, Pekkarinen S, Hopia A, Wähälä K, Heinonen M (2003) Processing of rapeseed oil: effects on sinapic acid derivative content and oxidative stability. *Eur Food Res Technol* 217:110–114
- Koubaa M, Mhemdi H, Vorobiev E (2016) Influence of canola seed dehulling on the oil recovery by cold pressing and supercritical CO<sub>2</sub> extraction. *J Food Eng* 182:18–25
- Krygier K, Sosulski F, Hogge L (1982) Free, esterified and insoluble phenolic acids II. Composition of phenolic acids in rapeseed flour and hulls. *J Agric Food Chem* 30:334–336
- Maheshwari PN, Stanley DW, Van De Voort FR (1980) Microwave treatment of dehulled rapeseed to inactivate myrosinase and its effect on oil and meal quality. *J Am Oil Chem Soc* 57:194–199
- Matthäus B (2012) Effect of canolol on oxidation of edible oils. In: Thiyam-Holländer U, Eskin NAM, Matthäus B (eds) *Canola and rapeseed: production, processing, food quality, and nutrition*. Taylor & Francis, Boca Raton, pp 317–328
- Mayengbam S, Khattab R, Thiyam-Holländer U (2013) Effect of conventional and microwave toasting on sinapic acid derivatives and canolol content of canola. *Curr Nutr Food Sci* 9:321–327
- Nacz M, Amarowicz R, Sullivan A, Shahidi F (1998) Current research developments on polyphenolics of rapeseed/canola: a review. *Food Chem* 62:489–502
- Rotkiewicz D, Zadernowski R (1997) Rape seeds dehulling. *Oilseed Crops* 18:493–504
- Shrestha K, De Meulenaer B (2014) Effect of seed roasting on canolol, tocopherol, and phospholipid contents, Maillard type reactions, and oxidative stability of mustard and rapeseed oils. *J Agric Food Chem* 62:5412–5419
- Siger A, Kachlicki P, Czubiński J, Polcyn D, Dwiecki K, Nogala-Kałucka M (2014) Isolation and purification of plastoquinone-8 for HPLC quantitative determinations. *Eur J Lipid Sci Technol* 116:413–422
- Siger A, Kaczmarek A, Rudzińska M (2015) Antioxidant activity and phytochemicals content in cold-pressed rapeseed oil obtained from the roasting seeds. *Eur J Lipid Sci Technol* 117:1225–1237
- Spielmeier A, Wagner A, Jahreis G (2009) Influence of thermal treatment of rapeseed on the canolol content. *Food Chem* 112:944–948
- Tuberoso CIG, Kowalczyk A, Sarritzu E, Cabras P (2007) Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. *Food Chem* 103:1494–1501
- Vaidya B, Choe E (2011) Effects of the seed roasting on tocopherols, carotenoids, and oxidation in mustard seed oil during heating. *J Am Oil Chem Soc* 88:83–90
- Wakamatsu D, Morimura S, Sawa T, Kida K, Nakai C, Maeda H (2005) Isolation, identification, and structure of a potent alkylperoxy radical scavenger in crude canola oil, canolol. *Biosci Biotechnol Biochem* 68:1568–1574
- Wijesundera C, Ceccato C, Fagan P, Shen Z (2008) Seed roasting improves the oxidative stability of canola (*B. napus*) and mustard (*B. juncea*) seed oils. *Eur J Lipid Sci Technol* 110:360–367

- Wroniak M, Andres A, Szterk A, Szymczak R (2013) Effect of seed dehulling on sensory and physical-chemical quality and nutritional value of cold-pressed rapeseed oil. *Food Sci Technol Qual* 5:90–106
- Wroniak M, Rękas A, Siger A, Janowicz M (2016) Microwave pretreatment effects on the changes in seeds microstructure, chemical composition and oxidative stability of rapeseed oil. *LWT Food Sci Technol* 68:634–641
- Yang M, Liu Ch, Huang F, Zheng Ch, Zhou Q (2011) Effect of dehulling treatment on the oxidative stability of cold-pressed low erucic acid rapeseed oil. *J Am Oil Chem Soc* 88:1633–1639
- Yang M, Huang F, Liu Ch, Zheng Ch, Zhou Q, Wang H (2013) Influence of microwave treatment of rapeseed on minor components content and oxidative stability of oil. *Food Bioproc Tech* 6:3206–3216
- Yang M, Zheng Ch, Zhou Q, Liu Ch, Li W, Huang F (2014) Influence of Microwaves Treatment of Rapeseed on Phenolic Compounds and Canolol Content. *J Agr Food Chem* 62:1956–1963
- Yoshida H, Takagi S (1997) Effects of seed roasting temperature and time on the quality characteristics of sesame (*Sesamum indicum*) oil. *J Sci Food Agric* 75:19–26
- Yoshida H, Takagi S, Mitsuhashi S (1999) Tocopherol distribution and oxidative stability of oils prepared from the hypocotyls of soybeans roasted in microwave oven. *J Am Oil Chem Soc* 76:915–920
- Zheng Ch, Yang M, Zhou Q, Liu Ch-S, Huang FH (2014) Changes in the content of canolol and total phenolics, oxidative stability of rapeseed oil during accelerated storage. *Eur J Lipid Sci Technol* 116:1675–1684