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Chemical composition and resistance to oxidation of high-oleic rapeseed oil pressed from microwave pre-treated intact and de-hulled seeds

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SUMMARY: The influence of a microwave (MV) pre-treatment (3, 6, 9 min, 800W) on the physicochemical properties of high-oleic rapeseed oil prepared from intact (HORO) and de-hulled seeds (DHORO) was investigated in this study. A control DHORO contained higher levels of total tocopherols and carotenoids, while higher concentrations of total phenolic compounds and chlorophylls were detected in the HORO. The MV pre-treatment caused a decrease in the unsaturated fatty acids content that was more evident for the DHOROs. The microwaving time significantly affected phytochemical contents and the color of both types of oils. A vast increase in canolol concentration was noticeable following 9 min of microwaving, which increased 506- and 155-fold in the HORO and DHORO, respectively. At the same time, the antioxidant capacity of oil produced from MV pre-treated seeds for 9 min was nearly 4 times higher than that of the control oil for both types of oils.

KEYWORDS: *Fatty acids; High-oleic rapeseed oil; Hulling; Microwaving; Oxidative stability; Phytochemicals*

RESUMEN: ***Composición química y resistencia a la oxidación de aceite de colza alto oleico prensado de semillas intactas y descascarilladas pre-tratadas con microondas.*** En este estudio se investigó la influencia del pretratamiento con microondas (MV) en las propiedades fisicoquímicas del aceite de colza alto oleico preparado a partir de semillas húmedas (HORO) y descascarilladas (DHORO) (3, 6, 9 min, 800W). El control DHORO contenía un nivel más alto de tocoferoles totales y carotenoides, mientras que se detectó una mayor concentración de compuestos fenólicos totales y clorofilas en el HORO. El pretratamiento de MV provocó una disminución en el contenido de ácidos grasos insaturados que fue más evidente para los DHOROs. El tiempo de microondas afectó significativamente al contenido fitoquímico y al color de ambos tipos de aceites. Se observó un gran aumento de la concentración de canolol después de 9 min de microondas, que aumentó 506 y 155 veces en el HORO y DHORO, respectivamente. Al mismo tiempo, la capacidad antioxidante del aceite producido a partir de semillas pretratadas de MV durante 9 min fue casi 4 veces mayor que la del aceite de control para ambos tipos de aceites.

PALABRAS CLAVE: *Aceite de colza alto oleico; Ácidos grasos; Descascarillar; Estabilidad oxidativa; Fitoquímicos; Microondas*

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1. INTRODUCTION

Rapeseed/canola now occupies the third position in rank order for the production of oils and fats after palm and soybean oil. EU-27, China and India dominate the production and consumption of this oil, whereas Canada is an important grower and exporter of rapeseeds (FAOSTAT, 2017). In the early 1960's Canadian plant breeders isolated rapeseeds with low eicosenoic and erucic acid contents. As a result, the past 50 years have seen significant growth in rapeseed production due to the introduction of rapeseed (canola) which is low in erucic acid (C22:1) and glucosinolates. Since then, rapeseed regained status in the market as a healthy vegetable oil. The term "rapeseed oil" is used for the original (erucic acid free) variety with around 10% linolenic acid. Presently, the fatty acid profile of rapeseed oil has been modified according to its health benefits, such as low linoleic acids (LLi), or to enhance nutritional and functional quality by introducing high-oleic acid (HOAR) or high-oleic/low linolenic acid (HOLLi) rapeseed varieties into the market. Rapeseed oil which contains more than 60% erucic acid (HEAR) has regained interest for biodegradable plastic, cosmetic, emollient industries and for biodiesel (Nath *et al.,* 2016).

The studies investigating the effect of low-erucic rapeseed (LEAR, canola) hulling are few and contradictory. Wroniak *et al.* (2013) found that conditioning rapeseed hulling decreased the amount of tocopherols and unsaturated fatty acids in the oil, while the effect of hulling on the oxidative stability of the oil was observed. Yang *et al.* (2011) reported that rapeseed hulling decreased the amount of tocopherols and sterols in the oil, but paradoxically increased the oxidative stability of the oil, as shown by a higher induction period in the Rancimat test. An unfavorable effect of hulling on the oxidative stability of the oil has also been demonstrated after the de-hulling of sesame seeds (Abou-Gharbia *et al.,* 1997).

The effect of soybean and sunflower seed microwave pre-treatment on the bioactive compound contents, oxidative stability and color change in the oil has been well documented (Anjum *et al.,* 2006; Yoshida *et al.,* 1999). The microwave pre-treatment of rapeseeds has been restricted to emphasize its impact on the phytochemical contents and oxidative stability Azadmard-Damirchi *et al.,* 2010, Yang *et al.,* 2013) or flavor characteristics (Zhou *et al.,* 2013).

This study was performed to provide information on the effect of hulling of high-oleic rapeseed on the physicochemical properties of the oil. De-hulling can improve the color, flavor and nutritional value of the oil, and therefore, the phytochemical contents and oil color change following rapeseed hulling were determined. Since high-oleic rapeseed oils are recommended for processes where high stability of the oil is required such as deep-fat frying, this work focused on the impact of the intact and de-hulled seeds' thermal pre-treatment by microwave on the extent of the oil's oxidation, measured by quantitative changes in fatty acid content, formation of primary and secondary oxidation products and the oxidative stability.

Therefore, the aims of this study were to (1) investigate the effect of hulling on the physicochemical composition of the resulting oil; (2) investigate the oxidative resistance to oxidation of the oil pressed from high-oleic rapeseeds.

2. MATERIALS AND METHODS

2.1. Experimental material

Seeds of high-oleic rapeseed were provided by the Złoto Polskie CLP (Kalisz, Poland). Seeds were harvested at optimum maturity, and did not contain any impurities or broken seeds. The moisture content of the rapeseeds was 6.1%.

2.2. Reagents

Analytical standards of tocopherols (> 95%), HPLC-grade n-hexane, methanol, acetonitrile (ACN), orthophosphoric acid, and 1,4-dioxane were provided by Calbiochem-Merck Biosciences (Darmstadt, Germany). KOH in methanol (1M), hexane, sodium methoxide (0.4N), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other solvents and chemicals used in this study were of analytical grade.

2.3. Sample moisturizing

The moisture content of the seeds was determined based on a precision weighing balance, using an Electronic Moisture Analyzer (Kern & Sohn GmbH, Germany). The seeds (batches of 500g) were sprayed with a pre-calculated amount of water, mixed thoroughly, sealed in polyethylene bags and equilibrated at 4 ± 2 °C for 72 h. Based on the results presented in our previous work (Rękas *et al.,* 2017a) a seed moisture level of 7.5% prevents seeds from overheating during microwave pre-treatment, whereas seed moisturizing prior to cold-pressing to the moisture content of 8.5% enables the highest oil yield. For this reason, the seeds were moistened twice: to the moisture content of 7.5% prior to microwave pre-treatment and before cold-pressing to reach the required moisture content of 8.5%. After 72 h of equilibration, the moisture content of the seeds was checked in order to verify whether homogeneous moisture distribution throughout the bulk of the seeds was reached.

2.4. Mechanical de-hulling

Mechanical hulling of the rapeseeds was performed using a shearing disc sheller equipped with cylindrical blades, developed by Anders (2003) following the method described in our previous work (Rękas *et al.,* 2017b).

2.5. Pre-treatment with microwaves

For each microwave (MV) pre-treatment, 500 g of seeds were placed in a glass beaker (16-cm diameter) inside the microwave (Model: NN-J155W). The seeds were exposed to microwave irradiation for 3, 6, and 9 min (2450 MHz, 800 W). Intact and dehulled high-oleic rapeseed samples without microwave radiation (0 min radiation time) were 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 homogenous sampling.

2.6. Cold-pressing

Pressing was carried out by applying the screw press method (Farmet, Czech Republic), where the temperature was kept below 40 °C. Once produced, the oil was stored at 4 °C overnight in the dark until analyzed.

2.7. HPLC analysis of tocopherols, plastochromanol-8 and canolol

Tocochromanols (α-, β-, γ-, and δ-tocopherol and plastochromanol-8) and canolol 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 Si-60 column (250 × 4.6 mm, 5 µm, 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^{-1} (for tocopherols and plastochromanol-8) and 2.0 min^{-1} (for canolol). Quantification of tocochromanols and canolol was conducted using data from the FLD with excitation/emission wavelengths of 295/330 nm and 280/325 nm, respectively. The plastochromanol-8 contents were assayed and calculated following the method described by Siger *et al.* (2014).

2.8. Analysis of total phenolic compounds

The total phenolic content was determined by the Folin-Ciocalteau colorimetric method (Koski *et al.,* 2002). Sinapic acid was used for calibration, and the results were expressed as milligram of sinapic acid per 100 g oil.

2.9. Identification and quantification of fatty acid methyl esters by GC-FID

Fatty acid methyl esters (FAMEs) were prepared using sodium methoxide (CH_3ONa, 0.4N) as a catalyst, following the method described previously (Rękas *et al.,* 2017b). The fatty acids were identified using a reference mixture of fatty acids methyl esters (FAME). Nonadecanoid acid (C19:0) was used as internal standard for the quantification.

2.10. Color indices

The total chlorophyll content (mg/kg) was quantified by spectrophotometry according to the AOCS Method (1997). The total carotenoid content was measured according to the BSI Method (1977).

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

The CIE Lab coordinates (L*, a*, b*) were directly read with a spectrophotometer (CM-3600d, Konica Minolta, Japan). Color difference (ΔE) was calculated as $\Delta E = [(L_0^*-L^*)^2+(a_0^*-a^*)^2+(b_0^*-b^*)^2]^{1/2}$, where L_0^*, a_0^*, and b_0^* are the color parameters of the control HORO and DHORO.

2.11. Oil oxidation indices

The peroxide value (PV), p-anisidine value (p-AnV), and K232 and K268 extinction coefficients, were measured following ISO standard methods (3960:2005; 6885:2008; 3656:2011).

Oxidative stability, expressed as the oxidation induction period (hours), was measured with the Rancimat apparatus (model 743 Metrohm Co, Herisau, Switzerland) using an oil sample of 2.5 g warmed to 120 °C, and an air flow of 20 l/h. All determinations were carried out in triplicate.

2.12. Antioxidant capacity

The radical scavenging capacity (RSC) of the oil sample was analyzed using the DPPH radical-scavenging following the method described by Tuberoso *et al.* (2007). The antioxidant capacities of the oil (TF), hydrophilic (HF), lipophilic (LF) fraction were measured spectrophotometrically at 517 nm (Spectronic Helios β UV-Vis, Thermo Electron Corporation, Waltham, MA, USA). The results were expressed as a Trolox equivalent antioxidant capacity (TEAC, mmol l^{-1}), using a Trolox calibration curve in the range of 0.02–4.00 mM.

2.14. Statistical analysis

A statistical analysis was carried out with Statistica v. 12 software (StatSoft, Inc., Tulsa, OK, USA). Statistical differences among the oil samples were estimated by applying one-way ANOVA and using the Tukey test at a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. The effect of microwave pre-treatment on the phytochemical contents

The contents of the individual tocopherol homologues in the HOROs and DHOROs prepared at the different MV conditions, as well as in the cold-pressed oil, are given in Table 1. A total of 4 tocopherol homologues (α, β, γ and δ) were identified in the rapeseed oil, which is consistent with previous studies (Yang *et al.*, 2013; Siger *et al.,* 2015). The total tocopherol contents in the control HORO and DHORO was 62.70 and 62.74 mg $100g^{-1}$, respectively, with the preponderance of γ-tocopherol (more than 58% of total tocopherols). The plastochromanol-8 (PC-8) content in the control DHORO was 2.78 mg $100g^{-1}$ and was slightly higher than in the control HORO (2.49 mg $100g^{-1}$). The favorable of effect low-erucic rapeseed on the tocopherol concentrations in the oil was demonstrated by the results obtained by Yang *et al.* (2011) and Wroniak *et al.* (2013).

Since tocopherols are thermal-sensitive, it was expected that the oil from MV seeds would have lower tocopherol concentrations. However, as the seeds' MV pre-treatment time increased, the total tocopherol content of the oil increased. A maximum total tocopherol concentration in the oil was achieved following 9 min of seed microwaving, where 70.03 and 71.19 mg·$100g^{-1}$ of total tocopherols were determined in the HORO and DHORO, respectively. However, α- and β-T were found to decrease with longer seed exposure to MV. At the same time, an increase in γ- and δ-T was noticed (Table 1). Moreover, the HOROs' tocopherols were more susceptible to degradation during seed MV than the DHOROs. After 9 min of MV, the levels of α-T were reduced to 24.7 mg·$100g^{-1}$ (3.4%) and 25.10 mg·$100g^{-1}$ (0.8%), for HORO and DHORO, respectively. The respective increase in γ-T was 44.60 mg·$100g^{-1}$ (22.4%) and 45.37 mg·$100g^{-1}$ (24%). After 6 min of MV, a 2-fold increase in PC-8 was noted, whereas extending MV time to 9 min did not cause a significant increase in this compound concentration in the HORO or DHORO. A reduction in α-T after seed and nut MV was observed by other authors (Yoshida *et al.,* 1999; Anjum *et al.,* 2006; Cai *et al.,* 2013). The roasting of rapeseeds (Siger *et al.,* 2015) and pumpkin seeds (Vujasinovic *et al.,* 2012) was found to increase γ-T concentration in the oil, while the roasting of pine nuts (Cai *et al.,* 2013) and sunflower seeds (Anjum *et al.,* 2006) resulted in a reduction in this compound. As shown by Moreau *et al.* (1999) a significant amount of γ-T in corn hulls formed complexes with proteins or was linked to phosphates or phospholipids. An increase in γ-T in the oil may be a result of heat-induced degradation of these bonds. Wijesundera *et al.* (2008) offered a possible explanation for the heat-induced increase in the levels of tocopherols, suggesting a possible co-elution of tocopherols with other compounds formed during rapeseed roasting. Although browning substances, resulting from Maillard-type reactions, are very polar due to active radicals, as suggested by McDaniel *et al.* (2012) it is possible that some Maillard browning compounds are oil soluble. This would partially explain the potential role of Maillard browning products in protecting the tocopherols from degradation.

Although the levels of total phenolics and canolol were higher in the control DHORO (0.41 and 0.76 mg $100g^{-1}$, respectively), with a longer seed exposure to MV, a greater increase in these compound concentrations was found in the HOROs (Table 2). After 6 and 9 min of MV of de-hulled seeds, the levels of canolol were 9- and 155-fold higher in DHORO than in the control DHORO. Intact seeds' MV for 6 and 9 min resulted in a 30- and 506-fold increase in canolol concentrations in the HOROs. A vast increase in canolol contents in the oil pressed from microwaved/roasted seeds was also reported by other authors (Yang *et al.,* 2014; Siger *et al.,* 2015). An increase in total phenolic compounds in the oil was also found for nut and oilseed roasting, although the changes in total phenolics in the oils were dependent on the roasting temperature and processing time (Vujasinovic *et al.,* 2012; Cai *et al.,* 2013). Generally, oilseed thermal pre-treatment causes evaporation of intracellular water, triggering chemical reactions that can change the lignocellulosic structure and promote protein denaturation, which may result in a greater availability of plant phenolic compounds in the matrix. This phenomenon was confirmed in a study by Yang *et al.* (2014) where MV pre-treatment applied to rapeseed yielded increased transfer of phenolic compounds from rapeseed to oil.

3.2. The effect of microwave pre-treatment on color changes in the oil

Chlorophyll and carotenoids, together with membrane proteins and membrane lipids, form a membrane-bound compartment called thylakoid. Crude rapeseed oil may contain as much as 95mg kg^{-1} total carotenoids. Unlike carotenoids, chlorophyll and its derivative (pheophytin) are not wanted in oils

TABLE 1. Tocochromanols, canolol and total phenolic compound concentrations (mg $100g^{-1}$) of high-oleic rapeseed oils produced from microwave pre-treated intact and de-hulled seeds

Oil source	**HORO**				**DHORO**			
Microwaving time (min)	**0**	**3**	**6**	**9**	**0**	**3**	**6**	**9**
Tocochromanols (mg·100g^{-1})								
α-Tocopherol	25.58 ± 0.07^{a}	25.40 ± 0.02^{a}	25.59 ± 0.03^{a}	24.70 ± 0.14^{a}	25.33 ± 0.02^{A}	25.81 ± 0.07^{A}	25.57 ± 0.11^{A}	25.11 ± 0.00^{A}
β-Tocopherol	0.12 ± 0.01^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.08 ± 0.01^{a}	0.11 ± 0.01^{A}	0.11 ± 0.01^{A}	0.10 ± 0.01^{A}	0.06 ± 0.01^{A}
γ-Tocopherol	36.44 ± 0.03^{a}	36.81 ± 5.17^{a}	36.32 ± 0.20^{a}	44.60 ± 0.24^{b}	36.60 ± 0.12^{A}	36.76 ± 0.07^{A}	37.31 ± 0.05^{B}	45.37 ± 0.12^{C}
δ-Tocopherol	0.58 ± 0.01^{a}	0.60 ± 0.03^{a}	0.69 ± 0.02^{a}	0.65 ± 0.01^{a}	0.72 ± 0.01^{A}	0.71 ± 0.01^{A}	0.74 ± 0.01^{A}	0.66 ± 0.02^{A}
Total tocopherols	62.70 ± 0.06^{a}	61.89 ± 0.23^{a}	62.68 ± 0.23^{a}	70.03 ± 0.40^{b}	62.74 ± 0.10^{A}	63.38 ± 0.14^{B}	63.71 ± 0.07^{B}	71.19 ± 0.10^{C}
Plastochromanol-8	2.49 ± 0.05^{a}	3.49 ± 0.05^{a}	5.46 ± 0.11^{b}	5.49 ± 0.03^{b}	2.78 ± 0.06^{A}	4.29 ± 0.04^{B}	5.68 ± 0.20^{C}	5.76 ± 0.08^{C}
Phenolic compounds (mg·100g^{-1})								
Canolol	0.32 ± 0.09^{a}	0.74 ± 0.09^{b}	9.59 ± 0.09^{c}	162.00 ± 0.74^{d}	0.41 ± 0.04^{A}	0.85 ± 0.17^{B}	3.64 ± 0.16^{C}	63.52 ± 4.27^{D}
Total phenolic compounds	0.57 ± 2.31^{a}	0.98 ± 3.02^{b}	11.45 ± 1.12^{c}	165.45 ± 5.05^{d}	0.76 ± 0.34^{A}	1.15 ± 4.31^{B}	5.65 ± 4.04^{C}	68.67 ± 3.34^{D}

(a-d) Denotes statistically significant differences ($p < 0.05$) within a group of oils pressed from intact seeds.
(A-D) Denotes statistically significant differences ($p < 0.05$) within a group of oils pressed from de-hulled seeds.

TABLE 2. Color development of high-oleic rapeseed oils produced from microwave pre-treated intact and de-hulled seeds

Oil source	**HORO**				**DHORO**			
Microwaving time (min)	**0**	**3**	**6**	**9**	**0**	**3**	**6**	**9**
Total chlorophylls (mg·kg^{-1})	1.08 ± 0.09^{a}	3.03 ± 0.09^{b}	4.13 ± 0.11^{c}	5.17 ± 0.13^{d}	0.61 ± 0.04^{A}	2.07 ± 0.12^{B}	2.75 ± 0.22^{B}	4.32 ± 0.17^{C}
Total carotenoids (mg·kg^{-1})	6.31 ± 0.31^{a}	7.98 ± 0.02^{a}	8.81 ± 0.12^{b}	8.63 ± 0.05^{b}	7.82 ± 0.34^{A}	7.99 ± 0.12^{A}	10.31 ± 0.04^{B}	10.02 ± 0.34^{B}
Browning index (λ=420 nm)	0.090 ± 0.03^{a}	0.144 ± 0.05^{bc}	0.166 ± 0.00^{cd}	0.196 ± 0.01^{d}	0.100 ± 0.00^{A}	0.145 ± 0.01^{B}	0.167 ± 0.09^{C}	0.176 ± 0.05^{D}
CIE L*a*b* coordinates†								
L*	96.99 ± 0.02^{a}	96.06 ± 0.01^{a}	95.55 ± 0.01^{b}	95.34 ± 0.00^{b}	97.21 ± 0.01^{A}	97.03 ± 0.02^{A}	96.15 ± 0.00^{B}	95.59 ± 0.01^{C}
a*	-3.31 ± 0.01^{a}	-3.66 ± 0.01^{b}	-4.15 ± 0.00^{c}	-4.84 ± 0.01^{d}	-3.06 ± 0.01^{A}	-3.16 ± 0.01^{A}	-3.57 ± 0.01^{B}	-3.81 ± 0.01^{C}
b*	27.82 ± 0.07^{a}	30.05 ± 0.01^{a}	49.86 ± 0.04^{c}	45.28 ± 0.03^{b}	29.88 ± 0.13^{A}	33.43 ± 0.03^{B}	47.87 ± 0.04^{C}	52.63 ± 0.11^{D}
ΔE	-	2.44 ± 0.03^{a}	22.10 ± 0.03^{c}	17.60 ± 0.02^{b}	-	3.56 ± 0.02^{A}	18.03 ± 0.03^{B}	22.82 ± 0.06^{D}

†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; ΔE color difference
(a-d) Denotes statistically significant differences ($p < 0.05$) within a group of oils pressed from intact seeds.
(A-D) Denotes statistically significant differences ($p < 0.05$) within a group of oils pressed from de-hulled seeds.

because they produce an undesirable green hue in the oil, in addition to the pro-oxidative effect of chlorophylls in oil exposed to light. The level of chlorophylls in the crude rapeseed oil may vary from 5 to 25 ppm (Przybylski, 2011). In our study, the control oils contained very low amounts of chlorophylls; however, the level of chlorophyll pigments in the DHORO was nearly 2-times lower (0.61 mg kg^{-1}) than in the control HORO (1.08 mg kg^{-1}). Extending the time of seeds' MV yielded a significant ($p < 0.05$) increase in the total carotenoid and chlorophyll contents (Table 2).

A color change in the oil was the most noticeable effect of rapeseed de-hulling. The DHORO had a bright yellowish color, suggesting that the coloring compounds, especially those responsible for the undesirable green hue, were significantly removed from the seeds by de-hulling (Abou-Gharbia *et al.,* 1997). By increasing MV time, an increase in the browning index and a distinct color change (ΔE) was noted (Table 2). Although higher browning index values were noted for the HOROs, the MV of de-hulled seeds resulted in a more noticeable oil color change, as indicated by the calculated color difference (ΔE). However, in both types of oils, intensification of the brown color increased with longer seed MV pre-treatment. Gradual oil darkening and higher CIE L*a*b* indices with increasing roasting/microwaving time of oilseeds and nuts was reported by other authors (Megahed, 2001; Anjum *et al.,* 2006; Cai *et al.,* 2013). According to those authors, color intensity increases with the formation of browning substances, as a result of Maillard-type non-enzymatic reactions and phospholipid degradation.

3.3. The effect of microwave pre-treatment on oxidative stability

High oleic rapeseed oil combines high oxidation stability with the lowest saturated fatty acid contents among other commercial edible oils (Merrill *et al.,* 2008). The fatty acid content (mg $100g^{-1}$) of both the control HORO and DHORO was typical for the high-oleic rapeseed – the dominant fatty acid was C18:1, followed by C18:2 and C18:3, in the concentrations of 76.58, 76.32; 10.65, 10.86; 4.80, 4.93 mg·$100g^{-1}$, respectively (data not shown).

The de-hulling of rapeseed prior to microwaving affected fatty acids' susceptibility to thermal degradation (Figure 1). After 6 min of MV, the percentage loss in PUFAs was 5.6 and 8.3%, for the HORO and DHORO, respectively. Seed MV for 9 min caused the highest degradation of PUFAs, where 11.9 and 14.5% of PUFAs had degraded, respectively. MV pre-treatment of the intact and de-hulled seeds was found to affect MUFA and SFA contents. After 9 min of MV, the amount of SFAs decreased from 6.63 to 6.53 mg·$100g^{-1}$ for HORO, and from 6.65 to 6.51 mg·$100g^{-1}$, for DHORO. The respective loss in MUFAs was within the range of 77.89–72.31 mg·$100g^{-1}$ and 77.92–70.95 mg $100g^{-1}$. As shown by Gracka *et al.* (2016), the loss in SFAs, MUFAs and PUFAs after roasting of low-erucic rapeseed was nearly 2-fold lower when compare to the fatty acid loss in the oil pressed from high-oleic rapeseed oil.

As shown in Table 3, the initial oxidative quality of the control DHORO was comparable to that of the control DHORO. After microwaving, the HOROs were more stable than the DHOROs, as reflected in PV, *p*-AV and the values of conjugated

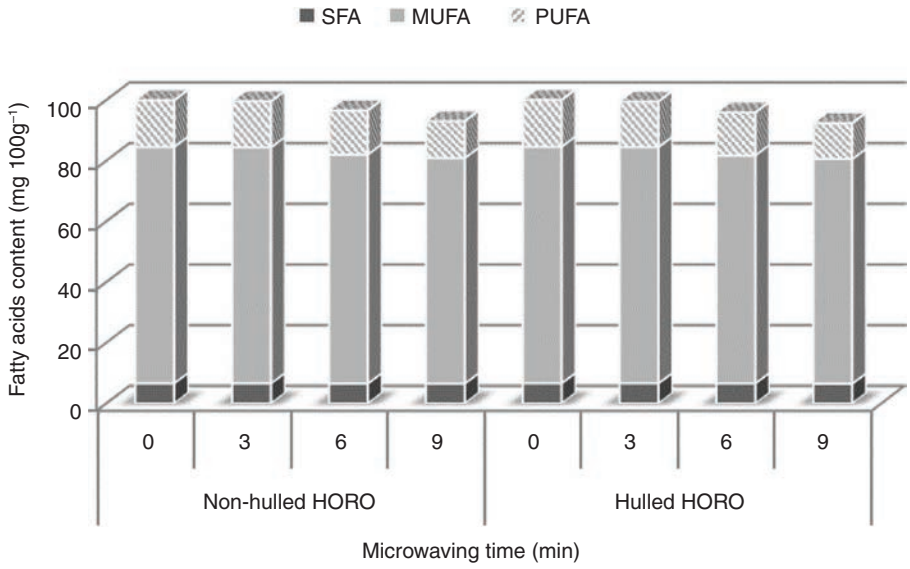

FIGURE 1. The effect of microwave pre-treatment on the changes in fatty acid contents (mg $100g^{-1}$) of high-oleic rapeseed oils.

TABLE 3. Oxidative stability parameters and antioxidant capacity (mmol TEAC l^{-1}) of rapeseed oils produced from microwave pre-treated intact and de-hulled seeds.

		Oxidative stability parameters*					Antioxidant capacity (mmol TEAC l^{-1})†		
Oil source	Microwaving time (min)	PV (meq O_2·kg^{-1})	*p*-AnV	K_{232}	K_{268}	IP (h)	HF	LF	TF
HORO	0	0.93 ± 0.09^{a}	0.17 ± 0.03^{a}	1.31 ± 0.046^{a}	0.067 ± 0.001^{a}	6.72 ± 0.01^{a}	0.62 ± 0.05^{a}	0.86 ± 0.04^{a}	1.54 ± 0.01^{a}
	3	1.25 ± 0.16^{ab}	0.13 ± 0.03^{a}	1.35 ± 0.052^{a}	0.074 ± 0.009^{a}	7.99 ± 0.08^{b}	0.98 ± 0.11^{b}	1.13 ± 0.04^{b}	2.21 ± 0.08^{b}
	6	1.31 ± 0.03^{b}	0.26 ± 0.05^{b}	1.41 ± 0.023^{b}	0.128 ± 0.013^{b}	8.74 ± 0.23^{b}	2.33 ± 0.04^{c}	1.32 ± 0.03^{c}	3.86 ± 0.04^{c}
	9	1.69 ± 0.18^{c}	1.26 ± 0.09^{c}	2.03 ± 0.014^{c}	0.565 ± 0.004^{c}	11.94 ± 0.31^{c}	5.17 ± 0.06^{d}	1.49 ± 0.09^{d}	6.89 ± 0.06^{d}
DHORO	0	1.05 ± 0.12^{A}	0.06 ± 0.03^{A}	1.28 ± 0.052^{A}	0.066 ± 0.003^{A}	5.69 ± 0.04^{A}	0.54 ± 0.01^{A}	0.94 ± 0.04^{A}	1.62 ± 0.04^{A}
	3	1.42 ± 0.09^{B}	0.25 ± 0.06^{B}	1.33 ± 0.025^{B}	0.072 ± 0.007^{A}	6.59 ± 0.23^{B}	0.93 ± 0.03^{B}	1.18 ± 0.07^{B}	2.36 ± 0.13^{B}
	6	1.51 ± 0.04^{B}	0.61 ± 0.07^{C}	1.42 ± 0.023^{C}	0.132 ± 0.002^{B}	8.49 ± 0.08^{C}	2.15 ± 0.09^{C}	1.28 ± 0.01^{BC}	3.52 ± 0.04^{C}
	9	3.12 ± 0.20^{C}	2.27 ± 0.01^{D}	2.58 ± 0.011^{D}	0.975 ± 0.011^{C}	10.58 ± 0.04^{D}	4.14 ± 0.12^{D}	1.72 ± 0.02^{C}	5.94 ± 0.02^{D}

*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) and whole oil (TF)
(a-d) Denotes statistically significant differences ($p < 0.05$) within a group of oils pressed from intact seeds.
(A-D) Denotes statistically significant differences ($p < 0.05$) within a group of oils pressed from de-hulled seeds.

dienes and trienes. Abou-Gharbia *et al.* (1997) found that oils from coated sesame seeds were more resistant to oxidation than those extracted from de-hulled seeds after roasting and microwaving. The results of the Rancimat test showed that hulling decreased the oxidative stability of the oil, as the induction period (IP) determined for the control HORO was higher (6.72 h) than that of the control DHORO (5.69 h). By extending MV time, a gradual increase in IP was observed, which is in agreement with studies employing rapeseed MV pre-treatment (Azadmard-Damirchi *et al.,* 2010; Yang *et al.,* 2013). The oxidative stability of the HOROs and DHOROs showed 2-fold higher IP after seeds' 9 min exposure to microwaves when compare to the control oil, and were 11.94 and 10.58 h, respectively. This special phenomenon of a vast increase of the oils' oxidative stability was also observed after different oilseed thermal pre-treatments (Wijesundera *et al.,* 2008; Vaidya and Choe, 2011; Vujasinovic *et al.,* 2012).

3.4. The effect of microwave pre-treatment on antioxidant capacity

The antioxidant capacity of the methanol soluble phase (HF) was higher for the control HORO, while a higher TEAC value of the insoluble in methanol fraction (LF) was calculated for the control DHORO (Table 3). On the whole (TF), the control DHORO showed a higher TEAC value (1.62 mmol·l^{-1}) than the control HORO (1.54 mmol·l^{-1}). As MV time increased from 3 to 9 min, the TEAC values of TF increased from 2.21 to 6.89 mmol·l^{-1}, for the HORO, and from 2.36 to 5.94 mmol·l^{-1}, for the DHORO.

In general, the rapeseed oil lipophilic fraction contains mainly tocopherols, carotenoids and phospholipids, whereas phenolic compounds are the major constituents of the hydrophilic fraction. The relative antioxidant activity *in vitro* of tocochromanol homologues varies greatly due to its dependence on a number of factors, including the composition of the system, the temperature, the structure of the lipid phase, and the concentration of tocochromanols (Falk and Munné-Bosch, 2010). Because of the complex interplay between the chemical and physical parameters of the lipid substrate and of the overall system, the order of antioxidant activity of tocochromanol homologues resulting from the balance between anti- and pro-oxidant activates, as well as the prediction of antioxidant activity of tocochromanols, is unreliable. As shown by Kamal-Eldin (2006), the pro-oxidant or antioxidant activity intimately depends on their concentration. The inhibition of vegetable oil oxidation is usually associated with a critical minimal concentration of antioxidants (60–70 mg·kg^{-1} of a tocopherol in olive oil). (Blekas *et al*., 1995). Studies by Goffman *et al*. (1999) revealed that the tocopherol contents in

oils (*Brassicaceae* family) are close to their optimal amounts needed for the stabilization of these oils.

The antioxidant activity of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule. Monophenols are less efficient antioxidants than polyphenols, the presence of a secondary hydroxyl group in the *ortho* or *para* position increases the antioxidant activity of hydroxylated phenolic acids; however, the presence of more than three hydroxyl groups in a given aromatic nucleus does not improve the antioxidant efficacy (Shukla *et al*., 1997). The presence of a CH=CH-COOH group in cinnamic acids (caffeic, *p*-coumaric, ferulic, sinapic acids) ensures greater efficiency of antioxidative ability than the COOH group in benzoic acids (protocatechuic, *p*-hydroxybenzoic, vanillic, syringic acids); however, a single COOH group exhibits a negative role in antioxidative activity (Cuvelier *et al*., 1992).

The heat pre-treatment of oilseeds leads to the formation of new antioxidants, including canolol and Maillard reaction products. Canolol (2,6-dimethoxy-4-vinylphenol) classified as phenolic compound has gained interest in recent years due to antioxidant, anticancer, anti-inflammatory, and antibacterial activities. There are a number of scientific evidences that canolol is a potent antioxidant and anti-mutagenic compound. Galano *et al*. (2011) reported canolol as similar hydroxyl radical scavenging agent to carotenes, Koski *et al*. (2003) demonstrated that the lipid peroxyl radical scavenging activity of canolol is higher than that of other compounds isolated from rapeseed oil, while Kuwahara *et al*. (2004) found canolol to be a good peroxynitrite scavenger.

Iimproved oxidative stability and antioxidant capacity of the mustard and rapeseed oils were also found to correlate well with increased contents of phosphorus and phospholipids (Shrestha *et al.,* 2014). Studies by Bandarra *et al*. (1999) revealed a high degree of synergy between phospholipids and tocopherols, most likely due to the occurrence of a simultaneous antioxidant mechanism involving Maillard compounds. Antioxidant synergism between tocopherols and other bioactive compounds, such as carotenoids and phenolic compounds, was confirmed by Kamal-Eldin (2006).

4. CONCLUSIONS

De-hulling high-oleic rapeseed prior to pressing significantly affected extractability of the bioactive compounds, no such effect was found when the oxidative state of the oil was analyzed. The applied microwave pre-treatment altered the contents of tocopherols, plastochromanol-8, total carotenoids, whereas a remarkable increase in the canolol concentration was noted with longer seed exposition to microwaves. After 9 min of seed MV pre-treatment, a marked increase in the oxidative stability was noted, which was approx. 2-fold higher in relation to the control oils prepared from intact and de-hulled seeds. At the same time, the antioxidant capacity of the oils produced from MV pre-treated seeds for 9 min was nearly 4-fold higher than that of the control HORO and DHORO. With the increase in MV pre-treatment time the formation of hydroperoxides and their degradation products was noticeable. Additionally, prolonged seed heating resulted in unsaturated fatty acid degradation, which was higher in DHOROs than in the HOROs. Although high-oleic rapeseed de-hulling in conjunction with microwaving enabled the production of oil which was high in antioxidant compounds, undesirable changes such as lipid oxidation, darkened color and altered flavor must be monitored to ensure high quality of the oil.

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Conflict of interest statement

The authors have declared no conflict of interest.

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