Influence of de-hulled rapeseed roasting on the physicochemical composition and oxidative state of oil

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Submitted: 01 August 2016; Accepted: 21 November 2016

SUMMARY: The effect of roasting time on the contents of bioactive compounds (tocopherols, phytosterols, phenolic compounds), antioxidant capacity and physicochemical properties of rapeseed oil pressed from de-hulled seeds was investigated. The de-hulled seeds were roasted at a temperature of 165 °C for 20, 40, 60, 80, and 100 min. The results of this study show that a roasting pre-treatment led to a gradual increase in canolol content (from 1.34 to 117.33 mg/100 g), total phytosterols (from 573.51 to 609.86 mg/100 g) and total carotenoids (0.82 to 2.41 mg/100 g), while only slight changes in the contents of tocopherols were noted. With the increase in roasting time a gradual increase in oxidative stability (from 4.27 to 6.85 h), and antioxidant capacity, seen mainly in the hydrophilic fraction of oil (from 0.32 to 2.30 mmol TEAC/l) was found. Although roasting resulted in the formation of primary and secondary oxidation products, the quality parameters of oils were within Codex Alimentarius limits.

KEYWORDS: Bioactive compounds; De-hulling; Oxidative stability; Radical scavenging activity; Rapeseed oil; Roasting

RESUMEN: Influencia del tostado de colza descascarillada sobre la composición fisicoquímica y el estado oxidativo del aceite. Se estudió el efecto del tiempo de tostado sobre el contenido de compuestos bioactivos (tocoferoles, fitosteroles, compuestos fenólicos), capacidad antioxidante y propiedades fisicoquímicas del aceite de prensado de semillas descascarilladas de colza. Las semillas descascarilladas se tostaron a una temperatura de 165 °C durante 20, 40, 60, 80 y 100 min. Los resultados de este estudio muestran que el pretratamiento con tostado condujo a un aumento gradual del contenido de canolol (de 1.34 a 117.33 mg/100 g), fitosteroles totales (de 573.51 a 609.86 mg/100 g) y carotenoides totales (0.82 a 2.41 mg/100 g). Sólo se observaron ligeros cambios en el contenido de tocoferoles. Con el incremento del tiempo de tostado se observó un aumento gradual de la estabilidad oxidativa (de 4.27 a 6.85 h) y se encontró capacidad antioxidante, observada principalmente en la fracción hidrofílica de aceite (de 0.32 a 2.30 mmol TEAC/l). Aunque, el tostado produjo formación de productos de oxidación primaria y secundaria, los parámetros de calidad de los aceites estaban dentro de los límites del Codex Alimentarius.

PALABRAS CLAVE: Aceite de colza; Actividad de barrido radical; Compuestos bioactivos; Descascarillado; Estabilidad oxidativa; Tostado

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1. INTRODUCTION

The EU is the world’s largest producer of rapeseed and its products. The leading countries in rapeseed production in the EU are Germany and France, followed by the U.K., Poland, and the Czech Republic (FAOSTAT, 2015). Besides the food use of rapeseed, its meal is used in the livestock sector as the EU is a leading producer and exporter of meat and dairy products. Recently, the main driver for the demand of oil is the biodiesel industry, as rapeseed oil has become the primary feedstock for biodiesel in Europe.

The seeds of rape consist of three basic components: the embryo, the endosperm and the seed coat (hull). Endosperm degenerates during seed maturation and the seed coat enwraps the embryo tightly. The embryo contains two pieces of cotyledons (which serve as food reserve structures), radicle and hypocotyl (Hu et al., 2013). The oil in this oilseed is distributed in spherosomes throughout the germ cell. Rapeseeds are composed of 38–50% lipid, 20–32% protein, and 10–15% crude fiber. These major rapeseed constituents are not evenly distributed throughout the rapeseed. The respective oil contents for hulls and kernels range from 10.6 to 16.4% (dry basis) and from 47.1 to 59.6%. The protein content of the hulls ranges from 17–18% (defatted dry basis), while the protein content in defatted kernels ranges from 46% up to 79%. Crude fiber contents range from 27.0 to 44.1% (dry basis) in de-fatted hulls and 3.0% to 12% in de-oiled kernels (Carré et al., 2016).

Today’s consumers have a different opinion concerning the cold-pressed rapeseed oil flavor. For some, it is valued for its unique fresh and mild taste resembling asparagus, cabbage or fresh green vegetables. Others find it unpleasant due to its pungent odor which is a consequence of the breakdown products of glucosinolate supervision (Brühl and Matthäus, 2008). An alternative may constitute rapeseed oil extraction from the hulled rapeseeds. Rapeseed de-hulling prior to pressing allows for improving the sensory characteristics of the oil (removal of hull pigments, reduction in phenolic acid tannin content responsible for the bitter and astringent after-taste and the dark color of crude oil) as well as the quality of the meal (increase in protein content, reduction in fiber content) (Carré et al., 2016). Studies conducted by Yang et al. (2011) investigated the effect of rapeseed hulling prior to cold-pressing on the composition and oxidative stability of oil. Guderjan et al. (2007) compared the application of pulsed electric fields on oil yield and phytochemicals of rapeseed oil pressed from hulled and non-hulled seeds. The results obtained in this study showed favorable effect of seed de-hulling on the extractability of bioactive compounds during pressing. Zhou et al. (2013), who studied the effect of rapeseed pre-treatment with de-hulling and microwaving on the flavor characteristics of cold-pressed rapeseed, found that a de-hulling pre-treatment could improve the flavor of the oil. Thermal rapeseed pre-processing techniques, such as roasting (Shrestha and De Melnauer, 2014; Rękas et al., 2015; Siger et al., 2015), microwave pre-treatment (Spilmeyer et al., 2009; Azadmard-Damirchi et al., 2010; Yang et al., 2013; Yang et al., 2014) showed the favorable effect of high temperatures on oil yield, the extractability of functional oil ingredients (tocopherols, phytosterols, carotenoids) and the formation of new compounds (canolol). The aim of the present study was to investigate the impact of rapeseed hulling in conjunction with different roasting times on the phytochemical contents, antioxidant capacity and oxidative stability of the oil.

2. MATERIALS AND METHODS

2.1. Experimental material

Seeds of the winter-type rapeseed, Bojan, were provided by the Plant Breeding Strzelce Ltd. Co. – IHAR Group, Poland. The seeds were harvested at optimum maturity, and did not contain any impurities or broken seeds. They were stored in paper bags under atmospheric conditions at 19 ± 1 °C.

2.2. Reagents

Analytical standards α-, β-, γ-, and δ-tocopherol (>95%), HPLC-grade n-hexane, methanol, formic acid, and 1,4-dioxane were purchased from Calbiochem–Merck Biosciences (Darmstadt, Germany). Phenolic acid standards, Sylon BTZ, 5α-cholestan (> 97%), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), α-, β-, γ-, and δ-tocopherols (DPPH), and (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Mechanical de-hulling

Mechanical de-hulling of rapeseeds was performed using a shearing 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 had a diameter of 140 mm, and was equipped with blades inclined at 45 degrees to the flat surface of the disk. De-hulled rapeseeds were subjected to pneumatic separation on a laboratory separator Petkus K-293 (PETKUS Technologie GmbH, Germany).

2.4. Roasting conditions

De-hulled rapeseeds were equilibrated at refrigerated temperature (4 ± 2 °C) in closed containers for 72 h to reach a moisture content of 7.5% by spraying.
the seeds with a specific amount of water. For each sample, 500 g of de-hulled rapeseeds were thinly and evenly spread out on the bottom of a glass beaker, tightly covered with aluminium foil, and heated for 20, 40, 60, 80, and 100 min inside an oven with forced ventilation (SUP-100, WAMED, 2600 W) maintained at a temperature of 165 °C. A de-hulled rapeseed sample without thermal treatment was used as a control sample. Following each heating run, the seeds were allowed to cool to ambient temperature and were thoroughly mixed to obtain a homogenous sampling. Then, the seeds were re-moisturized to reach required moisture level of 8.5% prior to pressing. Each experiment was performed in triplicate for all variants of roasting.

2.5. Oil extraction by cold-pressing

The oil was pressed with the use of screw press (Farmet, Czech Republic) at room temperature (20 ± 2 °C). During the pressing, the temperature was kept below 40 °C. After pressing the oils were collected, subjected to natural sedimentation (3 days) under refrigeration conditions (4 ± 2 °C) and decanted.

2.6. Analysis of biochemical composition of oils

To determine tocopherol (α, β, γ, and δ-tocopherol and PC-8), 200 mg of oil were 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) at a flow rate 1.0 mL·min⁻¹. Quantification of tocopherol was conducted using data from the FLD with excitation/emission wavelengths of 295/330 nm, respectively. The tocopherol content was assayed and calculated according to the method described by Siger et al. (2014).

Phytosterols were determined following the 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. Then, 700 μL of unsaponified fraction was transferred into a 1.5 mL vial and the solvent was evaporated to dryness under nitrogen. Dry residues were dissolved in 100 μL pyridine and silylated with 400 μL of Sylon BTZ. Derivatives of the sterols were separated on a gas chromatograph (Shimadzu, Japan) equipped with a FID detector, using a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm; Phenomenex Torrance, CA, USA). 1 μL of the sample was injected in splitless mode (setup: hydrogen carrier gas at 1.5 ml min⁻¹ flow rate, the detector temperature was set at 300 °C). The column temperature: 50 °C held for 2 min, ramped to 230 °C at 15 °C-min⁻¹, ramped to 310 °C at 3 °C-min⁻¹, held for 10 min. All sterols were quantified using 5α-cholestanol as internal standard. The identification was based on a GC-MSn laboratory sterol spectral library, as well as the online NIST mass spectra library.

The analysis was carried out using the Shimadzu HPLC system (Shimadzu, Japan), equipped with a Luna C18 reversed-phase column (4.6 × 250 mm; 5 μm, Phenomenex, Torrance, CA, USA), and a DAD detector. The solid-phase extraction (SPE) of phenolic compounds was carried out following the method presented by Siger et al. (2015). Gradient elution was used, combining solvent A (formic acid:H₂O 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⁻¹. The injection volume was 20 μL, 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). A quantitative determination of phenolic compounds was carried out by comparing the retention times and diode array spectral characteristics with the appropriate standards. HPLC/ESI/MS² analyses were performed to qualitatively identify phenolic compounds that differed in their retention times from the standards.

The isolation and identification of canolol was performed 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), following the method presented by Siger et al. (2015). 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⁻¹. 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-MS².

The total phenolic compound content was determined by the Folin-Ciocalteau method following procedure presented by Koski et al. (2002). In brief, an aliquot (0.2 mL) of methanolic extract was mixed with 0.5 ml of a Folin-Ciocalteau reagent and...
sodium carbonate solution (1 mL). After 60 min of reaction in the absence of light, the absorbance was measured at 725 nm. Sinapic acid was used as a standard, and the results were expressed in mg of sinapic acid equivalent/100 g of sample.

Total carotenoid pigments, expressed as β-carotene, were assessed spectrophotometrically 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.

2.7. Determination of color development of oils

The measurement of the oil color was conducted with a CM-3600d colorimeter (Konica Minolta, Japan), in a CIE L*a*b* system, using illuminant D65 and 10° observer angle. The values of the L*, a* and b* parameters allowed for the calculation of the absolute difference of the samples' color (∆E) after roasting pre-treatment compared to the control:

\[
\Delta E = [(L_0-L)^2+(a_0*-a*)^2+(b_0*-b*)^2]^{1/2}.
\]

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

2.8. Radical scavenging of oils (RSC)

To evaluate the antioxidant activity of the oils, a spectrophotometric analysis was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), following the method described by Tuberoso et al. (2007). The antioxidant activity of the 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 then they were shaken in a vibration mixer for 10 s, shaken in a rotary shaker for 30 min, and centrifuged at 700g 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 the 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 of 0.02–4.00 mM.

2.9. Analysis of oxidative stability of oils

The peroxide value (PV), p-anisidine value (p-AnV), and specific UV extinctions (K232 and K268 values) were determined in accordance with the ISO standard methods (ISO, 2005, 2008, 2011, respectively).

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

2.10. Statistical analysis

Data are expressed as Mean ± SD. To examine the effect of the microwave pre-treatment of rapeseeds on the crude oil variables studied, one-way ANOVA was used when the variables fulfilled parametric conditions, and the Kruskal-Wallis test when they were non-parametric. Correlations between variables studied were determined using Pearson’s correlation coefficient (r). Significance was established at a probability of p < 0.05, using the Statistica 12.0 software (StatSoft, Inc., Tulsa, OK).

3. RESULTS

3.1. Effect of de-hulled rapeseed roasting on the content of bioactive compounds

As can be seen in Table 1, α- and γ-tocopherol were the predominant tocopherol homologues in the analyzed oil samples, and their contents in the control oil sample were 25.13 and 38.66 mg/100 g, while the other tocopherol homologues, such as δ- and β-tocopherol, were present in much smaller quantities (0.61 and 0.08 mg/100 g, respectively). α-Tocopherol was thermal-sensitive, the content of which decreased to 21.02 and 21.21 mg/100 g with 60 and 80 min of roasting, respectively. However, the amount of α-T after 100 min of roasting was 25.54 mg/100 g, which was nearly the same as the amount of α-T in the control oil sample. On the other hand, a gradual increase in γ-T content was noted, as a result of a longer seed roasting time, reaching its maximum concentration of 46.71 mg/100 g after 100 min of roasting. The roasting pre-treatment of rapeseed prior to pressing significantly (p < 0.05) increased the content of plastochromanol-8 (PC-8) in the analyzed oil samples (Table 1). The seed roasting for 100 min resulted in an increase of the PC-8 content of 73.6%, compared to the control oil sample (increased from 2.42 to 4.20 mg/100 g).

The control rapeseed oil contained a total of 573.51 mg/100 g phytosterols, among which the dominant phytosterols were β-sitosterol, campesterol, and brassicasterol, accounting for 52%, 31%, and 14% of the total phytosterol contents, respectively (Table 1). The roasting pre-treatment of rapeseeds exerts a significant effect on the content of phytosterols in the oil. The respective increase in the concentration of brassicasterol, campesterol, and β-sitosterol following 100 min of...
roasting was 19.9%, 9.1%, and 1.8%, compared to the control. The amount of the remaining phytosterols, namely stigmasterol, Δ5-avenasterol, and cholesterol, remained practically unchanged in all roasting times.

The level of the total phenolic compounds in the control oil measured by the Folin-Ciocalteau method was 3.62 mg/100 g, while the total phenolic content calculated from HPLC-DAD was 1.61 mg/100 g. Roasting the seeds prior to pressing significantly increased the amount of total polyphenols in the analyzed rapeseed oil (Table 1). The results related to the changes observed in the individual phenolic compounds of the rapeseeds submitted to roasting are displayed in Table 1. The control rapeseed oil contained relatively low amounts of phenolic compounds, represented by canolol, trans-sinapic acid and its methyl ester, sinapine, ferulic acid and p-coumaric acid. The dominant phenolic compound was canolol, which accounted for 84% of the total phenolic content.

Along with increasing seed roasting time, only minor changes in the quantities of polar phenolics in the oil were found, while the amount of canolol increased significantly. The most pronounced changes in the canolol content occurred after rapeseed roasting for 60 min: the amount of this compound increased to 69.28 mg/100 g (over a 50-fold increase when compared to the control oil sample, and nearly a 4-fold increase in comparison with the canolol content in the oil pressed from seeds roasted for 40 min). Further roasting time prolongation resulted in a rapid canolol forma-

### Table 1. Tocochromanol, phytosterol and phenolic compound contents (mg/100 g) in rapeseed oil samples produced from de-hulled roasted seeds

<table>
<thead>
<tr>
<th>Roasting time (min)</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocochromanols (mg/100 g)</td>
<td></td>
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<tr>
<td>α-Tocopherol</td>
<td>25.13 ± 0.15a</td>
<td>25.29 ± 0.14a</td>
<td>25.84 ± 0.15a</td>
<td>21.02 ± 0.27b</td>
<td>21.74 ± 0.1b</td>
<td>25.54 ± 0.09a</td>
</tr>
<tr>
<td>β-Tocopherol</td>
<td>0.08 ± 0.02a</td>
<td>0.09 ± 0.04a</td>
<td>0.08 ± 0.02a</td>
<td>0.10 ± 0.02a</td>
<td>0.09 ± 0.03a</td>
<td>0.16 ± 0.21a</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>38.66 ± 0.17b</td>
<td>38.77 ± 0.07c</td>
<td>39.35 ± 0.24b</td>
<td>40.66 ± 0.21b</td>
<td>46.16 ± 0.09c</td>
<td>46.71 ± 0.22a</td>
</tr>
<tr>
<td>δ-Tocopherol</td>
<td>0.61 ± 0.04a</td>
<td>0.72 ± 0.03a</td>
<td>0.71 ± 0.05a</td>
<td>0.72 ± 0.04a</td>
<td>0.67 ± 0.02a</td>
<td>0.63 ± 0.01a</td>
</tr>
<tr>
<td>Total tocopherols</td>
<td>64.88 ± 0.20a</td>
<td>64.87 ± 0.09a</td>
<td>65.97 ± 0.12c</td>
<td>62.50 ± 0.43c</td>
<td>68.66 ± 0.07d</td>
<td>73.04 ± 0.51c</td>
</tr>
<tr>
<td>PC-8</td>
<td>2.42 ± 0.05c</td>
<td>2.46 ± 0.08a</td>
<td>3.55 ± 0.09b</td>
<td>3.86 ± 0.09b</td>
<td>4.42 ± 0.15a</td>
<td>4.20 ± 0.15a</td>
</tr>
<tr>
<td>Phytosterols (mg/100 g)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Cholesterol</td>
<td>1.77 ± 0.01a</td>
<td>1.76 ± 0.07a</td>
<td>1.77 ± 0.02a</td>
<td>1.75 ± 0.12a</td>
<td>1.74 ± 0.08a</td>
<td>1.71 ± 0.02a</td>
</tr>
<tr>
<td>Brassicasterol</td>
<td>77.86 ± 0.11a</td>
<td>76.02 ± 0.25a</td>
<td>81.18 ± 0.42a</td>
<td>86.32 ± 0.37a</td>
<td>89.35 ± 0.17a</td>
<td>93.42 ± 0.23a</td>
</tr>
<tr>
<td>Campesterol</td>
<td>176.08 ± 5.12a</td>
<td>175.37 ± 4.02a</td>
<td>179.02 ± 3.41b</td>
<td>183.93 ± 4.81c</td>
<td>188.92 ± 5.05d</td>
<td>192.10 ± 2.21c</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>2.89 ± 0.02a</td>
<td>2.82 ± 2.03a</td>
<td>2.88 ± 0.05a</td>
<td>2.91 ± 0.08a</td>
<td>2.92 ± 0.03a</td>
<td>2.88 ± 0.06a</td>
</tr>
<tr>
<td>β-Sitosterol</td>
<td>297.88 ± 5.02b</td>
<td>296.36 ± 3.97b</td>
<td>299.34 ± 4.04b</td>
<td>301.42 ± 6.03d</td>
<td>298.37 ± 5.32c</td>
<td>303.36 ± 8.11c</td>
</tr>
<tr>
<td>Δ5-Avenasterol</td>
<td>17.03 ± 0.03a</td>
<td>17.08 ± 0.12a</td>
<td>18.04 ± 0.20a</td>
<td>19.03 ± 0.02a</td>
<td>19.96 ± 0.06a</td>
<td>16.39 ± 0.05a</td>
</tr>
<tr>
<td>Total phytosterols</td>
<td>573.51 ± 4.15b</td>
<td>569.41 ± 3.39a</td>
<td>582.23 ± 4.01c</td>
<td>595.36 ± 5.11a</td>
<td>601.26 ± 4.07c</td>
<td>609.86 ± 7.16d</td>
</tr>
<tr>
<td>Phenolic compounds (mg/100 g)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Canolol</td>
<td>1.34 ± 0.04a</td>
<td>2.52 ± 0.09a</td>
<td>18.74 ± 0.10c</td>
<td>69.28 ± 0.13d</td>
<td>88.44 ± 0.19c</td>
<td>117.33 ± 0.32c</td>
</tr>
<tr>
<td>trans-Sinapic acid</td>
<td>0.20 ± 0.00a</td>
<td>0.23 ± 0.00a</td>
<td>0.25 ± 0.00a</td>
<td>0.33 ± 0.00a</td>
<td>0.47 ± 0.01a</td>
<td>0.40 ± 0.00a</td>
</tr>
<tr>
<td>Sinapic acid methyl ester</td>
<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
<td>0.02 ± 0.00a</td>
<td>0.02 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.02 ± 0.00a</td>
<td>0.02 ± 0.00a</td>
<td>0.02 ± 0.00a</td>
<td>0.02 ± 0.00a</td>
<td>0.04 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
</tr>
<tr>
<td>Sinapine</td>
<td>0.03 ± 0.00a</td>
<td>0.01 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
<td>0.09 ± 0.00a</td>
<td>0.12 ± 0.00a</td>
<td>0.15 ± 0.00a</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>0.02 ± 0.00a</td>
<td>0.01 ± 0.00a</td>
<td>0.01 ± 0.00a</td>
<td>0.01 ± 0.00a</td>
<td>0.02 ± 0.00a</td>
<td>0.02 ± 0.00a</td>
</tr>
<tr>
<td>Total phenolic compounds – HPLC</td>
<td>1.61 ± 0.01a</td>
<td>2.79 ± 0.05a</td>
<td>19.05 ± 0.04c</td>
<td>69.75 ± 0.06d</td>
<td>89.10 ± 0.03c</td>
<td>117.96 ± 0.13f</td>
</tr>
<tr>
<td>Total phenolic compounds – Folin-Ciocalteau</td>
<td>3.62 ± 0.26c</td>
<td>4.14 ± 0.75a</td>
<td>25.64 ± 1.54c</td>
<td>81.23 ± 4.34d</td>
<td>112.98 ± 3.15c</td>
<td>201.17 ± 8.51c</td>
</tr>
</tbody>
</table>

Mean values denoted by the same letter in rows do not constitute statistically significant differences at p < 0.05.
After 100 min of seed roasting, the amount of both groups of pigment increased by nearly 3-fold, when compared to the control oil sample.

3.2. Color development of rapeseed oil pressed from de-hulled seeds as a function of roasting time

The control oil sample was characterized by a light yellow color, without the typical green hue, due to the removal of hulls, which impart a greenish color to cold-pressed rapeseed oil. The browning index of untreated oil was 0.15. With the increase in roasting time from 20 to 100 min, an increase in the browning index (from 0.22 to 0.72) took place (Table 2). The lightness (L*) of the oils increased in the browning index (from 0.22 to 0.72) took place (Table 2). The lightness (L*) of the oils increased with roast time; however, only minor changes with respect to these color coordinates were seen. On the other hand, the rate of change in the yellow-blue color coordinate (b*) significantly increased with roasting time. The b* value for the control oil sample was 28.99, after 80 and 100 min of seed roasting and its value was nearly 2-fold higher than in the control oil sample. A significant negative correlation between the amount of carotenoids and the L* and a* color parameters was found (r = -0.948 and -0.929, respectively), whereas a strong positive correlation was noted for the b* parameter (r = 0.918). A visible oil color change from light yellow to light brown with the increase in roasting time was also assessed in terms of changes in the calculated color difference (ΔE) value (Table 2).

3.3. Effect of de-hulled rapeseed roasting on the DPPH radical scavenging activity of rapeseed oil

Table 3 shows the DPPH radical scavenging activities (RSC), as expressed by the TEAC values of the analyzed oils for both hydrophilic (HF) and lipophilic fractions (LF), as well as for whole oil (TF). The respective RSC values of LF, HF and TF of the control oil sample were 0.88, 0.32, and 1.34 mmol/L. As a result of prolonged roasting time, a significant increase in the radical scavenging activity of HF was recorded; while only slight changes in TEAC values were noted for LF. As the roasting time increased to 100 min, the TEAC values evaluated for HF, LF and TF were as follows: 2.30, 1.55, and 3.98 mmol/L, respectively. A statistical analysis showed a significant effect of increased content of the hydrophilic-like compounds and TEAC values of the HF of the oils studied. A significant correlation between antioxidant capacity and total phenolic contents was found (r > 0.9). The RSC value of HF was found to correlate best with the content of canolol (r = 0.959). The statistical analysis also showed a significant effect of the increased content of lipophilic-like compounds and TEAC values of LF in the oils. The Pearson correlation coefficient was r = 0.981, r = 0.952, r = 0.624, for phytosterols, carotenoids, and tocopherols, respectively.

3.4. Effect of de-hulled rapeseed roasting on the oxidative stability of rapeseed oil

De-hulled rapeseed roasting prior to pressing resulted in the formation of hydroperoxides, a primary lipid oxidation product. The lowest hydroperoxide level, measured by peroxide value (PV), was found in the control oil sample (0.79 meq O₂/kg), whereas the PV of the 100-min roasted sample reached a maximum value of 2.89 meq O₂/kg.

Grasas Aceites 68 (1), January–March 2017, e176. ISSN-L: 0017–3495 doi: http://dx.doi.org/10.3989/gya.0806162
Influence of de-hulled rapeseed roasting on the physicochemical composition and oxidative state of oil • 7

TABLE 3. DPPH radical scavenging activity (mmol TEAC/l) and oxidative stability parameters of rapeseed oil samples produced from de-hulled roasted seeds

<table>
<thead>
<tr>
<th>Roasting time (min)</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>0.32 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.25 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.56 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.98 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.30 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>LF</td>
<td>0.88 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.92 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.04 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.34 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.51 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.55 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TF</td>
<td>1.34 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.42 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.41 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.94 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.56 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.98 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Oxidative stability parameters†

| PV (mEq O₂/kg) | 0.79 ± 0.06<sup>a</sup> | 0.99 ± 0.15<sup>c</sup> | 1.34 ± 0.05<sup>a</sup> | 1.62 ± 0.07<sup>c</sup> | 2.45 ± 0.09<sup>a</sup> | 2.89 ± 0.13<sup>a</sup> |
| p-AnV            | 0.24 ± 0.08<sup>a</sup> | 0.28 ± 0.07<sup>a</sup> | 0.39 ± 0.08<sup>a</sup> | 0.49 ± 0.06<sup>a</sup> | 0.61 ± 0.03<sup>a</sup> | 0.70 ± 0.03<sup>a</sup> |
| K<sub>328</sub>   | 0.82 ± 0.03<sup>a</sup> | 0.90 ± 0.02<sup>a</sup> | 1.10 ± 0.01<sup>a</sup> | 1.38 ± 0.04<sup>a</sup> | 1.99 ± 0.05<sup>a</sup> | 2.41 ± 0.04<sup>a</sup> |
| K<sub>258</sub>   | 0.09 ± 0.02<sup>a</sup> | 0.15 ± 0.09<sup>a</sup> | 0.21 ± 0.04<sup>a</sup> | 0.28 ± 0.09<sup>a</sup> | 0.39 ± 0.06<sup>a</sup> | 0.44 ± 0.02<sup>a</sup> |
| IP (h)            | 4.27 ± 0.03<sup>a</sup> | 4.28 ± 0.15<sup>a</sup> | 4.62 ± 0.12<sup>a</sup> | 5.09 ± 3.53<sup>a</sup> | 6.47 ± 0.07<sup>a</sup> | 6.85 ± 0.07<sup>a</sup> |

*Antioxidant activity of: hydrophilic fraction (HF); lipophilic fraction (LF) and whole oil (TF).
†PV, peroxide value; K<sub>328</sub>, specific UV extinction at the indicated wavelength (nm); IP, induction period (h) determined by Rancimat test at 120 °C.
Mean values denoted by the same letter in rows do not constitute statistically significant differences at p < 0.05.

which was 3.6-fold higher than that of the control oil sample (Table 3). Although seed roasting prior to pressing altered the content of primary oxidation products considerably (p < 0.05), none of the oil samples reached the upper limit for PV of 15 meq O₂/kg. Along with prolonged seed roasting time, a simultaneous increase in the content of primary oxidation products was observed, resulting from the decomposition of hydroperoxides under elevated temperatures. The roasting of de-hulled rapeseeds significantly increased the level of aldehydes, assessed in terms of changes in the p-AnV (from 0.24 to 0.70). Roasting time in the range of 20–100 min resulted in the formation of conjugated dienes (K<sub>328</sub>) and trienes (K<sub>258</sub>), whose level increased from 0.82 to 3.56 and from 0.09 to 2.45, respectively (Table 3). The results of the oil stability evaluation based on the Rancimat test are given in Table 3. It was found that roasting time exerts a statistically significant effect (p < 0.05) on the induction period (IP) of the oils studied. The control oil sample was characterized by the lowest induction period (IP) of 4.27 h, and with increasing roasting time, a gradual increase in IP occurred. The IP was 4.28, 4.62, 5.09, 6.47, and 6.85 h of the oil pressed from seeds roasted for 20, 40, 60, 80, and 100 min, respectively.

4. DISCUSSION

The main constituents of rapeseed oil unsaponifiables are tocopherols and phytosterols (Przybylski, 2011). According to Codex Alimentarius crude rapeseed oil contains 4500–11300 mg/kg of phytosterols and 430–2680 mg/kg of tocopherols. Wijesundera et al. (2008), who studied the effect of rapeseed roasting at 165 °C for 5 min found that a thermal pre-treatment of seeds prior to pressing resulted in a modest increase in γ-T (10%) with a practically unchanged content of α-T. Similar results were presented by Siger et al. (2015), who reported that whole rapeseed roasting at 140, 160 and 180 °C for 5, 10 and 15 min increased γ-T (up to 37%) in seeds roasted for 15 min at 180 °C, when compared to the control oil, but no significant difference in the amount of α-T was found. On the contrary, Shrestha and De Melnauer (2014) found the rapeseed roasting pre-treatment at 180 °C (10–90 min) to increase the content of α-T and decrease the γ-T concentration by up to 6–7%, when compared to the control oil. In our study, rapeseed roasting pre-treatment resulted in an increase of up to 21% in γ-T, while the decrease in the concentration of α-T amounted to 16%. As Wijesundera et al. (2008) suggested, slight alterations in tocopherol levels in oils produced from roasted seeds may result from tocopherol co-elution in HPLC with other compounds formed during seed roasting. According to Matthäus (2013) it is possible that canolol acts as an antioxidant protecting tocopherols from degradation during the seeds’ thermal pre-treatment. Similar to tocopherol content, the amount of plastochromanol-8 increased gradually due to longer seed heating time (Table 1). This observation concurs with the results obtained by Shrestha and De Melnauer (2014), who found rapeseed roasting at 165 °C for 10 min to increase PC-8 significantly, whereas Siger et al. (2015) noted a decrease in the PC-8 content during rapeseed roasting at 140 and 160 °C for 5, 10 and 15 min.

Ghazani et al. (2014) found the effect of rapeseed oil production method on the level of phytosterols to be significant. The highest level of total phytosterols was found in solvent-extracted...
oil (940.8 mg/100 g), followed by oil produced by hot-pressing (836.5 mg/100), and cold-pressing (835.8-774.1 mg/100 g), while the lowest content of phytosterols was found in fully refined rapeseed oil (613.5 mg/100 g). By the application of roasting pre-treatment for 100 min we were able to increase the amount of total phytosterols by up to 6%, in comparison to the control oil sample. Whole rapeseed roasting for 1 h at 80, 100, 120, 140 °C was reported to cause a gradual increase in the total phytosterol content in the oil (Rękas et al., 2015). In contrast, Siger et al. (2015) found the roasting of rapeseed at 140, 160 and 180 °C (5–15 min), in general, to decrease the total content of phytosterols. In turn, Yang et al. (2013), who treated whole rapeseed with microwaves (0–8 min, 800W) noted that phytosterol contents in the rapeseed oil increased with increasing microwave time and with decreasing initial moisture content of the rapeseeds.

The level of total phenolic compounds detected in rapeseed oil depends heavily on the production procedure, rapeseed variety and ripening degree, in addition to the method of extraction and type of solvent used (Kozłowska et al., 1990). Yang et al. (2014) showed that the use of Folin–Ciocalteu reagent may result in an overestimation of total phenolic content by comparing the total phenolic content in rapeseed oil measured by the Folin-Ciocalteau method and calculated from UPLC data (28.53 mg/100 g vs. 19.94 mg/100 g), as it may react with amino acids (alanine, cysteine, glycine, tryptophan), sugars (fructose, glucose, sucrose) or easily oxidized substances. In comparison with other oilseeds, rapeseed contains comparatively high amounts of phenolic compounds, of which esterified phenolic acids are present in the largest quantities (Naczk et al., 1998). However, due to their hydrophilic nature, only a small proportion of phenolic acid is transferred to the oil (Yang et al., 2014). Unlike other phenolics, canolol, due to its molecular structure, shows better solubility in oil. As a result, canolol constitutes a dominant phenolic compound found in rapeseed oil (Kraljić et al., 2013; Shrestha and De Meulenaer, 2014; Yang et al., 2014; Siger et al., 2015). The canolol concentration in rapeseed oil pressed from roasted seeds greatly depends on the applied heating temperature, duration of heating and moisture content of the seeds. However, the available data in the literature considering the effect of rapeseed thermal pre-treatment on the formation of canolol is inconsistent. According to Spielmeyer et al. (2009) canolol is thermally unstable and undergoes degradation at temperatures above 165 °C, while Matthaus (2013) stated that substantial amounts of canolol precede rapeseed roasting at temperatures above 180 °C. Mayengbam et al. (2013) found that ground rapeseed roasting at 200 °C for 15 min increased the content of canolol up to 2000-fold, when compared to the control oil (0.003 to 6.671 mg/g). Yang et al. (2014) found that after a microwave exposure time of rapeseeds for 7 min (800 W) the amount of canolol in the seeds increased by more than 6 times (increase from 14.07 to 89.89 mg/100 g), while in the oil canolol content was 9-fold higher than that of unheated rapeseed oil (increase from 17.06 to 162.71 mg/100 g).

Carotenoid pigments present in crude oil possess antioxidant activity, especially in the light, in contrast to chlorophyll pigments, which act as pro-oxidants when exposed to light. Ghazani et al. (2014) found a 10-fold higher content of chlorophyll pigments in solvent-extracted rapeseed oil than in that of cold-pressed oil. The content of chlorophylls in rapeseed depends primarily on the degree of ripeness. Moreover, as shown by Kraljić et al. (2013), rapeseed variety is also an important factor. The de-hulling of rapeseeds leads to the removal of major parts of the fiber and a group of hull pigments such as chlorophylls (Niewiadomska, 1990). The amount of chlorophyll in the control oil obtained from de-hulled rapeseeds was 0.24 mg/kg, which is nearly 2 to 3 times lower in comparison with the chlorophyll pigment content in cold-pressed oil pressed from whole rapeseeds (Kraljić et al., 2013; Ghazani et al., 2014). Following 100 min of seed roasting the concentration of chlorophylls in the oil increased to 0.70 mg/kg, which is consistent with the results presented by Prior et al. (1991), who reported that heating rapeseeds at 80–100 °C for 30 min significantly affected chlorophyll content (6.5 mg/kg for cold-pressed oil, up to 47.3 and 67.8 mg/kg for roasting at 80 and 100 °C, respectively). The favorable effect of rapeseed pre-treatment on the extractability of carotenoid pigments was reported by Kraljić et al. (2013) and Rękas et al. (2015). In contrast, carotenoids were found thermally-unstable during the roasting (150 °C for 10, 20, 30, 40, 60 min) of pine nuts (Cai et al., 2013). A similar correlation between roasting pre-treatment (160 °C) and the amount of lutein content in mustard oil was presented by Vaidya and Choe (2010).

Color is an important criterion for cold-pressed oils. Hull removal allowed us to produce rapeseed oil characterized by a light-yellow hue; however, roasting of the seeds resulted in a color change to light brown. The darkening of oil with an increase in roasting time may result from the formation of Maillard type browning reaction products, the release of phospholipids (Shrestha and De Meulenaer, 2014) and furfural derivatives (Durmaz and Gökmak, 2010). Higher extractability of compounds possessing antioxidant activity released (tocochromanol, carotenoids) or generated (canolol) during the roasting process resulted in increased radical scavenging activity and oxidative stability of the resulting oil. Additionally, other factors, such as the formation of lipophilic Maillard type browning reaction products.
may be responsible for such effect. Uquiche et al. (2008) and Yang et al. (2013) suggested that the greater oxidative stability of oils obtained from roasted seeds may also result from inactivation of pro-oxidative enzymes, such as lipase, peroxidase, and lipoxygenase. Namiki (1995) and Shrestha and De Meulenaer (2014) pointed out that enhanced oxidative stability of roasted sesame oil and rapeseed oil results from the synergistic effect among different components with antioxidant activity. Moreover, phospholipids, released as a result of thermally-induced lipid bilayer degradation, may affect oil oxidation through the sequestration of trace pro-oxidant metals, such as iron (Choe and Min, 2006).

5. CONCLUSIONS

The results obtained in this study show that a de-hulled rapeseed pre-treatment for different times, ranging from 20 to 100 min, significantly affected the extractability of bioactive compounds. The applied roasting conditions did not alter the content of tocopherols or polar phenolic compounds; whereas a gradual increase in phytosterols, and oxidative stability; however, with the increase in roasting time, the formation of hydroperoxides and their degradation products was noticeable. Although de-hulled rapeseed roasting prior to pressing enabled the production of an oil with added value in nutritional terms, browning processes as well as changes in the sensory assessment are of great importance in order to produce high quality rapeseed oil.


Conflict of interest statement

The authors have declared no conflict of interest.

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Grasas Aceites 68 (1), January–March 2017, e176. ISSN-L: 0017–3495 doi: http://dx.doi.org/10.3989/gya.0806162


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