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ORIGINAL PAPER

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COLD-PRESSED AND HOT-PRESSED RAPESEED OIL: THE EFFECTS OF ROASTING AND SEED MOISTURE ON THE ANTIOXIDANT ACTIVITY, CANOLOL, AND TOCOPHEROL LEVEL

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ABSTRACT

Background. The paper looks at the levels of canolol, tocopherols and antioxidant activity in cold-pressed and hot-pressed rapeseed oils produced from seeds of various moisture levels (5%, 7.5%, and 10%). The paper also considers the effects of seed roasting on the levels of these compounds.
Material and methods. The material used for the tests was rapeseed cv. *Adrianna.* The quality of the oils obtained is determined using peroxide and acid values. The levels of canolol and tocopherols are analyzed using HPLC. The DPPH radical-scavenging activity method for oil samples and phenolic extract from oils was used.
Results. It has been demonstrated that the oils produced from rapeseeds with a 5% moisture content, and in particular from cold-pressed oils, were characterized by the lowest peroxide values. Cold-pressed oils produced from rapeseeds with a 5% moisture content were characterized by higher levels of tocopherols and plastochromanol-8. In the case of hot-pressed oils, the highest levels of tocopherols were found in oils produced from seeds with a 7.5% moisture content, and the greatest amount of PC-8 (more than 4 mg/100 g) was found in oils produced from seeds with a 10% moisture content. Hot-pressed oils have been shown to have higher levels of these compounds than cold-pressed oils. Both roasting and hot pressing led to an increase in the amount of canolol in the oils investigated. When analysing the antioxidant activity of the oils and phenolic extracts it was shown that phenolic compounds are responsible for approx. 10% of total antioxidant activity.
Conclusion. Various levels of biologically active compounds were shown to be present in the rapeseed oil obtained from raw materials of a varying moisture content. The type of pressing process (cold-pressing or hot-pressing) and whether the seeds have undergone roasting has also been shown to affect the resulting oil and the level of native antioxidants it contains.

Keywords: rapeseed, cold-pressed, hot-pressed, canolol, tocopherols, roasting

Abbreviations: PC-8 – plastochromanol-8, T – tocopherol, α-T – alpha-tocopherol, β-T – beta-tocopherol, γ-T – gamma-tocopherol, δ-T – delta-tocopherol.

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INTRODUCTION

Rapeseed oil is the third most produced oil, after palm oil and soybean oil. The amount of fatty acids in rapeseed oil satisfies nutritional requirements, as it has an adequate n-6/n-3 acid ratio of around 2:1 (Singh et al., 2002). The main unsaponifiable components in rapeseed oils are tocopherols, plastochromanol-8, and sterols. Tocopherols are known to be very efficient natural antioxidants. The antioxidant activity of the homologous tocopherols in vivo is as follows: α-T > β-T > γ-T > δ-T. In vitro, the activity is the reverse: α-T < β-T ≈ γ-T < δ-T (Eitenmiller and Lee, 2004). Plastochromanol-8 is considered to be a natural γ-T3 homologue, the only difference being that it has a longer side chain (Siger et al., 2014). According to Olejnik et al. (1997), the antioxidant properties of PC-8 are 1.5 times greater than those of α-T. Plant sterols form the majority of the unsaponifiable components of most plant oils. The overall quantity of sterols in '00' rapeseed oil varies between 0.45% and 1.13%. A characteristic feature of the *Brassica* genus is the level of brassicasterol, the presence of which allows an oil to be identified as rapeseed oil. Other sterols found in rape include β-sitosterol and campesterol, which together make up 80% to 88% of all sterols (Ratnayake and Daun, 2004).

Rapeseed is characterised by a high content of phenolic compounds, whose level is ten times as high as other oilseeds (Naczk et al., 1998). The most common phenolic compound is sinapic acid and its derivatives, especially sinapin, which constitutes 80% of the phenolic compounds in rapeseed. Sinapic acid also exists as glucopyranosyl sinapate. Only a small amount of sinapic acid, less than 16%, is present as a free sinapic acid (Khattab et al., 2010; Kozlowska et al., 1983; Siger et al., 2013; Thiyam et al., 2009). These substances are transferred to the oil to a small degree (Kraljić et al., 2013). 4-vinylsyringol (canolol), a product of the decarboxylation of sinapic acid formed during the pressing process or seed roasting (Koski et al., 2003). Aachary et al. (2014) demonstrated that the formation of primary and secondary oxidation products during deep-fat frying is significantly lower in oils fortified with canolol. Matthäus et al. (2014) showed a very strong effect of a canolol-enriched extract on the thermal stability of high-oleic canola oil during deep-fat frying.

The seeds of the presently cultivated varieties of rapeseed contain more than 40% fat, which leads to rapeseed's lower tolerance to humidity, as compared to cereal seeds (Niewiadomski, 1993). Improper storage of rapeseed may contribute to a decrease in its levels of bioactive compounds, and thus to a deterioration in the nutritive value of the oil produced. Under the Canada Grains Act, the maximum moisture level at which canola can be sold as straight grade (dry) is 10% (Canola Council of Canada, 1994). For storage periods longer than 5 months, canola should be binned at a maximum of 8% moisture (Mills, 1989). The Australian Oilseed Federation (AOF) Standards Committee has recently introduced a maximum moisture concentration at seed delivery of 8% (Mailer et al., 1998). Generally, in Poland, harvested seeds are dried to a moisture content of 7%, which is considered safe in terms of storage conditions (Rybacki et al., 2001). The moisture content of seeds in storage is usually neither uniform nor balanced over the period of storage. Often, seeds in the top layer are moister than those in deeper layers. Their moisture is also subject to variations, as a result of the diffusion and adsorption of water from the air by the seeds, and as a result of water absorption secreted during seed respiration (Niewiadomski, 1993). Gopalakrishnan et al. (1996) investigated the effects of storage (at room temperature and in a cold room) on chemical changes in lipids, including the levels of tocopherols in comminuted rapeseeds. They showed a 50% drop in the tocopherol levels of oil made from rapeseeds stored at 20°C for 10 days and at 5°C for 30 days. In turn, Goffman and Möllers (2000) investigated the effects of various temperatures (5°C, 20°C, and 40°C) and of oxygen availability during storage on the levels of tocopherols and plastochromanol-8 in intact rapeseeds and in oil pressed from these seeds. They observed losses of tocopherol content only at a temperature of 40°C, and also noted that throughout the experiment (24 weeks) the α-T homologue remained unchanged at all the temperatures tested. In contrast, the levels of γ-T decreased by 20% after 24-week storage, which was also illustrated by the α-T/γ-T ratio.

Although the majority of standards point to rapeseed moisture levels of 7–8%, we can, depending on the storage conditions, often meet different moisture levels. This study aims to examine the levels of various bioactive compounds in oil pressed from seeds of different

moisture levels. As is clear from the literature, the moisture of stored rapeseeds should be greater than 5% and less than 9%; we therefore chose to examine seeds with moisture levels of 5%, 7.5%, and 10%. In addition, little data is available on the native antioxidants (tocochromanols) in rapeseed oil, particularly considering the issue of seed moisture content. Another issue worth investigating is the influence of seed moisture on the levels of canolol in cold-pressed and hot-pressed oil from rapeseeds previously subjected to roasting at 160°C for 15 min and moistened to the appropriate moisture content (5%, 7.5%, or 10%) prior to pressing. The study also examined the antioxidant activity of the sample oils, and determined how the phenolic compounds present in the oils contribute to this antioxidant activity.

MATERIAL AND METHODS

Materials

The material used for tests was rapeseed *cv*. *Adrianna* obtained directly after harvest from the Złotniki Experimental Station owned by Poznań University of Life Sciences, Poland.

Seed roasting

The rapeseeds were roasted for 15 minutes at 160°C, according to the method presented in earlier work (Siger et al., 2015).

Preparation of seeds of various moisture levels

The seeds' moisture content was determined using an electronic moisture analyzer based on a precision weighing balance. A 5 g sample was dried at 115°C to constant mass and taken as a reference. The measurement accuracy of the analyzer is 0.05% w.b. (wet basis). The rapeseed had a moisture content of 5%. The amount of water to be added to the seed in order to obtain moistures of 7.5% and 10% was calculated using mass balance. Similarly, after roasting, the seeds had their water content measured, and this was found to have dropped to 2.12%. The seeds were then moisturized to reach the required moisture content (5%, 7.5%, or 10%) by spraying the seeds in batches of 1 kg with a specific amount of distilled water. After moistening, the rapeseeds were packaged in polyethylene bags and stored for 24 h in order to equalize the moisture content throughout the seed bulk. The following day, the seed moisture levels were checked. In this way, samples of unroasted and roasted seeds with moisture contents of 5%, 7.5%, and 10% were obtained. Oil was then pressed from these rapeseeds.

Cold-pressing and hot-pressing seeds

Rapeseeds (*Brassica napus*) were pressed using a Farmet Uno cold-pressing machine (Farmet, Czech Republic). The temperature inside the press was 60 ±10°C for cold-pressing and 90 ±10°C for hot-pressing, and the temperature of the oil produced was 39 ±1°C (cold-pressing) or 55 ±2°C (hot-pressing). The oil was pressed under ambient conditions of about 18°C. Once produced, the oil was centrifuged at 5000 rpm for 15 minutes and transferred directly to small, dark 100 ml bottles and stored at 4°C in the dark.

Peroxide value (PV) and acid value (AV) determination

The PV and AV of the rapeseed oil were determined using the standard methods from ISO 3960 (2007) and ISO 660 (2009).

Determination of tocopherols, plastochromanol-8, and canolol by NP-HPLC

Rapeseed oil (200 mg) was dissolved in *n*-hexane, made up to 10 ml, and transferred to vials for analysis. Tocopherols were qualitatively and quantitatively identified using a Waters HPLC system (Waters, Milford, MA) consisting of a pump (Waters 600), a fluorimetric detector (Waters 474), a photodiode array detector (Waters 2998 PDA), an autosampler (Waters 2707), a column oven (Waters Jetstream 2 Plus), and a LiChrosorb Si 60 column (250 × 4.6 mm, 5 µm) from Merck (Darmstadt, Germany). The mobile phase was a mixture of *n*-hexane with 1,4-dioxane (96:4 *v/v*). The flow rates were 1.0 ml/min (for tocopherols and PC-8) and 2.0 ml/min (for canolol). To detect the fluorescence of tocopherols and PC-8, the excitation wavelength was set at λ = 295 nm and the emission wavelength at λ = 330 nm. To detect the fluorescence of canolol, the excitation wavelength was set at λ = 280 nm and the emission wavelength at λ = 325 nm. Standards of α-, β-, γ- and δ-tocopherols (>95% purity) were purchased from Merck (Darmstadt, Germany). The plastochromanol-8 contents were assayed and calculated according to Siger et al. (2014).

Extraction of phenolic compounds from oils

The Chromabond® System (Macherey-Nagle, Germany) with a SPE column filled with diol (500 mg) (Supelco) was used to extract the phenolic acid fractions. The process consisted of four stages: 1. conditioning the column (5 ml methanol and 5 ml *n*-hexane); 2. adding the samples (2.5 g oil in 5 ml *n*-hexane and 5 ml *n*-hexane); 3. washing the column (5 ml *n*-hexane/ethyl acetate 90:10 *v/v*); and 4. eluting the phenolic acids with methanol to 5 ml.

Antioxidant activity

The DPPH radical-scavenging activity of the oil samples was measured according to Górnaś et al.'s method (2014). The reaction was initiated by mixing 3.97 mL of the DPPH ethyl acetate solution with 0.03 mL of oil or a phenolic compound of an extract from oil. Mixture absorbance was recorded following 30 min storage in the dark. The activity of the oil antioxidants in scavenging DPPH was calculated as follows:

$$\%\text{DPPH}^{\bullet}\text{ scavenging} = \left[\frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}}\right]$$

Total antioxidant capacity was also expressed as the Trolox equivalent on the basis of the standard curve $y = -0.0259x + 0.8524$ ($R^2 = 0.9995$). In order to compare the antiradical activity of oil samples, the antiradical power (*ARP*) was determined:

$$ARP = \frac{1}{(EC_{50})}$$

where: EC_{50} – volume extract needed to decrease the initial DPPH$^{\bullet}$ content by half, µl.

Statistical analysis

Results are presented as means ±standard deviations from three replications of each experiment. The differences between the mean values were determined by analysis of variance (ANOVA). The post-hoc analysis was performed using Tukey's test. All tests were considered significant at $p < 0.05$. Statistical analysis was performed using Statistica 10.0 software (StatSoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Changes in acid value and peroxide value

Cold-pressed oils from unroasted and roasted seed of different moisture contents. According to the Codex Alimentarius (1999), cold-pressed rapeseed oil should have an acid value *LK* < 4 mg KOH/kg, while its peroxide value should be *LOO* < 15 meq O_2/kg. The peroxide and acid values for the cold-pressed and hot-pressed oils produced from seeds with different moisture contents are presented in Figure 1. The oil produced from the seeds with a 5% moisture content showed the lowest peroxide values (0.28 meq O_2/kg and 0.19 meq O_2/kg for oils produced from unroasted and roasted seeds, respectively). This can be explained by the fact that thermally unstable hydroperoxides decomposed to secondary oxidation products and that is the reason for the lowest peroxide value compared to oils obtained from seeds with a higher moisture level. The oils produced from seeds with higher moisture levels were characterized by higher peroxide values (cold-pressed oil produced from unroasted seeds: 1.52–1.70 meq O_2/kg and cold-pressed oils produced from seeds roasted for 15 minutes at 160°C: 1.49–1.83 meq O_2/kg). The cold-pressed oils from unroasted seeds were characterized by acid values from 1.34 to 1.61 mg KOH/g, while the cold-pressed oils from roasted seeds had higher acid values of 1.57 to 1.66 mg KOH/g.

Hot-pressed oils from unroasted and roasted seeds of different moisture contents. The peroxide values for hot-pressed oils obtained from 5% moisture seeds showed higher peroxide values than cold-pressed oil (1.10 meq O_2/kg, and 0.8 meq O_2/kg for oil from unroasted and roasted seeds, respectively; Fig. 1). In the case of oils produced from seeds with a higher moisture content (7.5% and 10%), peroxide values were lower than in the case of cold-pressed oils. The acid values for hot-pressed oils in each case were higher than the values for cold-pressed oils. The acid values for hot-pressed oils produced from unroasted seeds ranged from 1.96 to 2.13 mg KOH/g. In the case of hot-pressed oils produced from roasted seeds, the values were 1.67 to 2.23 mg KOH/g. It was found that the acid value increases with the moisture content (Fig. 1).

One of the most important post-harvesting procedures for rapeseeds is drying, which affects the quality

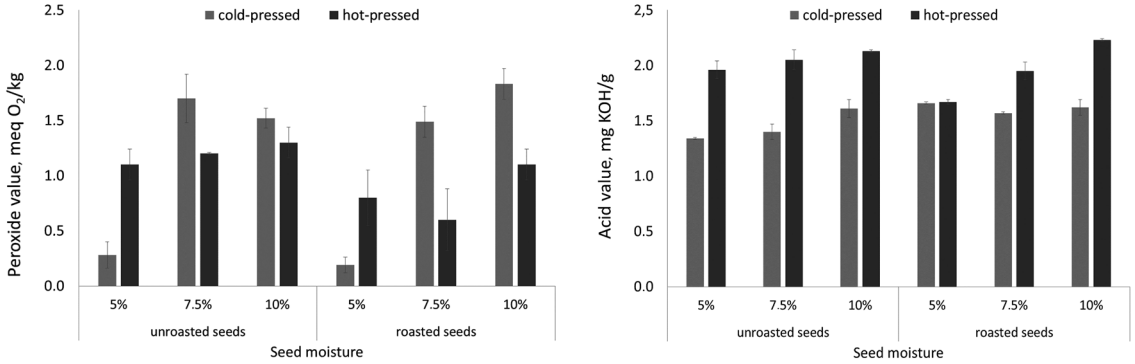

Fig. 1. Peroxide and acid values of cold-pressed and hot-pressed rapeseed oils obtained from roasted and unroasted seeds with different moisture levels

of their oil, their by-products (post-extraction pellets and pressings), and the costs incurred (Krasucki et al., 2002). Under European weather conditions, rapeseeds need to be dried after harvesting to reach 7% moisture content. Incorrect drying temperatures may affect the quality of the seeds (Gawrysiak-Witulska et al., 2009; Krygier et al., 1995). A temperature that is too high, or a too-long drying time, may lead to overdrying of the seeds, damaging the seeds and decreasing their mechanical resistance. The number of free fatty acids grows and the oxidation process of fat intensifies (Gawrysiak-Witulska et al., 2009). An increase in the drying temperature to above 93°C causes an increase in the content of free fatty acids in the oil (Pathak et al., 1991). Matthäus (2013b) draws attention to the fact that the moisture of seeds that are cold-pressed to produce oil also has a significant impact on the stability of the product. The oil obtained from seeds with a moisture content of 7% remained suitable for consumption for nine months, while the oil from seeds with 9% moisture lasted for only six months. Matthäus (2013b) also indicates that the moisture of seeds affects the sensory properties of the oils.

Tocochromanol contents

Cold-pressed oils from unroasted and roasted seeds of different moisture contents. The oils were tested to determine the tocopherol and plastochromanol-8 contents; the results are shown in Tables 1 and 2. The total level of tocopherols in the cold-pressed oils produced from unroasted seeds was 91.39 to 103.7 mg/100 g, and 92.0 to 97.79 mg/100 g in the case of oils produced from roasted seeds. It was found that the oils produced from seeds with differing moisture contents differ in terms of their levels of the various components. There was a statistically significant difference ($p < 0.0001$) between the oils produced from seeds with a 5% moisture content and the oils produced from seeds with higher moisture contents. The highest level of tocopherols, 103.7 mg/100 g, was found in the oil produced from unroasted seeds with a 5% moisture content. In the case of oil produced from roasted seeds with a 5% moisture content, the level of tocopherols was significantly lower – 97.79 mg/100 g ($p < 0.0001$). There were no statistically significant differences in the levels of tocopherols in the case of seeds with a 7.5% and 10% moisture content, as far as oils produced from unroasted seeds *vs*. oils produced from roasted seeds are concerned. When analyzing the levels of particular tocochromanol homologues, it was found that the most significant differences apply to the α-T homologue. The oil produced from unroasted seeds with a 5% moisture content contained the largest amount of the homologue (45.37 mg/100 g). Higher seed moisture levels of up to 10% led to a decrease of around 16% in α-T content. The oil produced from the seeds with a 7.5% moisture content contained 40.48 mg/100 g of the compound, whereas the oil produced from the seeds with a 10% moisture content had 38.16 mg/100 g of the compound. The oil produced from seeds with a 5% moisture content contained 57.08 mg/100 g of the γ-T homologue. Compared to

Table 1. Tocopherol levels in cold-pressed rapeseed oils obtained from roasted and unroasted seeds with different moisture levels

Oils	Tocopherol contents, mg/100 g					PC-8 content mg/100 g
	α-T	β-T	γ-T	δ-T	total	
Unroasted seeds						
moisture 5%	45.37 ±0.36^{c}	0.17 ±0.05^{a}	57.08 ±0.32^{c}	1.08 ±0.09^{a}	103.70 ±0.53^{d}	3.35 ±0.11^{b}
moisture 7.5%	40.48 ±0.21^{c}	0.12 ±0.06^{a}	53.24 ±0.29^{b}	1.02 ±0.05^{a}	94.87 ±0.29^{b}	2.54 ±0.13^{a}
moisture 10%	38.16 ±0.15^{a}	0.15 ±0.07^{a}	52.08 ±0.42^{a}	1.00 ±0.07^{a}	91.39 ±0.54^{a}	2.37 ±0.18^{a}
Seeds roasted at 160°C/15 min						
moisture 5%	42.38 ±0.22^{d}	0.16 ±0.09^{a}	54.17 ±0.25^{b}	1.07 ±0.10^{a}	97.79 ±0.35^{c}	3.14 ±0.16^{b}
moisture 7.5%	40.02 ±0.16^{b}	0.16 ±0.04^{a}	53.62 ±0.11^{b}	1.00 ±0.09^{a}	94.79 ±0.49^{b}	2.68 ±0.21^{a}
moisture 10%	38.31 ±0.33^{a}	0.13 ±0.06^{a}	52.51 ±0.48^{a}	1.05 ±0.08^{a}	92.00 ±0.66^{a}	2.68 ±0.17^{a}

Values (means ±SDs) bearing different superscripts are statistically significantly different ($P < 0.05$).

Table 2. Tocopherol levels in hot-pressed rapeseed oils obtained from roasted and unroasted seeds with different moisture levels

Oils	Tocopherol contents, mg/100 g					PC-8 content mg/100 g
	α-T	β-T	γ-T	δ-T	total	
Unroasted seeds						
moisture 5%	44.58 ±0.23^{a}	0.19 ±0.01^{a}	58.27 ±0.26^{a}	1.26 ±0.07^{a}	104.30 ±0.36^{a}	3.02 ±0.06^{a}
moisture 7.5%	48.87 ±0.14^{c}	0.18 ±0.01^{a}	66.26 ±0.24^{c}	1.31 ±0.12^{a}	116.63 ±0.27^{d}	3.11 ±0.09^{a}
moisture 10%	47.88 ±0.20^{b}	0.18 ±0.02^{a}	64.42 ±0.41^{b}	1.32 ±0.06^{a}	113.80 ±0.30^{c}	4.26 ±0.18^{b}
Seeds roasted at 160°C/15 min						
moisture 5%	47.27 ±0.22^{b}	0.17 ±0.01^{a}	63.35 ±0.39^{b}	1.15 ±0.03^{a}	112.10 ±0.60^{b}	3.30 ±0.25^{a}
moisture 7.5%	49.11 ±0.40^{c}	0.16 ±0.02^{a}	66.35 ±0.32^{c}	1.26 ±0.08^{a}	116.87 ±0.50^{d}	3.43 ±0.21^{a}
moisture 10%	47.34 ±0.26^{b}	0.17 ±0.03^{a}	63.56 ±0.24^{b}	1.34 ±0.07^{a}	112.40 ±0.07^{b}	4.32 ±0.24^{b}

Values (means ±SDs) bearing different superscripts are statistically significantly different ($P < 0.05$).

this, the 7.5% moisture seeds showed a 7% decrease in this compound (to 53.24 mg/100 g). For the seeds with a 10% moisture content, 9% less γ-T homologue was found (Table 1). In analyzing the level of tocopherols in the oil produced from roasted seeds, a similar relation was found between the amount of tocopherols in the oil and the moisture content of the seeds from which the oil was pressed. However, the quantities of α-T and γ-T were lower in the oil produced from the 5% moisture seeds. The levels of α-T and γ-T were lower by around 3 mg/100 g than in the oil produced from unroasted seeds with the same moisture level. No statistically significant difference was seen in the tocopherol levels of oils produced from roasted seeds with 7.5% and 10% moisture in comparison to the oils produced from unroasted seeds. The level

of plastochromanol-8 was significantly higher ($p < 0.0001$) in oils produced from seeds with a 5% moisture content (more than 3 mg/100 g). In oil produced from seeds with a 7.5% and 10% moisture content, the amount of PC-8 was around 2.5 mg/100 g. There was no statistically significant difference ($p > 0.05$) in the level of this compound found in oils produced from unroasted seeds and in oils produced from roasted seeds (Table 1).

Hot-pressed oils from unroasted and roasted seed of different moisture contents. Table 2 presents the data associated with the tocochromanol levels in the hot-pressed oils produced from seeds of various moisture contents. The tocopherol level in hot-pressed oil from 5% moisture seeds was 104.3 mg/100 g, similar to the amount in cold-pressed oil. The hot-pressed oil from seeds with 7.5% and 10% moisture had higher levels of tocopherols, at 116.63 mg/100 g (7.5% moisture content) and 113.80 mg/100 g (10% moisture content). The hot-pressed oil from 5% moisture seeds had more tocopherols when produced from roasted seeds (112.10 mg/100 g). For the oil from roasted seeds with 7.5% and 10% moisture content, no statistically significant difference ($p > 0.05$) was seen with the oil produced from unroasted seeds (Table 2). In analyzing the amounts of the various tocopherol homologues, it was found that for hot pressing, the highest values are obtained for 7.5% moisture content; in particular, the highest levels of α-T homologue (around 49 mg/100 g) and γ-T homologue (around 66 mg/100 g) were recorded. No statistically significant ($p > 0.05$) effect of seed roasting on the content of particular tocopherol homologues was identified. However, it was found that the hot pressing procedures have a statistically significant effect ($p < 0.0001$) on the level of tocopherols in oil. The hot-pressed oil produced from (roasted and unroasted) seeds with a 7.5% moisture content contained around 49 mg/100 g of α-T and 66 mg/100 g of γ-T, whereas the cold-pressed oil contained 40 mg/100 g of α-T (18% less) and 53 mg/100 g of γ-T (20% less) of these substances (Tables 1–2). It was found that in the case of hot-pressing, the amount of PC-8 in the oil increases with the water content of the seeds. The highest levels of PC-8, of over 4 mg/100 g, were found in oil produced from seeds with a 10% moisture content; the value was statistically significant ($p < 0.0001$) in comparison to the oil produced from seeds with a 5% and 7.5% moisture content. There was no significant difference between the oils produced from roasted and unroasted seeds (Table 2).

In analyzing cold-pressed and hot-pressed rapeseed oils and rapeseed oils extracted with an organic solvent, Ghazani et al. (2014) found that the solvent-extracted oil was characterized by the highest levels of tocochromanols (492.5 mg/kg). When they compared cold-pressed and hot-pressed oils, they did not find any statistically significant differences regarding the tocopherol levels. However, the hot-pressed oil contained 22.3–34.1 mg/kg more tocopherols. In their study, Wroniak et al. (2008) demonstrated that the total amount of tocopherols in hot-pressed oil is 20% greater than in cold-pressed oil. In analyzing the amounts of particular tocopherol homologues, it was determined that the hot-pressed oil contained 30% more α-T and 16% more γ-T than cold-pressed oil. Kraljić et al. (2013) reported that conditioning has a statistically significant effect, increasing the α-T contents of the oil; however, they found no significant effects of conditioning on the level of γ-T. In studies examining the levels of tocopherols in rapeseed oil produced from roasted seeds, some authors observed an increase in the amounts of particular tocopherols and plastochromanol-8 (Siger et al., 2015; Shrestha and De Meulenaer, 2014). Neither Wakamatsu et al. (2005) nor Spielmeyer et al. (2009) found any significant differences in the tocopherol content of canola oil from unroasted and roasted raw material. The lack of a decrease in tocopherol levels under the influence of high-temperature roasting of the rapeseed is often explained by the synergistic interaction of phenolic compounds (here, mainly canolol) – the amount of which increases in proportion to the temperature and roasting duration (Matthäus, 2013a; Siger et al., 2015). Other compounds may also reduce the losses of tocopherols during roasting. According to Fukuda and Nagashima (2005), during seed roasting, Maillard reactions occur and fat-soluble melanoidins may be extracted together with the oil. Antioxidant properties are also attributed to these compounds. However, according to Wijesundera et al. (2008), the increase in the levels of γ-T in oil from seeds roasted at 160°C for 5 minutes is possibly due to its coelution in HPLC with another component generated after roasting.

Changes in canolol levels

Cold-pressed oils from unroasted and roasted seed of different moisture contents. The first study of the amount of canolol − that is, of the product of the decarboxylation of sinapic acid − in cold-pressed rapeseed oil was conducted by Koski et al. (2003). The canolol content in the oils produced here was determined, and the results are shown in Table 3. The amount of canolol in cold-pressed oil produced from unroasted seeds with a 5% moisture content was 178.13 μg/g. In the case of oil produced from seeds with a 7.5% and 10% moisture content, the level of canolol was 179.50 and 177.12 μg/g respectively. Roasting caused the amount of canolol to increase in both the hot-pressed and cold-pressed oils. The levels of canolol in cold-pressed oil produced from roasted seeds with a 5% moisture content grew by 32% (235.39 μg/g) over that of the oil produced from unroasted seeds at the same moisture content. The level of canolol increased by 47% (263.59 μg/g) and 45% (256.85 μg/g) for oil produced from roasted seeds with 7.5% and 10% moisture content, respectively.

Hot-pressed oils from unroasted and roasted seed of different moisture contents. The amount of canolol present in hot-pressed oil has been found to increase compared to cold-pressed oils. Canolol levels are 16% higher (at 207.93 μg/g) in oil produced from seeds with a 5% moisture content, 20% higher in oil from seeds with a 7.5% moisture content (216.67 μg/g), and 18% higher in oil from seeds with a 10% moisture content (208.61 μg/g) (Table 3). A similar tendency was observed in the case of hot-pressed oils produced from roasted seeds. In this case, the increase in the amount of canolol compared to the oils produced from unroasted seeds reached 31% for oils produced from seeds with a 5% (273.05 μg/g) and 7.5% (285.0 μg/g) moisture content, and 35% for oils produced from seeds with a 10% moisture content (280.98 μg/g). In comparing the amount of canolol produced from roasted seeds by hot-pressing with that produced by cold-pressing, it was found that the hot-pressed oils contain 8–16% more canolol (Table 3).

Spielmeyer et al. (2009) found that the optimum temperature of seed roasting is 160°C and that the canolol content decreases if the temperature is higher. However, there is no confirmation of this finding in other research studies. Shrestha and De Meulenaer (2014) roasted rapeseeds for 10 to 90 minutes at a temperature of 180°C and demonstrated that the highest amount of canolol can be found in extracted oil produced from seeds roasted for 20 minutes. Siger et al. (2015) analyzed the amount of canolol in cold-pressed rapeseed oil produced from seeds roasted at 140°C, 160°C, and 180°C for 5, 10, and 15 minutes. The highest canolol levels were found in the oil produced from seeds roasted at 180°C for 15 minutes (these contained 50 times more canolol than in the control test). The amount of canolol in the oil produced from the seeds roasted at 160°C for 15 minutes was 244.76 μg/g for the PR46W20 cultivar. In the control test (using oil produced from unroasted seeds), the levels of canolol were 5.8 μg/g (Spielmeyer et al., 2009), 5.19 μg/g (Shrestha and De Meulenaer, 2014), and 11.54 μg/g (Siger et al., 2015). Kraljić et al. (2013) showed that conditioning the seeds prior to pressing the oil had a significant effect on canolol level. They found 3.8–7.8 μg/g canolol in the oil from nonconditioned seeds and 110.3–353.2 μg/g in the oil from conditioned seeds. In the case of the variety of rape studied here (Adrianna), the control oil from unroasted seeds was characterized by a significantly higher content of canolol (more than 170 μg/g). The oil-pressing procedure and the analysis

Table 3. Canolol levels in cold-pressed and hot-pressed rapeseed oils obtained from roasted and unroasted seeds with different moisture levels

Oil source	Canolol content, μg/g	
	cold-pressed	hot-pressed
Unroasted seeds		
moisture 5%	178.13 ±0.62^{b}	207.93 ±0.34^{a}
moisture 7.5%	179.50 ±0.25^{c}	216.67 ±0.42^{b}
moisture 10%	177.12 ±0.61^{a}	208.61 ±0.35^{a}
Seeds roasted at 160°C/15 min		
moisture 5%	235.39 ±0.32^{d}	273.05 ±0.36^{c}
moisture 7.5%	263.59 ±0.58^{f}	285.00 ±0.41^{e}
moisture 10%	256.85 ±0.47^{e}	280.98 ±0.26^{d}

Values (means ±SDs) bearing different superscripts are statistically significantly different ($P < 0.05$).

were conducted several times. The oil produced from the seeds of this variety of rape was also extracted using a Soxhlet extractor, and the amount of canolol remained high. It is probable that the variety of rape is of great significance for this phenomenon. Canolol is lost during the production of refined oil, but because of its lipophilic nature, it can be added back to the oil after being isolated from the pressing (Wakamatsu et al., 2005; Zacchi and Eggers, 2008).

Antioxidant activity

Cold-pressed oils from unroasted and roasted seed of different moisture contents. The antioxidant activity of tested oils (Table 4) shows an upward trend with an increase in the moisture content of the seeds used in oil pressing. It was also found that cold-pressed oils from roasted seeds exhibit a higher antioxidant activity. It is statistically significantly that the lowest antioxidant activity was recorded for oil from unroasted seeds with a 5% moisture content ($ARP = 13.86 \times 10^{-3}$). The other cold-pressed oils showed a higher antioxidant activity. Oil from unroasted seeds with a 7.5% moisture content had $ARP = 15.11 \times 10^{-3}$, while for seeds with a 10% moisture content $ARP = 14.92 \times 10^{-3}$. Seed roasting caused an additional increase in the antioxidant activity of cold-pressed oils, amounting to 15.58×10^{-3}, 17.66×10^{-3} and 17.57×10^{-3}, respectively, for oils produced from seeds with 5, 7.5 and 10% moisture contents (Table 4).

Hot-pressed oils from unroasted and roasted seed of different moisture contents. Table 4 presents the antioxidant activity of cold-pressed oils. Antioxidant activity increased with an increase in seed moisture content. Samples of hot-pressed oils from unroasted seeds with 5% and 7.5% moisture contents showed antioxidant activity from 14.76×10^{-3} to 15.35×10^{-3}; however, the difference was not statistically significant. A statistically significant increase in antioxidant

Table 4. Antioxidant activity of oils and phenolic compounds extracts obtained from oils determined by DPPH•

Oil source	Antioxidant activity of oils				Antioxidant activity of phenolic compounds extract from oil			
	cold-pressed		hot-pressed		cold-pressed		hot-pressed	
	DPPH• µM Trolox/g	ARP × 10^{-3}	DPPH• µM Trolox/g	ARP × 10^{-3}	DPPH• µM Trolox/g	ARP × 10^{-3}	DPPH• µM Trolox/g	ARP × 10^{-3}
Unroasted seeds								
moisture 5%	221.65 ±6.36^{a}	13.86 ±0.11^{a}	249.97 ±2.65^{a}	14.76 ±0.35a	21.19 ±1.25^{a}	1.32 ±0.35^{a}	22.33 ±2.98a	1.11 ±0.15^{a}
moisture 7.5%	261.86 ±2.72^{b}	15.11 ±0.06^{c}	250.07 ±2.55^{a}	15.35 ±0.11b	23.52 ±2.43^{a,b}	1.31 ±0.11^{a}	26.08 ±1.19b	1.28 ±0.11^{a,b}
moisture 10%	269.11 ±2.23^{c}	14.92 ±0.12^{b}	268.88 ±2.65^{b}	15.69 ±0.29b	24.87 ±1.88^{b}	1.30 ±0.29^{a}	30.42 ±1.38c	1.41 ±0.19^{b}
Seeds roasted at 160°C/15 min								
moisture 5%	273.77 ±3.35^{d}	15.58 ±0.14^{d}	275.56 ±2.65^{c}	17.26 ±0.35^{c}	23.58 ±1.04^{a,b}	1.60 ±0.25^{b}	29.86 ±1.58^{c}	1.55 ±0.15^{b,c}
moisture 7.5%	295.10 ±6.92^{e}	17.66 ±0.05^{e}	283.50 ±5.45^{c}	17.08 ±0.11^{c}	24.97 ±2.02^{b}	1.63 ±0.36^{b}	30.55 ±2.03^{c}	1.67 ±0.06^{c}
moisture 10%	301.41 ±5.89^{e}	17.57 ±0.09^{e}	293.42 ±3.42^{d}	17.68 ±0.29^{e}	26.09 ±2.03^{c}	1.66 ±0.41^{b}	37.52 ±1.04^{d}	1.88 ±0.02^{d}

Values (means ±SDs) bearing different superscripts are statistically significantly different ($P < 0.05$).

activity in this group was found for oil pressed from seeds with a 10% moisture content (*ARP* = 15.69 × 10^{-3}). Oils pressed from roasted seeds showed a significantly greater antioxidant activity in comparison to hot-pressed oils from unroasted seeds. Samples produced by hot-pressing from seeds with 5, 7.5 and 10% moisture contents showed activity ranging from 17.26 × 10^{-3} to 17.68 × 10^{-3} (Table 4). Recorded data show that both in cold- and hot-pressed oils antioxidant activity increased with an increase in seed moisture content, which was additionally enhanced by the applied roasting process. The increase in the antioxidant activity of oils from roasted seeds was correlated with increasing canolol content, as shown in Table 3, with the canolol content in roasted seeds being greater by approx. 30–40%.

Antioxidant activity of phenolic extracts from cold-pressed and hot-pressed oils. The antioxidant activity of the oils tested was influenced by the contents of native antioxidants: canolol, phenolic compounds and tocochromanols. In this study we also investigated antioxidant activity of the polar fraction (phenolic compounds) isolated from the non-polar matrix, such as oil, using the SPE technique. It was shown that in cold-pressed oils from seeds varying in their moisture contents the antioxidant activity of phenolic compounds was approx. 1.3 × 10^{-3} and did not differ statistically in oils from seeds with varying moisture contents (Table 4). In the case of cold-pressed oils from seeds which had been roasted a statistically significant increase was found for antioxidant activity – to 1.6 × 10^{-3}. In the case of hot pressing, a statistically significant effect ($p < 0.001$) was shown for seed moisture content on the antioxidant activity of phenolic extracts, which ranged from 1.11 × 10^{-3} to 1.41 × 10^{-3}. Extracts of phenolic compounds from hot-pressed oils from roasted seeds showed a higher antioxidant activity (from 1.55 × 10^{-3} to 1.88 × 10^{-3}). Summing up, it may be stated that phenolic compounds are responsible for approx. 10% of the antioxidant activity of the oils tested.

Water content in cold-pressed oils is relatively low, resulting in a small proportion of hydrophilic antioxidants in the total antioxidant contents. Moreover, the activity of individual phenolic compounds also varies. Studies indicate a significant correlation of contents of these compounds and antioxidant stability of these oils (Mińkowski et al., 2013; Szydłowska-Czerniak et al., 2008). Siger et al. (2008) also showed correlations between phenolic contents in oils and their antioxidant activity and free radical scavenging capacity. However, in another study, Espin et al. (2000) showed that such a dependence is not typical of all oils. Decker (2008) was of the opinion that the antioxidant activity of phenolic compounds depends on the number and position of hydroxyl groups in their structure, polarity, solubility and stability during processing. Thiyam et al. (2006) when comparing the antioxidant activity of sinapine and sinapic acid showed a higher activity of these compounds as well as a prooxidative effect of sinapine found in high concentrations. Vuorela et al. (2004) confirmed the antioxidant activity of phenolic extracts of rapeseed oil showing DPPH free radical scavenging at 60% and inhibition of hydroxyperoxide formation at 80%.

Canolol has a greater ability to prevent lipid oxidation than a number of other known antioxidants, such as tocopherols, ascorbic acid, β-carotene, rutin, and quercetin (Koski et al., 2003; Wakamatsu et al., 2005). Terpinc et al. (2011) assessed the antioxidant activity of canolol and compared it to that of sinapic acid and other phenolic acids and derivatives. Canolol's antioxidant activity exceeded that of sinapic acid by 15%. The results of the study showed the following descending order of antioxidant activity of the individual decarboxylation products of hydroxycinnamic acid: 4-vinylcatechol, 4-vinylsyringol (canolol), 4-vinylguaiacol, 4-vinylphenol. In their studies, Harbaum-Piayda et al. (2010) and Terpinc et al. (2011) demonstrated that the antioxidant activity of canolol, as determined by DPPH assay, was lower than that of sinapic acid. They have also shown that there is a synergistic effect between the different phenolic compounds in rapeseed oil. Such an effect is also confirmed by Wakamatsu et al. (2005) and by Spielmeyer et al. (2009), who reported no loss of tocopherols during the thermal processing of seeds and explained this as being due to the protective activity of canolol. Moltke-Sørensen et al. (2013) showed the high antioxidant activity of canolol in an oil-water emulsion, which in comparison to other compounds is ordered as follows: BHT > canolol > sinapine > canola extract > sinapic acid.

Table 5. Three factorial analysis of variance for antioxidant activity of bioactive compounds in rapeseed oil

Effect	Sum of squares (SS)	df	Mean of square (MS)	*F*-statistic	*p*-value
Pressed (P)	1	1	1	0.0	0.854627
Roasted (R)	10 129	1	10 129	563.8	$P < 0.00001$
Moisture (M)	4 785	2	2 393	133.2	$P < 0.00001$
P × R	290	1	290	16.1	0.000508
P × M	1 143	2	571	31.8	$P < 0.00001$
R × M	163	2	82	4.5	0.021121
P × R × M	284	2	142	7.9	0.002323
Error	431	24	18		

P – cold-pressed or hot-pressed, M – moisture (5, 7.5 or 10%).

Statistical analysis has shown that antioxidant activity depends on pressing, roasting, moisture content and their interactions (Table 5). A positive correlation between antioxidant activity and canolol content ($r = 0.754$; $p < 0.00001$) as well as total phenolic content ($r = 0.441$; $p = 0.01458$) was also found.

CONCLUSIONS

Various levels of biologically active compounds were shown to be present in the rapeseed oil obtained from raw materials of varying moisture content. The type of pressing process (cold-pressing or hot-pressing) and whether the seeds have undergone roasting have also been shown to affect the resulting oil and the level of native antioxidants it contains. The roasting of the seeds and their level of moisture both significantly affect the quality of the oil obtained. A statistically significant higher level of tocopherols has been shown in oils with a moisture content of 5% (for cold-pressed oils) or 7.5% (for hot-pressed oils). An inverse relationship was observed between the type of pressing and the seed moisture content in terms of plastochromanol-8, whose level in cold-pressed oils decreased with increasing seed moisture content, contrary to the hot-pressed oils. Cold-pressed oils obtained from unroasted rapeseed of the Adrianna variety were characterized by high levels of canolol. Both roasting and hot-pressing resulted in an increase in canolol content. When analysing the antioxidant activity of oils and phenolic extracts tested it was shown that phenolic compounds are responsible for approx. 10% of total antioxidant activity.

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