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Influence of Conditioning Temperature on the Quality, Nutritional Properties and Volatile Profile of Virgin Rapeseed Oil

Klara Kraljić[1]*, Tatjana Stjepanović[1], Marko Obranović[1], Milan Pospišil[2], Sandra Balbino[1] and Dubravka Škevin[1]

[1]University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, Zagreb, Croatia
[2]University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, Zagreb, Croatia

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*Corresponding author:
Phone: +38514605135;
Fax: +38514605072;
E-mail: kkraljic@pbf.hr

ORCID IDs: 0000-0002-8544-5433 (Kraljić), 0000-0001-5861-744X (Stjepanović), 0000-0001-7868-1182 (Obranović), 0000-0002-9972-3940 (Pospišil), 0000-0003-3298-6209 (Balbino), 0000-0001-9215-6245 (Škevin)

SUMMARY

Heating the rapeseed prior to the oil extraction is conducted to increase the oil yield but it can also induce changes of various components of the seed. These changes may affect the composition of the volatile and non-volatile compounds of produced virgin rapeseed oil. The aim of our study is to determine the impact of different conditioning temperatures (60, 80 and 100 °C) on the quality, nutritional value, aroma profile and sensory characteristics of virgin rapeseed oil. Conditioning the seeds at all three temperatures had no influence on the quality and major nutritional components (fatty acids and tocopherols) of the produced oil. However, temperature increase caused an exponential increase of canolol and significant changes in the aroma and sensory profile of the produced oil samples. The dominant volatile compounds of cold-pressed and virgin oil produced at 60 °C were enzymatic degradation products of glucosinolates (isothiocyanates and epithionitriles), responsible for pronounced seed-like flavour of these types of oil. Increasing production temperature deactivated enzymes and caused thermal decomposition of seed components and increment of nitriles, aldehydes, pyrazines and furanes, carriers of nutty and roasty flavour. These results can help producers to design virgin rapeseed oil with specific and desirable sensory characteristics.

Key words: rapeseed oil, seed conditioning, nutritional value, volatile components, sensory analysis

INTRODUCTION

Rapeseed oil is one of the most widely used types of oil in the world and it currently ranks third in the production quantity (*1*). Growing awareness about its nutritional value brought increase in food usage for almost 50 % in the past decade (*2*). Specific fatty acid composition, high percentage of unsaturated fatty acids and an ideal 2:1 ratio of ω-6 to ω-3 fatty acids have the highest impact on the nutritional value of rapeseed oil. Furthermore, significant amounts of sterols and tocopherols and a fair amount of polyphenols contribute considerably to its oxidative stability and nutritional value (*3,4*).

Majority of rapeseed oil today is placed on the market as refined rapeseed oil. This type of oil is characterized by a neutral odour and flavour, familiar and acceptable to the consumers. However, term 'refined' causes negative connotations among the consumers because of the belief that all of the aforementioned beneficial and nutritional components are removed or degraded during refining process. That is why nowadays another group of products, nonrefined types of oil, is of growing interest among the consumers. Nonrefined oil is produced only by mechanical procedures and can be placed on the market as cold-pressed or as virgin oil. The difference between these two types of oil is in the application of heat. When producing virgin oil, seeds are heated during conditioning phase, prior to pressing (*5*). Conditioning of the seed is conducted to improve oil yield, but it can also significantly increase the amount of bioactive compounds of virgin rapeseed oil. Heat-induced disruption of the cell improves extractability of tocopherols and sterols in the oil (*4,6,7*). However, conditioning of the rapeseed has the greatest impact on the increase of the phenolic compounds, especially canolol, a specific phenolic compound of rapeseed oil, formed by heat-induced decarboxylation of

sinapic acid (*8*). Shrestha and De Meulenaer (*9*) reported more than 150 times higher concentration of canolol in oil produced from conditioned seeds than in cold-pressed rapeseed oil.

Apart from the economical and nutritional aspects of the oil, conditioning of the seeds is known to have a great influence on the sensory characteristic of some types of virgin oil such as pumpkin, peanut or sesame oil (*10–12*). Good quality cold-pressed rapeseed oil has an expressed seed-like flavour characterized by a sensation resembling asparagus, cabbage, fresh green vegetable, sometimes with the sulfuric note (*13*). Aforementioned attributes are caused by specific volatile compounds formed during enzymatic degradation of glucosinolates (*14*). Applying the heat during production process causes inactivation of the enzymes and induces thermal degradation. These changes most likely affect sensory attributes of virgin rapeseed oil. That is why the aim of our study is to determine changes in nutritional value, volatile compounds and sensory characteristics caused by application of different conditioning temperatures during the production of virgin rapeseed oil.

MATERIALS AND METHODS

Reagents

Reagents used in this study were obtained from commercial sources: petroleum ether, diethyl ether, ethanol, isooctane, acetic acid, methanol, *n*-hexane, 2-propanol, acetonitrile, phosphoric acid, alkane standard solution (C_8–C_{20}), sinapic acid (Sigma-Aldrich Chemie, Taufkirchen, Germany), and FAME mix (C4-C24) (Supelco, Sigma-Aldrich, Merck, Bellefonte, PA, USA). Tocopherol homologues (α-, β-, γ- and δ-tocopherol) were purchased from Merck, Darmstadt, Germany. Plastochromanol-8 was given to us by Dr Jerzy Kruk from Jagiellonian University, Kraków, Poland.

Rapeseed oil production

Oil samples used in present research were either produced by cold pressing of unheated seeds (cold-pressed rapeseed oil) or by pressing seeds conditioned by heating at different temperatures to obtain virgin rapeseed oil. Rapeseed cultivar PR46W20 (Pioneer, Johnston, IA, USA) grown at the experimental field of the Faculty of Agriculture, University of Zagreb, Zagreb, Croatia, was used. Prior to each production procedure, 1 kg of seeds was ground using standard electric coffee grinder (Gorenje, Velenje, Slovenia). Cold-pressed oil was extracted using laboratory expeller press (Monforts & Reiners, Rheydt, Germany) with temperature not exceeding 40 °C. For virgin rapeseed oil production, ground rapeseeds were first heated for 30 min with constant stirring at 60, 80 or 100 °C. Conditioning of the seeds was carried out in the specially constructed heater with stirrer placed in the double-walled stainless steel container. Seeds were heated indirectly by thermal conduction, and the heating was achieved by placing the glycerol between double walls and heating it with electric heater. Heater was also equipped with temperature control that can maintain temperature at a set temperature ±2 °C. During this phase of oil production, portions of water were added totalling 80, 140 and 240 mL, respectively. Conditioned seeds were then pressed on the same laboratory expeller press used for cold pressed oil production. All produced oil samples were filtered through filter paper (Whatman grade 1, pore size 11 μm; Sigma-Aldrich Chemie, Merck) and stored in the dark glass bottles under nitrogen at room temperature. All analyses of the oil were performed within two weeks of the production.

Oil yield

Oil and moisture content of the seeds and cakes after oil pressing were determined using standard ISO methods (*15,16*). Moisture content was determined by drying 5 g of unground seeds, or ground cake, in metal vessel at 103 °C for 1 hour until constant mass was achieved. For the determination of oil content, standard method of extraction of non-polar component using petroleum ether was used. In brief, 10 g of seeds were ground in an electric mill (Gorenje), placed in extraction cellulose thimble, closed with cotton wool and placed into the Soxhlet syphoning-type extractor. The extractor was placed onto the 250-mL round flask and 150 mL of petroleum ether were added. Upper end of the extractor was attached to a water condenser. Assembled apparatus was placed on the electric heating bath (INKO d.d., Zagreb, Croatia) and extraction was carried out with reflux for 8 h. After that, greater part of the solvent was removed from the flask by distillation, and the rest of the solvent was removed by heating the flask in the oven at 103 °C for 60 min. Drying of the flask was repeated for 30 min until constant mass of oil was achieved. All analyses were performed in triplicates. The obtained data were used to calculate oil yield in each extraction process. Oil yield is defined as the percentage of oil extracted by each process on a total oil extractable basis.

Determination of oil quality parameters

Quality parameters of produced oil, free fatty acids (FFA) and peroxide value (PV), were determined using standard ISO methods (*17,18*). For FFA determination, 10 g of oil was weighed into the 250-mL Erlenmeyer flask. A volume of 50 mL of neutral mixture of ethanol (96 %) and diethyl ether (peroxide-free) in equal volumes was added to dissolve the test portion. Phenolphthalein was added as an indicator and the mixture was titrated with standard sodium hydroxide solution (0.1 mol/L) (*17*). PV was determined according to ISO 3960:2007 method (*18*). A mass of 5 g of the oil was dissolved in 50 mL of the glacial acetic acid/isooctane solution (3/2, *V/V*), then 0.5 mL of saturated potassium iodide solution was added, and closed Erlenmeyer flask was mixed for exactly 60 s. Water (30 mL) was added immediately to the flask, and liberated iodine was titrated with 0.01 mol/L sodium thiosulfate solution. A volume of 0.5 mL of starch solution (0.1 g/L) was added as an indicator. Each procedure was repeated three times for each oil sample.

Determination of fatty acid composition

Gas chromatography was used to determine fatty acid composition of produced rapeseed oil samples. Fatty acid methyl esters were prepared by transesterification with methanol according to ISO 5509:2000 method (*19*). A mass of 60 mg of oil was dissolved in 4 mL of isooctane and 200 µL of potasium hydroxide (2 mol/L) were added. Mixture was vigorously shaken using vortex (IKA-Werke GmbH & Co., Staufen, Germany) for 30 s and left for a few munutes at room temperature to react. Then, 1 g of sodium hydrogen sulphate monohydrate was added, mixed and clear supernatant containing methyl esters was transferred into the vial. The prepared methyl esters were injected (1 µL) into an Agilent Technologies 6890N Network GC system (Santa Clara, CA, USA) equipped with flame ionisation detector. Fatty acid methyl esters were separated on a DB-23 capillary column (60 m×0.25 mm×0.25 µm; Agilent Technologies). Helium was used as carrier gas with a constant flow of 1.5 mL/min. Injector temperature was set at 250 °C and detector at 280 °C. Owen temperature was programmed to increase by 7 °C/min from initial 60 °C to final temperature of 220 °C where it was held for 17 min. The split ratio was 30:1. Fatty acid methyl esters were identified by comparison of their retention times with those of the commercial standards. The content of each fatty acid is expressed as a percentage of total fatty acids, and the results are presented as the mean value of three parallel determinations.

Determination of tocopherols

Tocopherol content was determined following ISO 9936:2006 method (*20*). A volume of 50 µL of 1 % (*m*/*V*) oil solution in *n*-hexane was injected into a Varian HPLC system (Palo Alto, CA, USA) equipped with a fluorescence detector and LiChroCART Silica 60 column (250 mm×4.6 mm, 5 µm; Merck). Separation of tocopherols was performed at room temperature by isocratic chromatography using 0.7 % 2-propanol in *n*-hexane as a mobile phase. The flow rate of mobile phase was 0.9 mL/min. Tocopherols were detected at 295 nm excitation wavelength and 330 nm emission wavelength. Quantification of individual tocopherols was performed using calibration curves of α-, β-, γ-, and δ-tocopherols and plastochromanol-8. The analysis was performed in triplicate.

Determination of canolol content

Canolol content was determined using high-performance liquid chromatography (HPLC; Varian, Palo Alto, CA, USA) equipped with a UV detector, following the method we described in our earlier research (*7*). Canolol was identified by comparing its retention time and spectra with the ones of a compound identified as canolol in the above-mentioned research, and quantified by using calibration curve for sinapic acid. Results are given as mean value of three parallel determinations.

Determination of volatile components

Volatile components were isolated using headspace solid-phase microextraction (SPME) and their content and composition were determined using gas chromatography/mass spectrometry (GC/MS). Divinylbensene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (2 cm long) with 50/30 µm film thickness (Supelco, Sigma-Aldrich, Merck) was used. Rapeseed oil (10 g) was weighed into a 20-mL vial fitted with a silicone septum which was then placed in a heating block at 40 °C with constant magnetic stirring (Pierce Reacti-Therm Heating/Stirring Module, Artisan, Champaign, IL, USA). After 10 min of sample conditioning, SPME fibre was exposed for 30 min, and immediately desorbed in the gas chromatograph injector in a splitless mode at 260 °C for 1 min. Samples were analysed using an Agilent Technologies 6890N Network GC system coupled with an Agilent Technologies 5973 inert mass selective detector. Volatile compounds were resolved on a ZB-5 column (30 m×0.25 mm×0.25 µm; Phenomenex, Torrance, CA, USA). Owen temperature was held at initial temperature of 35 °C for 10 min, after which it was increased to 200 °C at 5 °C/min rate, followed by an increase to 250 °C at 20 °C/min. This final temperature was held for additional 5 min. The temperature of the ion source and the transfer line were 230 and 280 °C, respectively. Helium was used as carrier gas with a constant flow of 1.0 mL/min. Mass spectra were recorded in the 50–550 mass range. The *n*-alkanes (C_8–C_{20}) were run under the same conditions as the samples to calculate the Kovats index values. Identification of the compounds was based on the comparison of the mass spectra with the records in NIST mass spectral library (*21*) and by comparing Kovats index with the ones from the same library (*21*) or from the literature (*10,14,22*). The minimal s/n ratio for the integration of the peaks was 13. Each of the four samples of oil was analysed at least three times. Results of the volatile compound determination are expressed as peak area of individual component.

Sensory analysis

Sensory analyses were carried out by a panel of six panellists chosen and trained at our laboratory using a method described by Brühl and Matthäus (*13*). The panel consisted of two male and four female panellists, all non-smokers and aged between 30 and 50. Panellists were asked to evaluate colour, clarity and flavour which is described by attributes seed-like, nutty, roasty, burnt and rancid using a scale from 0 to 5 (zero representing the absence of an attribute and five representing highly expressed sensory characteristic). Oil samples (15 mL) were served to the panellists at room temperature, and water and unsalted bread were given to rinse their mouths between samples. Each sample was evaluated twice, at two different sessions.

Data analysis

All data, except for the sensory analysis, are expressed as the mean value of minimum three determinations±standard

deviation. Results of sensory analysis are presented as mean value of two parallel determinations. To determine the effect of conditioning temperature on the oil yield, oil quality and composition of volatile and non-volatile components, single factor analysis of variance (ANOVA) was performed followed by Tukey's honestly significant difference (HSD) test. A significance level of $p \leq 0.05$ was applied. Statistical analysis was performed using STATISTICA v. 10 (*23*).

RESULTS AND DISCUSSION

Conditioning of the seeds is a process designed to increase oil yield, thus having a beneficial economic effect on the oil production (*3*). Furthermore, this process can also improve nutritional value and alter sensory characteristic of the produced vegetable oil (*10,14,24*). Sensory characteristics probably have the greatest impact on the consumer choice whether to use a certain oil or not, and these characteristics are under the direct influence of volatile and non-volatile components formed in the seed during oil production, storage or usage. It is crucial to understand the changes that volatile and non-volatile components undergo during production in order to obtain high-quality virgin rapeseed oil with desirable sensory characteristics. The aim of our study was to determine the dynamics of these changes as a result of temperature alterations during conditioning process in the virgin rapeseed oil production.

Fig. 1 shows oil yield of cold-pressed and virgin rapeseed oil production. We extracted 72 % of available oil by cold pressing. Conditioning the seeds prior to oil pressing increased oil yield for 1.5–14.4 %. Data analysis showed that oil yield increase significantly depends on the temperature used during the conditioning process. This is because the addition of water and heat during the conditioning cause denaturation of seed proteins. As a result, small droplets of oil coalesce into larger ones that are easier to extract (*3*). Conditioning of the seeds at 60 °C gave only 2.5 % higher oil yield than cold pressing, probably because the used temperature was not high enough to cause a required degree of protein coagulation. On the other hand, conditioning the seeds at 100 °C gave even smaller increase in oil yield than cold pressing (1.5 %), probably because the seed was too dry after conditioning at 100 °C. To obtain adequate oil yield, moisture of the seed prior to the extraction should be 5–7 % (3). Although we added substantial amount of water during the conditioning at 100 °C (240 mL/kg), high temperature of the process caused evaporation of the water immediately after its addition. To obtain better oil yield at such high temperatures, one should consider altering the production process. The best oil yield was obtained after conditioning the seed at 80 °C for 30 min (86.6 %), which can be considered as an optimal temperature for virgin rapeseed oil production from an economical point of view.

Quality parameters of produced oil are shown in **Table 1**. All samples of oil produced in this research were of high quality. Free fatty acids (FFA), which indicate hydrolytic deterioration of oil, were far below Codex limits (*5*) of 2 % oleic fatty acid, an equivalent to an acid value of KOH of 4 mg/g of oil. Peroxide value (PV), an indicator of oxidation of fats and oils, of all produced oil samples was also below the limits given by the Codex (*5*). Applying the heat during conditioning had no significant influence on either of the determined quality parameters ($p > 0.05$).

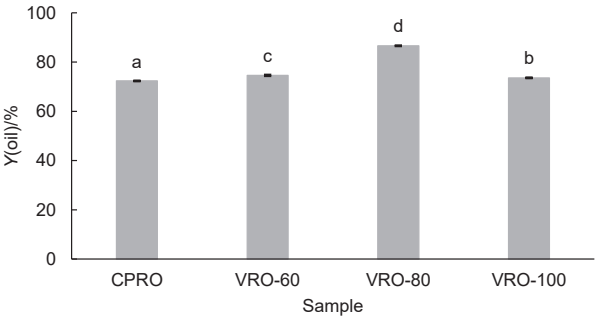

Fig. 1. Oil yield (Y) of cold-pressed rapeseed oil (CPRO) and virgin rapeseed oil (VRO) production. Numbers 60, 80 and 100 indicate temperature of the seed conditioning before pressing. Samples labelled with different lowercase letters are significantly different (Tukey's test, $p \leq 0.05$)

Table 1. Free fatty acids (FFA; expressed as percentage of oleic acid) and peroxide value (PV) of cold-pressed rapeseed oil (CPRO) and virgin rapeseed oil (VRO) produced after conditioning of the seeds at 60, 80 and 100 °C

Parameter	Sample			
	CPRO	VRO-60	VRO-80	VRO-100
w(FFA)/%	0.16±0.01	0.22±0.02	0.19±0.01	0.21±0.03
PV/(mmol/kg)	1.3±0.2	0.9±0.1	0.9±0.1	1.1±0.3

Dominant fatty acid in all produced oil samples was oleic fatty acid, followed by linoleic and linolenic acids, while saturated fatty acids accounted for about 7 % (**Table 2**). These results are in accordance with previous reports (*3,25*) and are within the limits given by Codex (*5*). Furthermore, conditioning had no significant influence on fatty acid composition of produced oil ($p > 0.05$), not even on polyunsaturated fatty acids which are susceptible to oxidation and make around 30 % of total fatty acids in produced rapeseed oil. Stability of fatty acids during the conditioning justifies the application of this process in the production of rapeseed oil because fatty acid composition has the greatest influence on its high nutritional value. According to the recommendations of US Food and Drug Administration (*26*), eating about 1.5 tablespoons of rapeseed oil daily may reduce the risk of coronary heart disease due to its unsaturated fatty acids, more specifically to an ideal 2:1 ω-6:ω-3 ratio of rapeseed oil. Other components that significantly contribute to the nutritional value of rapeseed oil are antioxidants (*3*). Rapeseed oil is rich in γ-tocopherol, followed by α-tocopherol and low amounts of δ-tocopherol (*27*). In addition, rapeseed oil contains plastochromanol-8, a chromanol derivative related to the tocopherols with antioxidant properties (*28*). Analysis of tocopherol content and composition of the obtained rapeseed oil are given in **Table 2**. α-Tocopherol (262–280 mg/kg), γ-tocopherol (404–418 mg/kg) and δ-tocopherol

(1 mg/kg) were found in all produced oil samples, while homologue of β-tocopherol was not detected. These results are in accordance with Codex limits (*5*) and are similar to the ones previously reported by Szydłowska-Czerniak *et al.* (*29*). Tocopherol homologues were not affected by heat during conditioning ($p>0.05$). However, conditioning had significant effect on plastochromanol-8 content, which increased with the used temperature ($p\leq0.05$). Furthermore, increase of the temperature during conditioning significantly increased canolol content of the produced virgin oil (**Fig. 2**). Canolol is a specific phenolic compound of rapeseed oil, formed by heat-induced decarboxylation of sinapic acid, a phenolic acid abundantly found in the rapeseed (*8*). Taking this into consideration, very low concentrations of canolol were found in the cold-pressed oil (4 mg/kg). Conditioning seeds at 60 °C doubled the concentration of canolol in the oil, but these two oil samples were not significantly different from each other and can be characterized as the oil with very low canolol content. Further increase of the temperature caused exponential increase of the canolol content in the produced oil. Virgin oil preconditioned at 80 °C had 16 times more canolol than the cold-pressed oil, while canolol content of the oil preconditioned at 100 °C was 74 times higher than in the cold-pressed one. The increase of canolol concentration with the used temperature is consistent with previous studies (*8,29*). Taking into consideration quality parameters and non-volatile components, fatty acids, tocopherols and polyphenols, which directly affect nutritional value of rapeseed oil, higher temperatures of conditioning prior to the oil extraction have more favourable effect. Increasing the temperature up to 100 °C led to the production of the virgin rapeseed oil rich in canolol and without any negative effects on fatty acids and tocopherols.

Table 2. Fatty acid and tocopherol composition of cold-pressed rapeseed oil (CPRO) and virgin rapeseed oil (VRO) produced after conditioning of the seeds at 60, 80 and 100 °C

Component	Sample			
	CPRO	VRO-60	VRO-80	VRO-100
	w(fatty acid)/%			
C16:0	4.8±0.0	4.8±0.0	4.9±0.0	4.8±0.0
C16:1	0.2±0.0	0.2±0.0	0.3±0.0	0.2±0.0
C17:1	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
C18:0	1.5±0.0	1.5±0.0	1.5±0.0	1.5±0.0
C18:1	60.9±0.0	61.2±0.0	60.5±0.0	60.9±0.0
C18:2	20.9±0.0	20.7±0.0	21.2±0.0	21.0±0.0
C18:3	9.6±0.0	9.6±0.0	9.7±0.0	9.6±0.0
C20:0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0
C20:1	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
C22:0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
	w(tocopherol)/(mg/kg)			
α-tocopherol	262±3	280±8	280±11	270±2
γ- tocopherol	405±1	418±12	404±17	417±20
δ- tocopherol	1±0	1±1	1±0	tr
plastochromanol-8	$(77\pm1)^{a}$	$(94\pm3)^{b}$	$(107\pm5)^{bc}$	$(113\pm1)^{c}$

Different lowercase letters in the row indicate significant difference between samples (Tukey's test, $p\leq0.05$). tr=traces (<0.5 mg/kg)

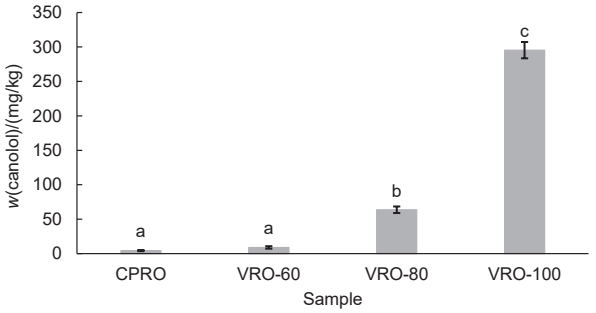

Fig. 2. Canolol content (*w*) of rapeseed oil samples produced by cold pressing (CPRO) or by pressing after conditioning (VRO) at different temperatures (60, 80 or 100 °C). Samples labelled with different lowercase letters are significantly different (Tukey's test, $p\leq0.05$)

Heating the seed prior to oil extraction can also affect the sensory characteristics of the produced virgin rapeseed oil, changing the characteristics from seed-like and nutty to roasty (*30*). Oil samples produced in our research were sensory evaluated by a panel and the results are given in **Fig. 3.** Conditioning had no effect on the colour and clarity of the produced oil and all four samples were given maximal values for these two parameters. The influence of the conditioning temperature on sensory characteristics can be observed in the changes of flavour of the oil. The strongest flavour of cold-pressed oil was seed-like, scoring 4.8 out of 5. Seed-like flavour was not affected

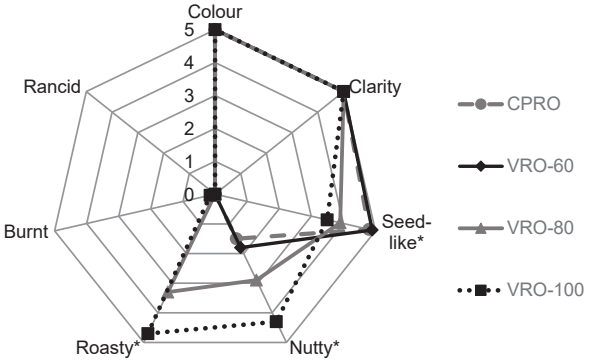

Fig. 3. Sensory characteristics of cold-pressed rapeseed oil (CPRO) and virgin rapeseed oil (VRO) produced after conditioning of the seeds at 60, 80 and 100 °C. *Conditioning temperature had significant influence on the sensory attribute ($p\leq0.05$)

by conditioning the seeds at 60 °C for 30 min. Additionally, both of these, cold-pressed and virgin oil preconditioned at 60 °C, were characterized by medium intensity of nutty flavour and an absence of roasty and burnt flavour. Although seed-like and nutty flavour were slightly more enhanced in the latter, data analysis showed that there is no significant difference in the sensory characteristics between these two types of oil. On the other hand, flavours of the virgin oil samples preconditioned at 80 and 100 °C were significantly different from those of cold-pressed and the one preconditioned at 60 °C, and also from each other. Increasing conditioning temperature decreased the intensity of seed-like flavour in the virgin oil preconditioned at 80 and 100 °C, simultaneously increasing nutty and roasty flavours of the produced oil. The decrease of seed-like flavour

and increase of nutty and roasty flavour were consistent with the used temperature. Burnt flavour was detected, but in a very low intensity (0.15), only in the virgin oil preconditioned at 100 °C, and rancid flavour was not detected in any of the produced oil samples.

It is well known that the differences in the sensory characteristics of different types of oil, especially aroma is directly under the influence of volatile components present in the oil (*31*). Volatile components detected and identified in the obtained cold-pressed and virgin oil are shown in **Table 3** (*10,14,21,22*). A large number of compounds were detected and they were grouped in 11 categories (isothiocyanates, epithionitriles, nitriles, other sulfur and nitrogen components, pyrazines, aldehydes, ketones, alcohols, furan derivatives, and other components that cannot be placed in any of the aforementioned groups). Amount of every compound present in the oil is expressed by area of its peak, and for each compound, Kovats index is included (**Table 3**).

As we mentioned before, crucial point of the oil extraction is a rupture of the cell structure (*3*). Because of this, naturally present glucosinolates come in contact with enzymes, resulting in a variety of volatile products including isothiocyanates, thiocyanates, nitriles, epithionitriles *etc.* (*32*). Isothiocyanates are the most common products of glucosinolate hydrolysis catalysed by the myrosinase (*33*). The 4-isothiocyanato-1-butene was the major volatile component of our cold-pressed oil, making 56 % of total volatiles. Wei *et al.* (*14*) also reported the same component as a dominant volatile compound

Table 3. Volatile components of cold-pressed rapeseed oil (CPRO) and virgin rapeseed oil (VRO) produced after conditioning of the seeds at 60, 80 and 100 °C

Component	Kovats index	Sample			
		CPRO	VRO-60	VRO-80	VRO-100
		Peak area·10^7			
Isothiocyanates					
1-isothiocyanatobutane*#	929[1]	(8.3±0.1)^b	(9.7±1.0)^c	nd^a	nd^a
4-isothiocyanato-1-butene*#	982[1]	(2744.6±124.0)^b	(4181.6±188.8)^c	(553.3±49.5)^a	(910. 9±104.9)^a
4-methypentylisothiocyanate*§	1161	(10.6±0.2)^b	(13.0±1.9)^c	nd^a	nd^a
Eprithionitriles					
1-cyano-3,4-epithiobutane*#	1118[2]	(132.3±1.5)^d	(29.1±6.5)^c	nd^a	(15.9±7.0)^b
1-cyano-4,5-epithiopentane*	1195	nd^a	nd^a	nd^a	(22.3±9.3)^b
Nitriles					
2-methyl-2-butenenitrile*	<800	(172.9±3.5)^a	(193.1±4.5)^a	(234.3±27.1)^a	(2680.6±145.5)^b
2,4-pentadienenitrile*	<800	nd^a	nd^a	(5.7±0.7)^a	(190.6±9.7)^b
2-methyl-5-hexenenitrile*	869	(73.1±6.5)^a	(107.6±7.9)^a	(168.9±28.9)^a	(2421.4±245.9)^b
5-methylhexanenitrile*	948	nd^a	nd^a	nd^a	(17.8±2.1)^b
heptanenitrile*§	953	(63. 7±0.7)^a	(59.6±5.0)^a	(58.5±9.4)^a	(178.2±2.2)^b
benzenepropanenitrile*§	1234	nd^a	nd^a	nd^a	(25.0±5.3)^b
Sulfur components					
carbonil sulfide*	<800	(39.4±2.0)^b	(40.7±1.9)^b	nd^a	(62.6±5.2)^c
ethanethiol*	<800	(21.4±1.7)^a	(15.1±3.0)^a	(14.1±5.2)^a	(283.3±15.0)^b
dimethyl sulfide*	<800	(62.4±2.9)^b	(26.9±8.4)^a	(34.4±4.2)^a	(54.7±9.4)^b
carbon disulfide*	<800	(114.5±9.5)^c	(7.7±1.4)^a	(87.4±9.5)^b	nd^a
dimethyl sulfone*§	915	nd	5.3±1.2	9.9±4.8	nd
4-ethyl-5-methyl thiazole*§	1079	(450.7±9.0)^c	(692.4±33.5)^d	(132.2±22.0)^a	(270.5±17.1)^b
Pyrazines					
2-methylpyrazine*§	820	nd^a	nd^a	nd^a	(135.7±3.1)^b
2,5-dimethylpyrazine*§	911	nd^a	nd^a	nd^a	(205.1±13.6)^b
2-ethylpyrazine*§	915	nd^a	nd^a	nd^a	(57.3±6.6)^b
2,6-dimethylpyrazine*§	916	nd^a	nd^a	nd^a	(78.3±3.7)^b
2-ethyl-6-methylpyrazine*§	996	nd^a	nd^a	nd^a	(58.0±12.6)^b
2-ethyl-3-methylpyrazine*§	998	nd^a	nd^a	nd^a	(51.2±4.5)^b
2-ethyl-5-methylpyrazine*§	999	nd^a	nd^a	nd^a	(79.0±7.6)^b
3-ethyl-2,5-dimethylpyrazine*#	1075[1]	nd^a	nd^a	nd^a	(67.9±6.9)^b
Other nitrogen components					
urea*	<800	nd^a	nd^a	nd^a	(159.7±23.5)^b
2,5-dimethylpyrroline*	<800	nd	6.9±1.4	nd	nd
isopropyl-1-butanamine*	806	nd^a	nd^a	nd^a	(26.6±1.8)^b
4-methyl-2-pyrrolidinone*	880	(20.4±1.9)^b	(34.5±7.7)^c	nd^a	nd^a
1-(1H-pyrrol-2-yl)ethanone*§	1063	nd^a	nd^a	nd^a	(10.5±1.1)^b

Table 3. Continued

Component	Kovats index	Sample			
		CPRO	VRO-60	VRO-80	VRO-100
		Peak area·10^7			
Furan derivatives					
furfural*§	828	nd[a]	nd[a]	nd[a]	(719.2±23.5)[b]
2-furanmethanol*§	858	nd[a]	nd[a]	nd[a]	(96.5±4.5)[b]
dihydro-2(3H-)-furanone*§	907	(6.8±1.9)[a]	(13.6±1.6)[a]	(12.9±4.1)[a]	(78.7±7.1)[b]
5-methyl-2-furfural*§	962	nd[a]	nd[a]	nd[a]	(177.6±11.5)[b]
5-ethyldihydrofuran-2(3H)-one*§	1049	(5.6±0.7)[ab]	(9.3±1.0)[c]	(5.5±3.7)[bc]	nd[a]
Aldehydes					
propanal*	<800	nd[a]	nd[a]	nd[a]	(42.6±2.9)[b]
3-methylbutanal*	<800	nd[a]	(1.5±0.1)[a]	(15.5±4.2)[b]	(39.1±4.1)[c]
2-methylbutanal*	<800	nd[a]	nd[a]	(23.3±4.4)[b]	(76.7±17.2)[c]
hexanal*§	801	(350.5±14.6)[b]	(179.2±2.4)[a]	(316.5±27.1)[b]	(248.2±9.5)[a]
heptanal*§	902	(57.3±6.0)[abc]	(44.6±3.1)[ab]	(69.9±5.4)[abc]	(75.1±11.8)[ac]
2-heptenal*§	960	10.3±3.4	8.4±0.4	14.6±2.0	nd
octanal*§	1003	(29.7±1.6)[a]	(34.6±6.8)[a]	(40.6±5.2)[a]	(57.4±4.9)[b]
phenylacetaldehyde*#	1042[3]	nd[a]	nd[a]	(10.6±2.1)[a]	(52.0±4.0)[b]
2-octenal*§	1058	nd	1.6±0.6	2.4±0.9	10.4±1.3
nonanal*§	1103	(21.7±0.9)[a]	(33.1±5.4)[a]	(50.2±6.0)[b]	(79.1±7.7)[c]
Ketones					
2,3-pentadione*	<800	nd[a]	nd[a]	(34.0±5.0)[a]	(99.7±12.5)[b]
2-heptanone*§	891	nd[a]	(1.4±0.4)[a]	(4.2±1.8)[a]	(21.0±4.4)[b]
6-methyl-5-hepten-2-one*§	987	23.2±8.5	38.1±2.6	30.7±8.8	36.7±2.8
3-octen-2-one*§	1039	(9.2±1.2)[abc]	(6.3±1.2)[ab]	(8.5±2.7)[abc]	(17.0±4.4)[ac]
1-phenylethanone*§	1065	(8.0±0.2)[b]	nd[a]	nd[a]	nd[a]
Alcohols					
2-methylbutanol*	<800	nd	4.9±0.5	2.9±1.7	nd
n-pentanol*	<800	(96.1±3.4)[c]	(73.8±8.9)[b]	(70.8±9.7)[b]	nd[a]
n-hexanol*§	874	(157.9±6.5)[b]	(114.0±19.9)[b]	(135.3±37.1)[b]	(16.1±2.2)[a]
3-hepten-1-ol*	880	nd[a]	nd[a]	nd[a]	(61.5±17.0)[b]
n-heptanol*§	976	(9.6±1.6)[b]	(17.7±2.0)[c]	(2.6±1.2)[ab]	nd[a]
2-methyl-6-hepten-1-ol*§	997	(56.0±5.2)[b]	(58.2±11.2)[b]	(43.0±8.7)[b]	nd[a]
2,5-dimethylcyclohexanol*§	1094	(6.8±2.1)[b]	nd[a]	nd[a]	nd[a]
Other components					
2,2,3,3-tetramethylbutane*	<800	18.0±3.2	5.4±4.7	12.3±2.3	6.2±1.6
2,2,4-trimethylpentane*	<800	(72.8±3.9)[b]	(35.2±7.1)[a]	(81.3±7.3)[b]	(28.3±4.8)[a]
limonene*§	1027	(11.0±2.5)[a]	(11.3±1.5)[ab]	(14.8±0.7)[b]	(18.4±0.9)[c]
2-methoxy-4-vinylphenol*§	1305	nd[a]	nd[a]	nd[a]	(23.6±6.9)[b]

nd=not detected
*The compound was identified by comparison of mass spectra with mass spectra from NIST 05 (*21*)
§The compound was identified by comparison of retention indices with the ones obtained from the NIST 05 mass spectra library (*21*)
#The compound was identified by comparison of retention indices with the ones in the literature ([1]Wei *et al.* (*14*), [2]Kato *et al.* (*22*), [3]Siegmund and Murkovic (*10*)). Different lowercase letters in the same row indicate significant difference between samples (Tukey's test, $p \leq 0.05$)

of cold-pressed rapeseed oil. Conditioning the seeds for 30 min at 60 °C further increased its content in the oil and 4-isothiocyanato-1-butene accounted for more than 68 % of total volatiles in the oil preconditioned at 60 °C. This increase was a result of prolonged contact of enzymes and glucosinolates during conditioning. Bones and Slupphaug (*34*) reported the temperature optima for rapeseed myrosinase to be between 70 and 75 °C, and for its inactivation temperatures higher than that should be used (*3*). The effectiveness of higher temperatures on myrosinase inactivation was also shown in our research. Increasing the conditioning temperatures to 80 and 100 °C decreased isothiocyanate content to 24 and 9 %, respectively. Data analysis showed that the oil preconditioned at 80 and 100 °C can be characterized with lower content of isothiocyanates and there was no significant difference between these two types of oil, while the cold-pressed and the one preconditioned at 60 °C were significantly different from the other two, and from each other (Tukey's test,

p≤0.05). Isothiocyanates are the components that have the biggest impact on the specific seed-like flavour of the rapeseed oil (*14*), and their high content in our cold-pressed and virgin oil preconditioned at 60 °C correlated with high scores for seed-like attribute of these two oil samples.

Nitriles and epithionitriles are also volatile products of glucosinolate hydrolysis by myrosinase, but in their formation epithiospecifier protein (ESP) is involved. ESP uses unstable thiohydroxymate generated by myrosinase to produce nitriles and epithionitriles. Due to its lower thermal stability than of myrosinase (*33*), it is to be expected that the cold-pressed oil has the highest content of epithionitriles and nitriles. Results of our research showed that the content of 1-cyano-3,4-epithiobutane was the highest in the cold-pressed oil and it decreased with the increase of temperature used for seed conditioning, with the exception of the oil preconditioned at 100 °C, where its content was higher than in that preconditioned at 80 °C. In addition, 1-cyano-4,5-epithiopentane was also detected, but only in the oil preconditioned at 100 °C. Similar to this, concentration of nitriles, with 2-methyl-2-butenenitrile, 2-methyl-5-hexanenitrile and heptanenitrile as dominant nitriles in the produced oil, increases with conditioning temperature, which was not in accordance with lower thermal stability of ESP. However, according to the literature, nitrile formation can also occur due to thermal decomposition of the glucosinolates (*33*), and Wei *et al.* (*14*) also reported an increase of nitriles in virgin rapeseed oil after microwave and thermal pretreatment of the rapeseed.

Except for isothiocyanates, nitriles and epithionitriles, other sulfur- and nitrogen-containing components were detected in the produced rapeseed oil. The 4-ethyl-5-methyl thiazole shows similar behaviour to isothiocyanates and is probably a product of myrosinase hydrolysis of glucosinolates. Except for 4-ethyl-5-methyl thiazole, relatively high content of carbon sulfide, ethanethiol, dimethyl sulfide and carbon disulfide was found in cold-pressed oil; however, their content changed in a completely opposite way than of 4-ethyl-5-methyl thiazole, indicating that they are a result of chemical rather than enzymatic process (*35*). According to Siegmund and Murkovic (*10*) and Mottram (*36*), these compounds are probably Strecker degradation products derived from methionine. Heating the seeds at 60 °C resulted in the evaporation of these products, which were already present in the seeds, and therefore in the cold-pressed oil. Increasing the conditioning temperature to 80 °C further decreased the content of carbonil sulfide and ethenethiol, but increased the content of dimethyl sulfide and carbon disulfide. Conditioning the seeds at the highest temperature resulted in acceleration of Strecker degradation reactions, which increased the amount of degradation products, except for carbon disulfide, which was not detected in the oil preconditioned at 100 °C. The differences in the compound behaviour can be attributed to the differences in their volatility and/or their stability at high temperatures, but in general, we can conclude that the increase in conditioning temperature leads to an increase in the amounts of Strecker degradation products.

Pyrazines, pyrroles, pyrrolines and pyrrolidines are the typical compounds formed in the Maillard reactions and are found among the volatiles of most heated foods (*36*). Formation of these components positively correlates with heating time and temperature (*10–12*). Eight pyrazines were detected during our research, but only in the headspace of the virgin oil preconditioned at 100 °C: 2-methylpyrazine, 2,5-dimethylpyrazine, 2-ethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-3-methylpyrazine, 2-ethyl-5-methylpyrazine, and 2-ethyl-2,5-dimethylpyrazine. Formation of pyrazines at temperatures higher than 100 °C was previously reported (*10*), and their presence is associated with sensory attributes such as roasty, nutty, woody or burnt (*37*). The presence of pyrazines in the oil preconditioned at 100 °C was the reason for higher scores for nutty and roasty attribute in sensory evaluation than for other produced oil samples, as well as the appearance of burnt flavour. Pyrroles also contribute to the typical roasty flavour of oil (*11*). As well as the pyrazines, 1-(1H-pyrrol-2-yl)ethanone was found only in the oil peconditioned at 100 °C, while 2,5-dimethylpyrroline and 4-methyl-2-pyrrolidinone were detected only in the oil produced at lower temperatures. Except for heterocyclic nitrogen compounds, isopropyl-1-butanamine and urea were also detected in the headspace of the oil peconditioned at 100 °C. These two components are probably products of amino acid degradation, *i.e.* Strecker degradation.

Furans (furfurals and furanones) are the most abundant products of Millard reaction and they occur in volatiles of all heated food. They are formed by degradation of carbohydrates and generally they give caramel-like, sweet and fruity characteristic to food (*36*). Furan derivatives have previously been reported in vegetable oil and although they are present in some cold-pressed types of oil, similar to other Millard reaction products, concentration of furans significantly increases with increase of temperature used during oil production (*10–12,38*). Furanones identified in the present research were detected in all produced oils while furfurals (furfural and 5-methy-2-furfural) and 2-furanmethanol were detected only in the oil preconditioned at 100 °C and for their formation higher temperatures were needed. In general, this oil sample significantly differed from other obtained oil samples in the amount of furan derivatives (p≤0.05) and they were, together with other Millard reaction products, responsible for its pronounced nutty and roasty flavour.

Ten aldehydes were identified in the produced rapeseed oil samples. They can be grouped into two groups, depending on their formation pathway: Strecker degradation products (3-methylbutanal, 2-methylbutanal and phenlyacetadehlde) and compounds derived from lipid oxidation (propanal, hexanal, heptanal, 2-heptenal, octanal, 2-octenal and nonanal). The amount of the Strecker degradation products increased with the increase of conditioning temperature, which was expected and consistent with previous findings (*10*). Dominant aldehyde in all produced oil samples was hexanal. It gives a green flavour to the oil and it is derived from linoleic acid already in

the seed (*39*). Abundancy of hexanal decreased with increase of the production temperature to 60 °C, probably due to evaporation of hexanal present in the seeds. Using conditioning temperature of 80 °C significantly increased peak area of hexanal but further increments of temperature significantly decreased its abundance. The 2-heptenal, which is also a product of linoleic fatty acid oxidation (*40*), showed similar behaviour, and this was probably due to volatility of these two aldehydes. Abundance of other detected aldehydes (propanal, heptanal, octanal, octenal, 2-octenal and nonanal) significantly increased with the increase of conditioning temperature, and in general, the virgin oil preconditioned at 100 °C can be characterized with higher amounts of aldehydes derived from lipid oxidation. Ketones detected in our research are also products of fatty acid oxidation, and their abundance in the oil was changing in a similar manner as aldehydes derived from lipid oxidation. Most of the detected aldehydes and ketones are responsible for oily and fatty odour of the oil, but some contribute to nutty (2-octenal, 2-heptanone and 3-octen-2-one), green (hexanal, octanal, 2-octenal and nonanal) and even rancid (hexanal and heptanal) flavour of the oil (*41*). Since rancid flavour was not detected in neither of the produced oil samples during sensory evaluation (**Fig. 3**), we can conclude that lipid oxidation during conditioning even at 100 °C was not expressed in such manner to cause sensory defects of the oil.

In addition to aldehydes and ketones, alcohols represent another characteristic group of fatty acid secondary oxidation products. In this research we detected seven alcohols: 2-methylbutanol, *n*-pentanol, *n*-hexanol, 3-hepten-1-ol, *n*-heptenol, 2-methyl-6-hepten-1-ol and 2,5-dimethylcyclohexanol. As a rule, the content of alcohol decreased with with the increase of temperature used for conditioning the seeds, except for 3-hepten-1-ol. Loss of alcohols at higher temperatures can partially be explained by volatility of these compounds, and partially by their oxidation and formation of the corresponding aldehydes (*10*).

Additionally, four other components were detected in produced rapeseed oil that cannot be placed in any of the aforementioned and discussed groups: 2,2,3,3-tetramethylbutane, 2,2,4-trimethylpentane, limonene and 2-methoxy-4-vinylphenol. Alkanes showed similar behaviour to Strecker degradation products. Abundance of limonene, a monoterpene common to many vegetal samples, increased with higher temperatures, which is in accordance with previous reports of its formation in peanut oil during roasting of seeds (*11*), and 2-methoxy-4-vinyphenol was found only in the oil preconditioned at 100 °C. This is expected since this oil has significantly higher canolol content than other produced samples, and 2-methoxy-4-vinyphenol is the most likely derived from canolol because of their similar molecular structure.

CONCLUSIONS

Conditioning the rapeseed prior to oil extraction had a favourable effect on the oil yield and its nutritional value, and it also completely changed sensory and aroma profile of the produced rapeseed oil. From an economical point of view, optimal conditioning temperature was 80 °C with a 14 % oil yield increase compared to cold pressing. Temperatures up to 100 °C and water addition during conditioning did not lead to any hydrolytic or oxidative deterioration of the oil, but heating at 80 °C, and especially at 100 °C, significantly increased the amount of bioactive compounds. Therefore, from the quality and nutritional point of view, higher temperatures had more favourable effect. However, the greatest impact of conditioning temperature was observed on the volatile components, and therefore on sensory profile of the produced virgin rapeseed oil. Cold-pressed and virgin rapeseed oil produced at 60 °C were characterized by volatiles that are products of enzymatic degradation of glucosinolates (isothiocyanates and epithionitriles) and had expressed seed-like flavour. Increasing the conditioning temperature to 80 and 100 °C caused inactivation of the enzymes and decrease in the intensity of seed-like aroma, but at the same time temperature increase caused thermal degradation of seed components and expression of nutty and roasty flavour. Results of this research, especially the ones about changes in the aroma and sensory profile, can help in production of high-quality rapeseed oil with desirable and very specific sensory attributes and ensure a greater selection of products for the consumers.

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