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THE QUALITY OF COLD-PRESSED RAPESEED OIL OBTAINED FROM SEEDS OF *BRASSICA NAPUS* L. WITH INCREASED MOISTURE CONTENT

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ABSTRACT

Background. The basic parameter influencing the quality of cold-pressed oil is the quality of seeds used for pressing. Adverse moisture content and storage temperature of rape seeds may affect the quality of oil obtained from them. This paper presents the effects of increased rapeseed moisture content on the quality of the oil pressed.

Material and methods. The material used for the tests was rapeseed (canola) cv. PR 46 W14. The moisture content of the seeds was adjusted to 10%, 12% and 20%, and the seeds were stored at room temperature for 14 days. The samples were then dried to a seed moisture equal to 7% and oil was pressed from them. Acid and peroxide values, as well as the content of water, tocopherols and phenolic acids, were determined. In addition, a sensory analysis of the oil samples was carried out, and structural changes in the association colloids in the oil were determined using a fluorescent probe.

Results. With the increase in seed moisture, the increase in peroxide and acid values of the analyzed oils was recorded. A slight decrease in tocopherol content and a significant increase in phenolic acid concentration, depending on the seed moisture content, was observed. Sensory analysis showed odor sensory profile changes that probably indicate microflora development.

Conclusion. The rapeseed moisture content has a crucial influence on the quality of oil obtained from them. Along with an increase in seed moisture, the possibility of developing undesirable microflora grows, which results in a deterioration in the quality of the obtained oil.

Keywords: rapeseed, oil quality, cold-pressed oil, tocopherol, phenolic compounds, moisture

INTRODUCTION

Rapeseed is one of the most important raw materials used in the manufacturing of vegetable oils in the world. It may be utilized for consumption, in the food industry or for non-food purposes. The production of rapeseed in the European Union has exceeded 20 million tons/year and accounts for about 30% of global oilseed production (Wawrzyniak et al., 2018). Rapeseed oil, due to its fatty acid composition, is considered to be one of the healthiest vegetable oils. It is characterized by a low content of saturated fatty acids (6–7%), with a high content of monounsaturated fatty acids, predominantly oleic acid (58–62%), and polyunsaturated fatty acids, represented by α-linolenic acid (n-3) 8–12% and linoleic acid (n-6) about 20%

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(Gugała et al., 2014; Wroniak and Ręka, 2016). It is also characterized by a fatty acid ratio of n-6:n-3 (equal to about 2:1), which is optimal from a nutritional point of view. Rapeseed and the oil produced from it are good sources of biologically active compounds, such as phytosterols, tocochromanols and phenolic compounds (Siger et al., 2017a; 2018a; Vlahakis and Hazebroek, 2000).

The content of sterols (phytosterols) in rapeseed oil is in the range of 4500 mg/kg to 11 300 mg/kg. The total amount of sterols in low erucic rapeseed oil ranges from 0.45% to 1.13% of the total lipids (Ratnayake and Daun, 2004). One of their most important biological functions is to lower blood cholesterol levels, especially of LDL. Phytosterols limit the bioavailability of cholesterol by competing with it for absorption in the small intestine (Gül and Amar, 2006). The main lipophilic antioxidants present in rapeseeds and rapeseed oil are tocopherols. They prevent lipid peroxidation, especially the autoxidation of polyunsaturated fatty acids. The activity of tocopherols is primarily determined by the ability to donate protons to lipid free radicals (Schneider, 2005). α-Tocopherol, being the predominant homologue in the green parts of plants, has a protective function in the photosensitizing apparatus against reactive oxygen species. On the contrary, seeds are dominated by γ-tocopherol, which protects against the autoxidation of polyunsaturated fatty acids (Azzi and Stocker, 2000; Hofius and Sonnewald, 2003).

Hydrophilic antioxidants include phenolic compounds. Rapeseed is characterized by a content of phenolics over 10 times higher than that in other oilseeds. The main phenolic compounds of rapeseed are sinapic acid and its derivatives. These substances have a high antioxidant potential. However, only small amounts of phenolics are released into the oil during pressing (Jun et al., 2014; Khattab et al., 2010; Koski et al., 2003; Siger and Józefiak, 2016; Thiyam et al., 2009). Native antioxidants inhibit autoxidation processes, which are the main cause of the deterioration of quality in vegetable oils. However, when considering the lipid autoxidation process, we should not only focus on the chemistry of the free-radical chain reaction, but also consider the location of molecules involved in the autoxidation reaction, especially at the interface of nanoemulsions (Budilarto and Kamal-Eldin, 2015). Water plays an important role in autooxidation processes. In addition to the acceleration of triacylglycerol hydrolysis (especially in the presence of lipases), it also participates in the formation of association colloids. These are micellar structures formed by surfactants in a non-polar environment in the presence of a small amount of water. Association colloids (e.g. reverse micelles) are considered to be active oxidation centers in bulk oils (Kittipongpittaya et al., 2016).

The quality of cold-pressed oil depends primarily on the quality of the raw material used for pressing (Matthäus, 2013; Wroniak and Chlebowska-Śmigiel, 2013). The technology of cold-pressed oils mainly involves oil extraction through pressing with an expeller press, and the removal of fine solid particles from the obtained oil through sedimentation or filtration. Fat is an excellent carrier of taste and flavor. For this reason, raw material defects and undesirable substances, such as hydrolysis products, pesticide residues, metal ions and microbial contaminants may reduce the sensory and nutritional quality of the final product as well as negatively affecting its safety. That is why it is very important to choose a raw material of sufficiently high quality, because in the cold pressing process there are no other stages besides physical methods through which the quality of the obtained oil may be improved (Wroniak et al., 2015). Some of the basic quality parameters of oils, which customers pay special attention to, are taste and flavor. Sensory analysis of cold-pressed rapeseed oil may easily indicate the quality of the product (Matthäus, 2013). The quality of the raw material is influenced above all by the post-harvest treatment of seeds, especially the temperature and humidity during storage. Improper storage conditions may be the reason for the germination of seeds and the growth of microorganisms. Consequently, an increase in metabolic processes in seeds may be observed. This ultimately leads to an unpleasant odor, the degradation of biologically active compounds (tocopherols, phytosterols, phenolic compounds), and thus also to the lowering of the nutritional value of oils (Faron and Tańska, 2013; Gawrysiak-Witulska et al., 2015; Matthäus and Brühl, 2008; Wroniak and Chlebowska-Śmigiel, 2013). According to Matthäus (2013), rapeseeds with 7% moisture content may be stored for up to 9 months without a negative impact on their quality. Storage of samples with a higher seed moisture content produces environments favorable

to the development of mold fungi, and significantly shortens the length of time they can be safely stored (Wawrzyniak et al., 2018).

There are not many literature reports describing how rapeseed storage conditions, like high humidity and elevated temperature, affect the quality of cold-pressed oil. Therefore, it was considered useful to investigate how storage of the raw material under unfavorable conditions (increased humidity) would affect the quality parameters of the cold-pressed oil obtained. From a nutritional point of view, it was important to determine how adverse storage conditions influence the content of bioactive compounds and the sensory profile of the oil.

MATERIALS AND METHODS

Chemicals

Phenolic acid standards were purchased from Sigma (St. Louis, MO). All tocopherol homologues (purity > 95% by HPLC) were obtained from Calbiochem-Merck Biosciences (Darmstadt, Germany). *n*-Hexane (HPLC-grade) and 1.4-dioxane (HPLC-grade) were purchased from Merck (Darmstadt, Germany). A fluorescent probe NBD-PE (N-(7-nitrobenz-2-oxa--1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3--phosphoethanolamine, triethylammonium salt) was acquired from Thermo Fisher Scientific (USA). All other solvents and chemicals used in this study were of analytical grade.

Materials

Rape seeds (*Brassica napus* L. – cultivar PR46W20) were obtained from the Swadzim Experimental Station owned by the Poznań University of Life Sciences, Poland (seeds were obtained from the 2018 crop). Before the experiment, the seeds were humidified to 10%, 12% and 20% water content. The control samples were seeds of rape with 7% moisture content. The seeds were stored at room temperature in closed polyethylene bags for 14 days. Prior to pressing, the seeds were dried at 60°C to a final water content equal to 7%.

Determination of seed moisture content

The seed moisture content was determined using an electronic moisture analyzer (MA150 Sartorius Mechatronics, Poland). The moisture analyzer used a reference standard prepared by drying a 5 g sample to constant mass at a temperature of 115°C. The measuring accuracy of the analyzer was 0.05% w.b. (wet basis). The moisture analyzer was calibrated using the oven method according to the AOCS Official Method Ba 2a-38, using the prepared reference as stated above.

Cold-pressed oil extraction

Rapeseed was pressed at room temperature with a Farmet Uno expeller (Farmet a.s., Czech Republic), with a temperature of 60 ±10°C inside the press. The temperature of the resulting oil was 39 ±1°C. The oil was centrifuged at 5000 rpm for 15 min, transferred directly to small dark bottles (100 ml) and stored at 4°C in the dark.

Water content in oil determination

Water content in the rapeseed oil was determined using the Karl Fischer method (ISO-8534:2008).

Fluorescence measurement of oil samples

An active surface fluorescent probe NBD-PE is a phospholipid analogue comprised of a fluorescent functional group covalently attached to a choline headgroup. It was incorporated into rapeseed oil and used to study the surface activity of minor components. The solution of NBD-PE in methanol was added to a conical flask with a capacity of 25 ml, then the methanol was removed by evaporation under a nitrogen stream at room temperature and 5 ml of oil was added. The samples were magnetically stirred at 1000 rpm for 12 h. The final concentration of the NBD-PE in the oil was 0.95 μM. Steady-state fluorescence emission spectra of NBD-PE were collected using a F-7100 Hitachi fluorescence spectrometer (Japan) equipped with 10 × 10 mm disposable polystyrene cuvettes, with an excitation and emission wavelength of 463 nm and 530 nm respectively.

Determination of peroxide value (PV) and acid value (AV)

The PV and AV of the cold-pressed rapeseed oil were determined using standard methods of ISO 3960:2001 (Animal and vegetable fats and oils – Determination of peroxide value) and ISO 660:1996 (Animal and vegetable fats and oils – Determination of acid value and acidity).

Determination of tocopherols and plastochromanol-8

Rapeseed oil (200 mg) was dissolved in *n*-hexane, made up to 5 ml in a volumetric flask and transferred to vials for analysis. Tocopherols were qualitatively and quantitatively determined using a Waters HPLC system (Waters, Milford, MA) consisting of a pump (Waters 600), a fluorimetric detector (Waters 474) and a photo-diode 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 had a mixture of *n*-hexane with 1,4-dioxane (96:4 v/v). The flow rate was 1.0 ml/min. For the fluorescence detection of tocopherols and PC-8, the excitation wavelength was set at $\lambda = 295$ nm and the emission at $\lambda = 330$ nm respectively. The content of plastochromanol-8 was assayed and calculated according to Siger et al. (2014).

Determination of phenolic compounds

Phenolic compounds were extracted from oil samples using the liquid/liquid extraction method. Extraction was carried out on 1 g of oil by adding 1ml of *n*-hexane and 5 ml of methanol. After vortexing for 60 min and centrifugation at 6000 rpm for 10 min at 4°C (centrifuge model 6K15, Sigma, Osterode am Harz, Germany), the alcoholic phase was recovered, centrifuged again at 9000 rpm for 5 min at 4°C and filtered through nylon filters (pore size 0.45 µm, Sigma-Aldrich).

HPLC quantification of phenolic compounds

Polar phenolic compounds were identified and quantified using high-performance liquid chromatography (HPLC, Waters, Milford, MA) with an XBridge™ C18 reversed-phase column (4.6 × 100 mm; 3.5 µm; Waters, Milford, MA). A gradient program was used, combining solvent A (acetonitrile:water 50:50 v/v) and solvent B (water adjusted to pH 2.7 with orthophosphoric acid) as follows: 0 – 50% A (60 min), 50 – 0% A (9 min). The flow rate was 1.0 ml/min. The injection volume was 10 µl, while the column temperature was maintained at 20°C. The signal was monitored at 200–600 nm using a diode array detector (DAD; UV-VIS Waters, Milford, MA).

Sensory profiling

Twenty well-trained panelists (8 men and 12 women) aged 20–42 participated in the analyses. Oil samples (20 ml) were kept in 50 ml closed beakers. Each panelist received four samples of oil. The evaluations were conducted at room temperature (20 ±2°C) and under normal lighting conditions for 1 h. For the odor evaluations, the panelists were requested to first record the intensities of the attributes perceived orthonasally, and then to describe the retronasal attributes. The panelists rated the samples, indicating the intensities of the attributes on an unstructured 5 cm scale with well defined anchor points from 0 (imperceptible) on the left to 5 (at saturation level) on the right.

Statistical analysis

Results are presented as means ±standard deviation from three replicates of each experiment. A *P*-value < 0.05 was used to denote significant differences between mean values determined by the analysis of variance (ANOVA) with the assistance of Statistica 13.0 (StatSoft, Inc., Tulsa, OK) software.

RESULTS AND DISCUSSION

The production of high quality cold-pressed rapeseed oil requires the use of suitable raw materials. The post-harvest factors of appropriate temperature and storage humidity are very important. These factors not only affect the efficiency of the pressing process, but also the quality of the resulting oil, which will be directly related to the company's financial profits. It is very important to ensure that the seed storage process takes place under strictly controlled conditions. This study examined how short-term improper storage conditions (14 days, increased humidity) affect the quality of the oil obtained, the content of water and biologically active compounds. The influence of unfavorable storage conditions on the sensory characteristics of the product was also analyzed. In the experiment, seeds with different humidity (7, 10, 12 and 20%) were stored for 14 days at room temperature (20°C). The next step was to dry the seeds at 60°C to 7% humidity. This humidity facilitates optimally efficient rapeseed oil pressing. At a humidity above 10%, the efficiency of oil extraction is reduced dramatically (Siger et al., 2017b).

Table 1 presents the results of water content determination in oil samples obtained from seeds stored at different moisture contents. In spite of the restoration of original moisture content in the raw material before the pressing process (7%), water content differences in the oils obtained were observed. The smallest content of water was recorded in oil obtained from seeds stored at 7% humidity (control sample) – 744 ppm. The increase in seed moisture during storage from 10% to 20% eventually resulted in a higher (statistically significant) water content in the oils obtained from them. These values were at the level 780–840 ppm (Table 1). Cold-pressed oils have a higher water content when compared to refined ones. In refined oils, the water content is about 300 ppm (Budilarto and Kamal-Eldin, 2015). Siger et al. (2017c) reported that the water content in cold-pressed oils also depends on the raw material from which the oil was obtained. For beech oil (*Fagus sylvatica* L.), the reported content was 911 ppm, whereas in the case of poppy seed oil (*Papaver* L.), the content was 831 ppm and 583 ppm for blue and white poppy, respectively. Chia seed oil (*Salvia hispanica* L.) contained 437 ppm of water, *Silybum marianum* L. seed oil – 779 ppm, and *Nigella sativa* seed oil – 358 ppm.

Table 1. Water content of cold-pressed oils from raw material of different technological quality

Cold-pressed rapeseed oil from seeds with moisture, %	Water, ppm
7 (control)	744 ±15[a]
10	780 ±21[b]
12	840 ±22[c]
20	827 ±14[c]

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

In the case of rapeseed storage, seed moisture should not exceed 10%. According to Canadian Grains Commission (1994), the rapeseed moisture content in trade should also not exceed 10%, and if stored for more than 5 months, it should be reduced to 8% (Canadian Grain Commission, 1994; Mills, 1989). Similar requirements exist in Australia, where the Australian Oilseed Federation (AOF) Standards Committee introduced the maximum value of rapeseed humidity in trade – 8% (Mailer et al., 1998). In Poland, seeds of rape are dried after harvesting to a moisture content in the range 6–7%. This is considered optimal for further storage, due to the high mechanical strength of stored seeds and resistance to microorganisms (Rybacki et al., 2001).

Seeds of rape due to their morphological and anatomical structure and high fat content are more sensitive to storage conditions than cereals. Storage of seeds at increased humidity leads to an increase in lipase activity, first native and then of microbiological origin. It results in an increase in the content of free fatty acids. As a result of their oxidation and degradation, the shelf life of the oil is shortened (Matthäus, 2013; Tańska and Rotkiewicz, 2003). The main oil quality parameters are acid value (AV) and peroxide value (PV). The AV and PV values for the analyzed rapeseed oil samples are given in Table 2. According to Codex Alimentarius, acceptable acid values of cold-pressed oils should not exceed 4 mg KOH/g of the oil. Analyzing the obtained results, it was found that the lowest acid value was established for oil obtained from seeds stored at a moisture content equal to 7% (2.53 mg KOH/g). A higher humidity of seed storage resulted in a statistically significant increase in the acid value in the obtained oils. For oils pressed from seeds stored at 10% and 12% humidity, these values were 2.86 mg KOH/g and 3.48 mg KOH/g respectively. A clearly higher acid value – 6.01 mg KOH/g was recorded for

Table 2. Peroxide and acid values of cold-pressed oils from raw materials of different technological quality

Cold-pressed rapeseed oil from seeds with moisture, %	Peroxide value meq O_2/kg	Acid value mg KOH/g
7 (control)	1.58 ±0.14[a]	2.53 ±0.11[a]
10	1.68 ±0.11[a,b]	2.86 ±0.12[b]
12	1.94 ±0.14[b]	3.48 ±0.14[c]
20	1.95 ±0.21[b]	6.01 ±0.17[d]

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

oil samples obtained from seeds stored at a moisture content of 20%. In this sample, intensive mold growth occurred, which was confirmed by the occurrence of a characteristic white mold on the seeds' surface. It was shown that the higher the humidity of stored seeds, the higher the acid value of the oil obtained. It has been proved that seeds with a moisture content of 12% and 20% no longer meet the requirements for the raw materials of oil production and are not suitable for processing in accordance with the PN-90 R-66151 standard, according to which the highest acceptable acid value for seeds is 3 mg KOH/g. Increased seed moisture increases the rate of seed respiration, which may contribute to triacylglycerol degradation and the formation free fatty acids. At a humidity below 8%, the respiration rate is low, while at higher humidity it increases drastically. This effect is even more pronounced when the seeds are infected by microorganisms (Matthäus, 2013). According to Mierzejewska et al. (2001), the quality of the fat fraction of stored rapeseed depends, inter alia, on the seeds' moisture content. The authors stated that lipases in seeds with 10% moisture content have achieved levels of activity two times higher than seeds with 6% moisture content. These observations may also be confirmed by considering the influence of water activity on the growth of microorganisms. Although determination of water activity in seed samples was not carried out in the current study, it may be partially identified on the basis of seed moisture content, based on data provided by Wawrzyniak et al. (2018). This article presents the relationship between water activity and the humidity of rape seeds depending on the ambient temperature. On this basis, it may be assumed that seed samples with 10%, 12% and 20% moisture had water activity equal to 0.70, 0.81 and above 0.90, respectively. Wawrzyniak et al. (2018) showed that water activity in seeds influences the rate of mold growth. At the temperatures 18ºC and 24ºC (temperature range used to store seeds in the current study), after the third day of storage in seeds with 0.9 water activity, mold growth was observed. In contrast, mold growth in the samples with 0.76 and 0.8 water activity was consistently low for over 20 days. In addition, acid values of oils pressed in this study (Table 2) match the results obtained by Wawrzyniak et al. (2018) for seeds with specific (defined) water activity. Both at 18ºC and 24ºC, seeds with water activity 0.9 were characterized by a very high rate of acid value increase at the very beginning of storage. Whereas, the acid value of other samples (water activity 0.76 and 0.80) was at a similarly low level. In addition to the acid value, the peroxide value is also a very important parameter showing the degree of fat oxidation. The maximal peroxide value acceptable in cold-pressed oils is 10 meq O_2/kg (Codex Alimentarius, 1999). The peroxide value recorded for oils obtained from seeds stored at humidity in the range 7–20% increased with increasing humidity. However, this increase was not as pronounced as for the acid value. For the control sample, oil obtained from seeds with a moisture content of 7%, the peroxide value was 1.58 meq O_2/kg. While for samples with 10%, 12% and 20% water content, PV were in turn equal to 1.68, 1.94, 1.95 meq O_2/kg respectively. The differences between recorded peroxide values were not statistically significant ($p > 0.05$). All obtained results of the peroxide value were within the acceptable standard. Faron and Tańska (2013) analyzed changes in the quality of rapeseed oil produced from seeds with a moisture content equal to 11% and 17%. The oil obtained from seeds containing 11% water had a peroxide value of 2.0 meq O_2/kg, while the oil from seeds with 17% moisture had a PV more than two times higher, at 4.12 meq O_2/kg. Wroniak et al. (2015) also confirmed that the quality of seeds affects the quality of the final oil. Oil obtained from rape seeds originating from various regions of Poland was characterized by varied peroxide values in the range 0.99 meq O_2/kg to 4.52 meq O_2/kg. Faron and Tańska (2013) investigated the effects of increased humidity on qualitative changes in seeds of rape. The authors proved that a seed moisture increase up to 17% causes adverse quality changes illustrated by increases in acid, peroxide and anisidine values, as well as intense mold growth. There is less data on rapeseed improper storage conditions and their impact on the quality parameters of oil obtained from such raw material. Wroniak and Chlebowska-Śmigiel (2013) focused mainly on the impact of rape seeds purity and the method of oil purification on the quality of cold-pressed oils. The authors point out that improper seeds storage may cause mold growth and mycotoxin production. In addition, it was stressed that the best oil purification method after pressing was centrifugation of the sediment, which produces better effects of solid

Table 3. Tocopherol contents of cold-pressed oils from raw materials of different technological quality

Cold-pressed rapeseed oil from seeds with moisture, %	Tocopherol contents, mg/100 g					PC-8 content mg/100 g
	α-T	β-T	γ-T	δ-T	total	
7 (control)	32.65 ±0.14^{c}	0.18 ±0.05^{a}	39.43 ±0.11^{c}	0.77 ±0.11^{a}	72.03 ±0.14^{c}	4.07 ±0.04^{a}
10	32.25 ±0.15^{b,c}	0.20 ±0.02^{a}	39.37 ±0.06^{c}	0.69 ±0.05^{a}	72.51 ±0.11^{c}	5.15 ±0.11^{b}
12	31.90 ±0.05^{b}	0.18 ±0.03^{a}	37.03 ±0.08^{b}	0.76 ±0.06^{a}	69.86 ±0.18^{b}	5.91 ±0.10^{c}
20	30.68 ±0.09^{a}	0.17 ±0.04^{a}	37.68 ±0.12^{b}	0.74 ±0.01^{a}	69.27 ±0.11^{b}	5.37 ±0.15^{b}

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

and microbiological contamination cleaning. This process ensures better oil quality. The quality parameters (AV and PV) of the oils under investigation significantly deteriorated with an increase in humidity of the stored seeds. The effects of increased humidity on the native lipophilic and hydrophilic antioxidants content were also examined.

The most important rapeseed oil antioxidants are tocochromanols, the content of which is presented in Table 3. The tested samples of rapeseed oils are characterized by the typical composition of individual homologues of tocopherols. The dominant tocopherols in the oils analyzed are α-tocopherol and γ-tocopherol, the content of which in the control sample was 32.65 mg/100 g and 39.43 mg/100 g, respectively. This is consistent with the reports of many other researchers who have confirmed the existence of the vast majority of these two tocopherol homologues in cold-pressed rapeseed oils (Koski et al., 2002; Siger et al., 2018b; Teh and Birch, 2013). A decrease in tocopherol content with an increase in seed moisture may be noticed by analyzing the results contained in Table 3. The oil pressed from seeds with 7% water content contained 72.03 mg/100 g of total tocopherols. In the case of oil from seeds stored at 10% humidity, their total content was 72.51 mg/100 g and did not show statistically significant differences when compared to the control. On the other hand, statistically significant differences were found for samples of oils obtained from seeds stored at 12% and 20% humidity, which contained 69.86 mg/100 g and 69.27 mg/100 g of tocopherols respectively. In this case, about a 4% decrease in tocopherol content was observed. Gawrysiak-Witulska at al. (2016) also noted a similar decrease in tocopherol concentration. The authors analyzed changes in quality of rape seeds stored at increased humidity and temperature, simulating the conditions prevailing in industrial silos. They stated, inter alia, that after 28 days of storage for seeds with a moisture content of 13% (at 25°C), 8–9% of the total tocopherol content was degraded. Other studies conducted by Gawrysiak-Witulska et al. (2018) show how, during the storage of seeds with an increased moisture content, self-heating of the seeds' mass occurred. This phenomenon resulted in a decrease in the level of bioactive compounds. After 13 days of sample storage, a 34% loss of tocopherols, 22% loss of sterols, and 33% decrease in total phenolic content was observed.

In rapeseed oil, plastochromanol-8 (PC-8) is also present, the content of which in the control sample was 4.07 mg/100 g. In the case of oils obtained from seeds stored at increased humidity, a statistically significantly higher content of this compound was found ($p < 0.05$). The highest level was recorded for oil pressed from seeds stored at 12% humidity, in which the content of this compound was equal to 5.91 mg/100 g – a 45% increase in concentration was observed (Table 3). The role of PC-8 and its behavior under stress conditions remain poorly understood. It has antioxidant functions that are mainly related to preventing photo-oxidative damage in leaves and oxidation processes in seeds. But we have incomplete knowledge of its response to stress factors such as salt and drought in both leaves and seeds (Kruk et al., 2014). Plastochromanol-8 is a fat-soluble, universal antioxidant, protecting intra-tissue lipids. In chloroplasts, α-tocopherol acts as the main antioxidant, together with a reduced form of plastoquinone-9 and its derivative plastochromanol-8 (Martinis et al., 2011).

It is needed by plant seeds during longer storage or drying, and also affects the proper development and maturation of the seed coat (in fresh seeds it does not play such a significant role) (Gruszka et al., 2008; Mène-Saffrané et al., 2010). PC-8 protects polyunsaturated fatty acids from oxidation during seed desiccation and quiescence (Kruk et al., 2014). Plastochromanol-8 takes part in the transport of sugars in the plant organs that do not participate in photosynthesis (Strzałka et al., 2009). Studies have shown that the content of PC-8 in these parts of plants is not affected by sun exposure, unlike other tocochromanols, whose synthesis is stimulated by light (Szymańska and Kruk, 2010).

Another group of native antioxidants present in rapeseed oil is phenolic compounds, the content of which is presented in Table 4. In the control sample of oil obtained from seeds with 7% moisture, only the presence of *trans*-sinapic acid (80.68 µg/100 g) was determined. In turn, in the samples of oil obtained from seeds with 10% and 12% moisture content, it may be observed that in addition to the increased *trans*-sinapic acid content (270.86 µg/100 g and 556.64 µg/100 g respectively), *p*-coumaric acid (24.47 µg/100 g and 24.37 µg/100 g respectively) and ferulic acid (13.02 µg/100 g and 24.71 µg/100 g respectively) were also detected. In contrast, in the 20% moisture sample, the presence of sinapine (24.17 µg/100 g) was also observed, in addition to an increased content of *trans*-sinapic acid (957.99 µg/100 g), *p*-coumaric acid (32.80 µg/100 g) and ferulic acid (53.43 µg/100 g). Analyzing these data, one can notice that along with the increase in seed moisture content during storage, the content of phenolic compounds present in the oil obtained from them also increased, despite the fact that the seeds' water content immediately before pressing was the same in each case (7%). The highest phenolic concentration increase was recorded for sinapic acid, the content of which increased almost 12 times in the oil obtained from seeds stored at 20% humidity, compared to the control (Table 4). The total content of phenolic compounds in the oil obtained from seeds with a moisture content of 10% was 308.35 µg/100 g (a 4-fold increase compared to the control sample). In the case of oils obtained from seeds containing 12% and 20% of water, the total content of phenolic compounds was 605.72 µg/100 g and 1068.39 µg/100 g respectively (almost 8-fold and 13-fold increases of phenolic concentration compared to the control sample respectively). Phenolics are polar compounds and during pressing, most of them remain in the expeller cake without being extracted into the oil. The seeds also contain small amounts of water, which are transferred into the oil during pressing. Thanks to this, the parts of phenolic compounds with a higher polarity may be dissolved in oil (Mińkowski et al., 2013). It may be assumed that in the samples with increased water content, microorganisms, mainly molds, that could damage the structure of the cell walls of the seeds, have developed. This could result in a higher degree of seed penetration by water. Hence, more effective dissolution of polar phenolic compounds in oil occurred. The effect of this phenomenon was an increased amount of phenolics in the oil samples obtained from seeds with a moisture

Table 4. Contents of phenolic compounds of cold-pressed oils from raw materials of different technological quality

Compounds	Retention time, min	Phenolic compound content of cold-pressed rapeseed oil from seeds with moisture µg/100 g			
		7 % (control)	10%	12%	20%
Sinapin*	14.856	–	–	–	24.17 ±0.18^{a}
p-Coumaric acid	25.447	–	24.47 ±0.21^{a}	24.37 ±0.14^{a}	32.80 ±0.11^{b}
Ferulic acid	27.832	–	13.02 ±0.15^{a}	24.71 ±0.16^{b}	53.43 ± 0.24^{c}
trans-Sinapic acid	30.361	80.68 ±0.14^{a}	270.86 ±0.41^{b}	556.64 ±0.35^{c}	957.99 ±0.52^{d}
Total		80.68 ±0.14^{a}	308.35 ±0.35^{b}	605.72 ±0.85^{c}	1 068.39 ±1.52^{d}

*Contents are expressed as sinapic acid equivalent.
Values (means ±SD) with different index letters are statistically significantly different ($P < 0.05$).

content equal to 20%. A similar tendency was also observed by Siger et al. (2018a) analyzing the impact of improper storage conditions on the changes in the content of phenolic compounds in rape seeds. The authors describe a decrease in phenolic compounds occurring in the bound form with a simultaneous increase of *trans*--sinapic acid content (by 63%). The authors argue that changes in the content of antioxidants in rape seeds stored under unfavorable temperature and humidity conditions are caused primarily by lipid autoxidation and the activity of hydrolytic and oxidative enzymes such as lipases and lipoxygenases. The degree of microbial contamination of rape seeds is usually high. Molds, yeast and bacteria may appear on the seeds' surface. To inhibit their growth, it is very important to maintain proper storage conditions, especially seed moisture (Wroniak and Chlebowska-Śmigielska, 2013). The relationship between the growth in microorganisms and the content of phenolic compounds in rape seeds may be confirmed by reports by Gawrysiak-Witulska et al. (2018), who argue that the increase in sinapic acid content may result from the protective properties of phenolic compounds against biotic stress or the defensive response of seeds against pathogens.

Rapeseed oil consists primarily of triacylglycerols (99%), traces of water and a small amount of so-called minor oil components such as: monoacylglycerols, diacylglycerol, phospholipids, sterols and free fatty acids. These are amphiphilic compounds that accumulate at the oil-water interface, forming micellar structures known as association colloids (Chaiyasit et al., 2007). Association colloids are considered by many researchers to be sites of intensive lipid autoxidation (Chen et al., 2011; Homma et al., 2015; Xenakis et al., 2010). An effective method of detecting association colloids, as well as changes in their structure, is the use of fluorescent probes, such as NBD-PE ((N-(7--nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine). Figure 1 shows the results of the NBD-PE probe fluorescence measurement in oils pressed from seeds containing different amounts of water during storage. There is a clear decrease in NBD-PE fluorescence intensity along with an increase in seed moisture. These changes are also correlated with the increase of water content in oil, as shown in Table 1. The exposure of the probe NBD group to a polar environment causes a fluorescence intensity decrease. NBD-PE is preferentially located

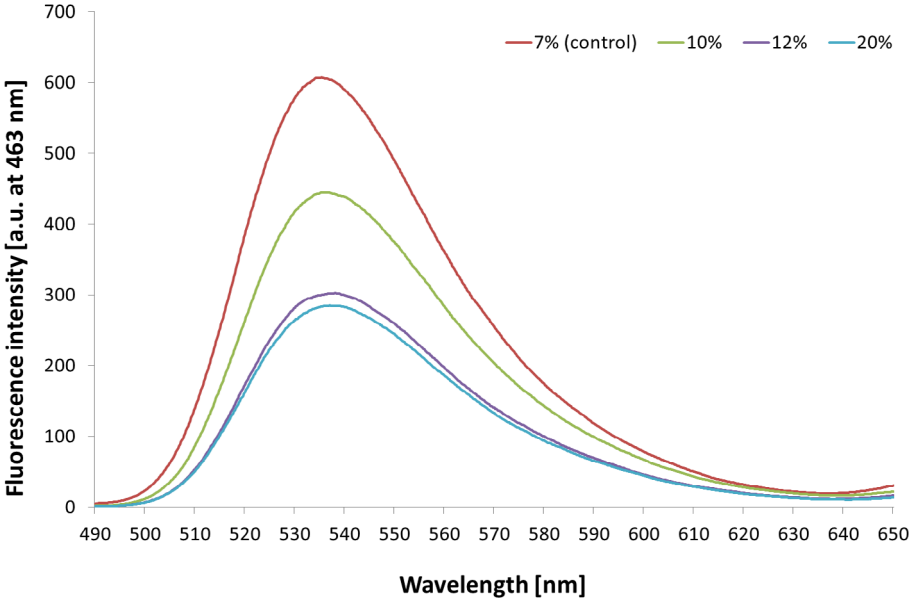

Fig. 1. Fluorescence spectra of NBD-PE probe in oil samples pressed from seeds with different moisture contents (excitation wavelength at 463 nm)

at the oil–water interface and orients the hydrophilic head group towards the water core. The imino group and/or oxygen molecule on the NBD probe could form H-bonds with water molecules, leading to fluorescence quenching. The observed fluorescence intensity decrease in the current study may have been caused by the increase in water content in oil and/or changes in the reverse micelle structure due to the formation of hydroperoxides and free fatty acids (as indicated by the increased peroxide and acid values respectively), as well as the significant increase in the content of phenolic compounds in oils pressed from seeds stored at a higher humidity. Changes in the micelle's structure as a result of the increasing concentration of amphiphilic compounds (hydroperoxides, free fatty acids, phenolic compounds) may have contributed to the increase of water available to the NBD-PE probe. This phenomenon causes probe fluorescence quenching. In this way, NBD-PE is used as a tool to monitor changes in the association colloid's structure. These changes may significantly affect the rate of oil autoxidation.

Autoxidation of edible oils containing polyunsaturated fatty acids is a significant problem in the food industry due to its direct influence on sensory and nutritional quality. In the sensory analysis, the attributes typical for rapeseed oil of good quality include seed-like, nutty, wood-like and astringent aromas. On the other hand, rancid, fusty, musty, roast, burnt and bitter aromas are considered as off-flavors. These negative attributes reveal a deterioration in the oil quality during storage or the poor quality of the raw material. Such aromas occur mainly as a result of the presence of volatile degradation products or are formed during an incorrect production process (Matthäus, 2013).

The oil samples obtained from the seeds stored in adverse storage conditions (high humidity) were also subjected to sensory analysis. The odor sensory profile of individual samples is shown in Figure 2.

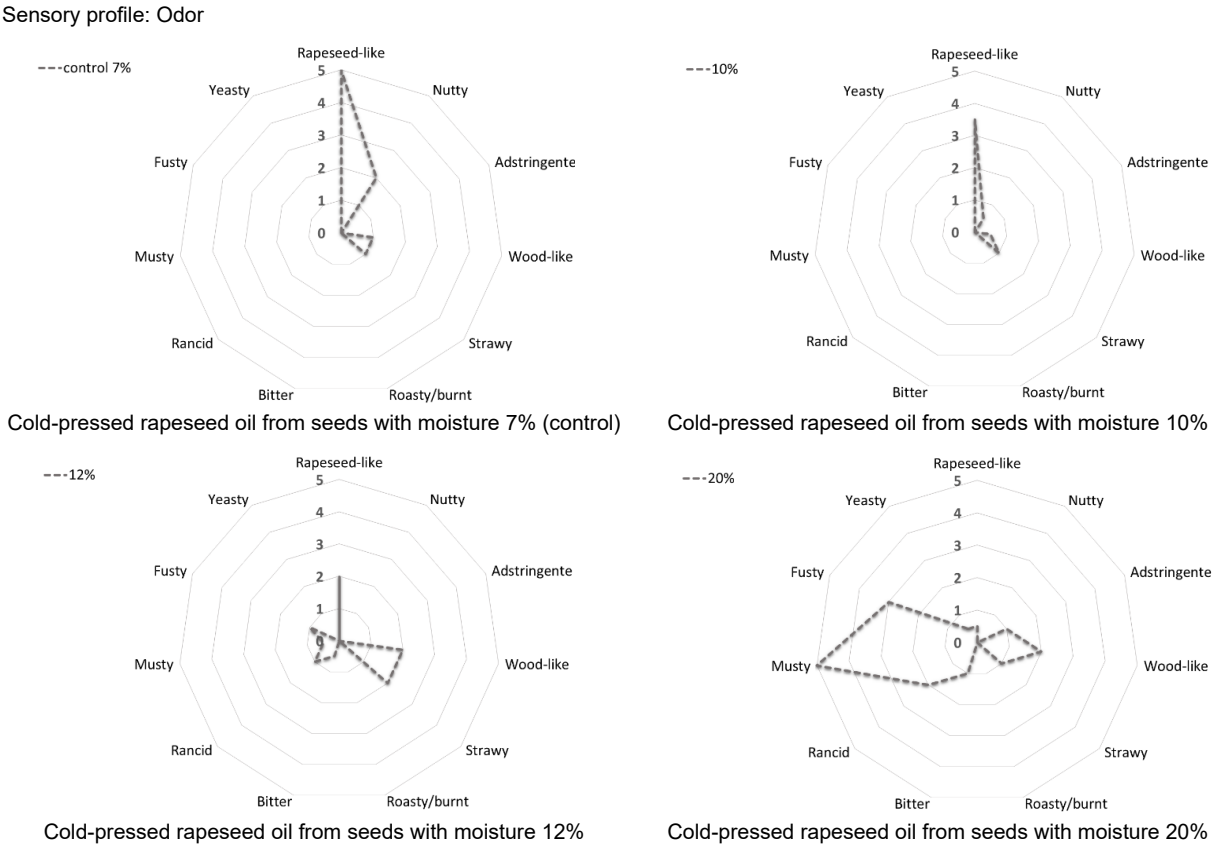

Fig. 2. Sensory quality of cold-pressed rapeseed oils from raw materials of different technological quality

Cold-pressed oil from seeds containing 7% water (control sample) was characterized by a typical rapeseed-like aroma, while a nutty aroma was less noticeable. The least expressed odors were straw-like and woody. Similar results of rapeseed oil sensory analysis were obtained by Rękas et al. (2016), specifying the dominant aromas as rapeseed-like and nutty. Siger and Michalak (2016) detected a distinct rapeseed-like aroma, as well as green and hay/grain odors in cold-pressed oil obtained from seeds containing 6% water. The oil sample obtained from seeds with a moisture content equal to 10% lost its characteristic rapeseed-like and nutty odors; still present were poorly perceptible straw-like and woody aromas. All other samples had an altered odor sensory profile, different from attributes typical for the control sample. In the oil samples obtained from seeds stored at a humidity of 12%, a loss of rapeseed-like odor was noticeable, but straw-like and woody became more expressed. Additionally, weak fusty and rancid odors appeared in this sample. This may indicate oil quality deterioration or confirm the use of poor-quality raw materials. In the oil sample obtained from seeds containing 20% water, the odor sensory profile was completely changed, compared to the control sample. The total loss of a typical rapeseed-like aroma was observed. The sensory profile was dominated by a musty odor, which confirmed the growth of mold in the raw material. In addition, fusty, bitter and wood-like aromas appeared. It should be emphasized that drying of the raw material to a 7% moisture content before pressing did not affect the sensory profile – an improvement in the sensory quality was not observed. Matthäus (2013) described research in which seeds of rape with a water content of 7%, 9% and 11% were stored for 9 months and the oil obtained from them was systematically analyzed. The oil pressed from seeds with a 7% moisture content maintained its characteristic rapeseed-like and nutty aromas throughout the entire storage period. In the oil samples pressed from seeds with 9% and 11% moisture content, fusty, musty and astringent odors appeared during storage, whereas the typical seed-like and nutty aromas became less perceptible. The high content of fat in rapeseed acts as sensory memory and "records" improper processing and conditions to which the raw material is subjected (Matthäus, 2013).

CONCLUSION

The rapeseed moisture content during storage has a crucial influence on the quality of oil obtained from them. Oils pressed from seeds with 10%, 12% and 20% moisture content had different quality parameters, even though the initial humidity of the seeds before pressing was the same in all cases (7%). With the increase in seed moisture, the quality characteristics of the oil obtained deteriorated, which was confirmed by the increased acid and peroxide values. In addition, changes in the bioactive compound content were observed, such as a slight decrease in tocopherol content and a significant increase in phenolic acid concentration. Changes in the content of those compounds depended on the moisture of the seeds from which the oil was pressed. The most pronounced changes were recorded for sinapic acid, the content of which increased almost 12 times in oil obtained from seeds with a water content equal to 20% when compared to the control. This increase may have been caused by the activity of fungal microflora growing in the mass of stored seeds. This was also confirmed by the sensory analysis of the oils, which indicated a loss of the characteristic rapeseed-like aroma in the oil from seeds with a 20% moisture content. Instead, the appearance of musty and fusty odors was recorded. Along with an increase in seed moisture, the possibility of undesirable microflora development grows. It results in a deterioration of the quality of the oil obtained. This study confirms that the storage conditions of seeds of rape have a crucial influence on the quality of oil obtained from them.

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