

1 Original Research Article

2 **INFLUENCE OF DIFFERENT STORAGE**

3 **CONDITIONS ON RAPESEED OILS QUALITY**

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5

6 **ABSTRACT**

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Aims: The aim was to determine the impact of various storage conditions on the stability of rapeseed oil, which determines the content of free fatty acids, peroxides, aldehydes, conjugated compounds and fatty acid composition

Study design: Storage experiment was performed in 6 months period; Quality of oils was analysed using standard methods. Fatty acid composition of oils was determined using gas chromatography. The data were analysed using Statistica v. 12.5 software.

Place and Duration of Study: Chair of Food Plant Chemistry and Processing, Olsztyn, Poland between December 2014 and July 2015.

Methodology: The material consisted of 24 commercial samples of rapeseed oils. Samples were stored in brown glass bottles for six months in different conditions - in the dark at +20°C, in a refrigerator at +4°C, and in a freezer at -20°C. The quality of rapeseed oil was determined by measuring the acid value, peroxide value and anisidine value. The fatty acid composition and the content of conjugated dienes and trienes were also analyzed.

Results: In control samples cold pressed oils were characterized by the highest acid value and the lowest anisidine and peroxide values. Cold pressed oil samples stored at -20°C had the highest acid value (an increase by 9.21%); however, unlike fresh oils, they also had the highest peroxide value (an increase by 5.17%). The greatest increase in the content of saturated fatty acids during storage was noted for refined hot pressed oils (6.33%). A permanent trend was an increase in the share of monounsaturated fatty acids, and a decrease in the share of polyunsaturated fatty acids. The share of MUFAs increased most following storage at 20°C for refined hot pressed oils (9.38%), and pressed and extracted oils (9.35%). In the same samples, the greatest decrease in the content of PUFAs also occurred by, respectively, 30.78% and 31.22%.

Conclusion: Higher temperatures are more conducive to oxidative changes in oils, which are indicated by higher values of all the analyzed distinguishing quality features of oils. Refined oils are more susceptible to these changes as in the process of purification a number of substances with antioxidant properties are removed.

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11 *Keywords: Rapeseed oil, oxidation, storage, quality, fatty acid composition*

1. INTRODUCTION

The oil obtained from rapeseed (*Brassica napus*) has been used in food technology and the catering industry for many years. In terms of global consumption it takes third place, after palm oil and soy oil. ~~Each year an~~ increase in the consumption of vegetable oils (including rapeseed) ~~ishas been~~ observed ~~since 1995~~. In the 2013/14 season global consumption reached 25.63 million tonnes, and for the 2014/15 season it is forecast to increase by 5.35% [1]. The largest producer of rapeseed for oil production is Canada. The size of the ~~rape~~~~rapeseed~~ harvest in this country in 2012 was 15.41 million tonnes. The second largest producer is China (14 million tonnes), and the third is India (6.78 million tonnes) [1,2].

Rapeseed oil is one of the most valuable vegetable fats, due to its nutritional properties. It is a rich source of mono- and polyunsaturated fatty acids, while saturated fatty acids are minor constituents [3]. Rapeseed oil is characterized by high levels of monounsaturated fatty acids (62%) and significant levels of polyunsaturated fatty acids (32%), and it contains very little saturated acids (6%) [4].

Oil from plant materials can be extracted by pressing or with solvents. Extraction with an organic solvent is used for raw materials with a fat content below 20%. Pressing is a method applied to materials with a higher oil amount. This process can be carried out either hot or cold. Cold pressing does not apply heat treatment to raw materials, in contrast to hot pressing, where rape seeds are pre-treated by heating [5]. Disadvantage of pressing is an expeller, that has relatively high fat residue. However, to increase the yield of the oil it can be further extracted with organic solvents. Oils obtained by cold pressing have better preserved natural properties, such as flavor [6].

Crude oils after hot pressing and / or extraction are often subjected to a refining process, which aims to remove undesirable components. It consists of degumming, neutralization, bleaching and deodorization [7]. Cold pressed oils and refined oils differ in their contents of many compounds, such as phenols. Factors affecting their antioxidant properties include the number and position of hydroxyl groups, and polarity, solubility, and stability during processes such as hot pressing or refining [8]. Cold pressed oils are rich in polar phenolic compounds [9]. Siger et al. [10] studied the contents of phenolic compounds in cold pressed and refined oils. All refined oils (rapeseed oil, soybean oil and sunflower oil) have shown lower contents of total phenolic compounds in relation to cold pressed oils, while in the case of phenolic acids, in most refined samples there was not found their presence at all. The differences in these contents are also reflected in variations in antiradical activity - in the case of refined oils this was higher. The participation of ~~non-triacylglycerol~~ compounds ~~which are not triacylglycerols~~ in cold pressed oils is higher than in refined oils [11].

The contents of compounds with antioxidant properties and their activity determines the progress of oxidative changes in oils during storage, and therefore their stability. These changes progress via autoxidation and / or are supported by rays of light. Responsible for this mechanism are various forms of oxygen, respectively, atmospheric triplet oxygen ($^3\text{O}_2$) and singlet oxygen ($^1\text{O}_2$). Triplet oxygen has two unpaired electrons that react with radical components of food, whereby with the participation of light and atmospheric oxygen singlet oxygen is formed [12]. Autoxidation can be divided into three stages. The first is initiation. This involves removing a hydrogen atom from the fat methylene group, thereby forming the alkyl radical. This is followed by propagation, namely the creation of peroxide radicals, which are capable of reacting with unsaturated fatty acids, resulting in the formation of hydroperoxides. Autoxidation ends with termination that is the formation of non-radical compounds [13]. During the induction time very small changes occur, but in the later stages of oxidation flavor changes are noticeable. In the molecules of unsaturated fatty acids removal refers to the least-bonded hydrogen atom. In polyunsaturated fatty acids it is an atom of a methylene group located between the two

65 unsaturated bonds. In molecules of monoene fatty acid hydrogen bonded to a carbon
66 atom adjacent to the double bond is removed [14, 15].

67 The changes described above cause quality flaws generally known as oxidative
68 rancidity. The second type of rancidity is hydrolytic rancidity, which consist of triglyceride
69 lipolysis, resulting in free fatty acids release. The endogenous lipase catalyzing this
70 reaction is present in rape seeds. Optimal pH for its activity is between 8 and 9 at 37°C
71 [16].

72 In this study, changes occurring during storage in rapeseed oil obtained from different
73 production methods was evaluated. Used storage conditions included: freezing,
74 refrigerating and room temperature. Most of the previous studies report the relationship
75 between antioxidant content or unsaturation degree and the length of shelf life. Zheng et
76 al [17] evaluated the changes occurring during storage in the contents of canolol and the
77 total phenolics, oxidative stability, and antioxidant capacity of rapeseed oil. Martin-
78 Polvillo ety al [18] investigated oxidative stability of sunflower oils differing in
79 unsaturation degree during long-term storage.

80 The aim of the study was to determine the impact of various storage conditions on the
81 stability of rapeseed oil, which determines the content of free fatty acids, peroxides,
82 aldehydes, conjugated compounds and fatty acid composition.

83 2. MATERIAL AND METHODS

84
85 The material consisted of 24 commercial samples of rapeseed oils (Table 1.) before) in
86 the endrange of shelf life. The oils were made by different manufacturers. The
87 differences between them consist in the process of production, the presence or absence
88 of added antioxidant, and type of package.

89 Table 1. Samples of rapeseed oils (production and purification methods, package
90 types and volumes).

<u>Oil sample</u>	<u>Method of production and purification</u>	<u>Time to the end of shelf life (months)</u>	<u>Type and volume of package (dm³)</u>
<u>1</u>	<u>Cold-pressed;</u> <u>unrefined</u>	<u>4</u>	<u>PET, transparent; 0.5</u>
<u>2*</u>		<u>8</u>	<u>Glass, brown; 0,5</u>
<u>3</u>		<u>5</u>	<u>PET, transparent; 0.5</u>
<u>4</u>		<u>14</u>	<u>Glass, brown; 0,5</u>
<u>5</u>	<u>1-step pressed;</u> <u>refined</u>	<u>10.5</u>	<u>PET, transparent; 1</u>
<u>6</u>		<u>10</u>	<u>PET, transparent; 0.5</u>
<u>20</u>		<u>11</u>	<u>Glass, transparent; 0.5</u>
<u>21</u>		<u>7</u>	<u>Glass, transparent; 0.5</u>
<u>22*</u>		<u>11</u>	<u>Glass, dark green; 0.75</u>
<u>7</u>	<u>Hot pressed and</u> <u>extracted; refined</u>	<u>10.5</u>	<u>PET, transparent; 1</u>
<u>8</u>		<u>6</u>	<u>Glass, dark green; 0.75</u>
<u>9</u>		<u>6</u>	<u>PET, transparent; 1</u>
<u>10</u>		<u>11</u>	<u>PET, transparent; 0.9</u>
<u>11</u>		<u>11.5</u>	<u>PET, transparent; 1</u>
<u>12</u>		<u>12.5</u>	<u>PET, transparent; 1</u>
<u>13</u>		<u>9</u>	<u>PET, transparent; 1</u>
<u>14</u>		<u>7.5</u>	<u>PET, transparent; 1</u>
<u>15</u>		<u>9</u>	<u>PET, transparent; 0.9</u>
<u>16</u>		<u>9.5</u>	<u>PET, transparent; 1</u>
<u>17</u>		<u>10.5</u>	<u>PET, transparent; 0.9</u>
<u>18</u>		<u>11.5</u>	<u>PET, transparent; 1</u>
<u>19</u>		<u>17</u>	<u>PET, transparent; 1</u>

	<u>23</u>	<u>10.5</u>	<u>PET, light green; 1</u>
	<u>24</u>	<u>9.5</u>	<u>PET, transparent; 0.9</u>

92 PET – Polyethylene terephthalate; * - Mixture of tocopherols was added

93 Control samples were analyzed at the beginning of the study. Samples were stored in
 94 brown glass bottles (volume 100 ml) for six months in different conditions - in the dark at
 95 + 20°C, in a refrigerator at + 4°C, and in a freezer at -20°C.

96 **Table 1. Samples of rapeseed oils (methods of production and purification).**

Method of production and purification	Sample number
Gold-pressed unrefined	1-4
1-step-pressed refined oil	5-6; 20-22
pressed and extracted refined oil	7-19; 23-24

98

99 The quality of rapeseed oil was determined by measuring the acid value [17,19], peroxide
 100 value [18,20], and anisidine value [21]. Acid value determined using PN – ISO 660: 1998,
 101 consisted of dissolving oil in the alcohol-ether mixture (1:1) and titrated with 0.1 M
 102 potassium hydroxide solution with phenolphthalein as an indicator [19]. Peroxide value
 103 was determined according to PN – ISO 3960: 1996 by dissolving the oil sample in
 104 chloroform, adding 80% acetic acid and saturated solution of potassium iodide, than
 105 titration of the released iodine with a 0.002 M sodium thiosulphate solution using starch
 106 solution as an indicator [20]. Measuring anisidine value was performed by
 107 spectrophotometric method at UNICAM UV / Vis UV2 according to PN-EN ISO 6885:
 108 2008. To the oil sample dissolved in hexane anisidine reagent (0.25% solution of p-
 109 anisidine in glacial acetic acid) was added. After 10 minutes from mixing, the absorbance
 110 change was measure at a wavelength $\lambda = 350$ nm [21]. The fatty acid composition [20,22]
 111 and the content of conjugated dienes and trienes [21,23] were also analyzed. Fatty acid
 112 composition was determined by GC gas chromatography [20(GC) [22]. Methyl esters
 113 were prepared according to the method of Zadernowski and Sosulski [22,24], and their
 114 analysis was carried out with a GC 121 8000 FISIONS chromatograph equipped with a
 115 DB-225 capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m), helium as the carrier gas,
 116 and a flame-ionization detector. Fatty acids were identified according to the retention
 117 time determined for fatty acid standards (Sigma-Aldrich, Poznań, Poland). In case of
 118 determining of content of conjugated dienes and trienes the oil (0.1 g) was placed into 25
 119 ml volumetric flasks and supplemented up to the volume with hexane. This was followed
 120 by measuring the absorbance of the solutions using a UNICAM UV / Vis UV2
 121 spectrophotometer at wavelengths of 233, 262, 268 and 274 nm versus hexane [21,23].

122 Oils were divided into groups, taking as a criterion the method of production and method
 123 of purification. Statistical analysis of the results was performed using STATISTICA
 124 StatSoft version 12.5 (Krakow, Poland). With this program PCA two analyzes was made:
 125 principal component analysis was made (PCA) and analysis of variance (ANOVA) with
 126 Duncan test for homogenous groups ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

Table 2 presents the average values of particular parameters for the analyzed oils. As compared to control samples, the acid value increased following storage. The highest value thereof was noted for oils stored at 20°C, which is due to the fact that the processes of hydrolysis occur more rapidly at higher temperatures. In control samples a higher content of conjugated dienes was noted, as well as the absence of conjugated trienes, whose presence was demonstrated in the stored samples. The composition of fatty acids also changed during storage. An increase in the share of saturated and monounsaturated fatty acids, and a reduction in the share of unsaturated ones can be observed here. Both control samples and stored samples were characterized by high variation in all analyzed parameters. ~~Fresh oils~~Before storage experiment the oil samples were ~~most~~very varied in terms of ~~the~~acid and anisidine values. ~~Variation (coefficient of variation equal 72% and 40%, respectively), between but variation~~ in those parameters decreased following storage (the variation coefficient decreased the most following storage at 20°C). The situation was opposite for the peroxide value, whose variation was lowest in control samples but increased following storage. The greatest increase in the variation of oils in terms of this parameter was noted following storage at 20°C (~~cv = 59.3%~~~~SD = 0.51~~).

Table 2. The acid, peroxide and anisidine values, the content of dienes, trienes and fatty acid composition in rapeseed oil, depending on the method of storage.

	Fresh (control samples)		Storage temperature [°C]					
			-20		4		20	
	x	evSD	x	evSD	x	evSD	x	evSD
Acid value [mg KOH/g]	0.2929 A	75.80.2 1	0.3434 A	52.90.1 8	0.3535 A	60.0.21	0.4444 B	45.50.2 0
Peroxide value [mEq O ₂ /kg]	1.0909 A	35.80.3 9	0.3434 B	50.0.17	0.5353 C	52.80.2 8	0.8686 D	59.30.5 1
Anisidine value [-]	1.9696 A	40.80.7 9	1.7575 A	34.30.6 0	1.8686 A	32.80.6 1	2.5353 B	30.40.7 7
Dienes [%]	0.2222 A	22.70.0 5	0.1414 B	28.60.0 4	0.1414 B	21.40.0 3	0.1717 C	23.50.0 4
Trienes [%]	0.0000 A	0.000 0	0.0303 B	33.30.0 1	0.0404 B	25.0.01	0.0404 C	50.0.02
Fatty acid composition [%]								
SFA	6.92		6.67		6.85		19.46	
MUFA	65.15		71.83		72.83		73.51	
PUFA	27.92		21.56		20.36		19.46	

SFA – Saturated Fatty Acids; MUFA – Monounsaturated Fatty Acids; PUFA – Polyunsaturated Fatty Acids; x – Average value; *ev* – Variation coefficient; *SD* – Standard deviation; A-D – Denotes statistically significant differences ($P \leq 0.05$); (n = 24 samples analyzed in triplicate)

The acid value in control samples ranged from 0.09 to 0.87 mg KOH/g oil. The lowest value of this parameter was noted for refined oil obtained by single pressing. In turn, the highest acid value was noted for unrefined cold-pressed oil. This is explained by the fact that during the oil refining process ~~a neutralization is applied in order to precipitate free fatty acids~~ are neutralized and removed from the oil in the form of soaps, ~~and separate them from the oil~~. The highest peroxide value (2.18 mEq O₂/kg) was noted for refined hot pressed oil; the lowest one for unrefined cold-pressed oil. In addition to the undesirable substances, many substances with antioxidant properties (e.g. tocopherols, carotenoids, sterols) are also removed from the oil during the refining process, which could be the reason for this situation. The anisidine value was highest (4.49) for a sample of refined oil, hot pressed with no chemical methods applied; the lowest (0.77) value of this parameter was noted for a sample of unrefined cold-pressed oil (Table 3). This situation is due to the application of refining, which removes antioxidants. The anisidine value indicates the presence of secondary products of lipid oxidation, e. g. aldehydes (especially 2-alkenale and 2,4-alkadienale), which are formed by decomposition of hydroperoxides [2325]. The principle of this method is the reaction of p-methoxyaniline (p-anisidine) with the aldehyde component. Products of this reaction are yellow and show a maximum absorbance at 350 nm [2426].

The values of particular parameters indicative of the stability of oils were averaged for particular oil groups. The method for obtaining oil and its purification was adopted as a criterion (Table 3). In control samples cold pressed oils were characterized by the highest acid value and the lowest anisidine and peroxide values. Similarly to control samples, cold pressed oil samples stored at -20°C had the highest acid value (an increase by 9.21%); however, unlike fresh oils, they also had the highest peroxide value (an increase by 5.17%). The anisidine value remained at the same level as for fresh products.

At a temperature of 4°C a slight increase in the acid value was noted for cold pressed oils (it remained the highest among all the analyzed oils). The peroxide value was about twice higher than for the oils stored at -20°C (Table 3). This indicates that the oxidative changes had progressed in the analyzed fats. The anisidine value remained at a level similar to that for control samples.

For the oils stored at room temperature the greatest changes in the analyzed parameters in relation to fresh oils were noted. The acid value increased by 9.21% for cold pressed oils, by 50% for hot pressed oils, and by 90% for oils obtained by hot pressing combined with extraction (Table 3). Iqbal and Bhangar [2527] also analyzed the storage stability of oils. After 24 days of storage they noted an increase in the oils' content of free fatty acids. Similar results were obtained by Kreivaitis et al. [2628]. For the rapeseed oils they analyzed, after 70 days of storage at a temperature of 70°C, the acid value increased, as compared to the baseline, by ~~approx-approximately~~ three times, ~~which indicates progressive-hydrolysis~~. The peroxide value decreased for both hot pressed and extracted oils, and those hot pressed only. In turn, the anisidine value for these product groups increased, which could be indicative of the completion of lipid oxidation and the formation of secondary products of this process other than radicals. They can include aldehydes, ketones, or short-chain carboxylic acids [2628].

No presence of conjugated trienes of fatty acids was found in control samples. For each oil group, regardless of the storage conditions, the average content of conjugated trienes was the same. The lowest one (0.01%) was noted for unrefined and cold pressed oils, while the highest one (0.05–0.06%) was noted for hot pressed oils or oils pressed, extracted, and purified by refining. Wanasundara et al. [2729] also studied the content of conjugated dienes and trienes in stored oils (rapeseed and soybean). The amount of these compounds in the samples was tested eight times in a period of thirty days of storage. In both types of oils content of conjugated dienes and trienes increased each measurement. ~~Therefore it can be concluded that during oil storage occur oxidative changes, in which these compounds are formed.~~ The results in the case of the dienes do not coincide with those obtained in this study. In addition, these authors noted a significantly higher content of dienes and trienes, probably due to the use of much higher storage temperatures (65°C).

During the formation of hydroperoxides from unsaturated fatty acids conjugated dienes are typically produced, due to the rearrangement of the double bonds. They can be created from oxydienes or hydroperoxydienes as a result of oxidation, where hydroxydienes are intermediates [2325]. Following storage the amount of conjugated dienes decreased for hot pressed oils and refined pressed and extracted oils. The greatest decrease (52%) in their content was noted after the storage of hot pressed oil samples at 4°C. For unrefined cold pressed oils the amount of conjugated dienes after storage either remained the same or increased. An increase in the value of this parameter by 27.27% was noted for samples stored at room temperature (Table 3).

~~Table 3. The average of acid, peroxide and anisidine values and content of dienes and trienes in rapeseed oil, depending on production method.~~

Storage temperature [°C]	Production method	Acid value [mg KOH/g]	Peroxide value [mEq O ₂ /kg]	Anisidine value [-]	Dienes [%]	Trienes [%]
Fresh	Cold-pressing	0.76	0.58	1.02	0.11	0.00
	1-step pressing*	0.18	1.24	2.49	0.25	0.00
	Hot-pressing + extraction*	0.20	1.18	2.03	0.23	0.00

-20	Cold-pressing	0.69	0.61	1.02	0.10	0.01
	1-step pressing*	0.23	0.23	2.02	0.13	0.05
	Hot-pressing + extraction *	0.28	0.30	1.85	0.15	0.04
4	Cold-pressing	0.79	0.99	1.18	0.11	0.01
	1-step pressing*	0.21	0.42	2.09	0.12	0.05
	Hot-pressing + extraction *	0.28	0.45	1.97	0.16	0.04
20	Cold-pressing	0.83	1.22	1.74	0.14	0.01
	1-step pressing*	0.27	0.81	2.78	0.15	0.05
	Hot-pressing + extraction *	0.38	0.79	2.66	0.19	0.04

225 * refined-oils
226

227 A number of correlative relationships were demonstrated; they are listed in Table 4. The
228 highest positive value of the correlation coefficient (0.76) was obtained following the
229 storage of oil at 4°C between the acid and peroxide values, as well as between the
230 content of conjugated trienes and the anisidine value. Strongly positive relationships
231 were also demonstrated for fresh oil between the anisidine value and the content of
232 conjugated dienes. In turn, the greatest negative relationship was found between the
233 acid value and the content of conjugated dienes (from -0.75 to -0.78) for all samples
234 following storage. A similar correlation in their study received Shahidi et al. [2830], who
235 studied animal oil derived from seals. This may explain the aforementioned fact that the
236 dienes are formed in parallel with the hydroperoxides.

237

238 Table 3. The average of acid, peroxide and anisidine values and content of dienes and trienes in rapeseed oil, depending on production
 239 method (6 months of storage).
 240

<u>Storage temperature [°C]</u>	<u>Production method</u>	<u>Acid value [mg KOH/g]</u>		<u>Peroxide value [mEq O₂/kg]</u>		<u>Anisidine value [-]</u>		<u>Dienes [%]</u>		<u>Trienes [%]</u>	
		<u>$\bar{x}$</u>	<u>SD</u>	<u>$\bar{x}$</u>	<u>SD</u>	<u>$\bar{x}$</u>	<u>SD</u>	<u>$\bar{x}$</u>	<u>SD</u>	<u>$\bar{x}$</u>	<u>SD</u>
<u>Fresh (control samples)</u>	<u>Cold pressing</u>	<u>0.76^A</u>	<u>0.11</u>	<u>0.58^A</u>	<u>0.10</u>	<u>1.02^A</u>	<u>0.18</u>	<u>0.11^A</u>	<u>0.01</u>	<u>0.00^A</u>	<u>0.00</u>
	<u>1-step pressing*</u>	<u>0.18^B</u>	<u>0.11</u>	<u>1.24^B</u>	<u>0.54</u>	<u>2.49^B</u>	<u>1.38</u>	<u>0.25^B</u>	<u>0.03</u>	<u>0.00^A</u>	<u>0.00</u>
	<u>Hot pressing + extraction*</u>	<u>0.20^B</u>	<u>0.06</u>	<u>1.18^B</u>	<u>0.28</u>	<u>2.03^C</u>	<u>0.43</u>	<u>0.23^C</u>	<u>0.02</u>	<u>0.00^A</u>	<u>0.00</u>
<u>-20</u>	<u>Cold pressing</u>	<u>0.69^A</u>	<u>0.20</u>	<u>0.61^A</u>	<u>0.05</u>	<u>1.02^A</u>	<u>0.30</u>	<u>0.10^A</u>	<u>0.01</u>	<u>0.01^A</u>	<u>0.00</u>
	<u>1-step pressing*</u>	<u>0.23^B</u>	<u>0.05</u>	<u>0.23^B</u>	<u>0.14</u>	<u>2.02^B</u>	<u>0.90</u>	<u>0.13^B</u>	<u>0.04</u>	<u>0.05^B</u>	<u>0.01</u>
	<u>Hot pressing + extraction*</u>	<u>0.28^B</u>	<u>0.05</u>	<u>0.30^B</u>	<u>0.13</u>	<u>1.85^B</u>	<u>0.43</u>	<u>0.15^C</u>	<u>0.03</u>	<u>0.04^C</u>	<u>0.01</u>
<u>4</u>	<u>Cold pressing</u>	<u>0.79^A</u>	<u>0.16</u>	<u>0.99^A</u>	<u>0.31</u>	<u>1.18^A</u>	<u>0.28</u>	<u>0.11^A</u>	<u>0.02</u>	<u>0.01^A</u>	<u>0.00</u>
	<u>1-step pressing*</u>	<u>0.21^B</u>	<u>0.04</u>	<u>0.42^B</u>	<u>0.21</u>	<u>2.09^B</u>	<u>0.98</u>	<u>0.12^A</u>	<u>0.04</u>	<u>0.05^B</u>	<u>0.01</u>
	<u>Hot pressing + extraction*</u>	<u>0.28^C</u>	<u>0.05</u>	<u>0.45^B</u>	<u>0.18</u>	<u>1.97^B</u>	<u>0.43</u>	<u>0.16^B</u>	<u>0.03</u>	<u>0.04^C</u>	<u>0.01</u>
<u>20</u>	<u>Cold pressing</u>	<u>0.83^A</u>	<u>0.20</u>	<u>1.22^A</u>	<u>0.48</u>	<u>1.74^A</u>	<u>0.22</u>	<u>0.14^A</u>	<u>0.02</u>	<u>0.01^A</u>	<u>0.00</u>
	<u>1-step pressing*</u>	<u>0.27^B</u>	<u>0.06</u>	<u>0.81^B</u>	<u>0.56</u>	<u>2.78^B</u>	<u>1.09</u>	<u>0.15^A</u>	<u>0.04</u>	<u>0.05^B</u>	<u>0.02</u>
	<u>Hot pressing + extraction*</u>	<u>0.38^C</u>	<u>0.04</u>	<u>0.79^B</u>	<u>0.51</u>	<u>2.66^B</u>	<u>0.67</u>	<u>0.19^B</u>	<u>0.04</u>	<u>0.04^C</u>	<u>0.01</u>

241 *- refined oils; SD – Standard deviation; A-D – Denotes statistically significant differences ($P \leq 0.05$)

242 | **Table 4. Correlation coefficients calculated for relationships between the parameters defining oils quality.**
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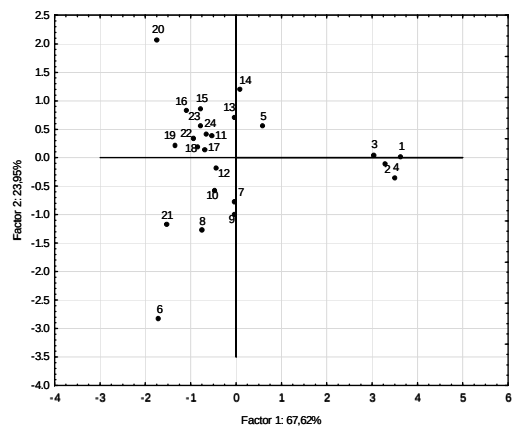
Parameter	Acid value	Peroxide value	Anisidine value	Dienes	Trienes	Parameter	Acid value	Peroxide value	Anisidine value	Dienes	Trienes
Fresh (control samples)						-20°C					
Acid value		-0.64*	-0.45*	-0.88*	-	Acid value		0.71*	-0.50*	-0.35	-0.78*
Peroxide value			0.08	0.53*	-	Peroxide value			-0.30	-0.20	-0.68*
Anisidine value				0.69*	-	Anisidine value				0.25	0.71*
Dienes					-	Dienes					0.41*
Trienes						Trienes					
4°C						20°C					
Acid value		0.76*	-0.48*	-0.31	-0.78*	Acid value		0.44*	-0.42*	-0.26	-0.75*
Peroxide value			-0.29	-0.05	-0.62*	Peroxide value			0.31	-0.13	-0.09
Anisidine value				0.29	0.76*	Anisidine value				0.32	0.72*
Dienes					0.28	Dienes					0.37
Trienes						Trienes					

244 | * - correlation coefficients statistically significant at $P \leq 0.05$

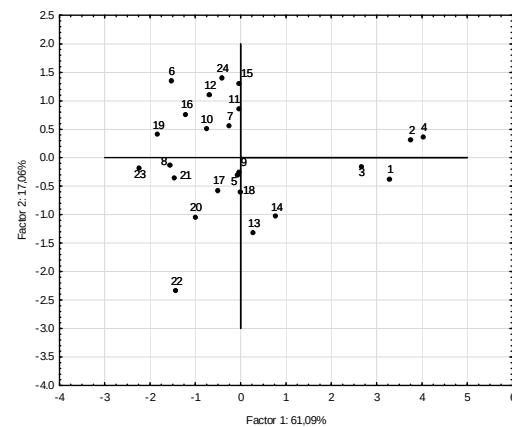
245 The PCA analysis diagrams (Figure 1) show the group with numbers from 1 to 4 isolated
246 from other samples. These are oils produced by cold pressing and not subjected to the
247 refining process. They differ from the other ones both in the control sample (Figure 1A)
248 and following storage under any conditions. In the control sample, in addition to the
249 above-mentioned ones, two oils produced by the hot pressing method (Nos 6 and 20)
250 also differed. Following storage at -20°C (Figure 1B), in addition to the cold-pressed
251 samples, one hot pressed sample (No 22) differed. As regards the samples stored at
252 4°C (Figure 1C), samples Nos 5 and 22 (cold pressing), as well as 18 and 13 (pressing +
253 extraction) can be distinguished. Following storage at 20°C (Figure 1D), in addition to
254 sample Nos 1 to 4, sample No 6 (hot pressing), as well as Nos 8 and 16 (pressing +
255 extraction) are distinguished. Figures (Figure 1 A-D) show that in the case of quality
256 samples stored in room temperature are much more diversified than fresh samples.

257 The composition of fatty acids (Table 5) changed in the analyzed oils during storage.
258 ~~One of the apparent trends was an~~ increase in the share of saturated fatty acids in hot
259 pressed oils was observed. On the other hand, the decrease in their content in both cold
260 pressed, and pressed and extracted oils was unexpected. ~~The greatest increase in the~~
261 ~~content of saturated fatty acids was noted for refined hot pressed oils (6.33%).~~ A
262 permanent trend was an increase in the share of monounsaturated fatty acids, and a
263 decrease in the share of polyunsaturated fatty acids. Morello et al. [31] investigated
264 changes in fatty acid composition of virgin olive oil during storage. They reported a
265 similar changes to those obtained in this study. Percentage of oleic acid increased, as a
266 consequence of the maintenance of the percentage of the saturated fatty acids and
267 decrease of the polyunsaturated ones, linoleic and linolenic acids. The share of MUFAs
268 increased most following storage at 20°C for refined hot pressed oils (9.38%), and
269 pressed and extracted oils (9.35%). In the same samples, the greatest decrease in the
270 content of PUFAs also occurred by, respectively, 30.78% and 31.22%. This is due to the
271 fact that acids with a greater number of double bonds in the molecule are more
272 susceptible to oxidation processes [2932]. In the refining process a significant proportion
273 of substances with antioxidant properties were removed from oils, which also contributed
274 to the decrease in the share of polyunsaturated acids. This is clearly visible in Table 6,
275 which sums the contents of fatty acids belonging to particular groups. It also presents the
276 ratio of n-6 acids to n-3 acids. After storage at -20°C this ratio did not change. The
277 greatest change was noted for oils stored at room temperature. For these oils this ratio
278 increased the most; however, this change did not significantly reduce the nutritional
279 quality of the oil. Simopoulos [3033] reports that the optimum ratio of n-6 acids to n-3
280 acids for humans ranges from 4/1 to 1/1. This ratio was maintained in most stored oils.
281 Only in refined oils produced by the single pressing method, stored at room temperature,
282 did the average ratio of n-6 acids to n-3 acids exceed 4/1.

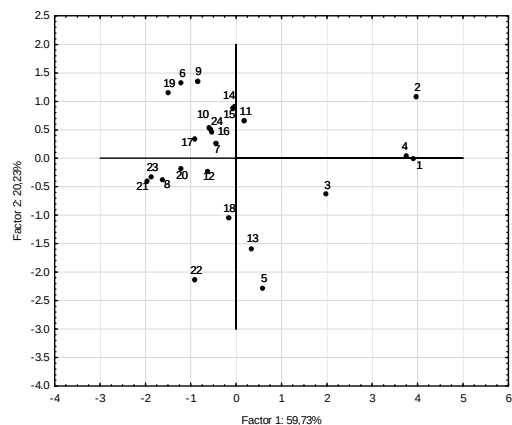
283 | A.



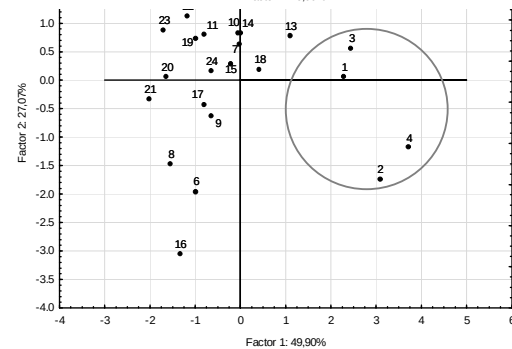
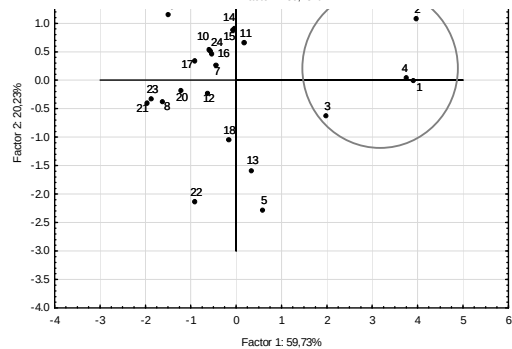
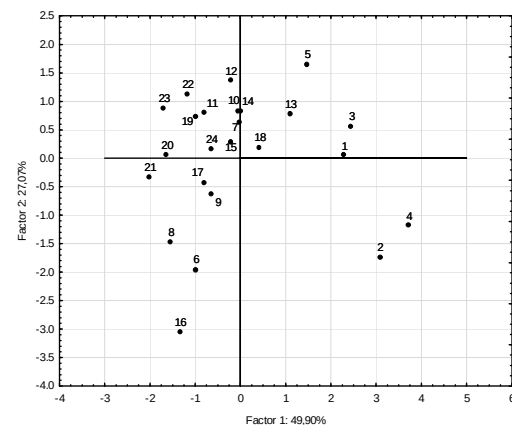
B.



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D.



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Figure 1. Diagrams of ~~principal~~principal component analysis (PCA) of quality features. Numbering corresponds to the samples shown in Table 1. (samples of cold pressed oils are marked in circles). Storage conditions of rapeseed oils – A: fresh; (control samples); B: -20°C; C: 4°C; D: 20°

329 | Table 5. The fatty acid composition in rapeseed oils, depending on the method of production.
330

Storage temperature [°C]	Production method	Fatty acid								
		palmitic	stearic	oleic	linoleic	linolenic	arachidic	eikozenoic	eicosenoic	others
Fresh (control samples)	Cold pressing	4.64	1.67	63.80	18.61	8.20	0.58	1.48		1.04
	1-step pressing*	4.60	1.55	62.95	19.27	8.74	0.55	1.38		0.96
	Hot pressing + extraction*	4.89	1.67	63.10	19.23	8.96	0.45	1.14		0.55
-20	Cold pressing	4.45	1.71	68.44	16.03	4.63	0.54	1.48		2.73
	1-step pressing*	4.57	1.74	67.05	16.67	4.88	0.51	1.35		3.22
	Hot pressing + extraction*	4.50	1.62	67.19	16.75	5.06	0.49	1.15		3.34
4	Cold pressing	4.48	1.77	68.94	15.36	4.38	0.52	1.44		3.13
	1-step pressing*	4.60	1.80	67.76	16.21	4.50	0.51	1.31		3.31
	Hot pressing + extraction*	4.66	1.68	68.26	16.02	4.39	0.51	1.22		3.33
20	Cold pressing	4.50	1.82	68.87	15.54	4.26	0.52	1.41		3.08
	1-step pressing*	4.70	1.72	69.13	15.63	3.76	0.70	1.24		3.12
	Hot pressing + extraction*	4.74	1.73	69.05	15.37	4.02	0.50	1.21		3.31

* - refined oils

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332

333 | Table 6. Amount of SFA, MUFA, PUFA and n-6/n-3 ratio of analysed oils.
334

Storage temperature [°C]	Production method	Fatty acids			
		SFA	MUFA	PUFA	n-6/n-3
Fresh (control samples)	~~cold~~ Cold pressing	6.88	66.31	26.80	3.47/1
	1-step pressing*	6.70	65.29	28.01	3.42/1
	Hot pressing + extraction*	7.01	64.80	28.19	3.31/1
-20	~~cold~~ Cold pressing	6.70	72.65	20.66	3.47/1
	1-step pressing*	6.83	71.62	21.55	3.42/1
	Hot pressing + extraction*	6.61	71.68	21.81	3.31/1
4	~~cold~~ Cold pressing	6.77	73.50	19.74	3.50/1
	1-step pressing*	6.92	72.38	20.71	3.60/1
	Hot pressing + extraction*	6.84	72.81	20.41	3.64/1
20	~~cold~~ Cold pressing	6.84	73.36	19.80	3.65/1
	1-step pressing*	7.12	73.49	19.39	4.15/1
	Hot pressing + extraction*	6.98	73.56	19.39	3.82/1

335 | * - refined oils

336 | 4. CONCLUSION

337 | Based on the obtained results it can be concluded that storage has an effect on the
338 | stability of rapeseed oil. The extent and severity of the effect depends on the storage
339 | conditions, the production method applied, and the purification of the oil. Higher
340 | temperatures are more conducive to oxidative changes in oils, which are indicated by
341 | higher values of all the analyzed distinguishing quality features of oils. Refined oils are
342 | more susceptible to these changes as in the process of purification a number of
343 | substances with antioxidant properties are removed. The production method is also
344 | important as during hot pressing the applied high temperature is also conducive to fat
345 | oxidation and reduces the stability of oils ~~at the very beginning~~.
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