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The Influence of Storage Conditions of Cold-Pressed Rapeseed Oil on Its Quality Parameters

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Abstract: Stored vegetable oil undergoes numerous processes, i.e., oxidation, hydrolysis, and thermal polymerization. As a result, its quality and organoleptic parameters deteriorate. The content of natural chlorophyll and carotenoid pigments determines the color of rapeseed oil. Almost imperceptible changes in the color of the oil may indicate the deterioration of its quality. Therefore, vegetable oils must be stored in the appropriate conditions to protect them against unfavorable factors leading to deterioration. This publication examines and describes the influence of storage temperature, type of packaging (clear glass, colored glass, PET), and presence of an oxygen-free atmosphere on the quality of stored cold-pressed rapeseed oil for three and six months. Changes in the following parameters were verified: the content of chlorophyll and carotenoid pigments, oil color (measured by the CIE Lab method), acid value, and radical scavenging activity (%RSA) by the ABTS (diammonium 2,2′-azinobis[3-ethyl-2,3-dihydrobenzothiazole-6-sulphonate) method. The decrease in the content of natural dyes was 7 to 87% after three months, depending on storage conditions, and after six months, from 12 to 97%. To determine which factors were responsible for the change in the physicochemical properties of the oil during storage, a principal component analysis (PCA) was performed.

Keywords: cold-pressed rapeseed oil; edible oil storage conditions; natural dyes; oxidative stability

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Citation: Marcinkowski, D.; Bochniak, M.; Wereńska, M.; Czwartkowski, K. The Influence of Storage Conditions of Cold-Pressed Rapeseed Oil on Its Quality Parameters. *Appl. Sci.* **2023**, *13*, 11746. https://doi.org/10.3390/app132111746

Academic Editor: Luca Fiori

Received: 3 October 2023
Revised: 23 October 2023
Accepted: 24 October 2023
Published: 26 October 2023

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1. Introduction

Due to their health-promoting properties, vegetable oils are essential to a balanced human diet [1–4]. Apart from food applications, vegetable oils and their derivatives are also used in synthesizing polymeric materials, robotics, and 3D printing [5–7]. Rapeseed oil is the world's third-most consumed vegetable oil, with an annual production of 32.63 million metric tons in 2022 [8]. This oil is characterized by a low content of saturated fatty acids (approx. 5–10%) and a high content of monounsaturated fatty acids (44–75%). It is also a rich source of vitamins A, D, E, and K [9,10]. Cold-pressed rapeseed oil has a favorable polyunsaturated fatty acid content due to the desired ratio of linoleic (n-6) to linolenic (n-3) acid of 2:1. This oil also contains tocopherols, sterols, polyphenols, and β-carotene. Consuming vegetable oils with a high content of unsaturated fatty acids is recommended, because large amounts of saturated fatty acids cause cardiovascular diseases [11,12]. Cold-pressed rapeseed oil is a high-quality edible oil that can be used as an addition to salads, for direct consumption, and sometimes for frying [13]. It is obtained by pressing rapeseed at a temperature below 50 °C. The technological process assumes only pressing and filtration of the oil, without further refining stages, i.e., degumming, winterization, bleaching, and deodorization [14].

The content of natural chlorophyll and carotenoid pigments determines the color of the oil. Almost imperceptible changes in the color of the cold-pressed oil may indicate a deterioration of its quality. Therefore, vegetable oils must be stored in appropriate

conditions to protect them against unfavorable factors (i.e., temperature, UV radiation, and oxygen) that could lead to deterioration [15,16]. Vegetable oils are stored in packaging that differs in volume and the material from which it is made. These include bottles made of colored and colorless glass and bottles made of plastics—most often polyethylene terephthalate (PET) [17]. This last group of packaging types is prevalent due to its high mechanical strength, low production price, and ability to form the desired shape quickly. The disadvantages of packaging made of plastic include the possibility of interactions with the oil [18,19]

Stored vegetable fat undergoes processes over time, i.e., oxidation, hydrolysis, and thermal polymerization, as a result of which its quality and organoleptic parameters deteriorate. Phosphatides, free fatty acids, volatile fragrances, waxes, and metal ions contained in oils are undesirable substances that adversely affect the physicochemical and sensory properties during the storage of rapeseed oil [20,21]. The most common form of deterioration of vegetable oils is their oxidation, which involves the spontaneous addition of oxygen molecules by unsaturated fatty acids, forming hydroperoxides. Oxidation of cold-pressed vegetable oils with atmospheric oxygen and without the participation of enzymes is called autoxidation, and its products can catalyze this process [22]. They result mainly from unfavorable changes in fatty acids, which are characterized by different positions of multiple bonds and the length of carbon chains. A cis configuration describes the fatty acids contained in fresh vegetable oils. The oxidative stability of an oil is the resistance to oxidation during its processing and storage. It can be expressed as the time required to reach the critical oxidation point, whether it is a sudden acceleration of the oxidation process or a sensory change [23–25].

Based on the literature analysis, we found that there is a lack of comprehensive studies on the impact of storage conditions on cold-pressed vegetable oils based on their quality parameters. Therefore, this publication aims to present the impact of changes in storage parameters (i.e., temperature, presence of oxygen, and type of packaging) on the quality of cold-pressed rapeseed oil during three- and six-month periods of storage. The publication examines and describes the influences of storage temperature, type of packaging (e.g., clear glass, colored glass, PET), and presence of an oxygen-free atmosphere on the quality of cold-pressed rapeseed oil stored for three and six months. Changes in the following parameters were verified: the content of chlorophyll and carotenoid pigments, oil color (measured by the CIE Lab method), acid value, and radical scavenging activity (%RSA by the ABTS method).

2. Materials and Methods

2.1. Raw Materials and Equipment

This study used raw winter rapeseed oil (variety "00", characterized by a low content of erucic acid and glucosinolates, GMO-free), cold-pressed at a temperature of 40 °C and filtered on a plate filter. The physicochemical properties of the obtained oil are presented in Table 1.

Table 1. Physicochemical properties of the rapeseed oil used in the research.

Parameters	Units	Rapeseed Oil
Acid value	mg KOH·g^{-1}	1.16
Density (15 °C)	kg·m^{-3}	942
Kinematic viscosity (40 °C)	mm^2·s^{-1}	37.3
Flashpoint	°C	241
Calorific value	kJ·kg^{-1}	38,213

Anhydrous methanol (>99%), ethanol (95,6%, v/v), and ABTS ($\geq$98%, v/v) were purchased from Sigma-Aldrich, Poznan, Poland. Absorption spectra were taken using a Metertech SP-830 PLUS spectrophotometer (Taipei, Taiwan). An oil color measurement via the CIE Lab method was performed using a Minolta CR 310 chromameter (Chongqing,

China) calibrated on a white standard. The initial values of each tested parameter were also measured for fresh oil, which was directly taken from the main tank.

2.2. Storage Conditions

The research verified the influence of the following factors on the change in oil quality parameters: the type of packaging, oil storage period, temperature, and presence of oxygen. Oil samples were stored in three types of 0.5 dm^3 containers, i.e., a bottle made of PET, a bottle made of colorless glass, and a bottle made of dark green glass. The oil was stored in an atmosphere of inert gas (nitrogen) and air. One batch of samples was held in a refrigerator at approximately 4 °C, without access to solar radiation, and the other was held in an air-conditioned room at a temperature of 25 °C with sunlight control. Each case was carried out in three containers under the same storage conditions. Figure 1 shows the analyzed rapeseed oil samples:



Figure 1. Samples were stored at 25 °C with free access to sunlight.

2.3. Measurement Methodology

2.3.1. Determination of the Content of Chlorophyll and Carotenoid Pigments

Measurements of chlorophyll and carotenoid pigment content were made using the method described by Wellburn [26] and PN-A-86934:1995 [27]. Approximately 1.5 g of the tested oil was dissolved in 10 mL of methanol; then, 2.5 mL of the obtained solution was taken and transferred to a spectrophotometric cuvette. UV-Vis absorbance measurements were performed in triplicate at wavelengths of λ = 470.0 nm, λ = 652.4 nm, and λ = 665.2 nm.

The obtained absorbance values were used to calculate the content of chlorophyll pigments and carotenoids in the tested oils. The following formulas were used for this Purpose (1)–(3):

$$chlorophyll\ a = 16.72 \times \mathrm{B} - 9.16 \times \mathrm{A} \quad (1)$$

$$chlorophyll\ b = 34.09 \times \mathrm{A} - 15.28 \times \mathrm{B} \quad (2)$$

$$carotenoids = \frac{1000 \times \mathrm{C} - 1.63 \times (1) - 104.96 \times (2)}{221} \quad (3)$$

where:

A—absorbance at λ = 652.4 nm
B—absorbance at λ = 665.2 nm
C—absorbance at λ = 470.0 nm

2.3.2. Measurement of Oil Color Using the CIE Lab Method

The analyzed oil sample was poured into a Petri dish so that the obtained layer was approximately 0.5 mL high. The prepared piece was placed onto a calibration plate, and then the color of each sample was measured three times. After each measurement, the head of the Minolta CR 310 chromameter was thoroughly cleaned to obtain reliable results.

2.3.3. Acid Value Measurement

Acid value (AV) was determined according to PN-EN ISO 660:2021. Then, 2.5 g of the analyzed oil and 15 mL of anhydrous ethanol were added to a 50 mL conical flask, and the resulting mixture was thoroughly mixed and heated at 60 °C for 20 min. After cooling, a few drops of phenolphthalein were added and titrated with potassium hydroxide solution at a concentration of 0.1 mol·dm^{-3} until the solution was peach-colored [28]. The *AV* was calculated from the following formula:

$$AV = 5.611 \cdot \frac{(a - b)}{c} \quad (4)$$

where:

a—volume of KOH used to titrate the oil sample (mL)
b—volume of KOH used to titrate the blank (mL)
c—mass of the oil sample (g)

2.3.4. Measurement of Radical Scavenging Activity

A solution of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt with a concentration of 7.00 mM and potassium persulfate with a concentration of 2.45 mM was prepared in a 10 mL volumetric flask. The solution was left in the dark for 12 h to generate the ABTS$^{\bullet+}$ radical. Afterward, 1 mL of the analyzed oil and 1 mL of anhydrous ethanol were added to the test tube, and the resulting solution was mixed thoroughly. A volume of 30 µL of the solution was then taken and transferred to a spectrophotometric cuvette. Then, a solution of the ABTS$^{\bullet+}$ radical was prepared in methanol so that the absorbance of electromagnetic radiation with a wavelength of λ = 734 nm reached 0.700.

Every 10 s, 3 mL of the prepared ABTS$^{\bullet+}$ radical solution was added to the previously prepared oil–ethanol mixture. The absorbance measurement was performed 10 min after adding the ABTS$^{\bullet+}$ radical solution. Subsequent measurements were performed every 10 s. Finally, the H_2O absorbance value was measured three times [29]. Three replicates were performed for each test sample, and the percentage change in *RSA* relative to the blank sample was calculated using Formula (5):

$$RSA\ (\%) = \frac{A_0 - A}{A_0} \times 100 \quad (5)$$

where:

A_0—absorbance of blank test (ethanol)
A—absorbance of tested sample

3. Results and Discussion

The filtered rapeseed oil was subjected to preliminary analyses, the results of which are presented in Table 2.

Table 2. Results of preliminary tests of rapeseed oil.

Chlorophylls (mg/kg)	19.9
Carotenoids (mg/kg)	83.4
L* [-]	96.013
a* [-]	−6.492
b* [-]	94.563
AV (mg_{KOH}/g)	1.119
ABTS$^{\bullet+}$ (%RSA)	1.77

The results obtained using the described methodology were averaged and converted into percentage changes with respect to the control sample according to the selected

parameters during the 3- and 6-month oil storage periods. The test results are collectively presented for each oil sample in Table 3.

Table 3. Percentage (%) changes in rapeseed oil parameters during storage.

Sample	Chlorophylls	Carotenoids	L*	a*	b*	AV	ABTS
PET, 3 months, N_2, 25 °C	−7.04	−6.14	−0.52	20.46	3.37	123.06	57.06
PET, 3 months, N_2, 4 °C	−7.04	−6.68	−2.57	40.10	3.29	59.87	27.68
PET, 3 months, Air, 25 °C	−73.24	−11.01	−1.55	17.79	1.80	166.76	67.80
PET, 3 months, Air, 4 °C	−7.04	−3.25	−2.80	31.19	2.82	67.02	49.72
PET, 6 months, N_2, 25 °C	−81.69	−13.72	−2.66	35.12	5.74	167.29	106.21
PET, 6 months, N_2, 4 °C	−12.68	−7.22	−2.96	60.58	4.94	89.81	77.97
PET, 6 months, Air, 25 °C	−83.10	−12.27	−2.62	51.68	4.27	221.36	119.77
PET, 6 months, Air, 4 °C	−12.68	−8.48	−3.31	52.42	4.39	102.41	70.06
Clear glass, 3 months, N_2, 25 °C	−87.32	−7.04	−1.58	24.15	2.31	125.29	50.85
Clear glass, 3 months, N_2, 4 °C	−8.45	−6.32	−2.53	31.59	4.68	50.09	29.38
Clear glass, 3 months, Air, 25 °C	−69.01	−22.38	−1.08	20.92	2.35	160.41	62.15
Clear glass, 3 months, Air, 4 °C	−19.72	−4.87	−2.65	35.66	3.84	71.58	53.11
Clear glass, 6 months, N_2, 25 °C	−97.18	−12.09	−2.70	40.28	2.03	155.85	98.31
Clear glass, 6 months, N_2, 4 °C	−21.13	−7.22	−3.03	51.11	4.85	103.31	85.31
Clear glass, 6 months, Air, 25 °C	−95.77	−35.38	−3.23	65.43	2.34	218.23	110.17
Clear glass, 6 months, Air, 4 °C	−21.13	−5.96	−2.92	48.91	3.85	108.04	66.10
Colored glass, 3 months, N_2, 25 °C	−21.13	−16.25	−0.39	8.90	1.58	93.66	36.16
Colored glass, 3 months, N_2, 4 °C	−12.68	−3.07	−1.25	23.58	2.79	27.52	11.86
Colored glass, 3 months, Air, 25 °C	−30.99	−17.69	−0.98	8.44	3.53	127.44	44.63
Colored glass, 3 months, Air, 4 °C	−4.23	−7.40	−1.22	28.23	2.43	66.31	39.55
Colored glass, 6 months, N_2, 25 °C	−57.75	−17.15	−1.89	33.70	3.53	140.57	85.88
Colored glass, 6 months, N_2, 4 °C	−14.08	−7.04	−2.86	60.77	3.65	71.76	71.19
Colored glass, 6 months, Air, 25 °C	−45.07	−28.70	−2.41	65.43	4.69	184.09	98.87
Colored glass, 6 months, Air, 4 °C	−15.49	−13.36	−2.00	48.91	3.63	81.05	50.85

Based on the obtained results, it was found that over time, the content of chlorophyll and carotenoid pigments decreased in cold-pressed rapeseed oil. The decrease in the content of natural dyes after three months was 7 to even 87%, depending on storage conditions, and after six months, from 12 to 97%. The analysis of the results shows that the reduction in the content of natural dyes was mainly influenced by the temperature at which the samples were stored. Higher oil storage temperature causes a faster decomposition of natural dyes, as was confirmed by research conducted by Roszkowska et al. [30]. Factors such as packaging and the presence of air are of secondary importance to the decrease in the content of natural dyes. Sikorska et al. [31] showed that long-term exposure of oil to highly oxygenated air accelerates the decomposition of natural dyes. However, the amount of oxygenated air was limited in the studies that reflected the actual method of storing rapeseed oil. On this basis, this factor can be considered as not affecting the autoxidation process of vegetable oils, which was also confirmed by Szydłowska-Czerniak [32].

The next parameter to be tested was colorimetric color measurement, for which the CIE Lab method was used. It has been shown that during storage, a decrease in the photometric brightness of the tested samples can be observed (parameter L*, meaning oil clarity). The factor influencing the decrease in oil clarity during storage is mainly the packaging in which the oil is stored. In a study conducted by Li et al., the color changes in stored rapeseed oil samples from different producers were examined and described. In the case of oil samples stored with free access to sunlight, it was found that the value of the L* (photometric brightness) parameter increased over time. This phenomenon can be explained by the higher rate constant of the decomposition of chlorophyll dyes than in the case of carotenoid dyes [33]. This research also observed that the decrease in oil photometric brightness may correlate with air in the packaging. Oils stored in a nitrogen atmosphere showed a less significant decrease in the L* parameter. Moreover, increases in the parameters a* (redness) and b* (yellowness) were observed. According to Kachel-Jakubowska [16], this may be

related to the decomposition of natural dyes. The increase in the a* parameter was solely associated with the breakdown of chlorophylls, which decompose under the influence of UV radiation. However, the simultaneous change in both parameters resulted from slower carotenoid degradation, as shown in previous studies [1]. Based on the above research, it can be concluded that cold-pressed vegetable oils are best stored away from sunlight.

According to CODEX ALIMENTARIUS [34], AV should not exceed 4 mg KOH/g of the tested oil; this determines the fresh oil. According to this criterion, temperature has the most significant impact on the oil's freshness. The tests showed that oils stored at 25 °C contained much higher quantities of free fatty acids than the other tested samples. The storage time is also essential because, as demonstrated over three months, the AV may even increase twice, with additional storage conditions remaining unchanged. Still, the rate of these changes depends mainly on the effect of temperature, as indicated by Maghsoudlou et al. [35]. Moreover, Wroniak et al. showed that the AV of storage cold-pressed rapeseed oil changes insignificantly during storage in a nitrogen atmosphere [36].

The last quality parameter to be examined was radical scavenging activity, which is understood as an increase in the activity of the $ABTS^{\bullet+}$ radical during oil storage. Research by Rabiej et al. [37] proved that as the temperature of storage of vegetable oils increases, their %RSA decreases, which was confirmed in this study. Samples stored at 4 °C show much lower oxidative activity of the $ABTS^{\bullet+}$ radical. Furthermore, Habeebullah et al. [38] showed that regardless of storage conditions, the %RSA of samples decreases with increasing storage time. This confirms the results obtained in this work. In order to clearly determine which factors mainly influence the changes in the physicochemical properties of oils during storage, a principal component analysis (PCA) was performed on the samples shown in Figure 2a,b.

The statistical analysis confirmed that two factors mainly influence the change in the physicochemical parameters of rapeseed oil during storage. The first is the storage temperature (blue field in Figure 2b), because the higher the temperature of the stored samples, the faster the degradation of the components dissolved in the oil. The second important factor is storage time (brown fields in Figure 2b). The analysis showed that samples stored in the same conditions, but for a shorter period, showed significantly better properties than samples stored for longer durations. Other factors affecting the change in oil parameters during storage are irrelevant. Chew [2] confirmed the obtained results and indicated that they are significant, especially for oils not subject to refining. To maximize the freshness and nutritional properties of oils, they should be stored in cool places as soon as possible after their production date. In turn, Dordevic et al. [39] indicated the production process parameters as another factor determining the rate of autoxidation of vegetable oils. They showed that cold-pressed and sedimented oil decompose much more slowly than oils pressed at elevated temperatures. Kreivaitis et al. [15] suggested adding natural antioxidants (e.g., plant extracts), which would slow down the autoxidation processes of oils and give them innovative sensory properties.

Further research on changes in the physicochemical properties of oils during storage should focus on the correlation between changes in the oxidative stability of the oil and changes in its fatty acid profile. Rezvankhah et al. [40] showed that the higher oxidative stability of the sample may have been caused by a greater degree of degradation of unsaturated fatty acids. However, Symoniuk et al. [21] showed that a higher content of unsaturated fatty acids in oils made them more susceptible to autoxidation and free radicals with the ABTS method. Moreover, they showed a correlation between oxidative stability and the content of α-linolenic fatty acid.

The statistical analysis confirmed that two factors mainly influence the change in the physicochemical parameters of rapeseed oil during storage. The first is the storage temperature (blue field on the right graph), because the higher the temperature of the stored samples, the faster the degradation of the components dissolved in the oil. The second important factor is storage time (brown fields on the right graph). The analysis demonstrated that samples stored in the same conditions, but for a shorter period of time,

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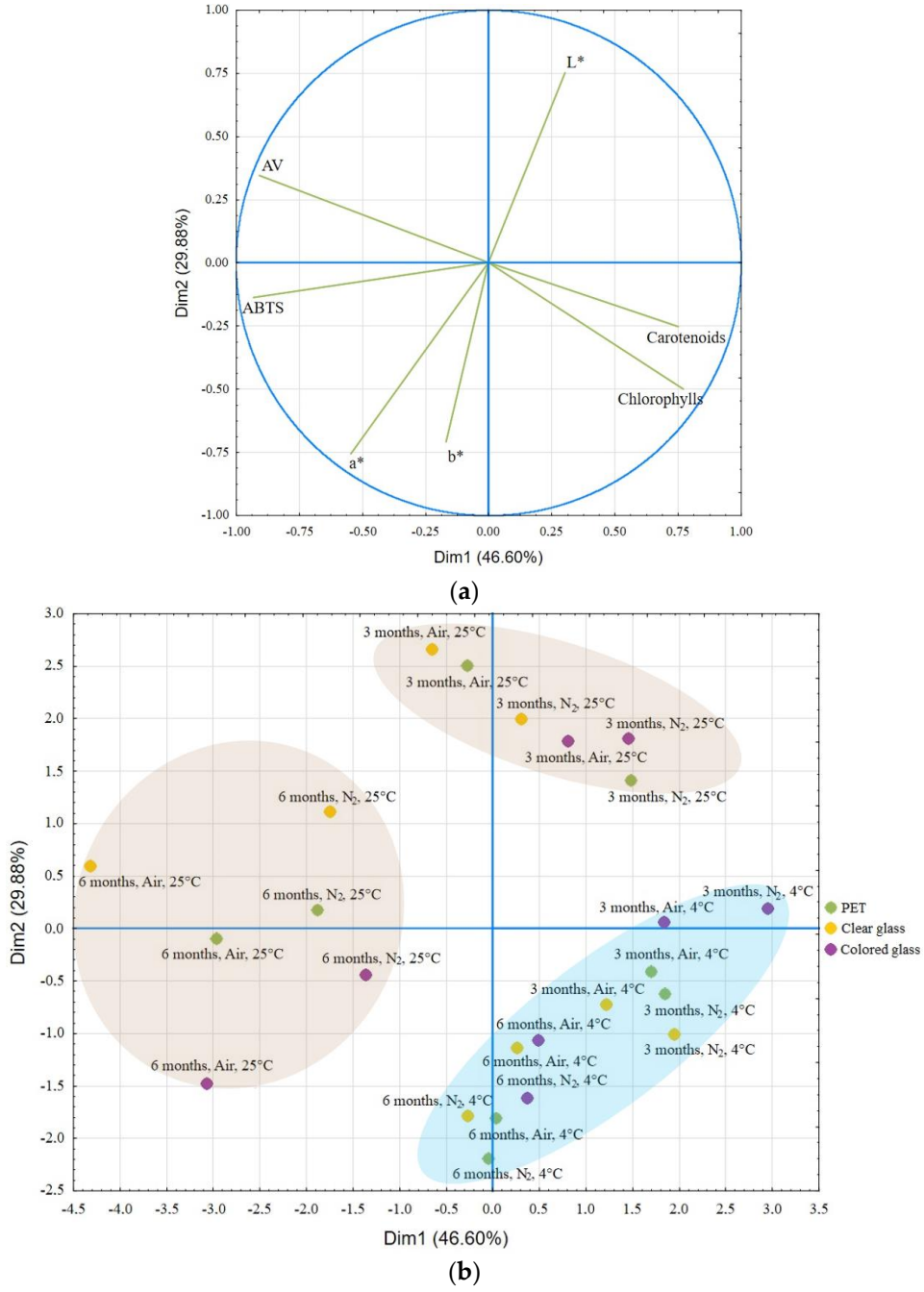


Figure 2. (**a**) PCA analysis of the tested rapeseed. Distribution of factors affecting the product. (**b**) PCA analysis of the tested rapeseed. Analysis of the obtained results.

4. Conclusions

The storage conditions of cold-pressed vegetable oils have a crucial impact on their quality. The research performed herein showed that changes in the physicochemical parameters of vegetable oils during storage are mainly influenced by two external factors: temperature and storage time. This is particularly important for the content of natural dyes used for the products. Oils stored in unsuitable conditions undergo autoxidation and other fat-degrading processes more quickly, and their consumption may negatively impact consumers, as they are often held in colored glass bottles. Still, as this research shows, the type of packaging has a marginal impact on the change in the properties of rapeseed oil over time.

Moreover, the influence of rapeseed oil storage conditions on the sensory properties of this product was noticed. The change in the color of oils determined by the CIE Lab method is probably associated with a change in other organoleptic values (e.g., taste, smell). Therefore, subsequent research should focus on assessing organoleptic changes in stored oils.

As shown in the scientific discussion, changes may also occur in the profiles of fatty acids, which are correlated, among others, with the acid value and oxidative stability of oils. The research results presented in this publication confirm that cold-pressed rapeseed oil should be stored at a reduced temperature, without exposure to solar radiation. Due to the increase in the share of cold-pressed oils on the market, this information is crucial for producers, sellers, and consumers. Therefore, they should not be stored and sold like traditional refined oils. Crude oils should be purchased fresh, directly from a manufacturer who has ensured their proper storage after production.

Author Contributions: Conceptualization, D.M. and K.C.; methodology, M.B. and M.W.; software, D.M. and K.C.; validation, D.M. and K.C.; formal analysis, D.M. and K.C.; investigation, M.B. and M.W.; resources, M.B. and M.W.; data curation, M.B. and M.W.; writing—original draft preparation, D.M.; writing—review and editing, K.C.; visualization, D.M. and K.C.; supervision, D.M. and K.C.; project administration, D.M. and K.C.; funding acquisition, D.M. and K.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest.

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