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Effects of Centrifugation on the Oxidative Stability and Antioxidant Profile of Cold-Pressed Rapeseed Oil during Storage

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Abstract: The recognition and growing consumption of cold-pressed rapeseed oil (CPRO) is due to by its unique, health-promoting properties; it is a rich source of omega-3 fatty acids and antioxidants. Nevertheless, the high content of unsaturated fatty acids and plant tissue particles makes CPRO more susceptible to oxidation. These pro-oxidant plant particles can be eliminated via centrifugation, thus improving the quality and stability of CPRO. Therefore, we aimed to determine whether the clarification of CPRO via centrifugation affects its quality parameters, pigment and phenolic profile, and antioxidant properties. These characteristics were analyzed and compared between centrifuged CPRO and CPRO clarified via natural decantation. Changes were monitored for 6 months of oil storage at 22 °C. Based on the results, no changes in the oxidation profile were found between centrifuged CPRO and decantated CPRO. When the storage is longer than 1 month, centrifugation is more beneficial with regard to delaying hydrolytic deterioration, while decantation provides slightly better pigment and polyphenol profiles. We did not observe that centrifugation improved the oil's antioxidant properties. Further research should be continued to establish the effects of centrifugation on CPRO quality, including parameters such as rapeseed quality, maturity degree, varieties, and stricter storage conditions.

Keywords: cold-pressed rapeseed oil; oxidative stability; antioxidant profile; centrifugation; storage

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1. Introduction

Rapeseed (*Brassica napus* L.) belongs to the cabbage family (*Brassicaceae*) and represents a third of the most important oilseed crops after oil palm and soybean [1]. According to the latest forecasts of the United States Department of Agriculture (USDA), the global production of vegetable oils in 2022/23 would amount to 219.8 million tons, and rapeseed provides over 14% of the global supply of vegetable oil [2]. Interestingly, several decades ago it was used primarily for industrial oil production [3]. Currently, rapeseed oil (RO) is one of the most commonly used edible oils in households and the food industry. Its appreciation and growing consumption are primarily due to its favorable nutritional composition, being rich in unsaturated fatty acids and bioactive compounds such as phenolic acids, phytosterols, flavones, and vitamin E. In particular, cold-pressed oils (CPO) successfully fit into the growing consumer interest in unprocessed foods, which are recognized as functional foods, for the various health-promoting attributes provided in comparison to their refined counterparts [4,5]. In addition, the production of cold-pressed oils is a chemical-free manufacturing process, which is in line with the growing interest of the food industry in the search for methods that minimize negative impacts on the natural environment [6]. Rapeseed oil is a well-established rich source of healthy fatty acids and many other health-promoting bioactive compounds [7]. According to the literature, triacylglycerols make up

94.4 to 99.1% of the total weight of rapeseed oil, while the remainder of non-glycerol components are present in much smaller quantities (1–3%) [4]. The unquestionable advantage of RO is the high content of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) (62% and 30%, respectively) compared to saturated fatty acids (7%) [8,9]. Such a composition of fatty acids contributes to decreasing the level of bad cholesterol (LDL) and thus, improves the ratio of good (HDL) to bad cholesterol. Moreover, rapeseed oil contains unique and nutritionally favorable linoleic (C18:2 n-6) to α-linolenic essential fatty acids (C18:3 n-3) ratio (2:1). In this respect, rapeseed oil is superior to sunflower, soybean, olive, safflower, poppy seed, corn, pumpkin seed, grape seed, sesame, and peanut oils, which contain an excess of linoleic acid [10–14]. Rapeseed oil is also known for its naturally occurring antioxidants such as polyphenols, tocopherols, and carotenoids [4]. On the other hand, the high content of unsaturated fatty acids makes rapeseed oil more susceptible to autoxidation [15]. Moreover, owing to the low degree of processing, cold-pressed rapeseed oil contains more oxidation-promoting components (metals, chlorophylls, and lipid peroxides) than its refined counterparts [16]. Oxidation negatively affects the quality of CPRO, leading to loss of nutritional value via degradation of bioactive compounds and the formation of anti-nutritional derivatives that are harmful to human health [17]. The oil oxidation leads to the gradual production of breakdown products; primary oxidation products are formed at the beginning of this process (peroxides, dienes, free fatty acids), then secondary products (carbonyls, aldehydes, trienes, malondialdehydes) and tertiary products are formed at the late stage of the oxidation process [18]. These compounds are characterized by high activity, and may significantly contribute to increasing the risks of chronic diseases, including cancer, as well as cardiovascular and neurological diseases [19]. Moreover, oxidation negatively affects the sensory qualities of CPO, impairing its flavor and aroma [20]. Throughout the oil oxidation process, primary oxidation products are subsequently broken down into secondary lipid oxidation products, such as alcohols, ketones, and aldehydes resulting in flavor deterioration [21]. Among edible vegetable oils, CPRO with a high content of unsaturated fatty acid may be particularly susceptible to oxidation [22,23]. Because of the low processing degree, CPO contains plant tissue-derived particles in suspension and water droplets, which can deteriorate the quality by promoting the oxidation and hydrolysis processes [24]. According to the *Codex Alimentarius*, the CPOs can be purified only by washing with water, settling, filtration, and centrifugation. The filtration procedure is most often carried out as a final step of the CPO elaboration process and is one of the commonly used stabilization processes for cold-pressed oils [25,26]. The downside of this oil clarification method is the possible loss of valuable CPO components such as phenols and carotenoids, and also changes in the color of the oil [27]. According to the literature, natural sedimentation followed by decantation can be more favorable than filtration in delaying oxidative deterioration and minimizing the loss of the healthy components of the oil [24]. On the other hand, waiting for the sediments to settle via gravity is time-consuming, and it often takes several months for the oil to clear. Besides, longer contact of the sediment with the oil can result in a faster oxidation rate in the CPRO. Furthermore, the effect of centrifugation on the oxidative status and antioxidant characteristics of CPRO remain underexplored.

Hence, this study aimed to evaluate the impact of centrifugation on the oxidative stability and antioxidant content of CPRO during 6 months of storage at room temperature (22 °C). In addition, the effects of centrifugation on the micro-nutritional components of CPRO, including phenols and pigments, were evaluated. For these purposes, the oxidative changes, phenol and pigment content, and antioxidant capacity were controlled in experimental centrifuged and non-centrifuged CPO samples over the study duration, statistically evaluated, and discussed to establish the impact of the centrifugation process on cold-pressed rapeseed oil.

2. Materials and Methods

2.1. Materials

The rapeseed (*Brassica napus* L.) was cultivated and harvested by a local producer from a farm in the town of Lanzhot, in the district of Breclav (Czech Republic). The seeds were first washed and dried and then stored or vacuum-packed to preserve their aroma and quality for further pressing. The oil was extracted via a screw press. Cold-pressed oil has not undergone a filtration procedure in order to preserve as many beneficial substances as possible. Oil was collected, subjected to natural sedimentation, and decanted. We obtained 5 L of raw cold-pressed rapeseed oil for this research purpose. Unfortunately, we do not have exact results regarding the yield. The oil was stored in the dark at an average ambient temperature of 22 °C until further investigation.

2.2. Methods

2.2.1. Preanalytical Oil Sample Preparation

Part of the CPRO was centrifuged at 6000 rpm for 5 min and at a temperature of 15–20 °C. Non-centrifuged, cold-pressed rapeseed oil (NC-CPRO) was stored in dark bottles and hidden from the effects of daylight at room temperature. The study room temperature ranged between 21 and 24 °C, averaging 22 °C.

Centrifuged CPRO (C-CPRO) was stored under the same conditions (Figure 1).

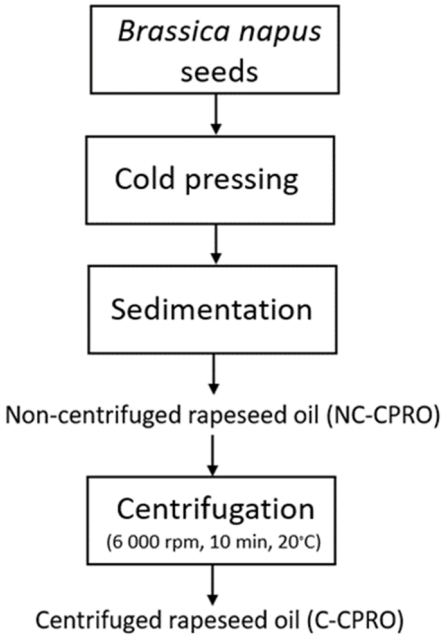

Figure 1. Flowchart to obtain rapeseed oil (N-CPRO) and centrifuged rapeseed oil (C-CPRO).

The oxidative status, antioxidant profile, and bioactive compounds of oil samples were observed for 6 months of storage. The CPRO samples were analyzed (each sample in at least triplicate) at three time points, namely at the beginning of the experiment duration (T0) then after 3 months (T1), and after 6 months (T2) of storage.

2.2.2. Chemical Characteristic

Peroxide Value

The peroxide value (PV) of the CPRO was estimated according to ISO 3960:2017 standard method. Briefly, 30 mL of chloroform and glacial acetic acid (2:3 ratio) were added to 5 g of CPRO and mixed. Then, 30 mL of dH_2O and 5 mL of starch solution were added to the mixture. The CPRO samples were titrated with 0.01 M $Na_2S_2O_3$ until the color of the

mixture changed. The dH_2O was used instead of CPRO in the blank sample. The peroxide value was estimated according to the following formula:

$$PV = 10 \times (V\ Na_2S_2O_3) - Vblank)/m$$

where V $Na_2S_2O_3$—the volume of $Na_2S_2O_3$ used for the titration of the sample (mL), Vblank—the volume of $Na_2S_2O_3$ used for the titration of the blank sample (mL), m—the amount of the sample (g).

Acid Value

The acid value (AV) of the NC-CPRO and C-CPRO was determined according to ISO 660:2009. Briefly, 50 mL of diethyl ether was added to 5 g of CPRO, and mixed with 1 mL of phenolphthalein solution that served as an indicator. The samples were titrated with 0.1 M KOH. The AV was estimated according to the following equation:

$$AV = (56.11 \times V \times c/m) \times 10$$

where V is the amount of the used KOH (mL), c is the concentration of the KOH (0.1 M), and m is the amount of the sample (g).

2.2.3. Pigments and Total Polyphenols

Chlorophyll and Carotenoid Content

The content of chlorophylls and carotenoids was evaluated spectrophotometrically, according to the protocol suggested by Kraljić et al. [28]. The chlorophyll amount was estimated using Cecil Instruments (CE7210) spectrophotometer. The oil samples were diluted with cyclohexane at a ratio of 1:1 (2 mL of the oil + 2 mL of the cyclohexane). The absorbance was measured at 670, 630, and 710 nm for chlorophylls and at a wavelength of 445 nm for carotenoids. Cyclohexane was used as a blank solution for a spectrophotometer. The concentration of chlorophylls and carotenoids was expressed as mg of pigment per kg of CPRO.

The equations below were used:

$$\text{chlorophyll (mg/kg)} = 34.53\ [A670 - 0.5\ (A630 + A710)]$$

$$\text{carotenoid (mg/kg)} = [383 \times A445]/10\ *$$

* 10: cuvette width in mm

Total Polyphenols

The content of polyphenols was analyzed according to the protocol suggested by Li et al. [29]. The content of total polyphenols was determined as gallic acid equivalent (GAE) per 1 mL of CPRO sample. Briefly, approximately 1 mL of an extract was introduced into tubes followed by 5 mL of 1Folin-Ciocalteau reagent and 4 mL of 7.5% Na_2CO_3. The tubes were vigorously mixed and incubated for 30 min. After incubation, absorbance was collected at 765 nm (CECIL spectrophotometer) against a blank (1 mL of dH_2O instead of the CPRO sample). The following equation was used:

$$P = y/3.0959$$

y—measured absorbance value.

2.2.4. Antioxidant Capability

FRAP (Ferric Reducing Antioxidant Power) Method

The FRAP working solution was prepared by mixing acetate buffer (pH 3.6) with TPTZ solution (10 mM, dissolved in 40 mM HCl) and $FeCl_3$ solution (20 mM) in a 10:1:1 ratio. Samples for measurement were prepared in dark tubes at a ratio of 180 µL of analyzed

extract, 300 µL of dH_2O, and 3.6 mL of working solution. All CPRO samples were allowed to stand in the dark for 8 min. Then, absorbance was measured at 593 nm against a blank (960 µL of FRAP reagent was mixed with 7.2 mL of dH_2O). The results are given as the amount of Trolox (µmol) in 1 g of CPRO [30]. The following equations were used:

$$FRAP = y/0.0045$$

y = measured absorbance value.

Determination of the Antioxidant Potential (DPPH)

Briefly, 1 mL of 0.1 mM DPPH solution was mixed with 3 mL of CPRO extract. The mixture was mixed vigorously and left in darkness for 30 min. The mixture was allowed to stand for 30 min in the dark and absorbance was recorded at 517 nm. Ethanol and dH_2O at a ratio of 1:1 were used as a blank [31]. The activity of the CPRO's antioxidants in scavenging DPPH was estimated as follows:

$$\text{DPPH inhibition (\%)} = ((\text{A DPPH—A sample})/\text{A DPPH}) \times 100$$

where A means absorbance.

ABTS Radical Scavenging Activity

First, about 12–16 h before the measurement, 10 mL of 0.007 M ABTS and 10 mL of 0.00245 M potassium persulfate solution were mixed and set aside. The solution was then diluted with EtOH to an absorbance of 0.70 at 734 nm. Subsequently, 20 µL of CPRO extracts were mixed with 1980 µL of ABTS solution, and the mixtures were left for 5 min in the dark. The absorbance was read at 734 nm against a blank and ethanol was used in place of the oil extract [32]. The percentage of inhibition was estimated using the following formula:

$$\text{ABTS (\%)} = (A0 - A1)/A0 \times 100\%$$

A0 is blank absorbance and A1 is the RO absorbance.

3. Results and Discussion

3.1. Hydrolytic and Oxidative Profile of NC-CPRO and C-CPRO

The hydrolytic and oxidative profile of CPRO in both centrifuged and non-centrifuged samples were assessed and compared with the available quality parameters established for edible fats and oils by the *Codex Alimentarius*. The hydrolytic and oxidative features of NC-CPRO and C-CPRO are summarized in Table 1.

Table 1. Hydrolytic and oxidative profile of NC-CPRO and C-CPRO during storage.

Months of Storage	NC-CPRO	C-CPRO	% of Mean Change after 3 Months of Storage * (NC-CPRO; C-CPRO)	% of Mean Change after 6 Months of Storage * (NC-CPRO; C-CPRO)
Acid Value [mg KOH/g of the oil]				
T0 (0 months)	12.44 ± 9.53^{a}	12.58 ± 7.42^{a}	-;-	-;-
T1 (3 months)	49.69 ± 0.82^{b}	20.63 ± 0.73^{a}	299.44;63.99	-;-
T2 (6 months)	83.59 ± 2.38^{c}	28.03 ± 1.57^{a}	-;-	571.95;122.81
Peroxide Value [meqO_2/kg of the oil]				
T0 (0 months)	1.77 ± 0.27^{a}	1.72 ± 0.33^{a}	-;-	-;-
T1 (3 months)	8.49 ± 0.13^{c}	10.08 ± 0.15^{d}	379.66;486.05	-;-
T2 (6 months)	29.89 ± 0.14^{b}	29.20 ± 0.29^{b}	-;-	1588.70;1597.67

Different letters (^{a}, ^{b}, ^{c}, ^{d}) indicate statistically significant ($p < 0.05$) differences between NC-CPRO and C-CPRO. * % of mean change compared to fresh oil at T0 time point.

In the first phase of the experiment, both NC-CPRO and C-CPRO samples were characterized by similar acidities and they were not statistically different. However, acidity values reported in our study exceeded acceptable Codex values; the acidity of NC-CPRO and C-CPRO samples measured at T0 was 12.44 mg KOH/g and 12.58 mg KOH/g, respectively. The acidity of cold-pressed oils, which is to be lower than 4.0 mg KOH/g oil, is one of the most rigorous quality indicators and is difficult to achieve. The proper rapeseed storage conditions in terms of humidity, temperature, light exposure, and storage time as well as proper seed quality in terms of moisture level or damage degree are prerequisites to obtaining an CPRO with a desirable hydrolytic profile. The AV is one of the most important parameters for the assessment of the edible oil's quality, freshness, or identification of its adulteration. The AV is determined as the mass of KOH required to neutralize free fatty acids present in 1 g of the oil. Consequently, the AV is a relative measure of the rancidity of the edible oils because free fatty acids are formed during the degradation of triglycerides [33]. In the present study, with the advancement of storage (T1–T2), increasing acidity was observed in both types of oils. An almost 4-fold increase in the acid number was recorded in the case of NC-CPRO (46.69 mg KOH/g), and a nearly 2-fold increase was noted for C-CPRO (20.63 mg KOH/g) after three months of storage duration. After six months of storage, a significant difference between the AV of the oils was observed. The acidity of the NC-CPRO reached the value of 83.59 mg KOH/g, and C-CPRO had an acidity of 28.03 mg KOH/g. Even though both oils did not meet the quality requirements already at the initial point of the experiment, the NC-CPRO showed significantly worse hydrolytic quality at the T1 and T2 time points of the experiment duration. The AV of NC-CPRO increased by 299.44% and 571.95% in T1 and T2 time points, respectively, compared to the value measured at the T0 and by 63.99% and 122.81% in C-CPRO samples. Thus, implying the beneficial effect of the centrifugation on the oil acidity during storage. A similar effect of centrifugation was noticed in a study performed by Wroniak et al. [34], where they provided evidence that the purification method affects the chemical and microbiological quality of CPRO. In their experiment, the centrifuged rapeseed oil showed higher quality than the oil after decanting. The average acidity values measured within the first week after pressing were 1.88 mg KOH/g and 1.59 mg KOH/g for decanted and centrifuged CPRO, respectively. After 6 months of storage, these values increased to 2.26 mg KOH/g (20.2% increase) in decanted CPRO samples and to 1.65 mg KOH/g in centrifuged CPRO, which means an increase in the initial value of 3.8% [34]. The same acidity value of 2.53 mg KOH/g was reported for centrifuged oil after decantation in a study performed by Rokosik et al. [35].

In addition to AV, the peroxide value (PV) is a frequently used parameter to indicate the lipids hydrolysis degree in the oil. Lipid hydroperoxides (LOOH) arise as a primary product of lipid oxidation, which may undergo decomposition into short-chain and sensory-active compounds [3,36]. According to the *Codex Alimentarius* standards, the PV of virgin fats and oils should be below 15 meqO_2/kg [37]. In the present study, fresh NC-CPRO and C-CPRO samples had acceptable peroxide values (respectively 1.77 meqO_2/kg and 1.72 meqO_2/kg). However, there were no significant differences between both types of CPRO samples. Subsequently, PVs grew after 3 months of storage in both sets of samples (NC-CPRO: 8.49 meqO_2/kg, and C-CPRO: 10.08 meqO_2/kg) but were still below the *Codex* cut-off. During the first 3 months of storage (T1), peroxides increased in both NC-CPRO (+379.66%) and C-CPRO (+586.04%) samples, with the largest increase in C-CPRO samples. This may be due to the initial and fluctuating levels of $^3O_2$ dissolved in the CPRO during the extraction and bottling stages. As storage progressed (T2), the peroxides of the NC-CPRO and C-CPRO samples exceeded the *Codex* guidance limit, the PVs reached 29.89 meqO_2/kg and 29.20 meqO_2/kg ($p < 0.05$), respectively. Peroxides are known to react quickly to form secondary products of lipid peroxidation, therefore results can vary greatly even in the same oil, and values from our CPRO can also be difficult to compare with those in the literature. However, a similar oxidation degree was reported in a study performed by Rokosik et al. [35], wherein the recorded value of fresh centrifuged CPRO

reached 1.58 meqO_2/kg. In the same study, they also found a relationship between the moisture of rapeseed and oil quality; an elevated level of the seed moisture resulted in worsening quality characteristics of the obtained oil, as evidenced by the increased PV and AV [35]. On the other hand, an opposite observation to our results was noticed in a study performed by Wroniak et al., wherein the primary oxidation degree of lipids was higher than in our CPRO samples overall; however, their values differed between decanted CPRO and centrifuged CPRO (average of 2.8 meqO_2/kg and 2.53 meqO_2/kg, respectively). Wroniak et al. also proved that the quality of rapeseed determines the quality of the final CPRO [34].

3.2. Pigments and Total Phenols Content in NC-CPRO and C-CPRO

The results of the analysis of the content of pigments and polyphenols are included in Table 2.

Table 2. Pigments and polyphenols content in NC-CPRO and C-CPRO.

Months of Storage	NC-CPRO	C-CPRO	% of Mean Change after 3 Months of Storage * (NC-CPRO; C-CPRO)	% of Mean Change after 6 Months of Storage * (NC-CPRO; C-CPRO)
Chlorophyll [mg/kg of the oil]				
T0 (0 months)	18.79 ± 0.20 ^{a,c}	18.41 ± 0.32 ^{a}	-;-	-;-
T1 (3 months)	18.90 ± 0.27 ^{c}	17.11 ± 0.34 ^{b}	0.59; −7.06	-;-
T2 (6 months)	18.90 ± 0.27 ^{a,c}	16.61 ± 0.53 ^{b}	-;-	0.59; −9.78
Carotenes [mg/kg]				
T0 (0 months)	180.75 ± 0.96 ^{a}	178.10 ± 3.67 ^{a}	-;-	-;-
T1 (3 months)	157.77 ± 1.83 ^{b}	159.10 ± 0.30 ^{b}	−12.71; −10.67	-;-
T2 (6 months)	144.39 ± 4.37 ^{c}	146.53 ± 4.54 ^{c}	-;-	−20.12; −17.73
Total polyphenols [mg GAE/g of the oil]				
T0 (0 months)	0.169 ± 0.088 ^{a,b}	0.147 ± 0.012 ^{a}	-;-	-;-
T1 (3 months)	0.167 ± 0.002 ^{a}	0.425 ± 0.008 ^{c}	−1.18; 189.12	-;-
T2 (6 months)	0.024 ± 0.001 ^{b}	0.011 ± 0.001 ^{d}	-;-	−85.80; −92.52

Different letters (^{a}, ^{b}, ^{c}, ^{d}) indicate statistically significant ($p < 0.05$) differences between NC-CPRO and C-CPRO. * % of mean change compared to fresh oil at T0 time point.

Chlorophyll has a dual role: in daylight, it can act as a pro-oxidant causing oxidative instability, color change, and generally diminishing the nutritional attributes of the oil, but on the other hand, it can also act as an antioxidant agent in the dark [38,39]. The content of chlorophyll in oils can be very diverse and depends on the degree of maturity of the seeds, harvesting conditions, and also depends on conditions of drying and storage. Chlorophyll degradation is a multistep catabolic process that occurs during leaf senescence and fruit and seed ripening. This process may be initiated by external factors such as reduced daylight, temperature changes, and others. The appearance of these factors may result in changes in gene expression, and protein synthesis/degradation which drive senescence. The generally accepted mechanism of chlorophyll breakdown involves conversion of chlorophyll b to chlorophyll a, removal of the phytol tail by enzyme chlorophyllase and central Mg atom, breakage of the chlorin ring, and final enzymatic degradation [40–42]. The oils produced from more mature seeds contain less chlorophyll, which is beneficial due to chlorophylls' pro-oxidative tendencies. Depending on the amount of chlorophyll present in the rapeseed, the resulting oil may have only a small amount of chlorophyll (from fully matured seed) to more than 100 mg/kg chlorophyll (from very immature or frost-damaged seed) [43–45]. According to a study by Yang et al., the chlorophyll content in CPRO is highly variable and its content is determined by the cultivar of rapeseed used. This study demonstrated a range from 0.9 mg/kg up to 51.0 mg/kg [46]. The values reported in our study for both NC-CPRO

and C-CPRO correspond to this range (Table 2). However, centrifugation did not change pigment content in C-CPRO compared to the NC-CPRO counterpart at the first time point of analysis (18.41 mg/kg and 18.79 mg/kg, respectively). A greater degree of chlorophyll consumption was observed only in C-CPRO, where chlorophyll content decreased over the study period by 7.1% at T1 and by 9.8% at T2 time points. Conversely, NC-CPRO samples displayed highly stable pigment contents after 3 and 6 months of storage (18.90 mg/kg for both T1 and T2 time points).

Carotenoids can suppress photooxidation in several ways: light filtering, $^1O_2$ quenching, inactivating the sensitizer, and capturing free radicals. In the presence of chlorophylls, carotenoids inhibit the progress of photooxidation via physical or chemical $^1O_2$ quenching [47]. The reduction of these antioxidant molecules during storage is a natural process and is also accompanied by a change in the color of the oil [48]. In the present study, we observed only a slight difference between the NC-CPRO and C-CPRO, however, this difference is not statistically significant and is not subject to further consideration. A similar observation was reported by Vidal et al. [49] in extra virgin olive oil (EVOO) samples; both chlorophylls and carotenoids decreased slightly after vertical centrifugation. We also did not notice a difference in the carotenoid reduction dynamic between centrifuged and not centrifuged oil samples during storage. In both types of samples, the carotenoid concentration decreased with similar dynamics; after 3 months of storage, the carotenoid content decreased in relation to fresh oil by 12.71% in NC-CPRO and by 10.67% in C-CPRO samples. Our results are consistent with the commonly observed loss of carotenoids in oils during storage [50]. Furthermore, the content of carotenoids depends on the type of rapeseed cultivar. Chew reports several studies with a considerable spread of values, e.g., 6.66–17.39 mg/kg [5]. A study by Yang et al. reports a range of 29.4–358.7 mg/kg, which also corresponds to the values found by us [46].

Cold-pressed rapeseed oil is a rich source of phenolic compounds, which serve as a protective barrier against oxidation [51]. Therefore, their presence in the oil is beneficial for human health and their amount has a positive effect on the shelf-life of the CPRO. In the present study, fresh NC-CPRO had a higher level of phenols in comparison to fresh C-CPRO samples, and this is consistent with the results obtained for pigments. With advanced storage time, total polyphenols decreased in the range of 0.169–0.024 mg/g and 0.147–0.011 mg/g, respectively in NC-CPRO and C-CPRO samples. The highest polyphenols reduction rate was recorded in C-CPRO than NC-CPRO samples at the T2 time point; the levels of polyphenols decreased by −92.52% and −85.80%, respectively.

The available literature sources indicate that some factors, such as cultivar varieties, agronomics, environmental conditions, or seed maturity can significantly affect the content of phenols in CPRO. Yang et al. determined the average content of total phenols in different canola cultivars in China. The range of the total polyphenolic compounds oscillated between 0.09 mg/g to a maximum of 1.04 mg/g. Most of the samples they evaluated ranged from 0.15 to 0.65 mg/g [46]. Based on literature repositories, there are some discrepancies in the results regarding the effect of the selected clarification method on the polyphenol content in cold-pressed oils during storage. For example, higher reduction rates were noticed in filtered than non-filtered oils, which had higher total phenol levels [52,53]. Conversely, other researchers reported negligible or no differences in phenols contents between filtered and non-filtered oils during storage [24,54]. There are insufficient data on the effects of centrifugation on the polyphenol profile in CPRO; therefore, our results are difficult to compare with the literature.

3.3. Antioxidant Properties of NC-CPRO and C-CPRO

In addition to macronutrients such as PUFA and MUFA, rapeseed oil contains protective micronutrients, such as the aforementioned phenolic compounds and carotenoids, e.g., [55]. These micronutrients may exert antioxidant activity on the organism to scavenge free radicals (FR) [56]. Highly active free radicals can be formed during physiologically normal metabolic processes or can come from the external environment, carried by air

pollutants or chemical waste [57]. The presence of an excess of FRs induces oxidative stress, causing unfavorable changes in lipids, proteins, and DNA damage and thus triggers a number of diseases, such as cancer, autoimmune disorders, Alzheimer's, and Parkinson's disease [58,59]. The body is known to have defense mechanisms against FR, including but not limited to specific enzymes, but there is strong evidence that a diet abundant in antioxidants can also greatly enhance these mechanisms [60]. High CPRO consumption may support these mechanisms by providing micronutrients that can quench FRs, as well as macronutrients such as omega-3 fatty acids that are substrates for the synthesis of resolution mediators [61]. The antioxidant potential of CPRO is higher than its refined counterpart due to the lower degree of raw material processing and therefore, preserves bioactive compounds in CPRO [62,63]. Nevertheless, FRs can also lead to the oxidation of unsaturated fatty acids that leads to the rancidity of oil and loss of health-promoting properties, and leads to harmful effects on the human body [63]. Therefore, it is recommended to track the free radical scavenging capacity of the active compounds contained in CPRO, because it has a significant impact on human health. Currently, chemical assays for assessing the antioxidant activity of foods are widely used because they are simple, quick, and cost-effective. However, it is recommended to perform analysis using more than one type of assay to assess the antioxidant capacity of a target, as no standardized methods are available yet [64]. Therefore, for the evaluation of antioxidant capability, three different methods were employed in our study, namely, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and FRAP assay. As shown in Table 3, the antioxidant properties of CPRO vary depending on the method used.

Table 3. Antioxidant properties of NC-CPRO and C-CPRO.

Months of Storage	NC-CPRO	C-CPRO	% of Mean Change after 3 Months of Storage * (NC-CPRO; C-CPRO)	% of Mean Change after 6 Months of Storage * (NC-CPRO; C-CPRO)
DPPH inhibition [%]				
T0 (0 months)	53.04 ± 1.90 ^{a}	53.48 ± 1.80 ^{a}	-;-	-;-
T1 (3 months)	46.34 ± 4.85 ^{a,b}	39.99 ± 6.38 ^{b}	−12.63; −25.22	-;-
T2 (6 months)	17.57 ± 2.63 ^{c}	15.73 ± 2.63 ^{c}	-;-	−66.87; −70.59
FRAP [μg/mL]				
T0 (0 months)	156.91 ± 10.92 ^{a}	139.34 ± 9.18 ^{a}	-;-	-;-
T1 (3 months)	194.33 ± 52.64 ^{a}	141.43 ± 15.74 ^{a}	23.85; 1.50	-;-
T2 (6 months)	46.21 ± 2.95 ^{b}	18.33 ± 1.07 ^{c}	-;-	−70.55; −86.85
ABTS [%]				
T0 (0 months)	2.40 ±0.11 ^{a}	2.50 ± 0.07 ^{a,b}	-;-	-;-
T1 (3 months)	3.23 ± 0.47 ^{b,c}	3.36 ± 0.22 ^{c}	34.58;34.4	-;-
T2 (6 months)	1.10 ± 0.37 ^{d}	1.94 ± 0.75 ^{a,b,d}	-;-	−54.17; −22.4

Different letters (^{a}, ^{b}, ^{c}, ^{d}) indicate statistically significant ($p < 0.05$) differences between NC-CPRO and C-CPRO. * % of mean change compared to fresh oil at T0 time point.

All three methods rely on a similar principle: an electron transfer from an antioxidant present in the CPRO sample and the reduction of a colored oxidant [65]. On the other hand, DPPH and ABTS protocols involve radicals that can be neutralized by antioxidants, while FRAP is a non-radical-based method, and the reduction of Fe^{3+} to Fe^{2+} is monitored. Furthermore, the ABTS radical is formed gradually, whilst DPPH is a stable radical itself [66]. Moreover, the affinities against the compounds present in the CPRO may differ between these methods. Differences may also result due to the fact that antioxidants present in CPRO vary in their effectiveness and mechanism of action due to their different molecular structures. Different chemical structures among antioxidants determine their intrinsic affinity and reactivity to free radicals and thus determine their antioxidant properties [67].

Depending on the mechanism of action, antioxidants can be divided into two groups, namely primary and secondary. Primary antioxidants are those that interrupt the oxidation chain by donating hydrogen or accepting radicals, forming more stable radicals. In this group are mainly substances that have a phenolic structure and include phytochemicals such as flavonoids, catechins, carotenoids, β-carotene, lycopene, diterpene, antioxidant vitamins, and minerals. On the other hand, secondary antioxidants delay oxidation by chelating metals, decomposing hydroperoxides, or eliminating reactive oxygen species. In this group, we found butyl hydroxyanisole (BHA), propyl gallate (PG), and butylhydroxytoluene (BHT) [67]. The aforementioned information suggests that the use of one analytical method cannot reflect the complete antioxidant profile of the oil, due to the heterogeneity of the mechanisms in which they act, as well as many other factors, such as temperature, concentration, the substrate susceptible to oxidization, the presence of synergistic and pro-oxidant compounds, and many other factors. All of these factors should be considered when choosing the appropriate method for the analysis of the antioxidant properties of selected substances.

Among available assays, the DPPH test is considered a robust and accurate method for assessing antioxidant capacity and it is often applied to the evaluation of edible oils [68]. The DPPH assay revealed a similar antioxidant capacity for both NC-CPRO and C-CPRO, and no significant differences were observed at the beginning of the experiment (53.04% and 53.48%, respectively). A further reduction was observed with increasing storage months. Specifically, the DPPH inhibition ability decreased from 53.04% to 46.34% in NC-CPRO samples and 53.48% to 39.99% in C-CPRO samples after three months of storage. The greatest scavenging activity reduction was observed in NC-CPRO and C-CPRO after six months of observation (17.67% and 15.73%, respectively). It can be observed that the level of antioxidant activity was at a similar level in both samples and its reduction had a similar pattern throughout the experiment.

Furthermore, the FRAP assay revealed only a slight difference in the antioxidant ability between both types of oil samples. At time point 0, a value of 156.91 μmol/mL was measured for NC-CPRO and 139.34 μmol/mL for C-CPRO. After 3 months of storage, an increase in the ferric-reducing power was noticed. In the case of NC-CPRO, the increase was to a value of 194.33 μmol/mL and C-CPRO to 141.43 μmol/mL. In the final phase of the experiment, the ferric-reducing power decreased by 70.55% and 86.85%, respectively, in the NC-CRPO and C-CRPO samples in comparison to the initial value. The FRAP method showed that NC-CPRO has a higher overall antioxidant potential than its centrifuged counterpart.

The opposite conclusion is suggested by the results of the analysis carried out by using the ABTS assay. The antioxidant capacity at the initial phase of the experiment was slightly higher in the case of the centrifuged oil in relation to the NC-CPRO (2.40% and 2.50%, respectively); however, this difference is relatively low. The protective effect of centrifugation on the oil's antioxidant properties is more pronounced over a longer storage time. After six months of the experiment, the antioxidant power was reduced only by 22.4% in C-CPRO with regard to its value at the T0 time point, while in the case of NC-CPRO, this antioxidant capacity was reduced by more than half.

Based on the results obtained from all three methods, predictably, the antioxidant power gradually decreased over the course of the experiment, in both types of oil samples. Summarizing the results obtained using all the methods, it can be concluded that centrifugation has no significant effect on the antioxidant activity of CPRO in this study. Minimal discrepancies in the results of individual methods can be explained in that these assays differ from each other in terms of reaction mechanisms [69].

4. Conclusions

According to our results, there were no differences in the oxidation profiles between CPRO subjected to clarification via centrifugation and the non-centrifuged counterpart during 6-month storage. For storage longer than 1 month, centrifugation is preferable in

order to delay hydrolytic degradation, while natural sedimentation-decantation provides a slightly better pigment profile and polyphenol content. Regarding antioxidant capability, we did not observe that centrifugation improved the oil's antioxidant properties. Thus, further research should be undertaken to establish the effect of centrifugation on CPRO quality, but also taking into consideration parameters such as rapeseed quality, different varieties, and degrees of maturity of rapeseed. Another issue worth considering in future research is the storage conditions of the oil included in the study. In the above experiment, the storage temperature was 22 °C. Stricter storage conditions (controlled by a specific methodology; OXITEST Oxidation Stability Reactor) such as high temperature may reveal the protective effect of centrifugation on CPRO stability.

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