Rest plant materials with natural antioxidants increase the oxidative stability of omega-3 rich Norwegian cold pressed *Camelina sativa* oil

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Abstract

Quality of Norwegian cold pressed camelina oil for human consumption and fish feed production, with special focus on plant rest materials, natural antioxidants and oxidation products are studied. Both crude oil with plant rest materials (CCO), and centrifuged oil (CO) were highly unsaturated with 39.4 % (w/w) α-linolenic acid. The oils were oxidatively stable for 12 months at long-time storage conditions at 4°C. Lipid oxidation was observed in CO, but not in CCO, between 6 and at 9 months at 20 °C. This difference was even higher at accelerated conditions at 40 °C with an increase in PV, AV and secondary oxidation products and decrease in tocopherols after 2 weeks in CO but not in CCO. This indicates natural antioxidants with stronger antioxidant capacity than tocopherols in association with the plant rest materials in CCO. The polyphenol rutin with antioxidative properties might be a good candidate but needs further studies.

Keywords

Omega-3 fatty acids, PV, AV, lipid oxidation products, tocopherols, polyphenols, *Camelina sativa*

INTRODUCTION

Camelina sativa oil is one of the richest vegetal sources of omega-3 fatty acids and might be a good new source of omega-3 fatty acids for feed and food in the future. The aquaculture production worldwide is expected to increase in the coming years, and at the same time, the availability of omega-3 rich fish oils on the world marked is stable but limited. This leads to a lack of the valuable omega-3 fatty acids for fish feed production by the aquaculture industry, but possibly also to produce omega-3 products for human consumption by the nutraceutical industry for the world population estimated to reach 9.7 billion in 2050 (UN).

Camelina, known as gold-of-pleasure, false flax, wild flax, linseed dodder, camelina, German sesame, and Siberian oilseed, is a flowering plant in the Brassicaeae family. Archaeological excavations in Europa and Scandinavia suggest that camelina was an important oil crop 2000 years ago[1]. Since then, it has been characterized as a weed species throughout Europe, but it was revived as a minor crop on a small scale in Europe and Balkan in the 20th century where camelina oil was used as a dietary oil, in herbal medicine and for technical purpose. Camelina seeds contain up to 45 % oil, and the oil contains about 50-60 % polyunsaturated fatty acids (PUFAs) where the omega-3 acid α-linolenic acid (ALA, 18:3) amount to 35-45 % of the fatty acids and the omega-6 fatty acid linoleic acid (LA, 18:2) 15-20 %[2-3]. Camelina is a low-input and short-seasoned oilseed crop widely distributed in Europe, Northern America and Central Asia[4] with good growing conditions in the northern countries including Norway[5].

The health benefits of the long-chained omega-3 PUFAs of marine origin, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), are well documented with favourable effects on a number of physiological conditions in the body, including protective effects on cardiovascular disease[6-7], inflammation[8] and a potential to inhibit certain types of cancers[9]. The predominant omega-3 fatty acid in vegetable oils is ALA, and it is well known that EPA and DHA

are synthesized through multiple steps from ALA in a pathway that is relative well conserved between different species[10] sharing the same enzymes[11-12]. This conversion is limited in several species. However, the conversion efficiency can be improved by different factors[13-14], and it has been shown that dietary camelina oil gives significantly higher serum concentrations of ALA, EPA and DHA, as well as decrease in serum cholesterol in hypercholesteraemic subjects[15]. In addition, it is also a growing body of scientific data supporting the idea that ALA may exert beneficial effects by other mechanisms rather than simply acting as a precursor for EPA and DHA[16-19], which make ALA, and camelina oil, even more interesting as a good omega-3 source.

Lipid oxidation is the main cause of loss of quality in food and feed containing PUFAs. Due to the high content of PUFAs in camelina oil, its oxidative stability is an important factor[20]. In a comparative study, camelina oil was found to be more stable towards oxidation than highly unsaturated linseed oil, but less stable than rapeseed, olive, corn, sesame and sunflower oil[21]. In addition to the healthy PUFAs, camelina seeds also contain other bioactive lipids as phytosterols, tochopherols, phenolics, glucosinolates and carotenoids[20, 22-23], some with antioxidative activity. High amount of these compounds are retained in the press cake during production of cold pressed camelina oil[24-25], but they have also been measured to different extent in cold pressed camelina oil.

Cold pressed oils refer to oils that are extracted by cold pressing plant seeds with a screw press or hydraulic press. Cold pressing is mostly used to extract oil from plant seed instead of conventional solvent extraction method because cold pressing does not require the use of organic solvent or heat. Studies on hemp, flax and canola oil show that cold pressing is able to retain bioactive compounds such as essential fatty acids, phenolics, flavonoids and tocopherols in the oils[26]. Studies on quality parameters in hexane extracted camelina oil[27] and deodorized cold pressed camelina oil[28] indicate that these processing conditions decrease the content of bioactive components and

oxidative quality of camelina oil. While several studies include characterization of bioactive lipids in oil and/or in press cake during production of cold pressed camelina oil[24-25, 29-30], only a few studies on processing conditions and the quality of camelina oil are available. As far as we know, studies on cold pressing conditions, filtration, amount of rest plant materials and oxidative stability of cold pressed camelina oil are missing. Thus, the overall aim of the present study was to increase the knowledge of quality and oxidative stability of Norwegian cold pressed camelina oil for human consumption and/or fish feed production, with special focus on plant rest materials, natural antioxidants, omega-3 and omega-6 PUFAs and oxidation products.

2.0 MATERIALS AND METHODS

2.1 Materials

Heptanoic acid ethyl ester was obtained from Fluka, Buchs, Switzerland. Phenolic standards were obtained from Merck, Kenilworth, NJ, USA. Fatty acids-, tocopherols-, sterols- and phenolic standards and other chemicals were obtained from Sigma-Aldrich, St.Louise, MO, USA. Crude cold pressed camelina oil with sediments of plant materials (CCO) was purchased by Norsk Matraps SA, Tomter, Norway. The oil was produced from Norwegian grown *Camelina sativa* crops by a conventional screw press at Askim Frukt og Bærpresseri, Askim, Norway. A clear yellow camelina oil (CO) with less plant materials, was obtained after centrifugation of CCO (Thermo Sientific SL 16R with TX-400 swing bucket rotor; 500 mL, 2000G, 30 minutes).

2.2 Storage conditions

For the oxidation stability test of CCO and CO, 100 mL oil samples were stored in glasses, dark, at 4 °C and 20 °C, with and without air exposure, for 0-12 months (long term storage conditions), or dark, at 40 °C, with air exposure, for 0-9 weeks (accelerated test conditions).

2.3 Fatty acid composition

The fatty acid composition of the oils was measured as fatty acid methyl esters using gas chromatography (GC) with flame ionization detector (FID). Briefly, oil samples were extracted with chloroform : methanol (2:1), dried and resolved in benzene, derivatized by adding methanolic HCL and 2,2-dimethoxy propan and analysed as methyl esters using capillary GC on a HP 6890 equipped with a BPX-70 column, 60 mx0.25 mm i.d, 0.25 um film (SGE Analytical Science Pty Ltd., Ringwood, Australia). The temperature program started at 70 °C for 1 min, increased by 30 °C /min to 170 ° C, by 1.5 °C /min to 200 ° C, and by 3 °C /min to 220 °C with a final hold time of 5 min. Peaks were integrated with HP GC ChemStation software (rev. A.0502) (Agilent Technologies, Little Falls, DE, USA) and identifies by use of external standards. The concentrations of individual fatty acids were expressed in percent of total fatty acids. All results are based on duplicate analysis.

2.4 Determination of fatty acid oxidation

2.4.1 Peroxide Value and Anisidine Value

The peroxide value (PV) and anisidine value (AV) were measured according to AOCS Official Method Cd 8-53 and Cd 18-90, respectively.

2.4.2 Volatile oxidation products

Volatile secondary oxidation products were analysed by Dynamic headspace/GC-MS as described by Olsen et al.[31] with small modifications of the method. Samples of 2 g oil were added 3 µL heptanoic acid ethyl ester (400 µg/mL) as internal standard and heated to 70 °C before they were purged with 100 mL/min nitrogen through a Drechsel-head for 30 minutes. Volatiles were adsorbed on Tenax GR (mesh size 60/80, Alltech Associates Inc., Deerfield, IL, USA). Trapped compounds were desorbed at 250 °C for 5 minutes in a Markes Unity/Ultra TD automatic desorber (Markes International ltd, Llantisant, England) and transferred to an Agilent 6890 GC System (Agilent, Palo Alto, CA, USA) with an Agilent 5973 Mass selective detector operated in electron impact (EI) mode at 70 eV. The chromatographic peaks were integrated with Agilent Chemstation

148 software, and compounds were identified based on the measured mass spectra by comparison to
149 the NIST 2011 Mass Spectral Library. The concentration of the individual volatiles was calculated
150 as ng per gram sample based on the added internal standard.
151
152 *2.4.3 Hydroxyalkenals*
153 A modified and in-house validated method based on Luo et al.[32] was used[33]. Samples of 1 g of oil
154 were added 500 ng internal quantification standards, deuterated 4-hydroxyhexenal(4-HHE)-D3
155 and 4-hydroxynonenal (HNE)-D3 (Cayman Chemical Company, Ann Arbor, Michigan, USA). Prior to
156 GC/MS analysis, pentafluorobenzyl- oxime-trimethylsilyl ether (PFB-oxime-TMS ether) derivatives
157 of the syn and anti-stereoisomers of the respective 4-hydroxyalkenals were generated during a
158 two-step derivatization followed by GC/MS analysis in negative ion chemical ionization (NCI)
159 mode. Repeatability of the analysis of the two 4-hydroxyalkenals measured in replicate oil samples
160 was within 8 %. Limit of quantification (S/N = 10) was 0.7 ng/mL oil and limit of detection (S/N = 3)
161 was 0.2 ng/mL.
162
163 **2.5 Determination of bioactive compounds**
164 *2.5.1 Tocopherols*
165 Oil samples (250 µL) and n-heptane (250 µL) were pipetted directly into HPLC vials prior addition
166 of tocopherolacetate as internal standard and BHT. Individual response factors were estimated
167 with use of external standards for α-, β-, γ- og δ-tocopherol. The HPLC method was based on
168 Panfili et al.[34] that was further developed and in-house validated[5]. An Agilent 1050 series HPLC
169 (Agilent Technologies, Santa Clara, CA, USA) was used for chromatographic separation of the
170 tocopherols, interfaced with a Shimadzu RF-551 fluorescence detector (Shimadzu UK Limited,
171 Buckinghamshire, UK) set to an excitation wavelength of 292 nm and an emission wavelength of
172 330 nm. Twenty microliters of sample were injected onto a Kromasil (silica) 250x4.6 mm column
173 packed with 5 µm silica packing material (Thermo Electron Corp., Waltham, MA, USA). Mobile

phase was run isocratically with 97.3% n-heptane, 1.8% ethyl acetate, and 0.9% acetic acid at a flow rate of 1.6 mL/min. Tocopherol isomer standards were used for identification and quantification was based on retention time and expected isomer pattern. Concentrations (µg/g oil) of individual tocopherols were obtained from duplicate analyses. Total tocopherol content was calculated as the sum of α, γ and δ-tocopherols.

2.5.2 Phytosterols

A modified method based on Toivo et al.[35] was used for analysing sterols in the oils[5]. Samples of oils (0.03 g) were weighed accurately in a 50 nL glass centrifuge tube, and internal standard (5 β-cholestan-3α-ol) was added. Samples were saponified with saturated kalium hydroxide in ethanol, followed by solvent extraction, evaporation and silylation. The sterols were analysed using GC/MS in both TIC and SIM in EI mode. Identification of compounds was done by using EI spectra of standard compounds. Concentrations (µg/g oil) of individual phytosterols were based on duplicate analyses.

2.5.3 Polyphenols

Samples of 1 g oils were dissolved in 10 mL heptane and extracted one time with 10 mL 80% aqueous methanol and two times with 80% aqueous methanol (5 mL). For analysis of free phenolic acids, the combined methanol phases were dried in a speed vac evaporator. The dry residue was re-dissolved in acidic Milli Q water (pH 2.0, 10 mL) and extracted with ethyl acetate (10 mL, 4 repetitions). The combined ethyl acetate phases were dried in a speed vac evaporator and re-dissolved in 25% aqueous methanol (1 mL). For analysis of bound phenolic acids, the heptane phase and the pellet of plant residue were centrifuged, and the heptane phase removed. The pellet was then washed with heptane (5 mL) two times and re-dissolved in 2M sodium hydroxide (10 mL) for overnight hydrolysis. The pH was adjusted (pH 1.3 -1.5) before extraction with ethyl acetate (10 mL, 4 repetitions). The combined ethyl acetate phases were concentrated in a speed vac

200 evaporator and the dry residue re-dissolved in 25 % aqueous methanol (1 mL). The phenolic acids
201 were identified and quantified by chromatographic comparison with standards, and their UV-vis
202 spectra in a Dionex UltiMate 3000 RS LC system with diode array detector. A reversed phase HPLC
203 separation was carried out on an Acquity UPLC BEHC8 Column (1.7 µm, 2.1 x 150 mm) at 50 °C.
204 The separation was performed by gradient elution with solvent A (1% aqueous acetic acid) and
205 solvent B (1% acetic acid in acetonitrile) at a flowrate of 0.450 ml/min and injection volume of 10
206 µL. The gradient program was 0-0.8 min: 5 % B; 0.8-1.2 min: 5-10 % B; 1.2-1.9 min: 10 % B; 1.9-2.4
207 min: 10-15 % B; 2.4-3.7 min: 15 % B; 3.7-4.0 min: 15-21 % B; 4.0-5.2 min: 21 % B; 5.2-5.7 min: 21-
208 27 % B; 5.7-8.0 min: 27-50 % B; 8.0-9.0 min: 50-100 % B; 9.0-11.5 min: 100 % B; 11.5-12 min: 100-
209 5 % B. The chromatograms were recorded at five wavelengths (260 nm, 280 nm, 320 nm, 360 nm,
210 and 520 nm, with a bandwidth of 5 nm). Phenolic standards were used for identification and
211 quantification was based on retention time and expected pattern. Concentrations (ng/g oil) of
212 individual phenolics were obtained from duplicate analyses.

213

214 **3.0 RESULTS**

215 **3.1 Characterization of cold pressed Norwegian Camelina oil**

216 The crude cold pressed camelina oil from conventional Norwegian grown *Camelina Sativa* (CCO)
217 used in this study, is highly unsaturated with 61.1 % (w/w) PUFAs and 29.2 % (w/w)
218 monounsaturated fatty acids (MUFAs) (Table 1). The main PUFAs are ALA and LA, constituting 39.4
219 % and 16 % (w/w), respectively. Oleic acid (18:1n-9, 16.9 %) and gondoic acid (20:1n-9, 10.6 %) are
220 the main MUFAs. The level of erucic acid (22:1n-9) is 1.2 % (w/w). Only 9.6 % (w/w) of the fatty
221 acids in CCO is SFAs. No differences in the fatty acid composition of CCO and CO were observed
222 (results not shown). There were only minor differences in tocopherol content between CCO and
223 CO (Table 2). The total content of tocopherols (α- and γ-tocopherols) in CCO and CO were between
224 649 - 676 µg/mg oil, with γ-tocopherol as the predominant one (639 ± 4.9 µg/g oil and 665 ± 3.5
225 µg/g oil, respectively in CCO). The total amounts of phytosterols are 5426 µg/mg oil in CCO and

5504 µg/mg oil in CO (Table 2). The predominant phytosterols are sitosterol and campesterol (2840 and 1390 µg/mg oil in CCO and 2897 and 1406 µg/mg oil in CO), and lower content the phytosterols (in the order of magnitude) avenasterol > cholesterol > brassicasterol > stigmasterol. Only minor differences in the levels of these phytosterols in CCO and CO are observed. The chromatogram of phenolic extracts from CCO and CO showed up to 27 clear peaks, and 5 of these were identified as the phenolics catechin, syringic acid, rutin, ferulic acid and sinapic acid. Different composition and levels of these identified phenolics in CCO and CO are observed (Table 3). While only low levels of free rutin (4.06 ± 0.50 ng/g oil), syrigenic (1.16 ± 0.06 ng/g oil) and sinapinic acid (0.20 ± 0.03 ng/g oil) were detected in CO, especially high level of rutin (228.65 ng/g oil), all as free rutin was detected in CCO. In addition, CCO contained both free and bound sinapic acid, catechin and syrigenic and a small amount of bound ferulic acid.

3.2 Storage stability of cold pressed Norwegian Camelina oil

3.2.1 Long time storage stability

The oxidative quality of CCO was high with almost no change in PV (≤6.0) or AV (1) during 12 months of storage at 4 or 20 °C in dark with or without air (Figure 1). The longtime stability of the oil with less rest plant materials, CO, was also high when stored in dark without air with almost no change in PV or AV during 12 months at 4 or 20 °C (Figure 1A). However, at 20 °C with air, both PV and AV in CO increase after 6 months storage and the values increase further at 9 and 12 months (Figure 1B). No changes in PV or AV are observed in CCO stored dark at 4 °C with air until 6 months. After that, only a small increase in AV, but not in PV, at 9- and 12-months storage is observed (Figure 1B).

3.2.2 The oxidation process at accelerated conditions

PV and AV

At accelerated conditions (dark, with air at 40 °C, for 9 weeks), CCO was oxidative stable until 4 weeks with no change in PV or AV, while CO was less stable with minor increase in both PV and AV already after 2 weeks (Figure 2). The PV increased from 7.1 to 17.7 meq peroxides/kg and the AV increased from 1.0 to 6.3 between week 4 and 9 in CCO. During this time (4-9 weeks) a considerable increase in both PV and AV were observed in CO (PV increased from 15.3 to 40.3 meq peroxides/kg and AV increased from 2.7 to 11.4).

Secondary oxidation products

Up to 180 volatile compounds are found in CCO and CO during accelerating storage conditions (dark, with air at 40 °C), and which are dominated by secondary lipid oxidation products, comprising from 50 to 90 %. For 9 weeks storage, the sum of volatile secondary oxidation products increased from about 250 ng/g oil to 1200 and 3000 ng/g oil in CCO and CO, respectively (Figure 3). Major oxidation products are in decreasing order 1-penten-3-ol > propanoic acid > 2tr,4cis-heptadienal > hexanal > 3, tr,5, tr-octadien-2-one > 2tr,4tr-heptadienal > propanal > 2, cis-pentenal > butanoic acid > 2-ethylfuran and > 1-penten-3-one (Figure 4). They show in general a non-linear increase with storage time, as shown in Figure 4, and the levels in CO are about twice as high as the levels found in CCO, which is like the observed difference in PV and AV levels. However, in both oils, the level of hexanal decreased up to 3 weeks storage, followed by increasing levels at weeks 6 to 9. In CCO the 2-ethylfuran and 2, cis-pentenal levels increased until week 6, and then decreased by week 9. The quantities of the non-volatile oxidation products 4-HHE and HNE generated from omega-3 and omega-6 PUFAs, also show a non-linear course with storage time at accelerated conditions (dark, with air at 40 °C) in CCO and CO (Figure 5). After 4 weeks there was a significant increase in 4-HHE formation up to 9 weeks. The levels in CO were about two to three times higher than levels found in CCO. The amount of 4-HHE increased from 0.03 to 0.85 µg /g in CCO and from 0.03 to 2.31 µg /g in CO over the 9 weeks storage, whereas HNE increased from 0.01 to 0.47 µg /g and 0.02 to 0.83 µg /g in CCO and CO, respectively.

Natural antioxidants

The levels of α-tocopherol and γ-tocopherol decreased in a non-linear course in both CCO and CO during 9 weeks with accelerated storage conditions (dark, with air at 40 °C) (Figure 6). However, the decrease in both α-tocopherol and γ-tocopherol was faster for CCO than for CO. The level of α-tocopherol decreased from 7.0 to 2.5 μg/g in CCO and from 12 to 0 in CO over the 9 weeks storage. The γ-tocopherol level decreased from 6390 to 520 μg/g and from 665 to 389 μg/g in CCO and CO, respectively. No changes in the levels of sterols or phenolic compounds were observed during accelerated storage conditions (dark, with air at 40 °C) (results not shown).

4.0 DISCUSSION

The present study shows that Norwegian cold pressed camelina oil might be a good plant-based choice to meet the rapidly growing demand for omega-3 fatty acids for food and feed, especially when the oil still contains plant rest materials with natural antioxidants after production. The Norwegian cold pressed camelina oil from conventional grown *Camelina Sativa* used in this study, is highly unsaturated and with a content of ALA close to 40% (w/w) it is a good omega-3 source.

The scientific data supporting the idea that ALA may exert beneficial effects by other mechanisms rather than simply acting as a precursor for EPA and DHA[16-19] is growing. However, the transformation of ALA to the well documented EPA and DHA with favourable effects on a number of physiological conditions in the body including protective effects on cardiovascular disease[6-7], inflammation[8] and a potential to inhibit certain types of cancers[9] is important. It is well known that the this transformation is limited in several species, but it is also known that this transformation made by multiple relative well conserved steps in different species[10] sharing the same enzymes[11-12], is efficiently improved by different factors[13-14]. In addition it has been shown that dietary camelina oil gives significantly higher serum concentrations of ALA, EPA and DHA, as well as decrease in serum cholesterol in hypercholesteraemic subjects[15]. The content of bioactive

303 compounds like tocopherols and phytosterols is high, with γ-tocopherol as the predominant
304 tocopherol and sitosterol and campesterol as the two main phytosterols. These values are in
305 accordance with earlier reported values in oils from conventional grown seeds of camelina
306 cultivars in Central and Northern Europe[21, 29, 36] and oil from Norwegian organic grown camelina
307 crops[5]. However, the Norwegian camelina oil from conventional grown camelina seeds has a
308 higher level of ALA and a lower level of total tocopherols compared to oils from Norwegian organic
309 grown camelina seeds[5]. Interestingly, the content of the long chain MUFA erucic acid (22:1 n-9)
310 with limited dietary intake recommendations is 1.2 % (w/w) and lower than in oils from organic
311 grown seeds (2.6-3.0 % (w/w)[5], both well below the permitted value of 5 %[37].
312
313 Cold pressed camelina oil is highly polyunsaturated, and increased lipid oxidation during 3 months
314 storage at room temperature with access of light has been reported[38]. In a long time storage
315 experiment (room temperature and day light) with cold pressed rapeseed oil and sunflower oil,
316 Wroniak et al.[39] reported increased oxidation in both oils after 30 days and increased storage
317 stabilities in oils flushed with nitrogen. The cold pressed Norwegian camelina oil in this study, both
318 the crude oil (CCO) and centrifugated oil (CO), were oxidatively stable in a long-time test in
319 darkness, with and without air at 4 °C for 12 months. Despite the high content of ALA nearly no
320 increase in primary peroxides (PV) or secondary oxidation products (AV) were observed at 4 °C.
321 CCO was also oxidative stable at 20 ^{o}C for 12 months. However, CO was more oxidative unstable
322 and elevated levels of PV and AV were observed between 6 and at 9 months at 20 °C. These results
323 indicate that the natural antioxidants in the oils in association with the plant rest materials,
324 protects the unsaturated fatty acids in cold pressed camelina oil during long time storage
325 conditions.
326
327 In an attempt to further understand the oxidative progress in cold pressed camelina oil and the
328 interference of plant rest materials, oils with more or less plant rest materials (CCO and CO) were

329 stressed in an accelerated oxidation test (dark, with air at 40 °C) and followed up by measurement
330 of lipid oxidation products and natural antioxidants during the oxidative progress. The stability of
331 Norwegian cold pressed camelina oil was highly dependent on amount of plant rest materials in
332 the oil at accelerated conditions. While a small increase in both PV and AV were observed in CO
333 after 2 weeks, CCO was more oxidative stable with no change in PV or AV until 4 weeks storage.
334 The centrifuged oil (CO) was less stable at accelerated condition and huge increases in PV and AV
335 between 4 to 9 weeks were observed. The increases in PV and AV in CCO during this period were
336 significantly lower. Most of the volatile secondary oxidation products showed a non-linear increase
337 with storage time, like PV and AV results, except for hexenal in CCO, which showed a decrease in
338 levels up to four weeks storage. The major volatile oxidation products which showed increasing
339 levels with storage time, showed significant positive correlations in the two oils and separately
340 with PV and AV numbers ($r>0.93$, $p<0.001$, $n=7$). The observed decrease in levels of hexanal up to
341 3 weeks storage, followed by increasing levels at weeks 6 and 9 in both oils is, however, difficult
342 to explain. This also applies to 2-ethylfuran and 2, cis-pentenal levels, which increased until week
343 6, and then decreased by week 9. This may possibly indicate some effects of interaction of these
344 compounds with the plant rest materials. Limited data exist in the literature on hydroxy-alkenals
345 in oxidized vegetable oils. Results reported from unoxidized unsaturated vegetable oils vary from
346 0.01 to 0.2 µg/g oil[40], which are in agreement with our results. Levels reported in oxidized oils vary
347 from 2.0 to 45 µg/g oil[40-43]. In one of the studies[40], oxidized rapeseed oil reached HHE and HNE
348 levels of respectively 14 µg/g and 15 µg/g oil after 11 days storage of 28 grams oil in 1.1 litre closed
349 vessels in dark at 37 °C, which are respectively about 5 and 15 times higher than our data. The
350 other reported numbers (soybean, sunflower, palm, corn, linseed, camelina and peanut oils) are
351 much higher than in the present study, since these data are from studies of deep-frying of oils,
352 which have been exposed to far higher temperatures (>180 °C). A 2.7 times higher level was found
353 for of HHE compared to HNE in CO after 9 weeks, which is in agreement with the study by Ma et
354 al.[43], who also found HHE levels to be higher than HNE in camelina oil after 3 hr incubation with air

355 at 180 °C. The levels of 4-HHE and HNE reflect the proportion of omega-3 and omega-6 fatty acids
356 in the oil (omega-3/omega-6 = 2.1) and in addition an increased autooxidation rate of the more
357 unsaturated omega-3 fatty acids than the omega-6 fatty acids. However, another accelerated
358 storage study of camelina oil kept at 60 °C for 30 days in air[44], showed the same levels for HHE and
359 HNE, i.e. about 0.6 µg/g oil. This HNE concentration corresponds to our HNE concentration in CO
360 after 6 weeks storage at 40 °C, which is surprising, since the formation of hydroxy-alkenals have
361 been shown to be temperature dependent, so higher levels would have been expected at higher
362 temperatures[45]. HHE and HNE levels showed significant positive correlation with AV values in CO
363 and CCO and separately ($r>0.97$, $p<0.002$), reflecting the AV as measure of secondary oxidation
364 products. CCO showed a lower 4-hydroxy-alkenal formation after 4-9 weeks storage compared to
365 CO with less plant rest materials. The lower levels of 4-hydroxy-alkenals found in CCO with more
366 plant rest materials, agreed with lower AV values and levels of volatile secondary lipid oxidation
367 products compared to the centrifuged oil (CO) and may support stabilization effect due to
368 presence of plant rest materials with antioxidant properties. Further, typical omega-3 fatty acid
369 (ALA) derived volatile secondary lipid oxidation products in CO and CCO correlated significantly
370 with 4-hydroxy-hexenal levels, like 2tr,4cis-heptadienal ($r>0.94$, <0.002, $n=7$) and 1-penten-ol
371 ($r>0.96$, <0.002, $n=7$). 4-hydroxy-nonenal levels in CO correlated significantly with typical omega-
372 6 fatty acid (LA) derived volatile secondary oxidation products like hexanal ($r=0.84$, <0.002, $n=7$)
373 and 2, tr-heptenal ($r=0.96$, <0.002). However, in CCO there was no significant correlation of these
374 volatile compounds with 4-hydroxy-nonenal levels, which may possibly also be due to some
375 interaction with the sediment of the oil.
376
377 Decreased levels of both tocopherols and phenolics in camelina oil during storage at accelerated
378 conditions have been reported[20]. In our accelerated experiment, the degradation of the
379 tocopherols was different in the CCO and CO. Both α-tocopherol and γ-tocopherol decreased in a
380 non-linear course in the two oils, but the decreases were faster in CO than CCO. This may indicate

presence of an antioxidant with stronger antioxidant capacity than tocopherols in CCO, probably associated with the plant rest materials since the press cakes after production of cold pressed camelina oil contain several bioactive compounds[24-25]. It is well known that the press cake from camelina contains phenolic compounds with antioxidative properties[46-47], and a drop in total phenolics parallel with drop in tocopherols has been observed during accelerated storage conditions of cold pressed camelina oil[20]. Catechin, syringic acid, rutin, ferulic acid and sinapic acid identified from the chromatograms of the phenolic extracts from CCO and CO were previously identified in camelina seeds, oil and/or press cake by others[46, 48]. Differences in both composition and levels of the phenolics were observed between CCO and CO. While only low levels (< 5 ng/g oil) of free rutin, syrigenic acid and sinapinic acid were detected in the centrifuged oil (CO), both free and bound sinapic acid, catechin and syrigenic acid and a high amount of free rutin, were detected in CCO with more plant rest materials. In addition, CCO contained a small amount of bounded ferulic acid. Most likely are these phenolics in CCO associated with the plant rest materials. Phenolics have antioxidant properties, but they are hydrophilic and less soluble in oils. However, knowledge accumulated during the last two decades emphasize that lipid oxidation cannot be explained merely by chemical reactions but also by considering molecular positions in space, especially at the interfaces of nano emulsions[49]. Thus, bulk oils are considered as water- in-oil nano emulsions rather than pure lipid phases. In this context, the interphase between plant rest residues and oil might contain micelles or liposomes containing both tochopherols and phenolics and explain the antioxidative effects of hydrophilic compounds as phenolics in CCO. It has also been reported that rutin has higher antioxidant activity in liposomes than tocopherols[50], but the explanation of the antioxidant process between tocopherols and phenolics like rutin in cold pressed camelina oil need further investigation.

The present study shows that Norwegian cold pressed camelina oil might be a good omega-3 rich oil for food and feed, especially if the oil contains some plant rest materials with natural

antioxidants. The results indicate natural antioxidants with stronger antioxidant capacity than tochopherols in association with the plant rest materials in crude camelina oil. These antioxidants are still unknown, but the phenolic rutin might be a good candidate since the crude oil (CCO) with plant rest material contained much higher level of free rutin than the centrifugated oil (CO) with less plant rest materials. However, other possibilities as glucosinolates and carotenoids need further studies.

ABBREVIATIONS USED

ALA, alpha-linolenic acid; AV, anisidine number; BHT, 2,6-Di-tert-butyl-4-methylphenol; CCO, crude camelina oil; CO, camelina oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FID, flame ionization detector; 4-HHE, 4-hydroxyhexenal; 4-HNE, 4-hydroxynonenal; HPLC, high-performance liquid chromatography; GC, gas chromatography; LA, linoleic acid; MUFA, monounsaturated fatty acid; MS, mass spectrometry; PUFA, polyunsaturated fatty acid; PV, peroxide number; SFA, saturated fatty acid; UN, United Nations

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582 **TABLES AND ARTWORK**

583 **Table 1.** Fatty acid composition (% (w/w)) and total saturated fatty acids (SFAs), monounsaturated
584 fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) of Norwegian crude camelina oil (CCO).

Fatty acid % (w/w)	CCO
14:0	0.1
16:0	5.8
16:1n-7	0.1
18:0	2.8
18:1n-9	16.9
18:2n-6	18.8
18:3n-3	39.4
20:0	0.9
20:1n-9	10.6
20:2n-6	1.7
20:3n-3	1.3
22:1n-9	1.2
24:1n-9	0.5
Total SFAs	9.6
Total MUFAs	29.2
Total PUFAs	61.1

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589 **Table 2.** Tocopherols (µg/g oil) and sterols (µg/g oil) in Norwegian camelina oil, crude cold pressed oil
590 (CCO) and centrifugated crude oil (CO) with less rest plant materials. Data (n=2) are shown with
591 standard deviation.

		CCO	CO
Tocopherols (µg/g oil)	α-tocopherol	9.5±0.7	11±4.2
	γ-tocopherol	639±4.9	665±3.5
Phytosterols (µg/g oil)	Cholesterol	356±3	369±6
	Brassicasterol	327±1	333±1
	Campesterol	1390±2	1406±10
	Stigmasterol	103±1	89±3
	Sitosterol	2840±50	2897±8
	Avenasterol	410±1	410±1

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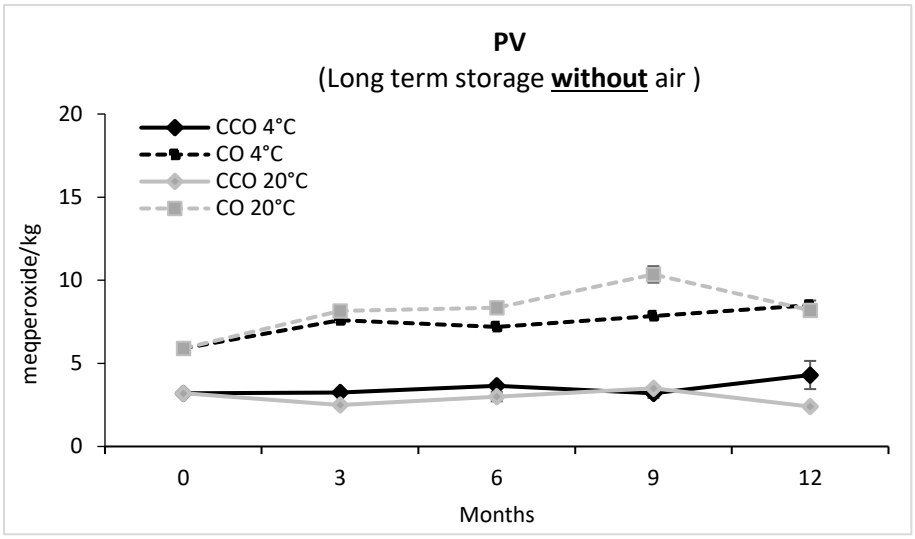
Table 3. Levels of identified free and bound phenolics (ng/g oil) in Norwegian camelina oil, crude cold pressed oil (CCO) and centrifugated crude oil (CO) with less rest plant materials. Data (n=2) are shown with standard deviation. n.d. = not detected.

	CCO		CO	
Phenolics (ng/g oil)	free	bound	free	bound
Catechin	0.56±0.04	1.09±0.21	nd	n.d.
Syringic acid	0.71±0.12	0.20±0.02	0.16±0.06	n.d.
Rutin	228.65±8.31	n.d.	4.06±0.50	n.d.
Ferulic acid	n.d.	0.17±0.06	n.d.	n.d.
Sinapic acid	3.68±0.73	0.11±0.08	0.20±0.03	n.d.



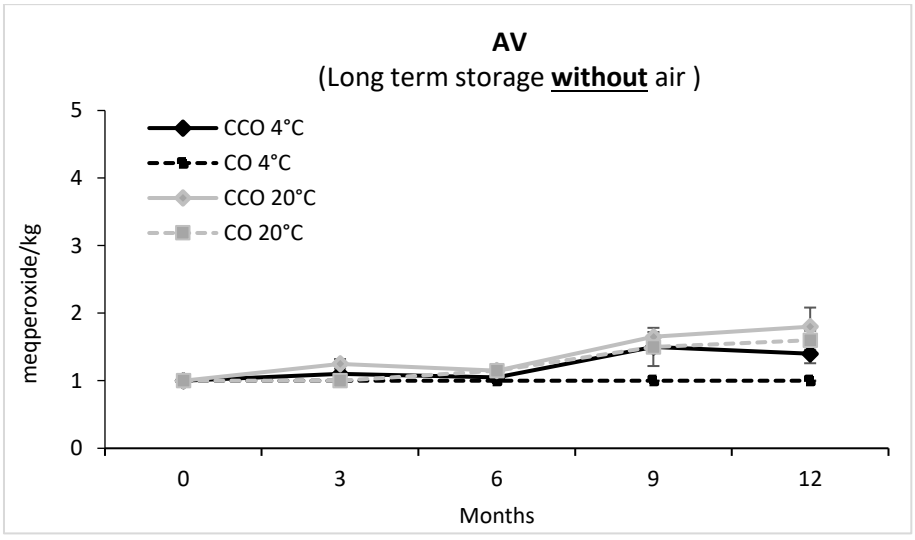
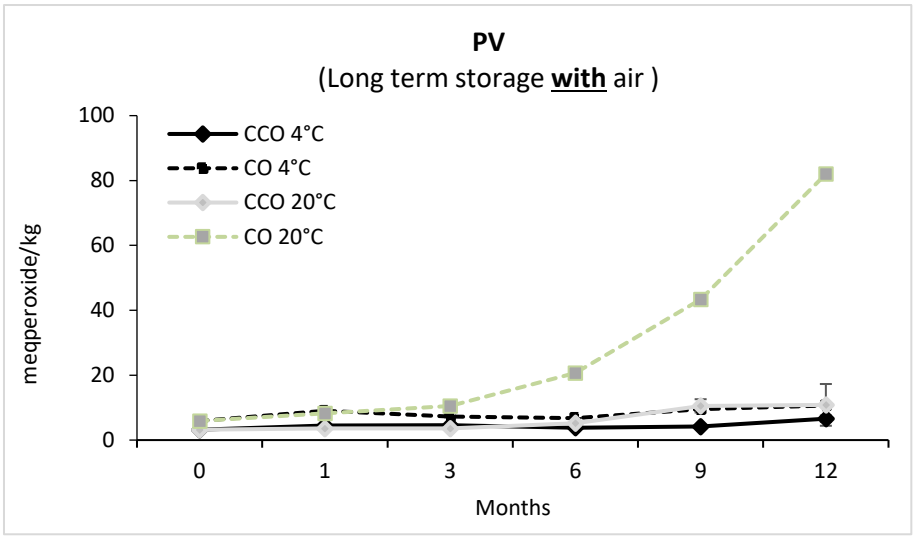


Figure 1A. Peroxide (PV) and Anisidine value (AV) in crude cold pressed camelina oil (CCO) and centrifugated camelina oil (CO) stored dark **without** air at 4 °C or 20 °C for 0-12 months. Data (n=2) are shown with standard deviation.



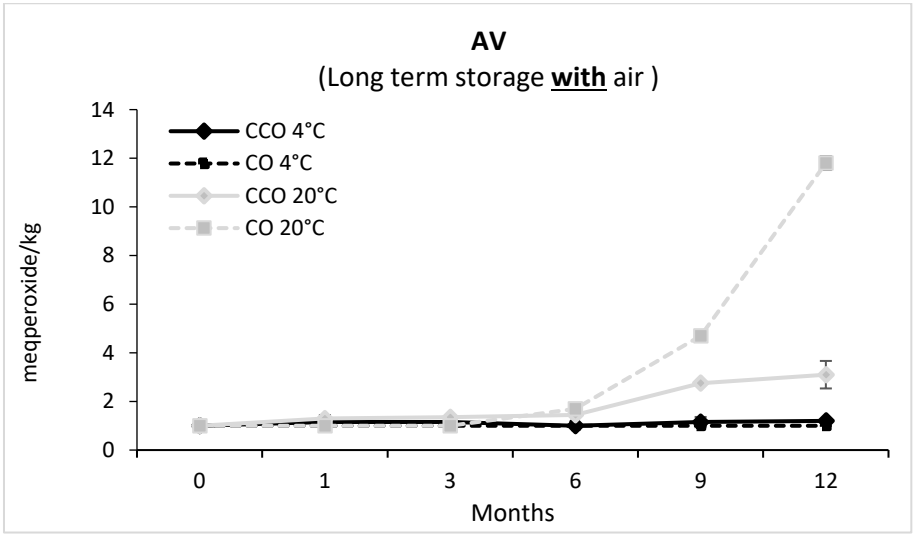
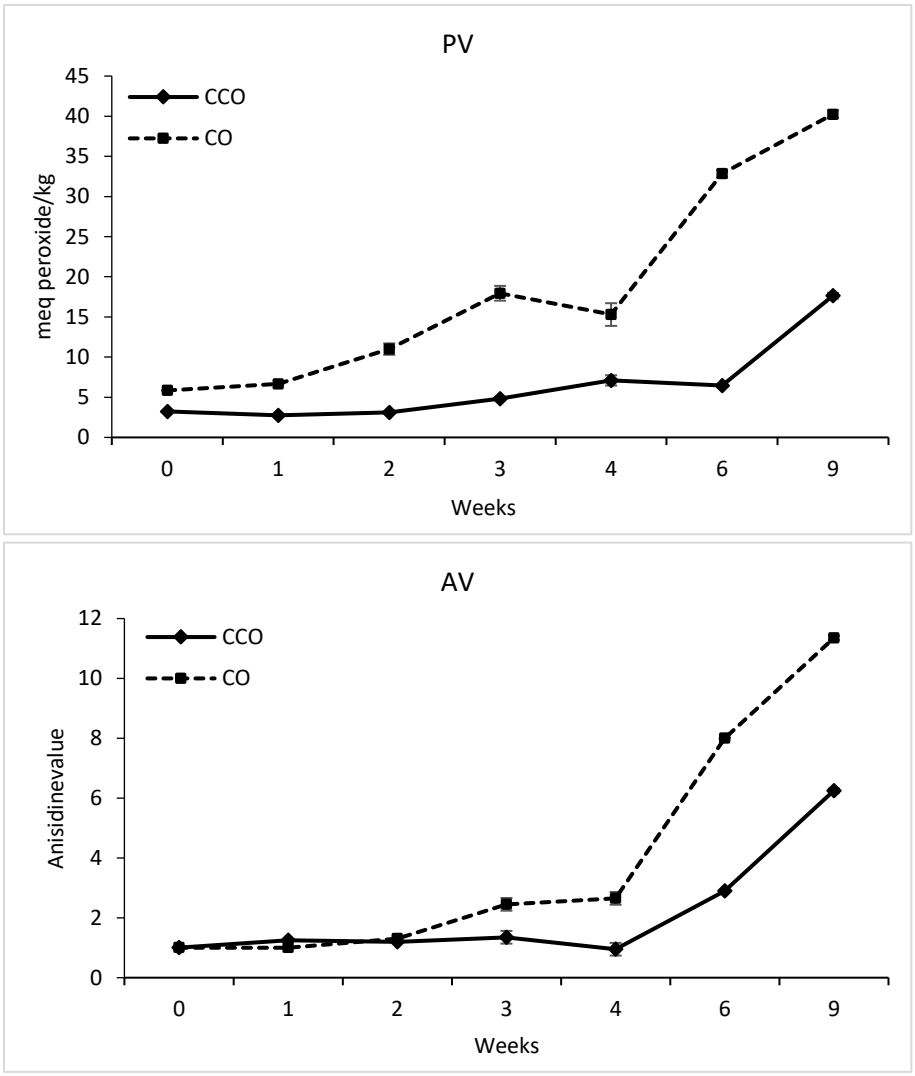


Figure 1 B. Peroxide (PV) and Anisidine value (AV) in crude cold pressed camelina oil (CCO) and centrifugated camelina oil (CO) stored dark **with** air at 4°C or 20°C for 0-12 months. Data (n=2) are shown with standard deviation.



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642 **Figure 2**. Peroxide value (PV) and Anisidine value (AV) in crude cold pressed camelina oil (CCO) and
643 centrifugated camelina oil (CO) stored dark with acess of oxygen at 40^{o}C for 0-9 weeks. Data (n=2) are
644 shown with standard deviation.

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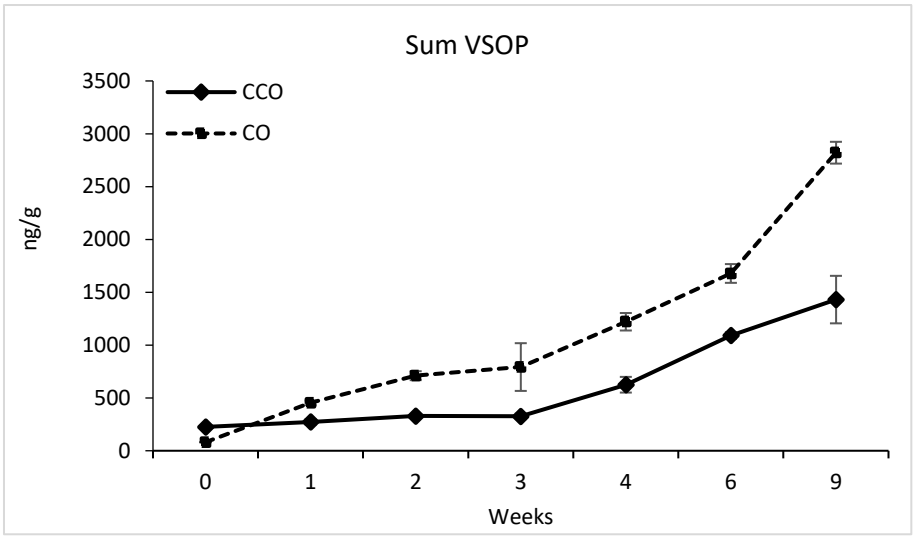
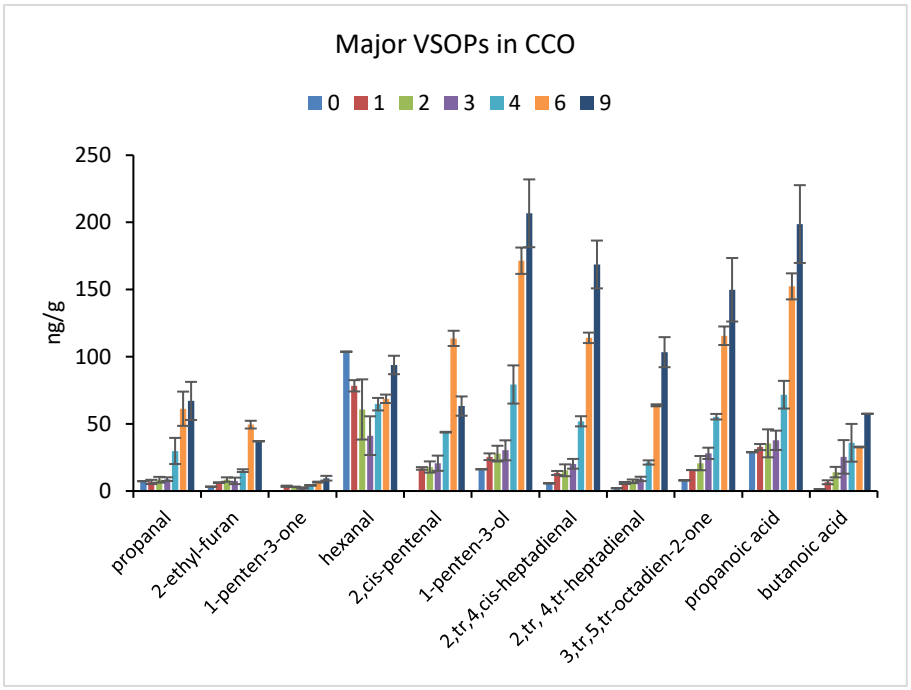


Figure 3. Sum volatile secondary oxidation products (VSOP, ng/g) in crude cold pressed camelina oil (CCO) and centrifugated camelina oil (CO) stored dark with acess of oxygen at 40°C for 0-9 weeks. Data (n=2) are shown with standard deviation.



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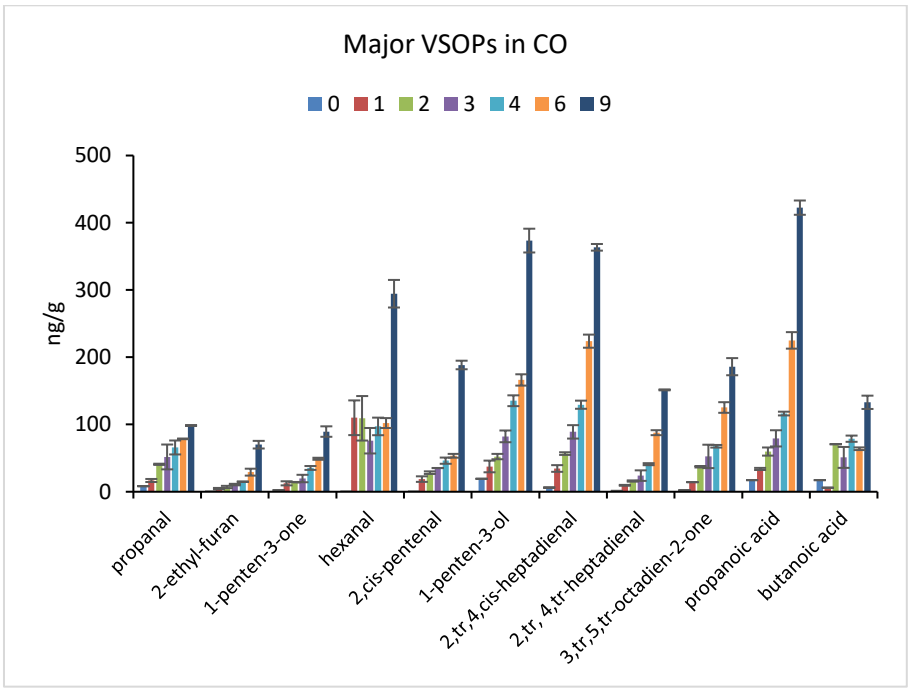

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663 **Figure 4**. Major volatile secondary oxidation products (VSOP, ng/g) in crude cold pressed camelina oil
664 (CCO) and centrifugated camelina oil (CO) stored dark with acess of oxygen at 40°C for 0-9 weeks. Data
665 (n=2) are shown with standard deviation.

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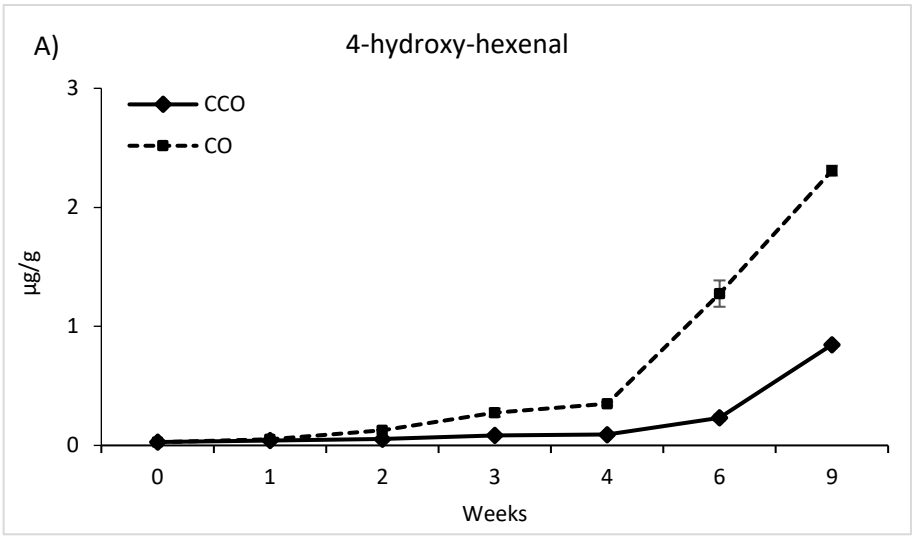

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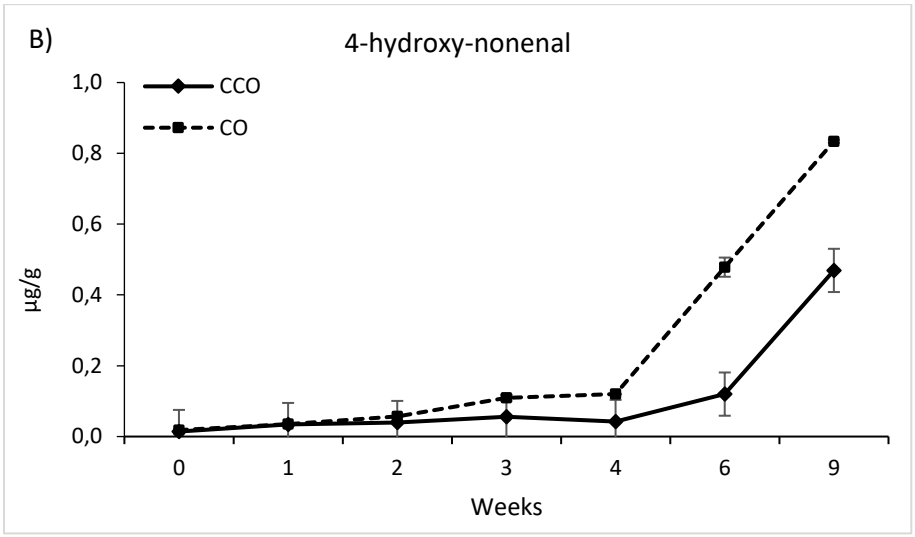

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673 **Figure 5**. **A)** 4-hydroxy-hexenal and **B)** 4-hydroxy-nonenal (secondary lipid oxidation products) (µg/g)
674 in crude cold pressed camelina oil (CCO) and centrifugated camelina oil (CO) stored dark with acess of
675 oxygen at 40 ^{o}C for 0-9 weeks. Data (n=2) are shown with standard deviation.

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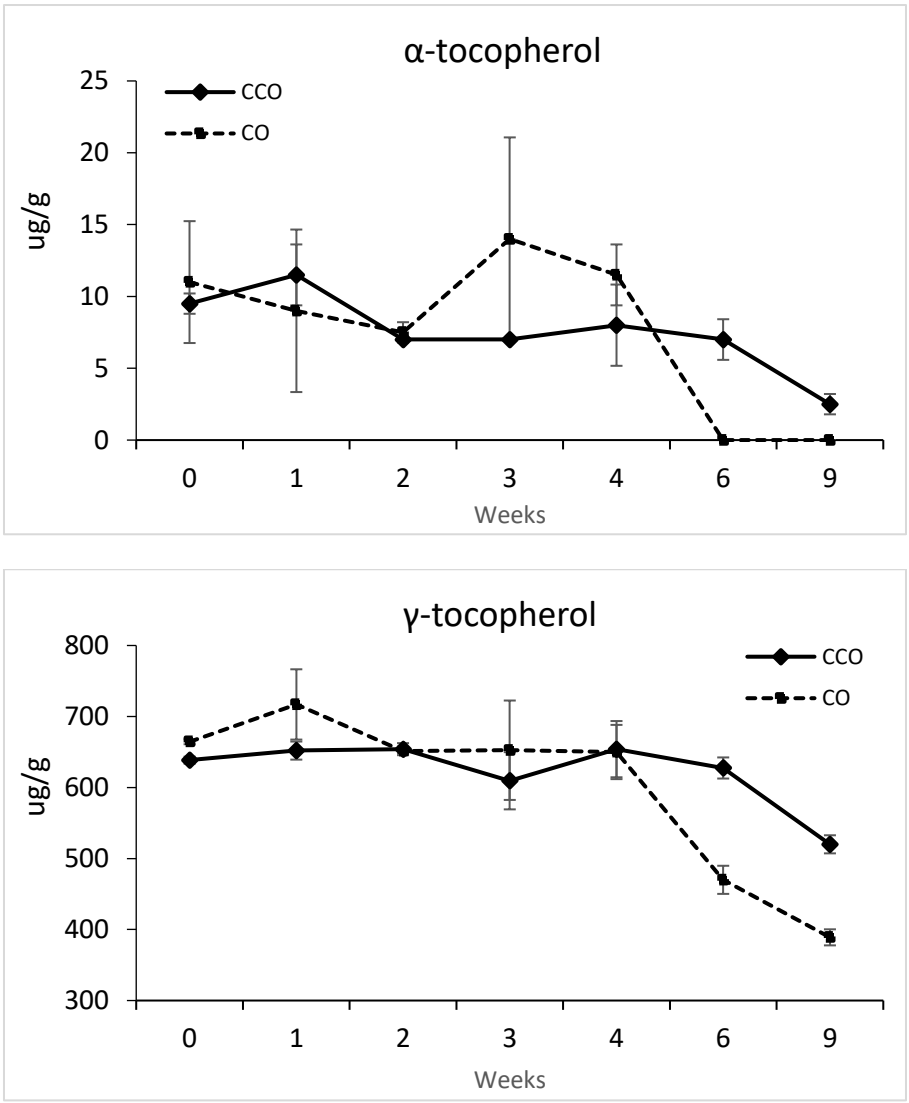

Figure 6. α-tochoperol and γ-tocopherol (µg/g) in crude cold pressed camelina oil (CCO) and centrifugated camelina oil (CO) stored dark with acess of oxygen at 40^{o}C for 0-9 weeks. Data (n=2) are shown with standard deviation.

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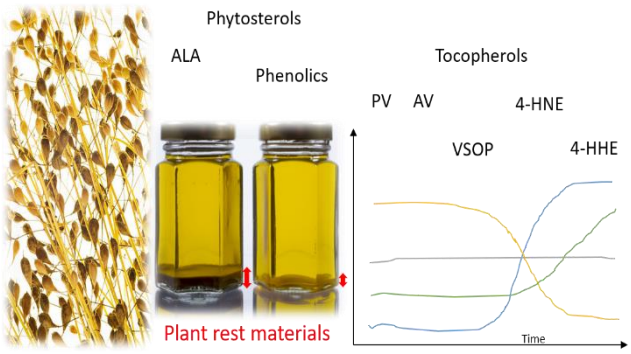
Phytosterols
ALA
Phenolics
Tocopherols
PV
AV
4-HNE
VSOP
4-HHE
Plant rest materials
Time