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) \( \alpha \)-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 20\(^{th}\) 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 \(\alpha\)-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\%\(^2\). 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\(^\text{10}\) sharing the same enzymes\(^\text{11-12}\). This conversion is limited in several species. However, the conversion efficiency can be improved by different factors\(^\text{13-14}\), 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\(^\text{15}\). 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\(^\text{16-19}\), 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\(^\text{20}\). 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\(^\text{21}\). In addition to the healthy PUFAs, camelina seeds also contain other bioactive lipids as phytosterols, tocohopherols, phenolics, glucosinolates and carotenoids\(^\text{20, 22-23}\), some with antioxidative activity. High amount of these compounds are retained in the press cake during production of cold pressed camelina oil\(^\text{24-25}\), 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\(^\text{26}\). Studies on quality parameters in hexane extracted camelina oil\(^\text{27}\) and deodorized cold pressed camelina oil\(^\text{28}\) 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, 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 Bærewisseri, Askim, Norway. A clear yellow camelina oil (CO) with less plant materials, was obtained after centrifugation of CCO (Thermo Scientific 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.\textsuperscript{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 software.
software, and compounds were identified based on the measured mass spectra by comparison to
the NIST 2011 Mass Spectral Library. The concentration of the individual volatiles was calculated
as ng per gram sample based on the added internal standard.

2.4.3 Hydroxyalkenals

A modified and in-house validated method based on Luo et al.\textsuperscript{32} was used\textsuperscript{13}. Samples of 1 g of oil
were added 500 ng internal quantification standards, deuterated 4-hydroxyhexenal(4-HHE)-D\textsubscript{3}
and 4-hydroxynonenal (HNE)-D\textsubscript{3} (Cayman Chemical Company, Ann Arbor, Michigan, USA). Prior to
GC/MS analysis, pentafluorobenzyl- oxime-trimethylsilyl ether (PFB-oxime-TMS ether) derivatives
of the syn and anti-stereoisomers of the respective 4-hydroxyalkenals were generated during a
two-step derivatization followed by GC/MS analysis in negative ion chemical ionization (NCI)
mode. Repeatability of the analysis of the two 4-hydroxyalkenals measured in replicate oil samples
was within 8 %. Limit of quantification (S/N = 10) was 0.7 ng/mL oil and limit of detection (S/N = 3)
was 0.2 ng/mL.

2.5 Determination of bioactive compounds

2.5.1 Tocopherols

Oil samples (250 µL) and n-heptane (250 µL) were pipetted directly into HPLC vials prior addition
of tocopherolacetate as internal standard and BHT. Individual response factors were estimated
with use of external standards for α-, β-, γ- og δ-tocopherol. The HPLC method was based on
Panfili et al.\textsuperscript{34} that was further developed and in-house validated\textsuperscript{5}. An Agilent 1050 series HPLC
(Agilent Technologies, Santa Clara, CA, USA) was used for chromatographic separation of the
tocopherols, interfaced with a Shimadzu RF-551 fluorescence detector (Shimadzu UK Limited,
Buckinghamshire, UK) set to an excitation wavelength of 292 nm and an emission wavelength of
330 nm. Twenty microliters of sample were injected onto a Kromasil (silica) 250x4.6 mm column
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. was used for analysing sterols in the oils. Samples of oils (0.03 g) were weighed accurately in a 50 mL 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
evaporator and the dry residue re-dissolved in 25 % aqueous methanol (1 mL). The phenolic acids were identified and quantified by chromatographic comparison with standards, and their UV-vis spectra in a Dionex UltiMate 3000 RS LC system with diode array detector. A reversed phase HPLC separation was carried out on an Acquity UPLC BEHC8 Column (1.7 µm, 2.1 x 150 mm) at 50 °C. The separation was performed by gradient elution with solvent A (1% aqueous acetic acid) and solvent B (1% acetic acid in acetonitrile) at a flowrate of 0.450 ml/min and injection volume of 10 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 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: 21-27 % 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: 100-5 % B. The chromatograms were recorded at five wavelengths (260 nm, 280 nm, 320 nm, 360 nm, and 520 nm, with a bandwidth of 5 nm). Phenolic standards were used for identification and quantification was based on retention time and expected pattern. Concentrations (ng/g oil) of individual phenolics were obtained from duplicate analyses.

3.0 RESULTS

3.1 Characterization of cold pressed Norwegian Camelina oil

The crude cold pressed camelina oil from conventional Norwegian grown Camelina Sativa (CCO) used in this study, is highly unsaturated with 61.1 % (w/w) PUFAs and 29.2 % (w/w) monounsaturated fatty acids (MUFAs) (Table 1). The main PUFAs are ALA and LA, constituting 39.4 % and 16 % (w/w), respectively. Oleic acid (18:1n-9, 16.9 %) and gondoic acid (20:1n-9, 10.6 %) are the main MUFAs. The level of erucic acid (22:1n-9) is 1.2 % (w/w). Only 9.6 % (w/w) of the fatty acids in CCO is SFAs. No differences in the fatty acid composition of CCO and CO were observed (results not shown). There were only minor differences in tocopherol content between CCO and CO (Table 2). The total content of tocopherols (α- and γ-tocopherols) in CCO and CO were between 649 - 676 µg/mg oil, with γ-tocopherol as the predominant one (639 ± 4.9 µg/g oil and 665 ± 3.5 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), syringenic (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 syringenic 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 DHA16-19 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 disease6-7, inflammation8 and a potential to inhibit certain types of cancers9 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 species10 sharing the same enzymes11-12, is efficiently improved by different factors13-14. 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 subjects15. The content of bioactive
compounds like tocopherols and phytosterols is high, with \( \gamma \)-tocopherol as the predominant tocopherol and sitosterol and campesterol as the two main phytosterols. These values are in accordance with earlier reported values in oils from conventional grown seeds of camelina cultivars in Central and Northern Europe\cite{21,29,36} and oil from Norwegian organic grown camelina crops\cite{5}. However, the Norwegian camelina oil from conventional grown camelina seeds has a higher level of ALA and a lower level of total tocopherols compared to oils from Norwegian organic grown camelina seeds\cite{5}. Interestingly, the content of the long chain MUFA erucic acid (22:1 n-9) with limited dietary intake recommendations is 1.2 % (w/w) and lower than in oils from organic grown seeds (2.6-3.0 % (w/w))\cite{5}, both well below the permitted value of 5 %\cite{37}.

Cold pressed camelina oil is highly polyunsaturated, and increased lipid oxidation during 3 months storage at room temperature with access of light has been reported\cite{38}. In a long time storage experiment (room temperature and day light) with cold pressed rapeseed oil and sunflower oil, Wroniak et al.\cite{39} reported increased oxidation in both oils after 30 days and increased storage stabilities in oils flushed with nitrogen. The cold pressed Norwegian camelina oil in this study, both the crude oil (CCO) and centrifugated oil (CO), were oxidatively stable in a long-time test in darkness, with and without air at 4 °C for 12 months. Despite the high content of ALA nearly no increase in primary peroxides (PV) or secondary oxidation products (AV) were observed at 4 °C. CCO was also oxidative stable at 20 °C for 12 months. However, CO was more oxidative unstable and elevated levels of PV and AV were observed between 6 and 9 months at 20 °C. These results indicate that the natural antioxidants in the oils in association with the plant rest materials, protects the unsaturated fatty acids in cold pressed camelina oil during long time storage conditions.

In an attempt to further understand the oxidative progress in cold pressed camelina oil and the interference of plant rest materials, oils with more or less plant rest materials (CCO and CO) were
stressed in an accelerated oxidation test (dark, with air at 40 °C) and followed up by measurement of lipid oxidation products and natural antioxidants during the oxidative progress. The stability of Norwegian cold pressed camelina oil was highly dependent on amount of plant rest materials in the oil at accelerated conditions. While a small increase in both PV and AV were observed in CO after 2 weeks, CCO was more oxidative stable with no change in PV or AV until 4 weeks storage. The centrifuged oil (CO) was less stable at accelerated condition and huge increases in PV and AV between 4 to 9 weeks were observed. The increases in PV and AV in CCO during this period were significantly lower. Most of the volatile secondary oxidation products showed a non-linear increase with storage time, like PV and AV results, except for hexenal in CCO, which showed a decrease in levels up to four weeks storage. The major volatile oxidation products which showed increasing levels with storage time, showed significant positive correlations in the two oils and separately with PV and AV numbers (>0.93, p<0.001, n=7). The observed decrease in levels of hexanal up to 3 weeks storage, followed by increasing levels at weeks 6 and 9 in both oils is, however, difficult to explain. This also applies to 2-ethylfuran and 2, cis-pentenal levels, which increased until week 6, and then decreased by week 9. This may possibly indicate some effects of interaction of these compounds with the plant rest materials. Limited data exist in the literature on hydroxy-alkenals in oxidized vegetable oils. Results reported from unoxidized unsaturated vegetable oils vary from 0.01 to 0.2 µg/g oil40, which are in agreement with our results. Levels reported in oxidized oils vary from 2.0 to 45 µg/g oil40-43. In one of the studies40, oxidized rapeseed oil reached HHE and HNE levels of respectively 14 µg/g and 15 µg/g oil after 11 days storage of 28 grams oil in 1.1 litre closed vessels in dark at 37 °C, which are respectively about 5 and 15 times higher than our data. The other reported numbers (soybean, sunflower, palm, corn, linseed, camelina and peanut oils) are much higher than in the present study, since these data are from studies of deep-frying of oils, which have been exposed to far higher temperatures (>180 °C). A 2.7 times higher level was found for of HHE compared to HNE in CO after 9 weeks, which is in agreement with the study by Ma et al.43, who also found HHE levels to be higher than HNE in camelina oil after 3 hr incubation with air
at 180 °C. The levels of 4-HHE and HNE reflect the proportion of omega-3 and omega-6 fatty acids in the oil (omega-3/omega-6 = 2.1) and in addition an increased autooxidation rate of the more unsaturated omega-3 fatty acids than the omega-6 fatty acids. However, another accelerated storage study of camelina oil kept at 60 °C for 30 days in air\textsuperscript{44}, showed the same levels for HHE and HNE, i.e. about 0.6 µg/g oil. This HNE concentration corresponds to our HNE concentration in CO after 6 weeks storage at 40 °C, which is surprising, since the formation of hydroxy-alkenals have been shown to be temperature dependent, so higher levels would have been expected at higher temperatures\textsuperscript{45}. HHE and HNE levels showed significant positive correlation with AV values in CO and CCO and separately (r>0.97, p<0.002), reflecting the AV as measure of secondary oxidation products. CCO showed a lower 4-hydroxy-alkenal formation after 4-9 weeks storage compared to CO with less plant rest materials. The lower levels of 4-hydroxy-alkenals found in CCO with more plant rest materials, agreed with lower AV values and levels of volatile secondary lipid oxidation products compared to the centrifuged oil (CO) and may support stabilization effect due to presence of plant rest materials with antioxidant properties. Further, typical omega-3 fatty acid (ALA) derived volatile secondary lipid oxidation products in CO and CCO correlated significantly with 4-hydroxy-hexenal levels, like 2tr,4cis-heptadienal (r>0.94, <0.002, n=7) and 1-penten-ol (r>0.96, <0.002, n=7). 4-hydroxy-nonenal levels in CO correlated significantly with typical omega-6 fatty acid (LA) derived volatile secondary oxidation products like hexanal (r=0.84, <0.002, n=7) and 2, tr-heptenal (r=0.96, <0.002). However, in CCO there was no significant correlation of these volatile compounds with 4-hydroxy-nonenal levels, which may possibly also be due to some interaction with the sediment of the oil.

Decreased levels of both tocopherols and phenolics in camelina oil during storage at accelerated conditions have been reported\textsuperscript{20}. In our accelerated experiment, the degradation of the tocopherols was different in the CCO and CO. Both α-tocopherol and γ-tocopherol decreased in a 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. It is well known that the press cake from camelina contains phenolic compounds with antioxidative properties, and a drop in total phenolics parallel with drop in tocopherols has been observed during accelerated storage conditions of cold pressed camelina oil. 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. 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, syringenic acid and sinapinic acid were detected in the centrifuged oil (CO), both free and bound sinapic acid, catechin and syringenic 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. 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 tocopherols 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, 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 tocopherols 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

**ACKNOWLEDGMENT**

We thank Frank Lundby, Nofima AS, Ås, Norway, for contribution in chemical analysis of sterols and hydroxyalkenals.
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REFERENCES


46. Terpinc, P.; Ceh, B.; Ulrih, N. P.; Abramovic, H., Studies of the correlation between antioxidant properties and the total phenolic content of different oil cake extracts. *Industrial Crops and Products* 2012, 39, 210-217.


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

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

Table 2. Tocopherols (μg/g oil) and sterols (μg/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.

<table>
<thead>
<tr>
<th></th>
<th>CCO</th>
<th>CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocopherols (μg/g oil)</td>
<td>α-tocopherol</td>
<td>9.5±0.7</td>
</tr>
<tr>
<td></td>
<td>γ-tocopherol</td>
<td>639±4.9</td>
</tr>
<tr>
<td>Phytosterols (μg/g oil)</td>
<td>Cholesterol</td>
<td>356±3</td>
</tr>
<tr>
<td></td>
<td>Brassicasterol</td>
<td>327±1</td>
</tr>
<tr>
<td></td>
<td>Campesterol</td>
<td>1390±2</td>
</tr>
<tr>
<td></td>
<td>Stigmasterol</td>
<td>103±1</td>
</tr>
<tr>
<td></td>
<td>Sitosterol</td>
<td>2840±50</td>
</tr>
<tr>
<td></td>
<td>Avenasterol</td>
<td>410±1</td>
</tr>
</tbody>
</table>
Table 3. Levels of identified free and bound phenolics (ng/g oil) in Norwegian camelina oil, crude cold pressed oil (CCO) and centrifuged crude oil (CO) with less rest plant materials. Data (n=2) are shown with standard deviation. n.d. = not detected.

<table>
<thead>
<tr>
<th>Phenolics (ng/g oil)</th>
<th>CCO free</th>
<th>CCO bound</th>
<th>CO free</th>
<th>CO bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>0.56±0.04</td>
<td>1.09±0.21</td>
<td>nd</td>
<td>n.d.</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>0.71±0.12</td>
<td>0.20±0.02</td>
<td>0.16±0.06</td>
<td>n.d.</td>
</tr>
<tr>
<td>Rutin</td>
<td>228.65±8.31</td>
<td>n.d.</td>
<td>4.06±0.50</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>n.d.</td>
<td>0.17±0.06</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>3.68±0.73</td>
<td>0.11±0.08</td>
<td>0.20±0.03</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
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.
**Figure 2.** Peroxide value (PV) and Anisidine value (AV) in crude cold pressed camelina oil (CCO) and centrifuged camelina oil (CO) stored dark with access of oxygen at 40°C for 0-9 weeks. Data (n=2) are shown with standard deviation.
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 access of oxygen at 40°C for 0-9 weeks. Data (n=2) are shown with standard deviation.
Figure 4. Major volatile secondary oxidation products (VSOP, ng/g) in crude cold pressed camelina oil (CCO) and centrifugated camelina oil (CO) stored dark with access of oxygen at 40°C for 0-9 weeks. Data (n=2) are shown with standard deviation.
**Figure 5.** A) 4-hydroxy-hexenal and B) 4-hydroxy-nonenal (secondary lipid oxidation products) (µg/g) in crude cold pressed camelina oil (CCO) and centrifugated camelina oil (CO) stored dark with access of oxygen at 40 °C for 0-9 weeks. Data (n=2) are shown with standard deviation.
Figure 6. α-tocopherol and γ-tocopherol (µg/g) in crude cold pressed camelina oil (CCO) and centrifugated camelina oil (CO) stored dark with access of oxygen at 40°C for 0-9 weeks. Data (n=2) are shown with standard deviation.