Changes occurring in phenolic content, tocopherol composition and oxidative stability of *Camelina sativa* oil during storage

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Abstract

Changes occurring during storage in the content of polar phenolic compounds, the composition of tocopherols (T), the presence of primary and secondary oxidative products and titratable acidity in oil obtained from the seeds of *Camelina sativa* were studied. In fresh oil the content of polar phenolic compounds amounted to 128 mg/kg (expressed as chlorogenic acid), the content of α-T was (41 ± 8) mg/kg, of γ-T (710 ± 19) mg/kg and of δ-T (12 ± 3) mg/kg. β-T and tocotrienols were not detected. In oil stored at 50 °C the concentration of total tocopherols decreased to a value of (440 ± 13) mg/kg in 15 days. In that time the content of polar phenolic compounds in the oil stored at 50 °C was reduced to 72% of its initial value. The content of polar phenolic compounds in oil stored at 65 °C for 15 days was reduced to 21% of its initial value. The content of polar phenolic compounds in the *C. sativa* oil investigated decreased linearly with peroxide value and with p-anisidine value. The antioxidative activity of polar phenolic compounds extracted from camelina oil was also elucidated. Analysis revealed that the phenolic extract obtained from camelina oil added to a model lipid system for a certain time significantly retarded the process of autooxidation.

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1. Introduction

*Camelina sativa* is a member of the *Cruciferae* family with the common names false flax or gold of pleasure. Slovenia is one of the few countries where the cultivation of false flax is still carried on, mostly in the Koroska region. The crop has several positive agronomic attributes. It is tolerant to insects and weeds, very adaptable to climate and soil type and does not require high inputs of nutrients and pesticides. The main product from false flax are the seeds with a content of about 40% oil on a dry mass basis.

Renewed interest in *C. sativa* was inspired by the recent search for natural antioxidants and for new vegetable sources of essential polyunsaturated fatty acids, particularly omega-3 fatty acids. Because of their many health promoting effects omega-3 fatty acids have been repeatedly recommended for dietary intake. Oil extracted from *C. sativa* seeds contains a high content of polyunsaturated fatty acids amounting 40–60% of total fatty acids (Abramović & Abram, 2005; Budin, Breene, & Putman, 1995; Zubr & Matthäus, 2002). The predominating acid, α-linolenic acid (18:n – 3), amounts to 30–40%. The content of linoleic acid (18:2 n – 6) is 15–25% and of oleic acid (18: n – 9) 10–25%. Camelina oil contains about 15% gondoic acid (20:1 n – 9) and about 3% erucic acid (22:1 n – 9). These two fatty acids are typical of oils that are obtained from seeds of plants belonging to the *Cruciferae* family. Because of its beneficial health effects (Eidhin, Burke, Lynch, & O’Beirne, 2003), the oil has potential promise for use in the production of health promoting foods. In their study Karvonen et al., 2002 found that a daily intake of 30 g of...
camelina oil for 6 weeks reacted beneficially regarding serum cholesterol level and serum fatty acid composition in human subjects.

Oxidation is the major cause of loss of quality in lipid foods. The two compositional factors of oils that determine their susceptibility to oxidation are their fatty acid composition and the presence of antioxidant compounds. Due to the high content of linolenic acid and other unsaturated fatty acids in camelina oil, its oxidative stability is an important factor. In some investigations (Eidhin, Burke, & O’Beirne, 2003; Matthäus, 2004) 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 oils.

Phenolic compounds are secondary products of plant metabolism that have functions in preventing insect predation, bacterial or fungal infections and resistance to the effects of exposure to UV light. In addition to tocopherols several phenolic compounds have shown potential for antioxidative activity (Pietta, 2000). Although interest in phenolic compounds is related primarily to their antioxidant activity, they also show important biological activity in vivo. It has been reported (Pietta, 2000) that the intake of several plant phenolic compounds reduces the risk of cardiovascular disease, carcinogenesis, thrombotic tendency, and inflammation. All these studies provide the basis for the present rapidly increasing interest for the use of natural antioxidants as functional food ingredients and/or as food supplements. When pressing oil from seeds, part of the phenolic compounds is transferred to the oil. Zubr and Matthäus (2002) and also Budin et al. (1995) analysed the nonpolar group of phenolic compounds, i.e. tocopherols, in camelina oil obtained from different cultivars originating from different localities in Central Europe, Northern Europe and Scandinavia. Though extensive research has been dedicated to identification of polar phenolic compounds from various natural sources (Rice-Evans, Miller, & Paganga, 1997), the content of polar phenolic compounds in camelina oil has not previously been investigated. Matthäus (2002) in his study performed on fat-free seeds of C. sativa found that the content of polar phenolic compounds amounted to 1.8 mg per gram of these seeds (expressed as gallic acid).

Many investigators have followed the relationship between the content of tocopherols or phenolics and the progress of oil oxidation during storage. Most studies have been performed on olive oil (Morello, Motila, Tovar, & Romero, 2004; Monteleone, Caporale, Carlucci, & Pagliarini, 1998; Okogeri & Tasioula-Margari, 2002) and some on rapeseed oil (Koski et al., 2002).

In the present investigation the content of polar phenolic compounds and tocopherol composition in camelina oil was determined. Further, the changes in concentration of tocopherols and the content of polar phenolic compounds in camelina oil with time and temperature of storage were followed. These changes were then correlated to the content of primary oxidative products and to the content of secondary oxidative products. The antioxidative activity of polar phenolic compounds that we extracted from camelina oil and added to a model bulk oil system was also elucidated.

## 2. Materials and methods

### 2.1. Materials

The camelina oil used in this study was produced from seeds of C. sativa plants grown in the year 2005 in the Koroska region, Slovenia. Camelina oil was obtained by the following procedure. Dried seeds were milled and mixed with water. The mixture obtained was steamed at temperatures ranging from 60 °C to 90 °C. After pressing, the oil thus obtained was filtered. According to the local oil producers, steaming of the seeds is necessary, as oil cannot be obtained from non-steamed milled seeds. This oil had an attractive yellow colour and distinctive mustard-like odour. Before the investigation the oil was held at 8 °C in darkness for 3 weeks.

All other chemicals and solvents were of analytical grade.

### 2.2. Storage conditions

Oil samples (70 ml) were transferred to transparent glass beakers (4 cm in diameter) covered with a watch glass and subjected for 44 days to storage in an oven at (50 ± 0.5) °C and for 18 days at (65 ± 0.5) °C. Samples were withdrawn periodically from the oven. These oil samples were subjected to determination of titratable acidity, tocopherol composition, content of total polar phenolic compounds, peroxide value and p-anisidine value.

### 2.3. Determination of titratable acidity

The titratable acidity was determined according to AOAC Official Method 940.28 (AOAC, 1999). The method is based on titration of a solution of oil in ethanol with potassium hydroxide solution. The values were given as percentage of oleic acid. These determinations were carried out in triplicate. The standard deviation for each determination was ±0.01%.

### 2.4. Determination of peroxide value and p-anisidine value

Oxidation rate was followed by periodic determination of the peroxide value (PV) and p-anisidine value (p-AV). PV was determined according to AOAC Official Method 965.33 (AOAC, 1999). The method is based on iodometric titration, which measures the iodine produced from potassium iodide by the peroxides present in the oil. PV was expressed as mmol O₂ per kg of oil. p-AV was determined according to IUPAC method 2.504 (IUPAC, 1987). The method is based on the spectrophotometric determination of products formed in the reaction between aldehydeic...
compounds in the oil and \( p \)-anisidine. Both determinations were carried out in triplicate. The standard deviation for each PV determination as well as of AV determination was less than 2%.

2.5. Extraction of polar phenolic compounds

Phenolic compounds were isolated according to the following procedure. Five grams of oil sample was weighed, dissolved in 50 ml hexane and transferred to a separatory funnel. Twenty milliliters of a methanol–water mixture (80:10 v/v) was added. After 3 min of shaking the lower methanol–water layer was removed. The extraction was repeated twice and the methanol–water phases were combined. The methanol–water extract was condensed in a rotary evaporator under vacuum at 40 \( ^\circ \)C. The dry residue was then diluted in 1 ml of methanol. The repeatability of the extraction of polar phenolic compounds was also determined. The extraction procedure described above was performed three times. The content of extracted phenolic compounds determined as described below for each parallel extraction procedure was reproducible within ±3%.

2.6. Determination of polar phenolic content

The content of total polar phenolic compounds in the oil was determined colorimetrically with Folin–Ciocalteu reagent using a slightly modified method by Gutfinger (1981). The reaction mixture contained 200 \( \mu \)l of methanolic–water extract diluted in methanol, 125 \( \mu \)l of freshly prepared diluted Folin–Ciocalteau reagent and 125 \( \mu \)l of 20% sodium carbonate solution. The final mixture was diluted to 1 ml with deionized water. The mixture was kept in the dark at ambient conditions for 30 min to complete the reaction. Then the absorbance at 765 nm was measured on a model 8453 Hewlett Packard UV–Visible spectrophotometer (Hewlett Packard, Waldbronn, Germany) with a 1 cm cell. Chlorogenic acid was used as a standard. Results are expressed as mg of chlorogenic acid per kg of oil. The reaction was conducted in triplicate and results were averaged.

2.7. Determination of tocopherol content

The content of tocopherols in oil was determined by high-performance liquid chromatography (HPLC) according to a standard method (ISO 9936, 1997). Analyses were performed on an Agilent 1100 Series HPLC system equipped with a BinPump G1312A binary pump, an ALS G1329A thermostatted autosampler, an ALSTherm G1330B autosampler thermostat, a COLCOM G1316A thermostatted column compartment, a Phenomenex Luna column (250 \( \times \) 4.60 mm) packed with silica (5 \( \mu \)m particle size), a FLD G1321A fluorescence detector operating at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. The mobile phase used was hexane-isopropanol (99.3:0.7 v/v) at a flow-rate of 1.0 ml/min. The isomers eluted in the order from \( \alpha \)-T to \( \delta \)-T, the retention time of \( \alpha \)-T being 6.6 min and the resolution factor between the \( \beta \)-T and \( \gamma \)-T peaks >2.0. For each of the four tocopherol isomers a five level calibration graph was constructed covering the concentration range from 10 to 1070 mg for each tocopherol isomer per kg of oil (mg/kg), with a linearity correlation coefficient, \( r \) for \( \alpha \), \( \beta \) and \( \gamma \)-T amounting 0.99996 and for \( \delta \)-T \( r \) = 0.99993. The exact concentration of each tocopherol stock standard which served as a basis for five working calibration standards was calculated from the measured absorbance of the standard and the known absorbance coefficients of the isomers. The calibration graphs were used to calculate the tocopherol concentrations. For each tocopherol isomer the LOQ was determined, as well as the expanded measurement uncertainty, \( U \) with a coverage factor of 2. The analyses were carried out in duplicate.

2.8. Determination of antioxidative activity of extracted phenolic compounds

The phenolic compounds were effectively extracted from camelina oil with aqueous methanol, which however is not suitable for food applications. For that purpose after drying of the methanol–water extract in a rotary evaporator, the dry residue was diluted in ethanol. The ethanolic solution of extracted phenolic compounds was added to commercially available safflower (\textit{Carthamus tinctorius}) oil (Sigma–Aldrich) that was free from antioxidative components, producing the desired final concentration of phenolic compounds. Safflower oil with 20 mg of phenolic compounds per kg of oil (SFO-phenolics) was exposed for 25 days to storage at \((50 \pm 0.5) ^{\circ} \)C in the dark. Samples were withdrawn periodically from the oven and subjected to determination of PV. For comparative purposes the PV of safflower oil without added antioxidant (SFO-control) and the PV of safflower with butylated hydroxytoluene (BHT) added at a concentration of 200 mg/kg of oil (SFO-BHT) were also determined.

2.9. Statistics

Duncan’s multiple range test was performed to evaluate the significance of differences between mean values. All computations were made using the statistical analysis system (SAS) program (SAS/STAT, 1999) with the general linear models (GLM) procedure.

3. Results and discussion

3.1. Titratable acidity of camelina oil

In fresh camelina oil the titratable acidity amounted to 0.54%. This parameter represents the content of free fatty acids present in fresh camelina oil and is a measure of the treatment of the seeds before and during pressing. Free fatty acids were probably formed by the hydrolytic activity
of lipolytic enzymes during preparation of the seeds for oil production. According to the Regulations on Edible Oils (2003), unrefined oils can have up to 3.0% of free fatty acids. The titratable acidity of camelina oil stored in the dark at 50 °C slightly increases with time. After 9 days of storage it amounted to 0.59%, on the 27th day it was 0.71% and after 44 days of storage reached a value of 0.74%. This increase could be explained by the formation of acids that originate from decomposition of hydroperoxides and oxidation of aldehydes.

3.2. Content of polar phenolic compounds in camelina oil

The content of polar phenolics (expressed as chlorogenic acid) in fresh camelina oil amounted to 128 mg/kg. The content of polar phenolic compounds in camelina oil has not previously been investigated. As rapeseed belongs to the same Cruciferae family as Camelina, we believe that a comparison is reasonable. In post-expelled crude rapeseed oil (Brassica napus) some investigators (Vuorela, Meyer, & Heinonen, 2003) determined the amount of polar phenolics extracted with 80% methanol by the Folin-Ciocalteau reagent. The content of polar phenolics found amounted to 439 mg/kg (expressed as sinapic acid). In post-expelled crude rapeseed oil (Brassica napus) Koski, Pekkarinen, Hopia, Wüählä, and Heinonen (2003) succeeded in determining the amount of polar phenolics as 1066 mg/kg (expressed as caffeic acid). It is known that a higher content of phenolic compounds is released from the seeds when the oil is extracted at higher temperatures and higher pressure. It has to be stressed that the rapeseed oil used in the investigations performed by Koski et al. (2003) and Vuorela et al. (2003) was obtained by pressing the seeds at 100 °C and high pressure. In virgin olive oils phenolic compounds have been investigated many times. Their contents reported in the literature are rather variable and for good-quality oils are usually in the range 200–1500 mg/kg (Hrncirik & Fritsche, 2004).

Fig. 1 represents the content of polar phenolics in camelina oil at different stages of storage in the dark at 50 °C and 65 °C. From Fig. 1 we can see that during storage the content of polar phenolics decreased, indicating their degradation. The content of polar phenolic compounds in the oil stored at 65 °C decreased more progressively. Under these experimental conditions after 15 days of storage the content of phenolics in the oil was 27 mg/kg, representing 21% of its initial value. As we can see from Fig. 1, when the oil was stored at 50 °C the content of phenolic compounds decreased less progressively. On the 15th day of storage the content of phenolics in the oil was 92 mg/kg. After 44 days of storage at 50 °C the content of these compounds in the oil was 58 mg/kg, representing 45% of its initial value.

The changes in the content of polar phenolic compounds in camelina oil with time of storage were compared to the changes in the content of primary oxidative products, i.e. hydroperoxides expressed as PV, and to the changes in the content of secondary oxidative products expressed as p-AV. Fig. 2 shows the content of phenolics in the oil stored at 50 °C and 65 °C in the dark at varying peroxide values. Before exposure to accelerated storage conditions, the PV of camelina oil amounted to 2.30 mmol O₂/kg. Compared to storage at 50 °C, PV in the oil stored at 65 °C rose more sharply and after 15 days reached a value of 28.2 mmol O₂/kg. At that time PV in the oil stored at 50 °C amounted 18.3 mmol O₂/kg. After 44 days of storage at 50 °C the PV in the oil reached a value of 40.7 mmol O₂/kg. The progress of oxidation in camelina oil in our study is comparable to that observed for camelina oil in the investigation performed by Eidhin, Burke, et al. (2003). They determined a PV of 33 mmol O₂/kg in camelina oil on the 14th day of storage at 65 °C.
From Fig. 2 we can see that the content of phenolics decreased linearly with peroxide value. This dependence may be described by the following relation:

\[
\text{phenolic content} = a_0 + a_1\text{PV}
\]

where \(a_0\) and \(a_1\) are regression parameters. The values for \(a_0\), \(a_1\) and the correlation coefficient, \(r\), obtained by linear regression analysis for the oil stored at 50°C, amounted \(a_0 = 128 \pm 1\), \(a_1 = -1.78 \pm 0.08\) and \(r = -0.996\). For the oil stored at 65°C: \(a_0 = 137 \pm 10\), \(a_1 = -3.6 \pm 0.6\) and \(r = -0.977\). The results of our investigation show a very good correlation between PV and the content of total phenolic compounds in camelina oil during storage. Deiana et al. (2002) followed the dependence of the content of phenolic compounds in camelina oil during storage. Deiana, Popova, and Tasioula-Margari (2005) found that during storage of virgin olive oil under diffused light at temperatures between 6°C and 18°C an almost 60% decrease in the content of total phenols occurred after 6 months, indicating their rapid degradation. At the same time, olive oil stored at 20°C showed a slower rate of reduction (a decrease of 30% of total phenols) with an increase of PV to 13 mmol O₂/kg.

Tsourlas, Georgiou, Koidis, and Boskou (2005) found that loss of phenolic compounds in virgin olive oil stored in closed bottles in the dark at 20°C occurred in parallel with an increase in PV. A significant decrease of phenol content in virgin olive oil after 12 months of storage in darkness at room temperature with subsequent loss of oxidative stability was also established in the study performed by Morello et al. (2004).

3.3. Tocopherols in camelina oil

The total tocopherol content determined in fresh camelina oil amounted (751 ± 27) mg/kg. Among the tocopherols we determined in fresh camelina oil γ-T was predominant at the level of (710 ± 19) mg/kg. The contents of α-T and δ-T were (41 ± 8) mg/kg and (12 ± 3) mg/kg, respectively. In our investigation the amount of β-T was less than the LOQ (<3.4 mg/kg). The excitation spectrum recorded in the range from 210 nm to 305 nm at a fixed emission wavelength of 330 nm in the chromatogram of a sample of fresh camelina oil was compared to the spectrum of the δ-tocotrienol standard, showing that there was no evidence of δ-tocotrienol in the camelina oil.

Zubr and Mattha¨us (2002) in their investigation found the content of total tocopherols in camelina oil to range from 695 mg/kg to 994 mg/kg, with γ-T predominating (651–922 mg/kg). The content of α-T was at the level of 14–46 mg/kg, the content of δ-T 15–30 mg/kg, while β-T and tocotrienols were not detectable.

The changes in tocopherol content in camelina oil during storage in the dark at 50°C were followed. Fig. 4 represents the residual levels of total tocopherols in oil at different stages of storage. The content of total tocopherols in camelina oil decreased and after 27 days of storage amounted to (291 ± 16) mg/kg. After 27 days of storage the content of γ-T in camelina oil was reduced to 40% of its initial value. At that time α-T in camelina oil was no longer detectable. The content of δ-T stayed practically unchanged and the difference due to storage was smaller than the stated expanded uncertainty. The lower stability of α-T in comparison to γ-T is due to the fact that α-T...
reacts faster with peroxy radicals formed in the process of autooxidation (Belitz & Grosch, 1999). It has been proposed when investigating the stability of tocopherols that \( \alpha\)-T is consumed first, followed by \( \beta\)-T and \( \gamma\)-T, and finally \( \delta\)-T, which is more stable and thus consumed more slowly. Koski et al. (2002) in his study performed on cold pressed rapeseed oil found that the content of \( \alpha\)-T in oil decreased from the value of about 200 mg/kg in fresh oil to zero within 7–11 days of storage at a temperature of 60 °C in the dark, while 5–10% of the initial value of about 600 mg/kg of \( \gamma\)-T was still present after 2 weeks of storage. Morello et al. (2004) found that \( \alpha\)-T was totally absent in olive oil after 12 months of storage at room temperature in the dark. At lower temperatures a slower rate of reduction of \( \alpha\)-T in virgin olive oil was observed (Okogeri & Tasioula-Margari, 2002). They reported that after 12 months of storage in darkness at temperatures between 6 °C and 18 °C \( \alpha\)-T decreased to 40%.

3.4. Antioxidative activity of polar phenolic compounds extracted from camelina oil

The antioxidative properties of the phenols extracted from camelina oil were also determined. For that purpose the ethanolic solution of extracted phenolic compounds was added to commercially available safflower (C. tincto-rius) oil that was free from antioxidative components. Fig. 5 shows the change in PV of SFO-phenolics (20 mg/kg of oil) stored at 50 °C in the dark. For comparison the changes in PV of the SFO-control and the changes in PV of SFO-BHT (200 mg/kg of oil) are also depicted. In Fig. 5 we can see that after a certain time of storage PV of SFO-phenolics increased less sharply than does the PV of SFO-control. On the 14th day as well as on the 20th day of storage PV of SFO-phenolics differed significantly (\( P \leq 0.001 \)) from PV of SFO-control. In Fig. 5 we can see that after the 14th day of storage the PV of SFO-phenolics increased more sharply than does the PV of SFO-BHT. In these days of storage the phenolic compounds were probably decomposed and thus their antioxidative activity declined. At the 25th day of storage PV of SFO-phenolics did not differ notably from PV of SFO-control.

Only a few studies have been published investigating antioxidative properties of polar phenolics from a vegetable oil source added to a bulk oil system. The varying oils and methods used complicate comparison. Van Ruth, Shaker, and Morrissey (2001) reported an antioxidative effect with regard to formation of primary and secondary oxidation products up to the 3rd day of storage at 60 °C for a methanolic extract of soybean oil added to linseed oil. A similar experiment with a methanolic extract of rapeseed oil added to purified rapeseed oil showed significant reduction in formation of primary oxidation products during 4 days of storage at 60 °C (Koski et al., 2003).

4. Conclusion

The titratable acidity of camelina oil stored in the dark at 50 ° slightly increased with time from 0.54% in fresh oil to a value of 0.74% at the 44th day of storage. In fresh camelina oil the content of polar phenolic compounds amounted to 123 mg/kg (expressed as chlorogenic acid). The content of polar phenolic compounds in the oil stored at 65 °C for 15 days was 27 mg/kg. In that time the content of polar phenolic compounds in the oil stored at 50 °C was 92 mg/kg. After 44 days of storage at 50 °C the content of these compounds in the oil was 58 mg/kg. During storage the content of polar phenolic compounds in camelina oil decreased linearly with peroxide value as well as with \( p\)-anisidine value. The total tocopherol content determined in fresh camelina oil amounted to 751 mg/kg. The contents of \( \alpha\)-T, \( \gamma\)-T, and \( \delta\)-T were 41 mg/kg, 710 mg/kg and 12
mg/kg, respectively. The presence of β-T and tocotrienols was not detected. In oil stored at 50 °C the content of total tocopherols decreased to a value of 440 mg/kg in 15 days. The phenolic compounds extracted from camelina oil when added to commercially available safflower oil resulted in significant reduction in formation of primary oxidation products during 20 days of storage at 50 °C.

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