Unique dietary oil from *Camelina sativa* seed

JOSEF ZUBR
The Royal Veterinary and Agricultural University
Department of Agricultural Sciences
Bülowsvej 13, 1870 Frederiksberg C
Denmark

Josef Zubr

ABSTRACT: Seed of false flax (Camelina sativa (L.) Crantz) is a source of edible oil with specific properties. Camelina oil (CO) was obtained from the seed by pressing. Laboratory analyses revealed the content of oil in seed of winter varieties (W) 41.8 ± 1.3 percent and in seed of summer varieties (S) 42.2 ± 0.46 percent on dry matter basis (DM). The content of linoleic acid 18:2n-6 (LA) in W was 12.4 ± 0.09 percent and in S it was 15.3 ± 0.43 percent of the total fatty acids (FA). The content of a-linolenic acid 18:3n-3 (ALA) in W amounted to 40.8 ± 0.16 percent and in S 36.8 ± 0.84 percent. The content of gondoic acid 20:1n-9 was 15.7 ± 0.11 percent in W and 15.4 ± 0.23 percent in S. Erucic acid 22:1n-9 was 3.10 ± 0.14 percent and 2.8 ± 0.10 percent in W and S, respectively. Tocopherols were found in amounts ranging from the mean of 806 μg/g in S to 1,021 μg/g in W. The content of the prevalent γ-tocopherol was 742 - 935 μg/g. Oxidative stability of the oil was examined and evaluated.

INTRODUCTION

Camelina sativa (L.) Crantz (CS) belongs to the Brassicaceae family. The plant is known as false flax and gold of pleasure. In European countries during the 19th and 20th centuries, CS was grown as an alternative oil-seed crop up to the1950s. In Southeastern Europe CS is still grown to a limited extent for home production of oil. The oil is used as a dietary supplement and a remedy in folk medicine (1). A search for new sources of n-3 FA turned a renewed attention to CS. Research with CS was carried out mainly in European countries (2-11). During the period 1995-98 an EU research project "Alternative oil-seed crop *Camelina sativa*" (AIR3-CT94-2178) was executed with participants from six EU countries. New knowledge was obtained about cultivation, qualitative evaluation and the exploitation of the products from CS. The crop can be grown environmentally friendly without the application of herbicides and pesticides (9). Main product from the crop is seed. The reddish-brown seed is small with 1,000 seeds weight of about 1.0 g. Mucilage in the coating of the seed exerts affinity to water. The seed possesses a distinct smell and taste. The content of oil in the seed is approximately 42 percent in DM. The oil is highly unsaturated. The content of LA is about 15 percent and the content of ALA amounts to about 38 percent of the total FA (8). The present investigation disclosed some specific properties of CO with large significance for exploitation of the oil in human nutrition.

MATERIALS AND METHODS

Within the framework of the EU research project 1995-1998, the crop was grown under identical cultivation practices simultaneously in Germany, Denmark, United Kingdom, Ireland, Sweden and Finland. Seed samples from the different locations were collected for analyses in Denmark. The oil for analyses was obtained from seed by pressing in KOMET CA 59 G. Analyses were carried out by authorized laboratories.
The content of oil in camelina seed was determined by the use of Pulsed Nuclear Magnetic Resonance Spectrometry (EØF L 328, 2.9.2.). Seed samples were analysed in Oxford NMR Instrument (Oxford Analytical Instruments, Abingdon Oxon, UK). The analyses for FA were carried out in GLC (IUPAC Standard Method 2.304). The GLC instrument Perkin Elmer XL (PTV, Shelton, CT) was equipped with PC column 50 m / 0.25 mm silar 88 coating 0.2 mm. The sample size was 0.2 μL (methyl ester), oven temperature 100-225 °C, detector 250 °C, PE data treatment Turbochrom.
The content of tocopherols (AOCS Official Method Ce 8-89) was determined in HPLC instrument Perkin Elmer, equipped with injection reodyne 7125 (sample 20 μL), pump LDC 3200 (1.0 mL / min), column Spherisorb S5W 250 mm / 4.6 mm, mobile phase hexan / isopropanol, detector Perkin Elmer fluorescence LS. Standard methods were used for analyses for free fatty acids (AOCS Official Method Ca 5a-40), peroxide value (AOCS Official Method Cd 8-53) and anisidine value (AOCS Official Method Cd 18-90).

Variety / Origin >	DK East	DK West	DE Mul	DE Pad
Winter	43.1	42.3	46.1	38.9
Summer	42.0	41.5	42.0	40.8
Variety / Origin >	**SE Ups**	**IR Car**	**Means**	**SEM**
Winter	37.2	43.1	41.8	1.31
Summer	44.1	42.7	42.2	0.46

Symbols: DE Mul = Müllheim, DE Pad = Paderborn, SE Ups = Uppsala, IR Car = Carlow, W = Winter varieties, S = Summer varieties, SEM = standard error of mean, n = 18.

Table 1. Content of oil in camelina seed (percent of DM).

RESULTS AND DISCUSSION

The present report makes an abridged account for recent progress in the knowledge about properties and qualitative evaluation of CO. Particular attention was paid to the properties of the oil with regard to enrichment of food with essential LA and ALA. Results from the coordinated research 1995-1998 (internal reports) were compared with data from different investigations. Relevant scientific evidence was reviewed.

Content of oil in camelina seed

The content of oil in CS seed is a primary qualitative parameter. Seed samples from six remote locations were analysed. The mean content of oil in seed from W with 41.8 percent was slightly lower than in S with 42.2 percent on DM basis. Large variation was found in the content of oil in seed from different locations (Table 1). Within 6 locations in EU countries, the highest content of oil (46.1 percent) was found in the seed of W from Southern Germany. The highest content of oil in S (44.1 percent) was

recorded in seed from Sweden. Incidentally, the seed of W from Sweden had the lowest content of oil among the samples from other locations (Table 1).

Camelina oil is a rich source of α-linolenic acid

Production of camelina oil

In practice, production of CO for human consumption is carried out by pressing from seed in a screw press. The press is capable of handling whole seed without any pre-treatment. When the oil is produced industrially on large scale, the seed prior to pressing must be crushed or milled. Under pressing in laboratory, the oil was heated to 35-45 °C by friction in the screw expeller. In order to eliminate lipases and to enhance the release of oil-soluble substances, such as tocopherols, phenols and phytosterols, the temperature of the expeller was maintained at 80 °C by additional electrical heating. Pressing from freshly harvested seed with a moisture of 9 percent yielded 29.8 ± 0.48 percent oil of the original seed weight (n=11). Pressing the seed stored for 12 months with moisture of 7 percent, without additional heating of the expeller, yielded 31.4 ± 0.31 percent oil of the original seed weight (n=5) . With additional heating (80 °C) the yield was 32.0 ± 0.37 percent oil (n=5). From seed stored 2 years in cotton bags (50 kg) at 5-25 °C with moisture of 6 percent, without additional heating, the yield of oil was 33.1 ± 0.29 percent (n = 17). The differences in temperature during pressing had a moderate effect on the yield of oil. Under processing the oil was protected against direct sunlight. The colour of the oil was yellow. Sensory testing revealed a distinct flavour resembling fresh cauliflower. The flavour was less intensive in oil obtained from the long term stored seed.

A high content of antioxidants preserves camelina oil against oxidation

Refining of camelina oil

Crude oil from freshly harvested seed contained a larger amount of impurities than the oil from stored seed. The impurities were removed by settling. Partial settling at 20 °C was achieved within two days (48 h). After vacuum filtration the oil was stored in sealed coloured bottles in darkness at a constant temperature of 5 °C. For special application (mixed fat, ice cream), the oil must be prepared by deodorization. In a laboratory batch deodorizer with 9 L oil, before starting deodorization the absorbed air was removed from the oil by vacuum. Deodorization temperature was kept at 200 °C for 2.5 h and was finally increased to 220 °C for 30 min. Vacuum in the deodorizer and in the adjacent condenser was 6-10 mm Hg. The vacuum was maintained during cooling to 50 °C. Optimum water input for steaming was about 25 percent v/v. The exhausting stripping vapour had a strong distinct smell. The condensate from the deodorizer was a white gel with a pale green surface. The smell was similar to that of the exhausting vapour. The deodorized oil was bright yellow in colour and was odourless and tasteless. The oil was stored in sealed containers in darkness at 5 °C. To avoid oxidation induced by light, CO must be distributed in light proof containers or coloured bottles (brown). By using different processing methods, experience was achieved with the treatment of crude oil by degumming, neutralization and bleaching. The conventional pre-treatment prior to deodorization caused a physical damage to the oil. The deodorized oil became cloudy and during storage separated a layer of sediments. The pre-treatment of crude oil before deodorization by a vacuum filtration only was sufficient.

FA	Origin >	DK East	DK West	DE Mul	DE Pad	SE Ups	IR Car	Means
16:0	W	5.2	5.4	5.3	5.6	5.6	5.4	5.4 ± 0.07
16:0	S	5.4	5.3	5.4	5.6	5.1	5.5	5.4 ± 0.07
18:0	W	2.2	2.2	2.1	2.2	2.5	2.4	2.3 ± 0.07
18:0	S	2.6	2.6	2.6	2.6	2.6	2.7	2.6 ± 0.02
18:1n-9	W	11.9	11.7	13.3	11.3	10.2	11.7	11.7 ± 0.41
18:1n-9	S	13.1	12.6	16.7	13.4	13.8	14.1	14.0 ± 0.59
18:2n-6	W	12.1	12.3	12.5	12.6	12.1	12.5	12.4 ± 0.09
18:2n-6	S	14.6	14.5	17.3	15.5	14.7	15.0	15.3 ± 0.43
18:3n-3	W	41.4	40.9	40.2	40.5	40.7	40.7	40.8 ± 0.16
18:3n-3	S	38.0	38.0	32.7	37.2	37.2	37.8	36.8 ± 0.84
20:1n-9	W	15.8	15.8	15.4	15.3	16.0	15.7	15.7 ± 0.11
20:1n-9	S	15.7	16.1	15.0	14.7	15.7	14.9	15.4 ± 0.23
22:1n-9	W	2.9	2.9	2.9	3.3	3.7	2.9	3.1 ± 0.14
22:1n-9	S	2.8	3.0	2.9	2.8	2.9	2.3	2.8 ± 0.10

Symbols: DE Mul = Müllheim, DE Pad = Paderborn, SE Ups = Uppsala, IR Car = Carlow, W = winter varieties, S = Summer varieties.

Legend: FA - 16:0 = palmitic, 18:0 = stearic, 18:1n-9 = oleic, 18:2n-6 = linoleic, 18:3n-3 = α-linolenic, 20:1n-9 = gondoic, 22:1n-9 = erucic. The content of the minor FA (0.1-1.6 %) 14:0, 16:1, 18:1n-7, 18:3 tr., 20:0, 20:2, 20:3, 22:0, 22:2, 24:0, 24:1, was omitted, n = 18.

Table 2. Content of fatty acids in camelina oil (percent of FA).

Content of fatty acids in camelina oil

The content of FA in CO depends mainly on the varieties and on the conditions under which the crop was grown. From the nutritional point of view, the most important is the content of LA (18:2n-6) and ALA (18:3n-3). In the seed of W and S the content of LA ranged from 12.1 to 17.3 percent and ALA ranged from 32.7 to 41.4 percent (Table 2). The oil from W had a lower content of oleic acid and LA while the content of ALA was significantly higher than in the oil from S (Table 2). The presence of gondoic acid with 14.7-16.1 percent is a curiosity of CO. The role of this FA in human metabolism is not known. The content of erucic acid was 2.3-3.7 percent. This was below the limit of 5.0 percent allowed in vegetable oils for human consumption (EU Commission, Subsidiary Legislation 231, 42, Erucic Acid Regulations, 2001). No substantial differences were found in the content of palmitic, stearic, gondoic and erucic acids between W and S. The content of saturated FA (SFA) ranged from 7.7 to 8.0 percent (Table 2). This was less than in flax oil with 10.5 percent SFA (12) and more than in rape oil with 6.2 percent SFA (13).

Variety / oil	α	γ	δ	P-8	Total	n / ref.
CS winter	23.5 ± 4.07	935 ± 72	25.0 ± 1.34	24.3 ± 4.46	1,008	15
CS summer	28.1 ± 6.15	742 ± 39	20.5 ± 2.33	14.9 ± 3.70	806	15
Rape	217	407	8	73	705	Local
Rape	237	391	15	82	725	[15]
Flax	121	510	33	137	801	[15]

Table 3. Content of tocopherols in selected oils (µg/g).

Variety / Origin >	DK East	DK West	DE Mul	DE Pad
Winter	0.18	0.18	0.13	0.22
Summer	0.19	0.34	0.28	0.17
Variety / Origin >	**SE Ups**	**IR Car**	**Means**	**SEM**
Winter	0.20	0.30	0.20	0.02
Summer	0.19	0.16	0.22	0.03

Symbols: DE Mul = Müllheim, DE Pad = Paderborn, SE Ups = Uppsala, IR Car = Carlow, SEM = standard error of mean, n = 18.

Legend: The oil was exposed to the temperature of 200 °C.

Table 4. Content of free fatty acids in camelina oil (percent of oil).

Content of tocopherols in camelina oil

A comparison of CO with commonly known vegetable oils revealed striking differences in the content of tocopherols. The total content of tocopherols in CO ranged from the mean of 806 µg/g in the oil from S to 1,021 µg/g in the oil from W. This was higher than in flax oil and rape oil (Table 3). Almost identical total content of tocopherols in CO (900 - 1,151 µg/g) was reported by Rode (1). Another investigation shows the total content of tocopherols in rape oil 915 µg/g, in sunflower oil 907 µg/g and in soy oil 1,865 µg/g (14). The content of α-tocopherol in CO (23.5-28.1 µg/g) was much lower than in flax oil (121 µg/g) and in rape oil with 217-237 µg/g (Table 3). The content of α-tocopherol in CO, amounting to 41 µg/g was reported (16). Hammond found 215 µg/g α-tocopherol in rape oil and 110 µg/g in soy oil. In sunflower oil, α-tocopherol was predominating with 860 µg/g (14). The content of β-tocopherol in CO was below 10.0 µg/g (omitted in Table 3). The content of the prevalent γ-tocopherol in CO ranged from 742 to 935 µg/g. This was more than in flax oil with 510 µg/g and in rape oil with 391-407 µg/g (Table 3). Abramovic et al. (16) found in CO 710 µg/g γ-tocopherol. Hammond reported the content of γ-tocopherol in rape oil 630 µg/g, in soy oil 1,280 µg/g and in sunflower oil 20 µg/g (14). The content of δ-tocopherol in CO (20.5-25.0 µg/g) was higher than in rape oil (8-15 µg/g), but was lower than in flax oil (Table 3). Abramovic et al. reported a content of 12 µg/g δ-tocopherol in CO (16). Plastochromanol-8 (P-8) content in CO (14.9-24.3 µg/g) was much lower than in flax oil and rape oil (Table 3). The content of tocotrienols was below 10 µg/g (omitted in Table 3).

Due to the high content of α-linolenic acid and antioxidants, camelina oil can be considered as a healthy food

Content of free fatty acids in camelina oil

Free fatty acids (FFA) represent the substrate for oxidation products. The content of FFA in oil is a measure of the enzymatic decomposition of triglycerides by the action of lipases (17). The activity of lipases, however, can be eliminated in advance by elevated temperature during production of the oil (18). In CO obtained by pressing in laboratory, the content of FFA was relatively small. The temperature of 80°C during pressing the seed apparently

eliminated lipases and thereby diminished the decomposition of triglycerides and the formation of FFA. In the oil produced from seed of different origin, FFA content ranged from 0.16 to 0.34 percent (Table 4). The small amount of substrate for hydroperoxides and for secondary oxidation products, such as aldehydes, ketones, alcohols, etc. (18), obviously predetermined the oxidative stability of the oil. Abramovic (10) reported 2.35 percent FFA in CO produced by the use of an ancient method.

Oxidative stability of camelina oil

The storage life of vegetable oils depends mainly on FA profile and on the presence of antioxidants. External factors such as temperature, access of air and light are also decisive. The high content of ALA in CO indicates a susceptibility of the oil to oxidation. This was confirmed by Rancimat test. However, the established testing with an induction period at 100 °C was misleading. The induction period in hours may correspond to a year at low temperature (19). Practical experience shows that a storage at 5 °C appears optimal. An exposure of natural CO to -15 °C for 2 days caused a coagulation of the oil. The cloudy appearance, however, was reversible at temperatures above freezing point. To verify the repeatedly observed oxidative stability of CO, natural oil was stored in sealed coloured glass bottles (500 mL) in darkness at a constant temperature of 5 °C for 2 years. The oxidation status of the oil was evaluated at the end of the storage by analyses and by sensory testing. At the end of storage, FFA content was 0.47 ± 0.03 % (n=6). Primary oxidation products were expressed by peroxid value (Pv) 7.8 ± 0.31 (n=6). The secondary oxidation products were quantified by anisidine value (Av) 2.3 ± 0.28 (n=6). Sensory test revealed the typical flavour, but markedly weaker than in fresh CO. A mild rancidity, described as a background pricking sensation, was detected. A sample of natural CO in sealed glass bottle (500 ml) was stored in darkness at 5 °C for 7 years. At the end of the storage, FFA content was 1.35 percent, Pv was 15.2 and Av was 2.6. In deodorized CO (500 ml), stored at identical conditions, was found 0.10 percent FFA, Pv was 18.2 and Av was 2.5. Sensory test disclosed a mild rancidity in both oils. It seems that a depletion of oxygen during the initial phase of storage inhibited further oxidation. To explain the unexpected oxidative stability of CO, available scientific documentation was reviewed. The long shelf life of CO can be explained partly by the presence of γ-tocopherol (20) and plastochromanol P-8 (21). Besides the tocopherols, in fresh CO polar phenolic compounds (128 μg/g expressed as chlorogenic acid) were found. When tested with safflower oil, the phenolic compounds retarded autoxidation (16). Unidentified phenolic compounds (400 μg/g) were found in CO originating from Southeastern Europe (10). In CO were also found considerable amounts of phytosterols such as sitosterol, campesterol, avenasterol and others (22, 23). Avenasterol is known as an effective antioxidant (24). It may be anticipated that the different antioxidants in CO provide a cumulative protection of the oil against oxidation. According to independent investigation, CO in closed bottles stored in darkness at 4 °C, maintained its original Pv 2.0 for 10 weeks. In closed and open bottles, kept at a temperature of about 20 °C, Pv during 10 weeks increased to about 18 and 23, respectively (23). The present investigation confirmed an important role of storage conditions in the control of autoxidation. Excluded access of light and air together with a low temperature (5 °C) are precautions with great significance for the shelf life of the oil.

CONCLUSIONS

Camelina oil is a rich source of LA and ALA as well as antioxidants. Due to the high content of essential FA (LA and ALA), the oil is highly unsaturated. The theoretically envisaged susceptibility of the oil to oxidation was found to be misleading. Under optimal conditions (darkness and low temperature) CO can be stored for years without loosing its nutritional quality. To prevent oxidation induced by light, the oil must be stored and distributed in light proof containers and dark coloured bottles. The high content of ALA, tocopherols, phenols and phytosterols, make natural CO suitable for nutritional enrichment of food. The oil can be used as a dietary supplement. Both the natural and deodorized CO are also applicable as ingredients in health promoting food products.

ACKNOWLEDGEMENTS

The project "Alternative oil-seed crop Camelina sativa" 1995-1998 (AIR-CT94-2178) was sponsored by the Commission of the European Communities. This report does not necessarily reflect the Commission´s views and in no way anticipates the Commission´s future policy in this area. The participation of the following institutions and laboratories is hereby acknowledged: Danish Institute of Agricultural Research, Foulum, Denmark; IFUL, Müllheim-Basel, Germany; Paderborn University, Laboratory Soest, Germany; Swedish Agricultural University, Uppsala, Sweden; Teagasc, Carlow, Ireland; BfEL, Institute for Lipid Research, Münster, Germany; Institute for Food Technology, Agro-Industrial Technology and Molecular Biotechnology, Kolding, Denmark; International Food Science Centre A/S, Lystrup, Denmark.

REFERENCES AND NOTES

1. J. Rode, "Study of Autochthon Camelina sativa (L.) Crantz in Slovenia", Breeding Res Aromat Medic Plants, pp. 313-318 (2002).
2. K.H. Müller, "Anbauwüdigkeit und Anbautechnik des Leidotters", Die Deutsche Landwirtschaft, 9, 2, pp. 65-67 (1958).
3. A. Nordestgaard, "Forsøg med stigende maengder kalksalpeter til dodder", Tidskrift for Planteavl, 64, 6, pp. 1024-1034 (1961).
4. C. Musnicki, "Cultivation experiments with winter false flax (Camelina sativa) in the Experimental Station Przybroda belonging to the Agricultural High School in Poznan", Pamietnik Pulawski, 5, pp. 15-32 (1962).
5. K. Adamczewski, "Nutrition needs of spring false flax (Camelina sativa)" Pamietnik Pulawski, 5, pp. 157-164 (1967).
6. R. Marquard, H. Kuhlmann, "Untersuchungen über Ertragsleistung und Samenqualität von Leindotter (Camelina sativa Crtz.)" Fette Seifen Anstrich, 88, pp. 245-249 (1986).
7. A. Bramm, M. Dambroth et al., "Analyses of yield components of linseed, false flax and poppy", Landbauforschung Völkenrode, 40, pp. 107-114 (1990).
8. J. Zubr, "New vegetable oil for food application", Agro Food Ind Hi-Tech, pp. 24-26 (1992).
9. J. Zubr, "Oil-seed crop Camelina sativa", Ind Crops and Prod, 6, pp. 113-119 (1997).
10. H. Abramovic, V. Abramovic, "Physico-Chemical Properties, Composition and Oxidative Stability of Camelina sativa Oil", Food Technol Biotechnol, 43, 1, pp. 63-70 (2005).
11. H.M. Paulsen, "Mischfruchtanbausysteme mit Ölpflanzen im ökologischen Landbau: 1. Ertragsstruktur des Mischfruchtanbaus von Leguminosen oder Sommerweizen mit Leindotter (Camelina sativa L. Crantz.)", Landbauforschung Völkenrode, 57, 1, pp. 107-117 (2007).
12. G.G. Rowland, R.S. Bhatty, "Ethyl Methanesulphonate Induced Fatty Acid Mutations in Flax", JAOCS, 67, 4, pp. 213-214 (1990).
13. K. Warner, E.N. Frankel et al., "Flavor and Oxidative Stability of Soybean, Sunflower and Low Erucic Acid Rapeseed oils", JAOCS, 66, p. 4 (1989).
14. E.W. Hammond, "Analyses Tocopherols and Tocotrienols", Lipid Tech, pp. 86-88 (1998).
15. Van M. Balz, E. Schulte et al., "Trennung von Tocopherolen und Tocotrienolen durch HPLC", Fat Sci Technol., 94, 6, pp. 209-213 (1992).
16. H. Abramovic, B. Butinar et al., "Changes occurring in phenolic content, tocopherols composition and oxidative stability of Camelina sativa oil during storage", Food Chem, 104, 3, pp. 903-909 (2007).
17. R.G. Jensen, "Detection and Determination of Lipase (Acylglycerol Hydrolase) Activity from Various Sources", Lipids, 18, 9, pp. 650-657 (1983).
18. E.R. Sherwin, "Oxidation and Antioxidants in Fat and Oil Processing", J Am Oil Chem Soc, 55, pp. 809-814 (1978).
19. J.B. Rossel, "Measuring resistance to oxidative rancidity", Lipid Tech, pp. 39-44 (1992).
20. J. Pokorny, "Natural antioxidants for food use", Trends Food Sci Technol, 2, pp. 223-226 (1991).
21. D. Olejnik, M.Gogolewski et al., "Isolation and some properties of plastochromanol-8", Nahrung, 41, 2, S 101-104 (1997).
22. V.K.S. Shukla, P.C. Dutta et al., "Camelina oil and its unusual cholesterol content", J Am Oil Soc, 79, pp. 965-969 (2002).
23. B. Matthäus, "Leindotteröl – ein altes Pfanzenöl mit neuer Zukunft", Ernährungs-Umschau, 51, 1, pp. 12-16 (2004).
24. M.H. Gordon, P. Magos, "The Effect of Sterols on the Oxidation of Edible Oils", Food Chem, 10, pp. 141-147 (1983).