Academy of Romanian Scientists *Volume 8, No. 2, 2019, pp. 24 - 34*
Annals Series on Biological Sciences ISSN ONLINE 2285 – 4177

ORIGINAL PAPERS

Research on the Purification of Camelina Oil Used in the Composition of Cosmetic Products

Simona **COPACI**[1, 3, *], Laura **OLARIU**[2, 3], Roxana **NITA**[2, 3], Diana **ENE**[2, 3], Ştefana **JURCOANE**[1, 3], Cristina Nicoleta **DĂNĂILĂ** (STOICA)[3, 5], Natalia **ROSOIU**[3, 4]

[1] University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Biotechnology, Romania

[2]SC BIOTEHNOS SA, Otopeni, Ilfov, Romania

[3]Academy of Romanian Scientists

[4]"OVIDIUS" University, Faculty of Medicine, Department of Biochemistry, Constanţa, Romania

[5]Doctoral School of Applied Sciences, Biochemistry/Biology, University Ovidius Constanța, Romania

* Corresponding author e-mail: copaci_simona@yahoo.com

Abstract

It is known the composition of camelina oil in fatty acids, mainly unsaturated fatty acids, in special linolenic acid, it can have many uses in the pharmaceutical industry, but also in the cosmetic industry. Camelina oil is obtained from Camelina seeds (Camelina sativa) through a cold pressing process. The quality of the oil, but also its use depends on the filtration and purification processes. The purpose of this project was to track and analyze the physico-chemical parameters of some oil samples obtained by pressing the seeds grown in the ecological system, kept under different temperature conditions.The evidence of understanding was obtained from the seeds of Camelina, the Mădălina variety, cultivates the Belciugatele farm, working point Moara Domnească (Găneasa village, Ilfov county) during 2017-2018. The samples kept maintaining the ambient temperature, in dark blue plastic (capacity 10l) filled with 5l oil, in parallel, there are experiments of keeping the refrigerator in the brown glass container, completely filled. After storage, impurity absorption treatments are applied with the addition of bentonite, zeolite, volcanic tuff, in small proportions. The physico-chemical parameters followed immediately after the sprinkling and purification, as well as after keeping under each temperature and encouraging conditions: appearance, color, odor, as well as the determination of the saponification, iodine, but also fatty acid composition. The results of the analyzes carry *out and* authorize the stabilization of the technological purification flow in the laboratory and the stabilization of the storage conditions of a purified oil, until they can be added in the cosmetic products.

Keywords: *Camelina sativa, filtration, purification, camelina oil, iodine index.*

Introduction

Camelina sativa is one of the oil plants of importance in obtaining biofuels (for aviation), but is increasingly used in other industries such as: pharmaceutical, cosmetic, but also in human nutrition. Camelina (*Camelina sativa* L. Crantz) is an annual plant of the *Brassicaceae* family (BUDIN & al. [1],; ZUBR [2]), which originates in North Europe and Central Asia. The main methods of oil extraction are mechanical extraction, solvent extraction and enzymatic extraction (Atabani, 2013), but also supercritical CO_2 extraction (Berti, 2016). The earliest method of extracting oil from seeds is hydraulic pressing, which has been used since 1795 and has appeared in Europe. Subsequently its location was taken over, mostly by screw presses (Rosenthal, 1996), and at present most commonly, eccentric manual presses or motor screw presses (Atabani, 2013), organic solvent extraction, organic extraction. Supercritical CO_2 (Berti, 2016). Mechanical extraction by hot or cold pressing does not require exceptional resources, but the oil obtained by this method must be subjected to further processing such as filtration or degumation (Atabani, 2013), and the choice of the correct pretreatment is very important to obtain a high yield. Solvent extraction first used in Europe in 1870 is widely used, and can be used to complete mechanical extraction. The efficiency of this method is very high, being a very good option for large-scale production. This method is influenced by several factors, namely: particle size, extraction solvent, temperature and agitation. In a study by Moser et al. In 2010, a yield of 30.5 wt% was obtained by drying the seeds with hexane and extracting by the Soxhlet method for 24 hours. Another study by Berti et al. in 2016, increased extraction efficiency was demonstrated by Soxhlet extraction compared to supercritical CO_2 extraction, respectively mechanical cold pressing. Also comparing Belayneh et al., in 2015 it obtained 35.9% efficiency through Soxhlet (with hexane for 6h), 29.9% by hot pressing and 31.6% by supercritical CO_2 extraction, the latter having a high efficiency in oil recovery, but the equipment is expensive. The enzyme extraction has the advantage that it does not use chemicals, it is not polluting, but it is time consuming. So far, no qualitative differences between the obtained oils have been revealed. The proportional representation of fatty acids is not equitable, so most are maintained by unsaturated, mono and polyunsaturated fatty acids, their proportion being greater than 55%. In a smaller percentage, respectively 9.1 - 10.8% saturated fatty acids are found (Toncea et al., 2013) [7]. Studies to date have shown that the highest amount of fatty acid in camelina oil is linolenic acid, known as omega 3. Other fatty acids are also found in oil, namely linoleic acid or omega 6, oleic, eicosanoic and erucic, these being in approximately equal quantities. Eicosanoic acid is rarely found in plants, which is

found in coconut oil and palm oil. An important characteristic based on this acid is that related to the classification of this oil into medium chain fatty acids (MCFA) (Righini et al., 2016) [5] Current studies have shown much lower stability of camel oil compared to other oils, such as rapeseed oil and sunflower oil. The analyzes were performed with both synthetic antioxidants and natural antioxidants (Frohlic et al., 2011)[8]. Studies for camelina oil have also targeted its antioxidant activity on the basis of the compounds present. Methanolic extract was analyzed based on several tests, such as: reduction power, 2,2-diphenyl-1-picrylhydrazyl (DPPH) test, beta-carotene whitening method and metal chelating activity analysis (Terpinca, 2012). A natural antioxidant that had very good results for stabilizing camelina oil was rosemary. A beneficial compound for the cosmetic industry identified in camelina oil is lecithin (Balayneh et all., 2018) [3]

Materials and methods

Materials

In the experiments carried out in the laboratory were used camelina seeds from the variety Mădălină (Romanian patent: Brevet ISTIS 504/2018-Camelina variety-Madalina) cultivated in an ecological system at the farm Moara Domnească (located 20 km from Bucharest,) Ilfov county.

The samples marked with numbers 1 and 2 were kept at room temperature, and samples 3 and 4 were kept in the refrigerator at 4 degrees Celsius. The oil was obtained after cold mechanical pressing using a vegetable oil extraction equipment called IEU-00 from the National Institute of Research and Development for Machinery and Facilities designed for Agriculture and Food Industry-INMA Bucharest. Prior to pressing, the seeds were conditioned to remove impurities using the ICS equipment from the same Institute. The oil obtained was left at room temperature for decanting for 48 hours. Following this stage, the clear oil was separated from the sediment.

Camelina oil purification method

The purification of this oil was performed for some samples in a single stage and for others in 2 stages. The adjuvants used were bentonite and zeolite or volcanic tuff. The working protocol used was aimed at removing as much of the impurities as possible to obtain an oil as pure as possible. For simple one-step purification, the samples were treated with bentonite and zeolite respectively at a concentration of 0.05%. Different from the simple step, the two-stage purification involved a pretreatment with 0.05% zeolite, respectively, followed by a subsequent filtration with 0.05% bentonite.

Sample 1

a) V1 = 90 mL

b) V1 + 10 mL = 100 mL

c) After centrifugation → V2 = 79 mL
d) V2 + 0.40 g bentonite
e) 30 minutes rest
f) pH before filtration = 5; pH after filtration = 5.5
g) V3 = 70 mL, oil color: light yellow, no deposits, clear

Sample 2
a) V1 = 90 mL
b) V1 + 10 mL = 100 mL
c) After centrifugation → V2 = 83 mL
d) V2 + 0.40 g bentonite
e) 30 minutes rest
f) pH before filtration = 4.5; pH after filtration = 5.5
g) V3 = 65 mL, oil color: light yellow, no deposits, clear

Sample 3
a) V1 = 50 mL, obtained from pressing 200 g of Mădălina variety seeds
b) V1 + 10 mL = 60 mL
c) After centrifugation → V2 = 48 mL
d) V2 + 0.22 g bentonite
e) 30 minutes rest
f) pH before filtration = 5, pH after filtration = 5.5
g) V3 = 40 mL, oil color: dark yellow, no deposits, opaque

Sample 4
a) V1 = 53 mL, obtained from pressing 200 g of seeds of the Mădălina variety
b) V1 + 10 mL = 63 mL
c) After centrifugation → V2 = 48 mL
d) V2 + 0.402g zeolite
e) 30 minutes rest
f) pH before filtration = 5, pH after filtration = 5
g) V3 = 31 mL, oil color: dark yellow to green, no deposits, opaque, a few bubbles of water

1.1. Determination of fatty acid content in camelina oil

The fatty acid composition was determined using a GC-MS-MS (TRACE 1310/TSQ 8000 Evo) equipment with a TG-5SILMS column (30 m x 0.25 mm x 0.25 μm) and helium as carrier gas. It was used an injection volume of 0.5 μl, a split rate of 1:100 and a solvent delay of 3 min.The temperature in the injector was 250^0C. The oven temperature program started at 170^0C for 2 minutes, and after two successive ramps it reached 240^0C, where it stood for 14 minutes. In the MS transfer line the temperature was 280^oC, while in the ionization source a 200^0C value was reached. Identification and quantification of fatty acids was

carried out by esterification in two steps. First, the oil samples were treated with methanolic NaOH solution (0.5 ml of 0.5 M concentration), 5 ml methanol and 5 ml hexane. The reaction has been achieved in the GC headspace termostat, at 70^0C, for 15 min. The second treatment applied to the samples consisted in addition of 1 ml of 14% metanolic BF3 solution and completing the esterification, under the same temperature and shaking conditions, for 5 minutes. The quantification of fatty acids was done using a certified mix solution of methylated fatty acids purchased from LCG (product code LA 90-1275). Except methyl oleate and methyl linolenate, for which the signal identification was done selectively, using the characteristic ions (fragment 292 for methyl linolenate and fragment 264 for methyl oleate), the other compounds were calibrated using usual total ions scaning.

Determination of tocopherols

The γ-tocopherol identification was performed using an Agilent Technologies 1260 HPLC coupled with a DAD detection system; the chromatographic column used was Poroshell 120 EC-C18, 50 x 3 mm, 2.7 μm. The column thermostat was set at 30^0C, and the mobile phase, a methanol: phosphoric acid solution (0.1%) 95: 5, had a flow rate of 0.5 ml / min; the injection was 10 ul. The reference solution, represented by y-tocopherol in methanol, approximately 100 μg / ml, was injected at least 2 times before sampling. The acquisition time was 15 min and detection was done at λ = 280 (4) nm. Data acquisition and processing were performed with OpenLab / ChemStation software.

Determination of physical and chemical characteristics

All determinations were performed according to the European Pharmacopoeia, following the methods in the table 1.

Table 1. Methods of analysis used

Nr	**Parameter**	**Associated method from European Pharmacopoeia (9.0 Edition)[20]**
1	Clarity and degree of opalescence; degree of coloration	-
2	Loss on dying	2.2.32
3	Odor	2.3.4
4	Peroxid value	2.5.5.
5	Acid value	2.5.1.
6	Refractive index	2.2.6.
7	Relative density	2.2.5.
8	Saponification value	2.5.6.
9	Iodine value	2.5.4.

Results and discussions

Oil purification

Sample 1 - pretreatment with 0.05% bentonite + 0.05% bentonite treatment

Sample 2 - pretreatment with 0.05% zeolite + 0.05% bentonite treatment

Sample 3 - no pretreatment + bentonite treatment 0.05%

Sample 4 - no pretreatment + treatment with 0.05% zeolite

The treatments applied were aimed at removing the impurities existing in the samples. Analyzing the appearance and color, the oils generated as a result of these treatments were within the limits of accessibility, namely the yellow color and the appearance- clear or slightly opalescent liquid.

Determination of the fatty acid content in Camelina oil

In the composition of camelina oil two fractions can be identified: a saponifiable one represented by tocopherol and sterols, as well as an unsaponifiable part respectively by fatty acids. The distribution of fatty acids can be seen in table 2. Most uses of camelina oil have as their point of interest fatty acids. Camelina sativa oil is known to be rich in polyunsaturated fatty acids. It has a high content of omega-3, namely linolenic acid, compared to other vegetable oils: canola oil-11.1%, rapeseed oil-10%, soybean oil-6.8%, corn oil-1 % (EIDHIN and others [13]).

In a study conducted by DOMIL [15] it reported the following values for camelina oil obtained from seeds grown in the Romania-Banat region: palmitic acid (C16: 0) -4.5 g / 100 g oil, linoleic acid (C18 : 2) -16.3 g / 100 g oil, linolenic acid (C18: 3) -17.5 g / 100 g oil, oleic acid (C18: 1) -14.0 g / 100 g oil, stearic acid (C18: 0) -1.85 g / 100 g oil. Popa [14] reported the following data on camelina oil composition: linolenic acid 28.0% -50.3%, linoleic acid-15.2% -22.4%, oleic acid-14.9-18.7%, eicosenoic-11.6% -17.5%, erucic 1.6% -4.2%-acid. The data obtained by us were comparable with the data obtained in the literature, namely: palmitic acid 6.02-6.34%, linoleic acid 17.51-17.81%, linoleic acid + oleic acid 46.68-48.56% , linoleic acid 15.34-16.42 g / 100 g oil, linolenic acid 24.45-28.35 g / 100 g oil.

Determination of tocopherols

In order to have the highest quality contribution, but also high emollient properties, γ-tocopherol must be positive, ie present (Table 2). Only one type of tocopherol, namely γ-tocopherol, could be determined in the literature. For the samples analyzed by us, only two of them had in their composition γ-tocopherol and certain oil samples 3 and 4. For samples 1 and 2 the absence of γ-tocopherol may be due to storage under ambient temperature conditions for about 2 years.

Table 2. The chemical composition of camelina oil

		Results			
Physico-chemical characteristics	**Conditions of admissibility**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**
Fatty acid content,%					
- Myristic acid	Min. 0,01	0,05	0,05	0,04	0,04
- Palmitoleic acid	Min. 0,02	0,05	0,06	0,05	0,05
- Palmitic acid	Min. 4,5	6,23	6,34	6,05	6,02
- Linoleic acid	Min. 13,5	17,51	17,7	17,79	17,81
- Linolenic + oleic acid	Min. 43,0	46,68	46,93	48,12	48,56
- Vaccine acid	Min. 0,5	1,51	1,49	1,40	1,42
- Stearic acid	Min. 1,5	2,58	2,53	2,45	2,43
- Cis-11.14 Acid - eicosadienoic	Min. 0,5	1,24	1,22	1,20	1,17
- 11-eicosenoic acid	Min. 13,0	17,44	17,35	17,1	16,86
- 13-eicosenoic acid	Min. 0,1	0,90	0,91	0,65	0,61
- Eicosanoic acid	Min. 1,0	1,57	1,60	1,52	1,47
- 13-docosenoic acid	Min. 1,5	3,33	3,23	3,08	3,02
- Behenic acid	Min. 0.05	0,23	0,22	0,19	0,19
- Nervic acid	Min. 0.05	0,29	0,28	0,27	0,26
Linoleic acid, g/100g oil	Min.13.5	15,5	15,34	16,22	16,42
Linolenic acid, g/100g oil	Min. 17,5	24,45	24,99	28,03	28,35
Identification of γ-tocopherol	positive	negative	negative	**positive**	**positive**

Determination of physical and chemical characteristics

The values for the evaluated parameters can be found in table 3. Until now, camelina oil has not been well individualized in cosmetic products, so analyzing it as a newly introduced ingredient, we do not have a well-defined standard in the European Pharmacopoeia either, but nor in the American Pharmacopoeia (USP). As there is no data available for this oil, it will be compared with the oils used and standardized for the cosmetic industry.

The four samples met the admissibility characteristics for the physical parameters. The oil obtained from all 4 samples presented liquids with a clear and

weak opalescent appearance, in the case of the ecological Mădălina sample without pretreatment + 0.05% zeolite treatment. The analyzed indices respected the limits allowed for vegetable oils, minus the peroxide index that had values 9 times higher than the limit allowed for oil samples with pretreatment (188.59 meq O_2 / kg respectively 188.56 meq O_2 / kg) and approximately 3 times higher in the case of the sample without pretreatment + treatment with 0.05% bentonite (37.78 meq O_2 / kg). For the sample without pretreatment + treatment with 0.05% zeolite the peroxide index was within the allowed limits, its value being 9.44 meq O_2 / kg. The peroxide index is an indicator that assesses the quality and stability of the oils, being a measure of the extent to which rinsing reactions have occurred (CODEX STAN 210). There is a peroxide limit of maximum 10 meq O_2 / kg set by SON (Standard Organization of Nigeria) (2000) and NIS (Nigerian Industrial Standard) (1992). (ZAHIR & al. [10]). Regarding refractive indices, they have values slightly higher than the minimum value mentioned in the Ullmann Encyclopedia of Industrial Chemistry, (1995) [9], for camelina oil-1.4698, respecting the admissibility conditions. The four samples recorded relative densities at 20^0C close to values and in the range 0.9170-0.9240 g / cm^3 mentioned by KARLESKIND [11], for the relative density of Camelina oil. The saponification index and the iodine index, respectively, recorded opposite values. The highest value of the saponification index was determined in sample 2- 183.95 mg KOH / g, and the lowest value in sample 4 - 180.21 mg KOH / g. For the iodine index we have 147.28 g I / 100 g assigned to sample 4, and 141.82 g I / 100 g determined in sample 2. The reference range used for these samples was 130-170 g I / 100 g respectively. 160-200 mg KOH / g. According to Ullmann's Encyclopedia of Industrial Chemistry, (1995) [9] the iodine value for cameline oil is 127-155 g / 100 g, and the saponification value is 180-190 mg KOH / g oil. The saponification value represents a measure of the average molecular weight of the fatty acids in the oil, while the iodine value is a measure of the unsaturation in a vegetable oil. The lower the iodine value, the greater the stability of oxidative storage. ZAHIR et al. [10]). Given the general characterization parameters, the results obtained by us are comparable to those determined by ABRAMOVIČ & ABRAM [12] for Camelina sativa oil from Slovenia: density at 20^0C being 0.9207 ± 0.0001 g / cm^3, index of refraction at 25^0C with the value 1.4756 ± 0.0001, the value of the peroxide 2.38 ± 0.01 meq O_2 / kg, the value of the acid -6.2 ± 0.1.

Even though there is no complete description of camelina oil for use in cosmetics, the data obtained especially for sample 4 (without pretreatment + 0.05% zeolite treatment) are comparable to the vegetable oils used in cosmetic composition.

Table 3. The chemical and physical characteristics of camelina oil

Physical chemical characteristics	**Conditions of admissibility**	**Results**			
		Sample 1	**Sample 2**	**Sample 3**	**Sample 4**
Aspect	Clear or slightly opalescent liquid	Clear liquid	Clear liquid	Clear liquid	slightly opalescent liquid
Color	yellow	yellow	yellow	yellow	yellow
Odor	Plant specific	Plant specific	Plant specific	Plant specific	Plant specific
Relative density, d_{20}^{20}	0,9100-0,9300	0,9278	0,9270	0,9233	0,9232
Refraction index, n_D^{20}	1,4700-1,4800	1,47751	1,47753	1,47747	1,47748
Acidity index, mg KOH / g	Max 10	1,462	1,409	1,730	1,997
Iodine index, gI / 100g	130-170	145,02	141,82	146,70	147,28
Saponification index, mg KOH / g	160-200	183,08	183,95	181,63	180,21
Peroxide index, meq O2 / kg	Max 10	**188,59**	**188,56**	**37,78**	9,44

Conclusions

From table 3 it is observed that samples 1, 2 and 3 do not correspond to the parameter "Peroxide index", variant 4 purified by the addition of zeolite being appropriate.

From table 2 it is observed that sample 4 has a higher content in unsaturated acids being considered the optimal purification variant.

References

[1] Atabani A.E., Silitonga A.S., Ong H.C., MahliaT.M.I., Masjuki H.H., Badruddin I. A., Fayaz H., 2013, Nonedible vegetable oils: A critical evaluation of oil extraction, fatty acid compositions,biodiesel production, characteristics, engine performance and emissions production, Renewable and Sustainable Energy Reviews 18: 211–245

[2] H. ABRAMOVIČ, V. ABRAM. Properties of Camelina sativa Oil.Food Technol. Biotechnol., 43(1): 63–70, (2005).
[3] Belayneh. D. H., Wehling. R. L., Cahoon. E., Ciftcia. O. N., 2015. Extraction of omega-3-rich oil from Camelina sativa seed using supercritical carbon dioxide. The Journal of Supercritical Fluids, Volume 104, Pages 153-159
[4] Berti M., Gesch R. , Eynck C., Anderson J., Cermak S., 2016, Camelina uses, genetics, genomics, production, and management,Industrial Crops and Products 94:690-710
[5] Budin, J. T., Breene, W., Putnam, D.H., (1995). Some Compositional properties of Camelina (Camelina sativa L.Crantz) seeds and oils. JAOCS 72, 3, 309-315.
[6] G. DOMIL, L. PÎRVULESCU, I.M. POPESCU. The study of camelina oil characteristics. Research Journal of Agricultural Science, 47(4): 55-58, (2015)
[7] D.N. EIDHIN, J. BURKE, D. O'BEIRNE. Oxidative Stability of 3-rich Camelina Oil and Camelina Oil-based Spread Compared with Plant and Fish Oils and Sunflower Spread. JFS: Sensory and Nutritive Qualities of Food, vol. 68, no. 1, pp.345-353, 2003
[8] Fröhlich, A.,O'Dea, G., Hackett, R., O'Beirne, D., Ni Eidhin, D., Burke, J., 2012. Stabilization of Camelina Oil with Synthetic and Natural Antioxidants. Journal of the American Oil Chemists' Society, Volume 89, Issue 5, pp 837–847
[9] A. KARLESKIND, AFECG, Manuel des corps gras, vol. 1, A. Karleskind, J-P Wolff, eds., Technique et Documentation-Lavoisier, 1992
[10] Popa, A.L, Drumea, V., Niţă, R.A., Florea, M.A., Olariu, L., Jurcoane, Ş., Cristea. S., A physico-chemical characterization of oil from Camelina sativaseeds grown in Romania, Romanian Biotechnological Letters, Vol. , No. x, 2106
[11] Righini D., Zanetti F., Monti A., 2016, The bio-based economy can serve as the springboard for camelina and crambe to quit the limbo, OCL 23(5) D504
[12] Rosenthal A., Pyle D. L., Niranjan K. ,1996, Aqueous and enzymatic processes edible oil extraction, Enzyme and Microbial Technology 19:402-420
[13] Terpinca, P., Polaka, T., Makucbc, D., Poklar Ulriha, N., Abramovič, H., 2012. The occurrence and characterisation of phenolic compounds in Camelina sativa seed, cake and oil. Food Chemistry Volume 131, Issue 2, Pages 580-589
[14] TONCEA I, NECSERIU D.,PRISECARU T.,BALINT L-D.,GHILVACS M-I., POPA M., (2013) The seed's and oil composition of Camelia – first

romanian cultivar of camelina (Camelina sativa, L. Crantz), Romanian Biotechnological Letters.

[15] Ullmann's encyclopedia of industrial chemistry,Vol A 10, Fats and oils, VCH, Weinheim 1995, Retrieved from http://www.dgfett.de/material/physikalische_eigenschaften.pdf

[16] Zubr, J., (2003). Qualitative variation of Camelina sativa seed from different locations. Industrial Crops and Products, 17,161-169

[17] E. ZAHIR, R. SAEED, M. A. HAMEED, A. YOUSUF. Study of physicochemical properties of edible oil and evaluation of frying oil quality by Fourier Transform-Infrared (FT-IR) Spectroscopy. Arabian Journal of Chemistry, 10: S870-S876, (2017).