Biologically active components in *Madia sativa* seed oil

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Abstract Biologically active components in lipids (fatty acids, phospholipids, sterols and tocopherols) from three varieties of *Madia sativa* seeds introduced in Bulgaria (BGR 457 and BGR 458 with German origin, and BGR 459 with US origin) were investigated. Glyceride oil in the seeds was found to be 36.6, 34.2 and 35.4%, respectively. Total phospholipid content was 2.4, 1.7 and 2.6% and the main classes were phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine. The amount of sterols in the oil was 0.3% for all samples and the major component was β -sitosterol, followed by campesterol and stigmasterol. Total tocopherols in the oils were 768, 795 and 856 mg kg⁻¹, respectively and α -tocopherol predominated (more than 70.0%). Fatty acid composition of triacylglycerols and sterol esters was also established. Main fatty acids in triacylglycerols were linoleic (47.5–50.5%), oleic (30.2–32.4%) and palmitic acids (13.0–13.5%). The content of saturated fatty acids (palmitic and stearic) in sterol esters (40.1–50.9%) was significantly higher than in triacylglycerols (18.3–19.4%).

Keywords *Madia sativa* · Glyceride oil · Phospholipids · Sterols · Tocopherols · Fatty acids

Introduction

Despite the biodiversity of plant genetic resources the agriculture uses negligible part of them. The adaptation of the alternative crops that could be a source of valuable products for people is one of the objectives of scientific research teams worldwide. Underexplored and underestimated are number of potentially useful crops, including *Madia sativa*.

Madia sativa (Asteraceae) is a plant cultivated in North and South America and the seeds were main food for some North American Indian tribes. They can be roasted and ground then eaten dry, mixed with water, or combined with cereal flours. The seeds contain about 41% of edible oil and 28% of the latter can be extracted by pressing (Facciola 1998). On the other hand, Rusinek et al. (2012) reported that the oil content of *Madia sativa* seeds obtained by cold deep expression was 37.06%. Recently these seeds have been proven to be an alternative potential source of vegetable glyceride oil (Zardini 1992), especially in America and some European countries (mainly in Germany). According to Angelini et al. (1997) the average amount of the glyceride oil in *Madia sativa* seeds was 31.0% with the highest value of 42.0%. Linoleic acid (52.0–72.4%) predominated in fatty acid fraction followed by palmitic and oleic acid (Schmeda-Hirschmann 1995; Angelini et al. 1997; Acevedo et al. 2012). Despite that, there is scanty information about the content and composition of bioactive compounds such as tocopherols, phospholipids and sterols in *Madia sativa* seed oil. On the other hand, these substances have a significant role in oxidative stability, nutritive and technological properties of the oils.

Crop production system of *Madia sativa* in Bulgaria is still under development. Studies on the potential benefits of their cultivation include part of the present research. The

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information on the lipid composition of *Madia sativa* seeds grown in Bulgaria is limited whence they are not used for oil production in the country. The aim of the present study was to investigate the content and composition of the biologically active compounds (phospholipids, free and esterified sterols, tocopherols, and fatty acids of the triacylglycerols and sterol esters) as well as oxidative stability of the seed oils from three varieties of *Madia sativa* cultivated in Bulgaria.

Materials and methods

Chemicals

All solvents and reagents were analytical grade and were used without additional purification. Reference phospholipids and fatty acid methyl esters were purchased from Fluka, tocopherol isomers and individual sterols were from Merck.

Samples

The investigated three accessions of *Madia sativa* seeds (BGR 457, BGR 458 and BGR 459) were selected from different backgrounds and morphology. They differ in plant's height and architectonic, size and yield of seeds per unit area. BGR 457 and BGR 458 are local varieties from Germany while BGR 459 is American cultivar. The plant material was introduced in Bulgaria and grown in the region of Plovdiv (Central Southern Bulgaria), crop 2014. Prior to use for analysis, the seeds were air dried for 72 h at 25 °C. The moisture content was determined by AOAC (2016) and the results for varieties BGR 457, BGR 458 and BGR 459 were 5.1 ± 0.1 (mean $\pm$ SD), 4.8 ± 0.3 , and 5.3 ± 0.2 , respectively.

Oil content

The seeds (50 g sample) were air-dried and the oil was extracted with n-hexane in *Soxhlet* for 8 h (ISO 659 2014). The solvent was partly removed in rotary vacuum evaporator, the residue was transferred in pre-weight glass vessels and the rest of the solvent was removed under stream of nitrogen to a constant weight to determine the oil content.

Phospholipids

Another part (10 g) of air-dried seeds was subjected to Folch extraction (Folch et al. 1957). Polar lipids were isolated from the total lipids by column chromatography according to Christie (2003). The phospholipid classes were isolated by a variety of two-dimensional TLC on

20 cm $\times$ 20 cm glass plates with 0.2 mm Silica gel 60 G layer impregnated with aqueous $(\text{NH}_4)_2\text{SO}_4$ (1 g in 100 mL water). In the first direction the plate was developed with chloroform:methanol:ammonia, 65:25:5 (by volume) and in the second—with chloroform: acetone:methanol:acetic acid:water, 50:20:10:10:5 (by volume) (Schneider and Daum 2006). The individual phospholipids were detected and identified by spraying with specific reagents according to Christie (2003): Dragendorff test (detection of choline-containing phospholipids); Ninhydrin spray (for phospholipids with free amino groups), and Schiff's reagent (for inositol containing phospholipids). Additional identification was performed by comparing the respective R_f values with those of authentic commercial standards subjected to Silica gel TLC under identical experimental conditions. The quantification was carried out spectrophotometrically at 700 nm after scraping the respective phospholipid spot and mineralization of the substance with a mixture of perchloric acid and sulphuric acid, 1:1 (by volume) (ISO 10540-1 2014).

Sterols

The oil (200 mg precisely measured) was applied on 20 cm $\times$ 20 cm glass plates (ca. 1 mm thick Silica gel G layer) and developed with n-hexane:acetone, 100:8 (by volume). Free ($R_f = 0.4$) and esterified sterols ($R_f = 0.8$) were detected under UV light by spraying the edges of each plate with 2',7'- dichlorofluorescein, then they were scraped, transferred to small glass columns and eluted with diethyl ether. The solvent was evaporated under a stream of nitrogen and the residue was weighed in small glass container to a constant weight. Thereafter free sterols were subjected to gas chromatography (GC), without derivatization. Sterol esters were hydrolyzed with ethanolic KOH, sterols were extracted with hexane and purified by TLC under the above conditions prior the GC analysis. Sterol composition was determined on HP 5890 gas chromatograph equipped with 25 m $\times$ 0.25 mm DB—5 capillary column and flame ionization detector. Temperature gradient from 90 °C (hold 2 min) up to 290 °C at a rate of change 15 °C min⁻¹ and then up to 310 °C a rate of 4 °C min⁻¹ (hold 10 min); the injector temperature was 300 °C and the detector temperature was 320 °C. Hydrogen was the carrier gas at a flow rate 0.8 mL min⁻¹; split 50:1. Identification was confirmed by comparison of retention times with those of a standard mixture of sterols (ISO 12228-1 2014).

Tocopherols

Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) on a Merck-

Hitachi instrument equipped with 250 mm × 4 mm Nucleosil Si 50-5 column and fluorescent detector Merck-Hitachi F 1000. Operating conditions were as follows: mobile phase of n-hexane:dioxan, 96:4 (by volume), flow rate 1.0 mL min⁻¹, excitation 295 nm, emission 330 nm. 20 µL 1% solution of crude oil were injected. Tocopherols were identified by comparing the retention times with those of authentic individual tocopherols. The tocopherol content was calculated on the base of tocopherol peak areas in the sample versus tocopherol peak area of standard tocopherol solution (ISO 9936 2016).

Fatty acids

Fatty acid composition of triacylglycerols and sterol esters was determined by GC after transmethylation of the respective sample with 2N methanolic KOH at 50 °C according to Christie (2003). Fatty acid methyl esters (FAME) were purified by TLC on 20 cm × 20 cm plates covered with 0.2 mm Silica gel 60 G layer with mobile phase n-hexane:acetone, 100:8 (by volume). GC was performed on a gas chromatograph equipped with a 30 m × 0.25 mm × 25 µm (I.D.) capillary EC 30-Wax column and a flame ionization detector. The column temperature was programmed from 130 °C (4 min), at 15 °C min⁻¹ to 240 °C (5 min); injector and detector temperatures were 250 °C. Hydrogen was the carrier gas at a flow rate 0.8 mL min⁻¹; split was 50:1. Identification was performed by comparison of retention times with those of a standard mixture of FAME subjected to GC under identical experimental conditions (ISO 12966-1 2014).

Oxidative stability

Oxidative stability of the oils was determined by measuring of induction period using conductometric detection of volatile compounds. Rancimat apparatus Methrom 679 was used at 100 °C and an air flow rate 20 L h⁻¹ (ISO 6886 2016).

Statistics

The measurements were done in triplicate. The results are presented as mean value with the corresponding standard deviation (SD).

Results and discussion

Oil content in the investigated varieties of *M. sativa* seeds and biologically active components in them are presented in Table 1.

The seeds of *M. sativa* contained a significant amount of glyceride oil (34.2–36.6%). These values were higher than data reported earlier by Schmeda-Hirschmann (1995) (26.0%) and close to quantities established by Copeland and McDonald (2012) (32.0%) and Angelini et al. (1997) (average value for 3 years of 31.0%). The oil content was higher than that of some common and widely used oil such as corn (3.0–5.0%), cotton (16.0%) or soybean (18.0%) and close to that of sunflower (35.0–45.0%) and safflower (38.0–48.0%) oils (Gunstone et al. 2007).

Phospholipid content (1.7–2.6%) was higher than that of most plant oils—corn, sunflower, safflower where the quantity was 0.4–0.9%, but identical with rape seed oil (1.0–3.0%) (O'Brien et al. 2004). Sterol and tocopherol content were similar to those found in other plant oils (CODEX STAN 210 2015).

Table 2 presents the results from the comparative analysis of phospholipid composition.

Almost all phospholipid classes were identified in the oils. Phosphatidylcholine was the main component followed by phosphatidylinositol and phosphatidylethanolamine. The amount of phosphatidic acids, lysophosphatidylcholine and lysophosphatidylethanolamine varied from 3.1 to 6.2%. This can be explained with the processes of hydrolysis in the oil and different stages of biosynthesis of the different phospholipids.

The quantitative and qualitative composition of the free and esterified sterols is given in Table 3.

Expectedly, free sterols comprised about 70% of the total sterols. All components were found in both free sterols and sterol esters and the differences were only quantitative. β -Sitosterol (60.0–73.9%) was the main component in both free and esterified sterols of the oil from all three varieties. Stigmasterol was considerably higher in the free sterols (11.1–15.2%) than in sterol esters (1.7–2.5%) at the expense of the lower content of β -sitosterol. The amount of campesterol in free sterols was the same as in the esterified sterols and was similar to that in CODEX STAN 210 (2015) for vegetable oils. A

Table 1 Content of glyceride oil in *M. sativa* seeds and phospholipids, sterols, tocopherols in the oils

Compounds	Variety		
	BGR 457	BGR 458	BGR 459
Oil in the seeds (%)	36.6 ± 0.5	34.2 ± 0.2	35.4 ± 0.4
Phospholipids (%)	2.4 ± 0.1	1.7 ± 0.1	2.6 ± 0.2
Sterols (%)	0.3 ± 0.05	0.3 ± 0.05	0.3 ± 0.05
Tocopherols (mg kg ⁻¹)	768 ± 10	795 ± 15	856 ± 17

Table 2 Composition of phospholipid fraction of *M. sativa* seeds

Phospholipids (% of total)	Variety		
	BGR 457	BGR 458	BGR 459
Phosphatidylcholine	34.1 ± 0.5	45.3 ± 0.7	42.2 ± 0.4
Phosphatidylinositol	18.5 ± 0.2	18.2 ± 0.5	23.8 ± 0.3
Phosphatidylethanolamine	12.6 ± 0.4	11.8 ± 0.5	13.4 ± 0.2
Phosphatidic acids	5.0 ± 0.1	3.5 ± 0.2	3.1 ± 0.5
Phosphatidylserine	3.3 ± 0.3	2.5 ± 0.4	1.5 ± 0.2
Lysophosphatidylcholine	6.2 ± 0.2	4.7 ± 0.3	3.4 ± 0.4
Lysophosphatidylethanolamine	6.2 ± 0.2	4.1 ± 0.1	3.4 ± 0.3
Monophosphatidylglycerol	5.8 ± 0.5	4.1 ± 0.2	3.8 ± 0.3
Diphosphatidylglycerol	4.0 ± 0.3	2.9 ± 0.4	2.7 ± 0.2
Others	4.3 ± 0.4	2.9 ± 0.3	2.7 ± 0.1

Table 3 Individual composition of sterol fraction from *M. sativa* seed oils

Sterols (% of total)	Variety					
	BGR 457		BGR 458		BGR 459	
	Free	Esterified	Free	Esterified	Free	Esterified
Cholesterol	0.4 ± 0.03	0.8 ± 0.05	0.3 ± 0.01	1.3 ± 0.2	0.3 ± 0.02	1.3 ± 0.1
Campesterol	16.6 ± 0.5	15.4 ± 0.4	14.5 ± 0.5	15.7 ± 0.3	15.9 ± 0.5	16.8 ± 0.7
Stigmasterol	12.9 ± 0.6	1.7 ± 0.2	15.2 ± 0.3	2.5 ± 0.1	11.1 ± 0.4	2.0 ± 0.2
Δ ⁷ -Campesterol	0.7 ± 0.1	1.0 ± 0.2	0.7 ± 0.05	1.4 ± 0.2	0.6 ± 0.1	0.7 ± 0.05
β-Sitosterol	62.0 ± 0.2	73.9 ± 0.3	60.0 ± 0.5	71.9 ± 0.7	69.5 ± 0.5	71.7 ± 0.4
Δ ⁵ -Avenasterol	2.5 ± 0.1	3.0 ± 0.3	4.0 ± 0.2	3.2 ± 0.2	1.3 ± 0.3	3.3 ± 0.2
Δ ⁷ -Stigmasterol	1.0 ± 0.04	1.2 ± 0.2	2.0 ± 0.1	1.2 ± 0.3	0.6 ± 0.1	1.0 ± 0.2
Δ ⁷ -Avenasterol	3.9 ± 0.3	3.0 ± 0.1	3.3 ± 0.2	2.8 ± 0.4	0.7 ± 0.1	3.2 ± 0.2
Ratio free: esterified sterols	78.3:21.7		78.3:21.7		71.4:28.6	

remarkable difference in the cholesterol content was established between free and esterified sterols. Its content in the sterol esters (0.8–1.3%) was significantly higher than in free sterols (0.3–0.4%). These results are in agreement with those reported for seed oils from Bulgarian varieties of sunflower (Marekov et al. 2010) and safflower (Zlatanov et al. 2015). The other sterol components were presented in insignificant quantities or as traces in all investigated oils.

Tocopherol composition of the oils is shown in Table 4.

There were differences in tocopherol composition in all investigated seed oils. The tocopherol fraction in seed oil from variety BGR 457 was presented only by tocopherols (α-, β- and γ-tocopherol), while in BGR 458 and BGR 459 considerable amounts of tocotrienols were detected. α-Tocopherol (more than 74.0%) was the main component in all tocopherol fractions of the oils, while β-tocopherol and γ-tocopherol were presented in small quantities.

Fatty acid composition of triacylglycerols and sterol esters of the investigated seed oils is shown in Table 5.

Table 4 Tocopherol composition of *M. sativa* seed oils

Tocopherols (% of total)	Variety		
	BGR 457	BGR 458	BGR 459
α-Tocopherol	96.2 ± 0.6	88.3 ± 0.3	74.4 ± 0.5
α-Tocotrienol	— ^a	—	3.5 ± 0.1
β-Tocopherol	2.8 ± 0.2	2.8 ± 0.4	5.2 ± 0.2
β-Tocotrienol	—	0.8 ± 0.1	2.5 ± 0.3
γ-Tocopherol	1.0 ± 0.3	2.2 ± 0.2	6.0 ± 0.5
γ-Tocotrienol	—	4.5 ± 0.3	5.1 ± 0.2
δ-Tocopherol	—	1.4 ± 0.1	3.3 ± 0.3

^a Not detected

Fatty acid composition of triacylglycerols of the three varieties of *M. Sativa* seed oils was similar. Linoleic acid was the main component, followed by oleic acid and unsaturated acids predominated in all oils (more than 80.0%). Palmitic and stearic acid, which were the main saturated fatty acids, were also detected in reasonable

Table 5 Fatty acid composition of triacylglycerols and sterol esters of *M. sativa* seed oils

Fatty acids (% of total)	Triacylglycerols			Sterol esters		
	BGR 457	BGR 458	BGR 459	BGR 457	BGR 458	BGR 459
C _{12:0} -Lauric	0.2 ± 0.05	0.2 ± 0.03	0.2 ± 0.04	0.5 ± 0.03	0.6 ± 0.1	0.6 ± 0.05
C _{12:1} -Lauroleic	— ^a	—	—	1.1 ± 0.2	0.5 ± 0.1	0.7 ± 0.2
C _{14:0} -Myristic	0.1 ± 0.03	0.2 ± 0.05	0.1 ± 0.03	0.8 ± 0.1	0.8 ± 0.05	1.0 ± 0.1
C _{14:1} -Myristoleic	0.1 ± 0.04	0.1 ± 0.02	0.1 ± 0.02	0.8 ± 0.05	0.3 ± 0.04	0.4 ± 0.02
C _{15:0} -Pentadecanoic	—	—	—	0.2 ± 0.01	0.3 ± 0.03	0.3 ± 0.05
C _{16:0} -Palmitic	13.5 ± 0.5	13.0 ± 0.4	13.4 ± 0.6	27.2 ± 0.2	29.5 ± 0.5	27.8 ± 0.3
C _{16:1} -Palmitoleic	0.2 ± 0.04	0.1 ± 0.01	0.2 ± 0.03	0.2 ± 0.03	0.1 ± 0.02	0.2 ± 0.05
C _{17:0} -Margaric	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.3 ± 0.02	0.4 ± 0.05	0.3 ± 0.03
C _{18:0} -Stearic	4.2 ± 0.3	5.2 ± 0.2	4.0 ± 0.4	9.1 ± 0.2	15.8 ± 0.5	12.1 ± 0.3
C _{18:1} -Oleic	32.3 ± 0.5	32.4 ± 0.7	30.2 ± 0.3	44.4 ± 0.4	42.9 ± 0.5	47.3 ± 0.3
C _{18:2} -Linoleic	48.1 ± 0.2	47.5 ± 0.4	50.5 ± 0.5	12.8 ± 0.4	4.7 ± 0.1	6.5 ± 0.2
C _{18:3} -Linolenic	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	—	—	—
C _{20:0} -Arachidic	0.5 ± 0.05	0.6 ± 0.04	0.4 ± 0.02	1.1 ± 0.02	2.1 ± 0.05	1.6 ± 0.03
C _{20:1} -Gadoleic	0.2 ± 0.03	0.3 ± 0.05	0.2 ± 0.03	0.4 ± 0.01	0.6 ± 0.03	0.3 ± 0.02
C _{20:2} -Eicosadienoic	—	—	—	0.2 ± 0.05	—	—
C _{22:0} -Behenic	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.9 ± 0.2	1.4 ± 0.2	0.9 ± 0.1
C _{22:1} -Erucic	0.3 ± 0.05	0.1 ± 0.03	0.4 ± 0.03	—	—	—
Saturated	18.7	19.4	18.3	40.1	50.9	44.6
Unsaturated	81.3	80.6	81.7	59.9	49.1	55.4

^a Not detected

amounts. These results were consistent to fatty acid composition reported earlier by Angelini et al. (1997).

The total amount of the saturated fatty acids (40.1–50.9%) mainly palmitic and stearic in sterol esters was about two times higher than in triacylglycerols (Table 5). On the other hand, the quantity of unsaturated linoleic acid in esterified sterols was considerably lower than in triacylglycerols. The quantity of the other saturated acids (lauric, myristic, pentadecanoic, margaric, arachidic and behenic acid) and monounsaturated oleic acid was slightly higher than in triacylglycerols. Overall, the fatty acid composition of triacylglycerols was different from sterol esters, because the content of saturated fatty acids in the latter was significantly higher.

The data about the ratio of saturated and unsaturated fatty acids was in agreement with the data reported earlier for fatty acid composition of triacylglycerols and sterol esters of seed oils from sunflower (Marekov et al. 2010) and safflower (Zlatanov et al. 2015). According to Munshi et al. (1983) the differences between fatty acid composition of triacylglycerols and sterol esters might due to different phases of the biosynthesis of those compounds.

Oxidative stability of *M. sativa* seed oils was 9.9 h (BGR 457), 10.3 h (BGR 458) and 12.5 h (BGR 459). These results were similar to that of oils from seeds of Bulgarian varieties of sunflower which were from 12.1 to 16.3 h (Zlatanov et al. 2008). The oils from the three

varieties of *M. sativa* had approximately the same oxidative stability which could be explained by their identical fatty acid composition, as well as close content of tocopherols, which were the main antioxidants in the lipids. The seed oil from variety BGR 459 had the highest oxidative stability which could be explained with its higher tocopherol content (856 mg kg⁻¹).

Overall, the small differences observed in the composition of the biologically active components in the lipids of the investigated seeds due mostly to their different origin and morphology.

Conclusion

The present study determines the investigated varieties of *M. sativa* seeds grown in Bulgaria (BGR 457, BGR 458, and BGR 459) as a potential source of glyceride oils rich of biologically active components. The seeds of all three varieties are favorable for production of vegetable oil whose oxidative stability is similar to that of sunflower oil-linoleic type. The information about biologically active substances such as polyunsaturated fatty acids, tocopherols and phospholipids may be useful for determination of nutritional value of these oils. Despite some small differences in the composition of their components all accessions of *Madia sativa* are valuable sources of healthy glyceride

oils for human consumption and can be used in Bulgarian food and cosmetic industry in the future.

References

- Acevedo F, Rubilar M, Shene C, Navarrete P, Romero F, Rabert C, Jolivet P, Valot B, Chardot T (2012) Seed oil bodies from *Gevuina avellana* and *Madia sativa*. *J Agric Food Chem* 60:6994–7004
- Angelini L, Moscheni E, Colonna G, Belloni P, Bonari E (1997) Variation in agronomic characteristics and seed oil composition of new seed crops in central Italy. *Ind Crops Prod* 6:313–323
- AOAC - Association of Official Analytical Chemist (2016) Official methods of analysis, 20th edn. AOAC, Washington, DC
- Christie WW (2003) Lipid analysis, 3rd edn. The Oily Press, Bridgwater
- CODEX STAN 210-1999 (2015) Standard for named vegetable oils. Revision: 2001, 2003, 2009. Amendment: 2005, 2011, 2013 and 2015
- Copeland LO, McDonald M (2012) Principles of Seed Science and Technology, 4th edn. Springer Science & Business Media, New York
- Facciola S (1998) Cornucopia—a source book of edible plants, 2nd edn. Kampong Publications, Vista
- Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226:497–509
- Gunstone FD, Harwood JL, Padley FB (2007) The lipid handbook, 3rd edn. Chapman Hall, London
- ISO 10540-1:2014. Animal and vegetable fats and oils. Determination of phosphorus content. Part 1: colorimetric method
- ISO 12228-1:2014. Part 1: animal and vegetable fats and oils. Determination of individual and total sterols contents. Gas chromatographic method
- ISO 12966-1:2014. Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters. Part 1: guidelines on modern gas chromatography of fatty acid methyl esters
- ISO 659:2014. Oilseeds. Determination of oil content (Reference method)
- ISO 6886:2016. Animal and vegetable fats and oils. Determination of oxidative stability (accelerated oxidation test)
- ISO 9936:2016. Animal and vegetable fats and oils. Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography
- Marekov I, Zlatanov M, Angelova-Romova M (2010) Sterol composition of oils from Bulgarian varieties of sunflower. *Bulg J Agric Sci* 16(2):158–162
- Munshi SK, Sukhija PS, Bhatia IS (1983) Lipid biosynthesis in developing kernels of almond (*Prunus amygdalus*). *Phytochemistry* 22(1):79–83
- O'Brien RD, Farr WE, Wan PJ (2004) Introduction to fats and oils technology, 2nd edn. AOCS Press, Champaign
- Rusinek R, Rybczyński R, Tys J, Gawrysiak-Witulska M, Nogala-Kałucka M, Siger A (2012) The process parameters for non-typical seeds during simulated cold deep oil expression. *Czech J Food Scis* 30(2):126–134
- Schmeda-Hirschmann G (1995) *Madia sativa*, a potential oil crop of Central Chile. *Econ Bot* 49(3):257–259
- Schneider R, Daum G (2006) Analysis of yeast lipids. In: Xiao W (ed) Yeast protocols (Methods in molecular biology), vol 313, 2nd edn. Humana Press, Totowa, pp 75–84
- Zardini E (1992) *Madia sativa* Mol. (Asteraceae-Heliantheae-Madinae): an ethnobotanical and geographical disjunct. *Econ Bot* 46(1):34–44
- Zlatanov M, Antova G, Angelova-Romova M, Damyanova B, Momchilova S, Marekov I (2008) Comparative characterization of vegetable oils from Bulgarian varieties of sunflower. *Food Process Ind Mag* 57(3):45–47
- Zlatanov M, Antova G, Angelova-Romova M, Momchilova S, Dimitrova R, Marcheva M (2015) Detailed characterization of lipids in safflower varieties grown in Bulgaria. *La Rivista Italiana delle Sostanze Grasse* 92(4):279–288