

Characterization of *Onosma bracteosum* Hausskn. & Bornm. and *Onosma thracicum* Velen. Based on Fatty Acid Compositions and α -Tocopherol Contents of the Seed Oils

Tamer Özcan

Istanbul University, Faculty of Science, Department of Biology, Division of Botany,
034116 Istanbul / Turkey (E-mail: tameroz@istanbul.edu.tr)

Abstract

Turkey is one of the important centres of origin for genus *Onosma* (*Boraginaceae*) with about 95 species including 48 endemics (ca.50%). A very limited number of investigations for fatty acid patterns and α -tocopherol contents of the seed oils were reported in this genus. Some differences were observed in total oil (18.8-24.0%) and α -tocopherol contents (1.66-46.03%) between species. Major unsaturated fatty acids were α -linolenic (38.70-41.05%), linoleic (16.13-18.38%) and oleic acids (11.86-12.96%) respectively. Palmitic (6.32-7.71%), γ -linolenic (6.36-6.92%) and stearic (2.15-2.32%) acids showed considerable levels. Other fatty acid concentrations were at minor concentrations below 1% of the seed oils. Total oil content in addition to oleic and α -linolenic acids quantified at higher levels in endemic *O. bracteosum*. The other fatty acids and α -tocopherol were observed at higher concentrations in *O. thracicum*. Some variations were examined in quantities, total percentages and the ratios of saturated and unsaturated fatty acids as additional chemotaxonomic markers. Differences for whole series of fatty acids were not significant between species but, significantly difference was found based on six calculated ratios of the fatty acids ($p < 0.05$). Investigated *Onosma* species could be evaluated as the alternative wild sources for the production of essential fatty acids (EFA) including α -linolenic (ω -3), linoleic and unusual γ -linolenic (ω -6) acids.

Keywords: α -Tocopherol, Chemotaxonomy, Fatty acid, Industrial product, *Onosma*, Seed oil
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Introduction

Genus *Onosma* consisting of over 150 species is typical of xeric habitats as perennial, usually suffruticose or biennial herbs that has its greatest species diversity in southeast Europe and Asia Minor, and is known to include several serpentine endemics, especially in the southern Balkans (Stevanovic et al. 2003). Anatolia is an important centre of origin for *Onosma* comprising about 95 species, 48 of which and 1 variety are endemic for Turkey (Riedl 1978; Güner et al. 2000; Riedl et al. 2005). Additionally, as a new species, *Onosma riedliana* was described recently (Binzet and Orcan 2007). The genus *Onosma* presents considerable taxonomic problems (Tutin et al. 1972). Indumentum of leaves and stem in the

identification, sectional and subsectional delimitations are of great importance. Shape and colours of corolla, size of calyx and number of flowers in cymes and nutlets morphology are some other key features. The systematic and evolutionary aspects of *Onosma* are still poorly known. It was reported that the present classification appears to be partly artificial and in need of modern biosystematic revision (Riedl 1978). A limited number of studies in detail were declared for taxonomy of Turkish *Onosma*. Some anatomical and ecological properties (Selvi and Bigazzi 2001; Akçin and Engin 2005), the structure and patterns of epidermal cells of the nutlets (Akçin 2007), palynological (Binzet and Orcan 2003) and caryological (Teppner 1988) features were

reported to reveal significant taxonomic differences between *Onosma* species. A remarkable genetic differentiation between populations of *Onosma* was found by means of Amplified Fragment Length Polymorphism (AFLP) fingerprinting technique (Mengoni et al. 2006). Pyrrolizidine alkaloids were also reported to be powerful plant defence compounds against vertebrate herbivores and insects (Wink and Roberts 1998), and have been used as chemotaxonomic markers in *Boraginaceae* (El-Shazly et al. 2003). On the other hand, fatty acid compositions of seed oils exhibit some useful patterns in the chemotaxonomic delineations and phylogeny of the wide plant groups (Tetenyi 1974; Aitzetmüller 1995). Seed oil fatty acid patterns and the occurrence of unusual fatty acids were highly correlated with plant genera and the pattern differences were often rather small at specific level (Aitzetmüller and Ivanov 1997). The family *Boraginaceae* is well known as a source of unusual fatty acids, γ -linolenic and stearidonic acids valuable in taxonomic and phylogenetic relations in addition to medicinal and nutraceutical interest (Velasco and Goffman 1999; Guil-Guerrero et al. 2001). Tocopherols, as efficient natural antioxidants have also important chemotaxonomic significance, and the infrafamilial variability of its content and composition in *Boraginaceae* were reported (Velasco and Goffman 1999). A limited number of data were published for *Onosma* from this aspect (Kleiman 1964; Barclay 1974; Tétényi, 1974; Papageorgiou and Assimopoulou 2003). In Turkey, a few *Onosma* species were investigated for the quantities of these traits (Bağcı et al. 2004; Erdemoğlu et al. 2004; Özcan 2008).

The objective of the present study was to evaluate the variability and chemotaxonomic significance of fatty acid compositions and α -tocopherol contents between species in addition to their alternative wild source potential in medicine and nutrition.

Material and Methods

Sampling of seed materials

Seed specimens of *Onosma bracteosum* Hausskn. & Bornm., endemic for Turkey and *Onosma thracicum* Velen. were collected from 25-30 individual plants for each species growing in their native habitat at ca. 600m in *Juniperus-Quercus* scrub, distributed in B1 (Kaz Mountain) and A1(E) (Istranca mountain) according to the grid system in Flora of Turkey. Collected specimens were transported to the laboratory in polypropylene bags and kept in deep-freezer (-18°C) until the analysis was performed.

Determination of oil and fatty acids

Total oil content was detected with a Tecator Soxtec System HT. Powdered seed material from each species (3g) was added to an oil cartridge (W1). 25–50 ml diethyl ether ($C_4H_{10}O$) was placed in a weighed extraction pot (W2) and extraction was carried out for 15 min. with rinsing for 30–45 min. The extracted seed meal was thoroughly air dried to remove traces of solvent in the system, and dried at 105 °C. The pots were placed in a desiccator, cooled, and weighed (W3). The oil amount (%) was calculated using the equation: % Oil = $((W3 - W2)/W1) \times 100$. The IUPAC standard method for preparation of the fatty acid methyl esters was used (IUPAC 1979). The methyl esters of 20 fatty acids were quantified by use of a Perkin Elmer AutoSystem XL Gas Chromatography equipped with an SP-2330 fused-silica capillary column (30m, 0.25mm i.d., 0.20 μ m film thickness). Injector and flame-ionization detector (FID) temperatures were 240 and 250°C, respectively. Oven temperature was held at 120°C for 2 min then increased at 5°C/min and held at 220°C for 15 min. Helium (10 psi) was used as carrier gas. Sample injection, split flow and split ratio were 0.5 μ l, 75 ml/min, 1/50 respectively. Identification and quantification of fatty acid methyl esters (area percent) by comparing the relative retention times of the peaks were accomplished with those of authentic standards (Sigma Code No: 189-19).

Determination of α -tocopherol

Extraction and preparation of the samples for the determination of α -tocopherol were performed according to the procedure of Manz and Phillipp (1988) and AOAC (2000). High-performance liquid chromatography (HPLC) system was operated with an eluent of % 97 n-hexane (Merck no 104391), % 31.4-Dioxane (Merck no 103115). Helium as the carrier gas was used at a flow rate of 1 ml/min. Injection volume was 50 μ l. Detection was carried out

with a fluorescence detector F-1000 (Merck) at 293/326 nm excitation/emission wavelength using silicagel packet column, 25cm x 4.6mm i.d. Quantifications were accomplished by comparing the area of peaks with authentic standards (Merck).

A statistical package program (SSPS 10.0) was used for the multivariate analysis of the experimental results.

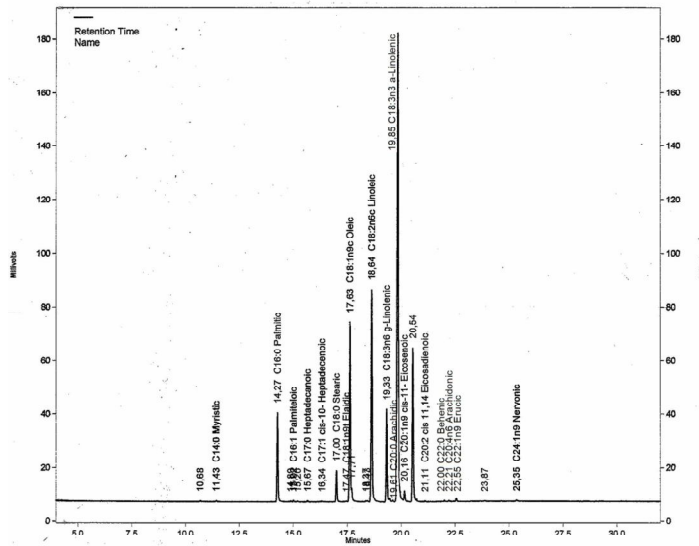


Figure 1. GC spectrum of the fatty acids in seed oil of *O. bracteosum*

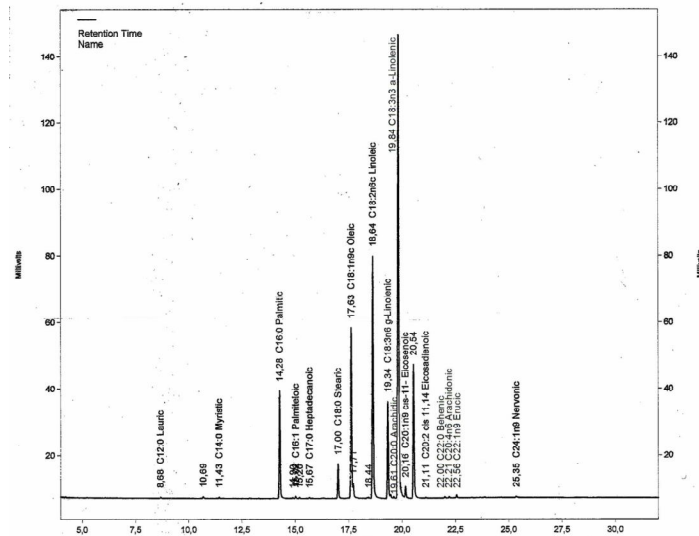


Figure 2. GC spectrum of the fatty acids in seed oil of *O. thracicum*

Results

Some differences were observed for total oil contents (18.8-24.0%) in the investigated species. The concentrations of α -tocopherol exhibited dramatically different values (1.66-46.03%). The quantities and some proportions of saturated, mono-unsaturated and poly-unsaturated fatty acid groups resulted in characteristic profiles. Obtained data for the concentrations and the ratios were documented in Table 1. Major unsaturated fatty acids were α -linolenic (38.70-41.05%), linoleic (16.13-18.38%) and oleic (11.86-12.96%) acids respectively. Palmitic (6.32-7.71%), γ -linolenic (6.36-6.92%) and stearic (2.15-2.32%) acids showed the higher levels. Other fatty acid concentrations were at minor concentrations below 1% of the seed oils. *O. bracteosum* (Fig. 1) and *O. thracicum* (Fig. 2) exhibited the characteristic GC spectrum of examined fatty acids. Total oil content, oleic and α -linolenic acids quantified at higher levels in endemic *O. bracteosum*. The other fatty acids and α -tocopherol were observed at higher concentrations in *O. thracicum*. The variations in total percents of fatty acid groups and some ratios were also observed between the species. Except for mono-unsaturated fatty acids, the total contents of saturated, unsaturated and poly-unsaturated fatty acids were determined at relatively higher percentages in *O. thracicum*. The samples examined included six proportions of fatty acids as additional traits, the ratios of unsaturated to saturated fatty acids, α -linolenic to γ -linolenic acid and α -linolenic to linoleic acid in *O. bracteosum*; poly- to mono-unsaturated fatty acids, linoleic to γ -linolenic acid and ω -6 to ω -3 fatty acids in *O. thracicum* exhibited relatively higher values. Differences for whole series of fatty acids was not significant between species ($p > 0.05$). But, significantly difference was found based on six calculated ratios of the fatty acids ($p < 0.05$). Correlations are also significant at 0.01 level for all examined traits. *Onosma* species could be evaluated as the alternative wild sources of α -

linolenic acid up to 41% (ω -3) in addition to linoleic and unusual γ -linolenic (ω -6) acids.

Discussion

Fatty acid compositions and α -tocopherol contents in seed oils together are a powerful tool for determining the systematic position and phylogenetic relationships of wider range of plant groups. *Boraginaceae* is a best source for the production of a set of unusual fatty acids in the seed oils having valuable chemotaxonomic significance. In general, our observations for fatty acid quantities and total oil contents are in agreement with the published results carried out on the limited number of *Onosma* species (Kleiman 1964; Barclay 1974; Tetenyi 1974; Velasco 1999). Major fatty acids are α -linolenic, linoleic and oleic acids in the literatures, as observed in present study. Similarly, palmitic, stearic and γ -linolenic acids show parallel results compared to our work, reflecting the characteristic of the genus. But, there is some difference in their concentrations between species. Up to now in Turkey, a limited number of *Onosma* species including *O. armeniacum*, *O. polioxantha*, *O. sericeum* and *O. bulbotrimum*, *O. halophilum* (Bağcı et al. 2004; Erdemoğlu et al. 2004; Özcan 2008) were analysed for fatty acid compositions and some differences between species determined for major fatty acids. The larger range of concentrations in α -linolenic acid among investigated *Onosma* species is considerable. Palmitic, linoleic and γ -linolenic acids show relatively small variations. It would be useful to evaluate these fatty acid data as a total marker set in taxonomical considerations. On the other hand, some of the ratios obtained of the fatty acids as relatively stable and significant parameters may reflect the genotypic divergency explaining phylogenetic associations in *Onosma*. In this study, we examined two species, *O. thracicum* and *O. bracteosum* as an endemic and morphologically variable species declared in LR(lc) category of IUCN (Ekim et al. 2000). They are from subsection *Asterotricha* having stellate-hairy

foliar tubercles as common characteristic. These perennial species have also several flowering stems and sterile rosettes. But, some different morphological characteristics of basal and cauline leaves in addition to calyx, corolla and the nutlets were described in the flora of Turkey. Additionally, reticulate pattern of nutlet surface (Akçin 2007) and anisocytic and anomocytic stomata cells were reported for *O. bracteosum* (Akçin and Engin 2005) as anatomical taxonomic characters. The similarity of these species for fatty acid compositions and total oil contents may result from the genotypic characteristics in addition to their similar ecological preferences.

Characteristic discrimination of *Onosma* populations distributed in different climatic and geographic conditions may be accomplished based on fatty acid patterns of the seed oils. In the seed oil of *Boraginaceae* or *Onagraceae*, it has been reported that environmental factors such as temperature and the soil types can affect the concentrations of γ -linolenic acid (GLA) and other polyunsaturated fatty acids (PUFAs) (Gunstone 1992). In some studies, these factors do not seem to affect significantly the profiles found in *Boraginaceae* (Guil-Guerrero et al. 2001) and the other plant families (Saffarzadeh et al. 1999). But, geographic differences were reported to affect the proportion of linoleic and α -linolenic acid (Johansson et al. 1997). Seeds from some *Echium* species collected from different locations and times were analyzed with similar results for the fatty acid profiles (less than 1% variation of the GLA content) and reported that some unusual fatty acid concentrations could be used for discrimination of *Echium* species based on geographic distribution, parallel with the genetic data (Guil-Guerrero et al. 2001). Parallel total oil contents were observed in examined species growing in similar mountain conditions. The amount of seed oil may be affected much more from environmental conditions than that its fatty acid profiles are determined ultimately from genetical factors. Total oil contents were studied in some Cruciferae species cultivated for three years with findings that oil amounts

were affected with climatic conditions of each year, producing decreasing levels of seed oils correlated with dry seasons (Angelini et al. 1997). The higher level of seed lipid contents in cold climate conditions were reported for different plant groups, but fatty acid quantities exhibited constant profiles generally (Saffarzadeh et al. 1999). Temperate variety oils were reported to be less saturated because of natural selection of oils with a higher energy storage capacity in northern latitudes and remain liquid at a lower temperature (Deferne and Pate 1996), as observed in present study. Substitution of ALA by GLA was reported to raise the melting point of storage lipids (Guil-Guerrero et al. 2001). Fatty acid profiles in any seed oil may provide a selective advantage for the seed germination in particular environmental conditions. Relatively lower levels of GLA (6.3-6.9%) in examined *Onosma* species may be useful in keeping the liquid characteristic of the seed oils for germination in mountain conditions.

From the nutritional point of view, valuable contents of essential fatty acids were determined considering daily dietary reference intakes of α -linolenic (0.5–1.6 g/day) and linoleic (4.4–17.0 g/day) acids for life stage groups (FNB 2002). Investigated *Onosma* species exhibited very high concentrations of α -linolenic acid, upto 41% (ω -3 fatty acid), and could be evaluated for the alternative wild sources of this essential fatty acid.

As lipophilic antioxidants synthesized exclusively by photosynthetic organisms, tocopherols in seed oils are the predominant group of vitamin E active compounds. It was found mainly in leaves and seeds (Munne-Bosch and Alegre 2002). Scavenging of reactive oxygen and inhibition of lipid peroxidation are the antioxidant function for tocopherols (Kruk et al. 2005). The content and the composition of tocopherols are further important criteria for the assessment of seed oils. Different seed oils express certain differences in the tocopherol composition. As a powerful chemotaxonomic tool, the consistent presence of tocopherol patterns was suggested

to determine delimitations at a specific level (Goffman and Galletti 2001). Tocopherol patterns in *Boraginaceae* were also reported to be used to confirm phylogenetic and taxonomic relations (Velasco and Goffman 1999). α -tocopherol levels may be used as a consistent chemotaxonomic tool for specific segregation of *Onosma*. The variation of the tocopherols was to be relatively small and probably independent of environmental influences resulting from different cultivation sites (Matthaus and Özcan 2005). Remarkable differences of α -tocopherol contents between two *Onosma* species may imply different pathways of accumulation and enzymatic activity for the synthesis. On the other hand, γ -tocopherol in non-photosynthetic tissues frequently predominates and is reported to be involved in the prevention of auto-oxidation of poly-unsaturated fatty acids (Munne-Bosch and Alegre 2002). As specific antioxidants, tocopherols may have the function of protecting stored lipids from oxidation in dry seeds. Considerably different contents of α -tocopherol in two *Onosma* species may reflect genotypic characteristics resulting from different seed viability in the same storage conditions.

Tocochromanols cannot be produced in humans and animals and therefore, vitamin E is an essential dietary component. Major sources for tocopherol in human nutrition are plant oils. Based on dietary reference intakes (DRIs 2004), *Onosma* seed oils, especially *O. thracicum* exhibited considerable levels of α -tocopherol (46 mg/100g) on the basis of daily adequate intakes (AIs) and recommended dietary allowances (RDAs) of life stage groups for vitamin E with 4-15 mg/day.

There is great potential in Anatolia considering the large diversity of *Onosma* species for development of the genotypes with high product quality. Large scanning and the evaluation of the seed oil quality in Anatolian population of *Onosma* have also importance for further selection of entries with high oil and essential fatty acids (EFAs) contents. Protection of the natural populations of this genus,

preparing germplasm collections and a databank of seed oil are needed.

Table 1. The values of examined parameters in the seed oils of both *Onosma* species

<i>Parameters</i>	<i>O. bracteosum</i>	<i>O. thracicum</i>
C12:0 Lauric	-	0.068
C14:0 Myristic	0.063	0.085
C16:0 Palmitic	6.323	7.717
C16:1 Palmitoleic	0.096	0.154
C17:0 Heptadecanoic	0.079	0.077
C17:1 cis-10-Heptadecenoic	0.060	-
C18:0 Stearic	2.151	2.320
C18:1n9c Oleic	12.964	11.864
C18:2n6c Linoleic	16.134	18.380
C18:3n6 g-Linolenic	6.367	6.924
C20:0 Arachidic	0.119	0.159
C18:3n3 a-Linolenic	41.050	38.703
C20:1n9 cis-11-Eicosenoic	0.929	0.972
C20:2 cis 11,14 Eicosadienoic	0.048	0.071
C22:0 Behenic	0.065	0.078
C20:4n6 Arachidonic	0.065	0.088
C22:1n9 Erucic	0.223	0.204
C24:1n9 Nervonic	0.118	0.120
Saturated fatty acids	8.681	10.345
Unsaturated fatty acids	76.261	77.558
Mono-unsaturated	14.390	13.314
Poly-unsaturated	61.871	64.244
Unsaturated / saturated	8.784	7.497
Poly-/monounsaturated	4.299	4.825
α -linolenic / γ -linolenic	6.447	5.589
Linoleic / γ -linolenic	2.534	2.654
α -linolenic/ Linoleic	2.544	2.105
ω -6 / ω -3	0.548	0.653
Total oil (g/100g)	24.00	18.80
Vitamin E (mg/100g)	1.66	46.03

Each value for fatty acids and α -tocopherol are the average from duplicate determinations

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