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FATTY ACID AND TOCOCHROMANOL PATTERNS OF SOME *ISATIS* L. (BRASSICACEAE) SPECIES FROM TURKEY

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Abstract

During investigations of new sources of higher plant lipids, seed fatty acid compositions and the tocopherol contents of some *Isatis* sp. (*Isatis cappadocica* subsp. *steveniana, I. kotschyana, I. candolleana, I. spectabilis, I. glauca* subsp. *glauca* and *I. kozlowskyi*) (Brassicaceae) were investigated by using GC and HPLC system. Some of the species are endemic to Turkey. All the *Isatis* species showed the same pattern of fatty acids. Linolenic and erucic acids were found as the abundant compounds. Tocochromanol derivatives of the seed oil showed more differences between *Isatis* species. Alpha, beta, gamma and delta tocopherols were the main compounds. Total tocopherol contents of *Isatis* species studied were found higher than the total tocotrienols. Polyunsaturated fatty acid concentration of *Isatis* genus patterns were found higher than most of the family and genera patterns in higher plant groups.

Introduction

Isatis L. (Brassicaceae) is represented by about 40 taxa in the genus (Davis, 1967, 1988; Güner *et al*., 2000). It is reported as an extremely difficult genus, centred in Irano–Turanian region in Turkey. The species are exceptionally variable, even in the fruit, which provides the most valuable diagnostic characters (Davis, 1967). Individual glucosinolates and fatty acids are widely recognized as constituents of systematic interest in Brassicaceae (Seigler, 1981). Study of the fatty acid composition of the seed oils of some wild plants in Spain, have a commercial value, fatty acid composition was found quite variable, but in general, besides the main fatty acids (oleic, linoleic and linolenic acid), erucic and petroselinic acids were the principal fatty acids in Brassicaceae and Apiaceae (Vioque *et al*., 1994). Tocopherols are a group of closely related derivatives of the phenolic tocol marked by extensive ring alkylation (Larson, 1988). The relative content of individual tocopherols is known to be characteristic of the seed oil of different cultivated plants. For example; the germ oil of wheat (*Triticum aestivum*) contains 60% of alpha tocopherol, the seed oil of *Salvia* sp., contains 30-70% gamma tocopherols (Bagci *et al*., 2004), alpha-gamma tocopherols were also comprised of most of the oils in some Fabaceae genera patterns (Bagci *et al*., 2004). *Brassica napus* contains ca. 65% of gamma tocopherol and 35% of alpha–tocopherol (Appelqvist, 1972; Goffman *et al*., 1999). Goffman *et al*., (1999) investigated the significance of tocopherols as chemotaxonomic markers and their relationships with oil content and fatty acid profile in a collection of 91 species of the family Brassicaceae and individual tocopherols were found to have great taxonomic value in the Brassicaceae.

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The objective of the present study was to determine the fatty acid, tocopherol, tocotrienol and plastochromanol–8 content of *Isatis* species viz., *I. cappadocica* subsp. *steveniana* (Trautv) Davis, (endemic to Turkey), *I. kotschyana* Boiss. & Hohen, *I. candolleana* Boiss., (endemic to Turkey); *I. kozlowskyi* Grossh., *I. spectabilis* Davis., *I. glauca* Aucher ex Boiss. subsp. *glauca* from Turkey firstly and to monitor the chemotaxonomic relationships in the genus and family patterns. While some *Isatis* spp., have been investigated from different regions in the world (Miller, 1965; Blatger, 1993), there is no information about the fatty acids and tocochromanols contents and chemotaxonomic significance of this genus in Turkey. In addition during the course of this study, it was aimed to characterize fatty acids and tocochromanols, to establish the taxonomic value and to do contributions as the renewable resources of FA patterns in this genus.

Materials and Methods

Fatty acid composition and tocochromanol content of the *Isatis* sp. patterns from Turkey were analysed by GC and HPLC. The results are shown in Tables 1-2.

Seed samples: In this research, following plant seeds were collected from natural habitats in Eastern Anatolian region of Turkey. *I. cappadocica* subsp. *steveniana* (Trautv) Davis, Van–Kurubaş passage, 2300m. Özçelik-6339; *I. kotschyana* Boiss. & Hohen, near the Van Lake, 1750 m., Özçelik-6292; *Isatis candolleana* Boiss. Bayburt Kop Mountains, 2800m. Özçelik - 6257; *I. kozlowskyi* Grossh., Erzurum–Horasan, 2000 m. Özçelik–6213; *I. spectabilis* Davis., between Tortum-Dumlu (Erzurum), 1700m. Özçelik-6438; *I. glauca* Aucher ex Boiss. subsp. g*lauca,* Uzundere (Tortum), 1000 m. Özçelik-6433.

Oil Extraction and preparation of fatty acid methyl esters (FAME): Impurities were removed from the seeds and the cleaned seeds were ground using a ball mill into powder. Lipids were extracted with heptane in a straight through extractor. The triglycerides were transesterified to methyl esters with potassium hydroxide in methanol according to ISO *method 5509* (DGF, 1989).

Capillary GLC: Fatty acid methyl ester composition was determined on two different gas chromato-graphs, Hewlett-Packard HP5890 (A) and HP6890 (B), each equipped with a fused silica WCOT capillary and FID: The results were retested with HP- 5973–N GC- MS.

A. Silar 5 CP, 50 m. x 0.25 mm ID, 0.24 μm film thickness, nitrogen as carrier gas, 1:50 split ratio, pressure 160 kPa, oven temp.: 5 min isothermal at 163°C, then 163 to 205^0C at 1^0C/min; Inj.= 230^0C, Det. 260^0 C.

B. DB-23, 60 m x 0.32 μm (J&W), 0.25 μm film thickness, hydrogen as carrier gas, 1:50 split ratio, pressure 69 kPa, oven temp.: 1 min isothermal at 80°C, then 80 to 150°C at 25^0C/min than 150 to 240°C at 3^0C/ min, 5 min isothermal, PTV-Inj. 80°C, 12°C/s to 250°C, 5 min isothermal, Det. 250^oC.

Data analysis was done with a chromato-integrator D 2500 (Merck-Hitachi) and a Chemstation integration software, respectively. Peak identification was achieved by comparison of relative retention times with those obtained from test mixtures of known composition on two different columns.

Table 1. Fatty acid composition of some *Isatis* sp., from Turkey. Data shown are peak area % from GLC.

Isatis sp.	Fatty acid Components										
	14:0	16:0	16:1 Δ7	16:1 Δ9	17:0	18:0	18:1 Δ9	18:1 Δ11	18: 2 Δ9,12	18: 3 Δ9,12, 15	20:0
I. cappadocica subsp. *steveniana*	0.1± 0.03	3.7±0.20	0.14±0.03	0.14±0.07	0.07±0.0	1.37±0.00	13.3±0.03	1.16±0.08	14.1±0.10	32.6±0.0	1.2±0.00
I. kotschyana	0.1±±0.08	3.6±0.05	0.08±0.01	0.22±0.03	0.07±0.01	1.02±0.01	18.8±0.06	1.59±0.01	9.37±0.12	26.8±0.0	0.1±0.01
I. candolleana	0.1±0.07	3.7±0.71	--	0.11±0.02	--	1.56±0.07	14.5±0.07	1.13±0.02	15.86±0.09	31.2±0.1	1.20±0.02
I. spectabilis	0.1± 0.06	3.5±0.03	0.67±0.00	0.30±0.02	0.05±0.03	1.07±0.02	13.53± 0.02	2.52±0.01	9.95±0.2	26.2±0.2	1.06±0.03
I. kozlowskyi	0.1±0.03	3.8±0.08	0.05±0.01	0.16±0.1	0.04±0.02	1.08±0.02	13.20± 0.01	1.28±0.0	8.94±0.23	31.9±20.1	1.14±0.00
Isatis glauca subsp. *glauca*	0.1±0.02	3.9±0.09	0.11±0.03	0.26±0.08	0.05±0.0	1.21 ± 0.1	14.6±0.03	1.72±0.0	10.2±0.08	29.8±0.3	1.01±0.02

Table 1. (Cont'd.).

Isatis sp.	20:1	22:0	22:1	24:0	24:1 Δ15	TSFA	TUSFA	Oil content (%)
I. cappadocica subsp. *steveniana*	7.54±0.00	1.28±0.02	15.12±0.07	0.57±0.01	2.73±0.04	8.4±0.09	86.9±0.4	24.7
I. kotschyana	9.91±0.02	0.57±0.00	19.75±0.05	0.33±0.00	1.9±0.00	6.7±0.10	88.4±0.07	22.2
I. candolleana	7.56±0.03	1.12±0.07	14.2±0.04	0.58±0.02	2.67±0.02	8.31±0.05	87.2±0.00	26.1
I. spectabilis	9.06±0.04	0.63±0.03	23.6±0.03	0.42±0.1	2.43±0.01	6.81±0.00	88.3±0.04	22.4
I. kozlowskyi	8.38±0.01	0.66±0.00	20.8±0.08	0.42±0.00	3.12±0.00	7.17±0.04	87.9±0.08	31.2
Isatis glauca subsp. *glauca*	9.12±0.02	0.77±0.02	18.8±0.0	0.28±0.0	2.76±0.00	7.26±0.00	87.4±0.2	26.4

TSFA: Total saturated Fatty acid, TUSFA: Total unsaturated FA.

Table 2. Tocochromanol (tocopherol and tocotrienol) composition of some *Isatis* sp., from Turkey. Data shown are peak area (%) from HPLC.

Isatis sp.	Tocopherol and Tocotrienol components										
	α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	P – 8	TToc	TT_3
Isatis cappadocica subsp. *steveniana*	5.53±0.02	--	91.39±0.04	0.90±0.00	--	--	--	--	2.18±0.1	97.82±0.04	--
I. kotschyana	5.65±0.03	88.36±0.04	0.22±0.00	0.25±0.00	--	0.10±0.03	5.43±0.01	--	--	94.48±0.02	5.53±0.01
I.candolleana	4.72±0.00	--	68.7±0.06	0.80±0.01	1.11±0.01	--	--	2.10±0.01	3.45±0.02	74.22±0.03	3.21±0.02
I. spectabilis	--	--	--	78.10±0.04	--	--	14.45±0.00	4.50±0.01	2.95±0.04	78.10±0.04	18.95±0.04
I. kozlowskyii	0.45±0.04	0.29±0.00	74.96±0.02	14.58±0.03	8.30±0.02	--	--	--	1.42±0.03	90.28±0.00	8.30±0.03
I. glauca subsp. *glauca*	91.72±0.00	1.70±0.04	4.36±0.00	--	1.91±0.02	--	--	0.09±0.00	0.22±0.01	97.78±0.02	2.0±0.01

T: Tocopherols, T3: Tocotrienols, P-8: Plastochromanol – 8, TToc.:Total tocopherol, TT3: Total tocotrienols

Tocochromanol analysis: Tocochromanols were determined by high-performance liquid chromatography (HPLC) according to the procedure of Balz *et al*., (1992). An aliquot of a solution of 50 mg oil in 1 ml heptane was injected in an HPLC system *via* a Rheodyne valve with a sample loop volume of 20 µl. Tocopherols were separated on a LiChrospher 100 Diol phase, 5 µm particle size (Merck, Darmstadt, Germany). HPLC column 25 cm x 4.6 mm ID with an additional guard column 4mm long and 4 mm ID, filled with LiChrospher Si 60, 5 µm particle size. The system was operated with an eluent of heptane/tert.-butyl methyl ether (96+4v/v) and detection by a fluorescence detector F-1000 (Merck, Darmstadt) at 295 nanometer excitation wavelength and 330 nm emission wavelength.

A D-2500 Chromato Integrator (Merck, Darmstadt) was used for data aquisition and processing. Calibration was done by external standards with α-, β-, γ- and δ-tocopherol (Calbiochem, Bad Soden, Germany). Tocotrienols were calculated with the same response factors as the corresponding tocopherols and plastochromanol-8 was calculated with the same response factor as gamma-tocopherol (Balz *et al*., 1992).

Results and Discussion

In this study, the fatty acid composition and tocochromanol derivatives, α-, β-, γ and γ- tocopherol and α-, β-, γ and γ- tocotrienols and plastochromanol-8- were determined in *Isatis* species naturally growing in Turkey. The results of the fatty acid analysis and the oil yield are shown in Table 1. The results for tocopherol and tocotrienol contents of the studied samples are shown in Table 2.

The total oil yield of the species studied reached 22.2-31.8 (wt%) of seed. The extracted seed oil of *Isatis* species contained significant amounts of α-linolenic (between 26.6-32.62%), oleic (13.3–18.8%) and linoleic acids (8.94-15.86%) which were the major usual fatty acids in *Isatis* species studied. High concentrations of these fatty acids were reported in *I. aleppica* (20.0, 23.0, 6.0%) (Miller, 1965); *I. tinctoria* (27.4, 17.7, 15.2%) (Blatger, 1993); *I. tinctoria* (28.5, 23.8, 12.6%), (Iba, 1992); *I. glauca* (29.4, 11.1, 13.4%) (Scrimgeour, 1976) respectively. Linoleic and oleic acids (13.2-18.8%) concentrations also were found as very similar to each other for these fatty acids (linolenic, oleic and linoleic acids). It is reported that from the genus *Aethionema* (Brassicaceae)*; Aethionema grandiflora* contained these FA in 63.60, 10.9, 11.80 percentages (Goffman *et al*., 1999), *A. saxatile* contained 56.4, 14.9, 8.7% (Goffman *et al*., 1999), from genus *Alyssoides; Alyssoides utriculata* contained 55.5, 13.5, 16.7% (Goffman *et al*., 1999), from genus *Arabidopsis; Arabidopsis thaliana* contained 18.0, 14.0, 27.0% respectively (Taylor, 1995). These fatty acids showed variation among the Brassicaceae genera patterns.

Palmitic and stearic acid concentrations of the *Isatis* species studied were found very low. They were between 3.5–3.9% and 1.02-1.56% respectively. Palmitic acid also was reported higher in *I. aleppica* (9.0%) and in *I. tinctoria* (6.0%) (Micolajczak, 1961; Miller, 1965). While variation for total tocopherol content and components were extremely wide, total fatty acid content and compositions of *Isatis* sp., were determined as very similar.

High concentration of gadoleic and erucic acid (20:1 and 22:1) was found very interesting. All of the *Isatis* species studied showed high 20:1 fatty acids (7.54-9.91%), and also 22:1, (14.2–23.6%). Both FA were reported at high level in some other *Isatis* sp., 10.0% and 23.0% in *I. aleppica*, 9.7% and 20.9% in *I. tinctoria*; 10.9% and 23.3% in *I. glauca* (Miller, 1965; Blatger, 1993; Scrimgeour, 1976) respectively. It is reported that,

the erucic and petroselinic acids in Brassicaceae and Apiaceae seed oil were principal fatty acids (Vioque *et al*., 1994; Bagci, unpublished). Erucic (24.6-30.5%), linolenic (17.7-27.7%), linoleic (13.9–24.6%) and oleic acids (12.3-21.8%) were determined major fatty acids in genus *Coincya* (Brassicaceae) (Vioque *et al*., 1993). However, gadoleic acid (20:1) was reported at very low level in the some genera in family Brassicaceae like *Aethionema, Alyssoides, Arabis* (Taylor 1995; Goffman *et a*l., 1999). It comprises high percentage of FA in some genus patterns like in *Arabidopsis thaliana* (22.0%) (Taylor 1995). 24:1 unsaturated fatty acid component of the *Isatis* sp. were also found higher (1.9-3.12%) in polyunsaturated fatty acid (PUSFA) like in *Sinapis alba* (2.1%; *Conringia orientalis* (4.4%), *Heliophila longifolia* (27.1%), *Iberis umbellata* (4.4%) (Brassicaceae) (Goffman *et al*., 1999) than the other family and genera patterns mentioned above.

It is possible to say that the polyunsaturated fatty acid (PUSFA) concentrations were found very high when compared with the other family and genera patterns eg., Boraginaceae (Velasco & Goffman, 1999; Bağcı *et al*., 2004), Vochysiaceae (Marco *et al*., 2002), *Pinaceae* (Wolf *et al*., 2001, Bağcı & Karaağaçlı, 2004), *Salvia* and *Astragalus* genus patterns (Bağcı *et al*., 2004, Bagci, 2006).

In general, the sum of all saturated fatty acids (SFA) in *Isatis* species seed oil were between 6.7-8.4% and the amount of unsaturated fatty acids (USFA) were between 86.90-88.26% (Table 1). This means that the shelf life of *Isatis* sp., seed oil is limited due to the high amount of unsaturated fatty acids, which are easily oxidised (Table 1).

High amounts of individual main fatty acids may be useful to assess chemotaxonomic relationships among the plant taxa, but unusual fatty acids are even more useful and important to elucidate chemotaxonomic relationships between some genera and families, because the occurrence of unusual fatty acids in seeds is often correlated to plant families (Aitzetmuller, 1993). *Isatis* patterns studied in here showed similar fatty acid composition both qualitative and quantitatively. High concentrations of the 18:3, 20:1, 22:1 and 24:1 fatty acids may be give some clues on the chemotaxonomic relationships in Brasssicaceae. Species of the genus *Lesquerella* S.Wats., and *Physaria* A. Gray within the Brassicaceae family, have seed oils containing hydroxy fatty acids (Engeseth & Stymne, 1996; Hayes, 1995; Dierig, 1996). In most *Lesquerella* species, either lesquerolic (14-hydroxy-eicosa-11-enoic), auricolic (14-hydroxy-eicosa-11, 17-dienoic) or densipolic (12-hydroxy-octadeca-9, 15-dienoic) acid dominates in the seed oils (Engeseth & Stymne, 1996).

The tocochromanol (tocopherol and tocotrienol) profile of *Isatis* sp., showed differences among the species studied in here. Except *Isatis spectabilis*, other *Isatis* sp., showed high tocopherol amount. While plastochromanol -8- was found in the all of the *Isatis* sp., studied, it was not found in the seed oil of *Isatis kotschyana.* The tocochromanol contents of *Isatis* sp., studied were showed more differences both qualitative and quantitatively in view of the main compounds (Table 2).

While in *Isatis cappadocica* (91.39%) and in *I. koslowski* (74.96%) gamma tocopherol was very high, beta tocopherol was abundant in *I. kotschyana* (88.36%), alpha–tocopherol was in *I. glauca* subsp. *glauca* (9.72%) and delta tocopherol was also highest in *I. spectabilis* (78.1%). In general, total tocopherol contents (TToc) of the studied *Isatis* sp. were found higher than the tocotrienol ($TToc_3$).

It is possible to say that high concentration of tocopherol and tocotrienols may be characteristic in infrageneric or infrafamilial level in plant families, variation among the species may be originated from the ecological conditions, stability of the seed oil, mineral intake of the plants etc., besides the genetic differences. Total tocopherol contents were

positively correlated with linolenic and negatively correlated with eicosenoic and erucic acid. A positive correlation between tocopherols content and linolenic acid also had been revealed in rapeseed mutants (Röbbelen & Thies, 1980; Goffman *et al*., 1999). Some Brassicaceae genera patterns collected from different Botanical garden in Germany displayed great variability for tocopherols content and profile. Based on the classsification of Schulz (1936), it is possible to conclude that the tocopherols have a significant taxonomic meaning, at least as important as that of the fatty acids have, while the fatty acid analyses results showed very high congruency among the *Isatis* species studied both qualitatively and quantitatively.

Tocopherols and plastochromanol–8 with the addition of fatty acids possess an important chemotaxonomic value for the genus *Linum* L., (Velasco & Goffman, 2000) and the tocochromanols (Velasco & Goffman, 1999; Goffmann *et al*., 1999a) have chemotaxonomic importance in *Boraginaceae* and *Brassicaceae*. Some clues were obtained to determine the degree to which fatty acids can contribute to delimit taxonomic classes within the family. Differences in fatty acid patterns illustrate some chemotaxonomic relationship between studied family members. However, further studies are required to confirm the results obtained from this study particularly on the family pattern all over the world.

More successful results have been obtained when the fatty acid analysis has been restricted to smaller plant groups, as in the investigations of Stone *et al*., (1969), Hohn & Meinschin (1976), Aitzetmuller *et al*., (1999) and Bağcı & Özçelik (2001). The occurrence of this fatty acid components in some plants, may have practical consequences with respect to genetic engineering or plant breeding for renewable lipid resources and it may attract significant interest in regard to natural product chemistry, plant chemotaxonomy and evolution. Further studies and more family patterns, however, are needed to determine the degree to which fatty acids can contribute to delimit taxonomic classes within this family.

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