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STUDIES ON LIPID PROFILES OF *VITIS VINIFERA* (GRAPE) AND *LACTUCA SCARIOLA*(OIL LETTUCE) SEED OILS IN COMPARISON WITH THOSE OF SOYBEAN OIL

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ABSTRACT

Two new oils, namely, *Vitis vinifera* (from red grape pomaces) and *Lactuca scariola* (oil lettuce, var *oleifera*, crop) were studied for their fatty acids, triacylglycerol molecular species, tocopherols and sterols patterns. The results were compared with those of soybean oil taken as a representative of common edible oils. It was found that red grape seed oil contains higher amount of linoleic acid 67.9%, whereas lettuce seed oil and soybean oil contain 61.1 and 49.0% respectively. Concerning the triacylglycerol profile, grape seed oil contains higher amounts of LLL and LLO than those of the two other oils. Tocopherol analysis of red grape seeds (in pomaces) oil showed the presence of alpha- and gamma- tocotrienols and lower amounts of alpha- and gamma- tocopherols. In lettuce seed oil, alpha-tocopherol was found to be the main tocopherol component. On the other side, the sterol pattern of lettuce seed oil showed a comparatively higher amount of 7-stigmasterol and avenasterol. It was concluded that the seeds of grape pomaces (produced in tonnages) and of oil lettuce can be used as potential sources of oils for edible purposes.

INTRODUCTION

In the frame of a screening analysis progamme designed for searching for non- conventional sources of oils and due to the interest in plant by-products for reducing environmental problems, two sources have been selected, namely, seeds sieved out of grape pomaces (*Vitis vinifera* family *vitaceae*) and seeds of oil lettuce crop (*Lactuca scariola*, var. *oleifera* family *compositae*). Tonnages of grape pomace resulting from mechanical pressing of grapes (red and white Ginaclis varieties) containing grape seeds (17-20% oil), are annually produced as a by-product from the distilleries industry. On the other side, oil lettuce crop was cultivated as an experimental trial in Aswan region and high promising yields of seeds, containing about 38-40% oil were obtained. In continuation of these trials, the crop was cultivated in the National Research Centre Experimental Station (season 2000) to be used as a new source of oil.

Very few studies (fatty acid and sterol composition) on lettuce oil of two Lactuca species, namely, scariola and saligna have been carried out (EL-Din, *et al.*, 1987 and Rafi *et al.*, 1991). The oil of grape seeds of domestic white and red varieties of grape pomaces were investigated only for fatty acid composition by UV spectroscopy (EL-Mallah, *et al.*, 1971), whereas other authors were concerned with the elucidation of fatty acid composition and determination of some triglycerides of the grape seed oils from different locally cultivated varieties but using argentation and partition TLC (EL-Zeany *et al.*, 1982a, 1982b). It is worthy to mention that the previous studies on lipids composition of grape seed oils of pomace seeds (Ginaclis), with the

exception of triacylglycerols composition, were conducted (EL-Shami, *et al.*, 1992 and EL-Mallah, and Murui, 1993).

The objective of the present work was to elucidate the lipids profiles of red grape pomace seed (new cultivated variety) and lettuce seed oils that have not been hitherto reported. Therefore, methods of analysis, namely, GLC and HPLC have been used for achieving high accuracy to obtain more reliable data about the above mentioned oils, for the possibility of using them among the other common edible oils. Fatty acids, triacylglycerol molecular species, tocopherols and sterols patterns of the two oils were compared with those of soybean seed oil taken as representative of a common edible oil.

MATERIALS AND METHODS

Material:

Oils from both grape (*Vitis vinifera* family *vitaceae*) and oil lettuce (*Lactuca scariola* var. *oleifera* family *compositae*) seeds were subjected to investigation. Samples of grape pomaces; obtained as a pressing by- product of a new variety of red grape seed, were obtained from the Egyptian Vineyard and Distilleries Company (Ginaclis, Alexandria) season 2000. A representative seeds sample, sieved out, from the air dried pomaces was subsequently ground and extracted. Meanwhile, oil lettuce seeds were obtained from the cultivated crop in the Experimental Agriculture Station of the National Research Centre of Egypt (season 2000), the seeds from the harvested crop were dried prior to grinding and solvent extraction. Also, a sample of soybean (Glycine max) of the season 2000, was obtained from the department of Oilseed crops, Ministry of Agriculture, and its oil composition was compared with those of the two mentioned oils.

Thus, solvent extraction of the ground seeds and beans was accomplished by using chloroform- methanol (2:1 v/v) and the obtained oils were subjected to analysis.

Methods:

Fatty acid Pattern:

The oil was converted into methyl esters via transesterfication with 5% methanol hydrochloric acid (Chrisite, 1973). Transestrification reaction was monitored with the help of TLC using silica gel G plates and n-hexane: diethylether: acetic acid (80:20:1 v/v/v) as developing solvent.

Hewlett Packard- HP 5890A gas chromatograph was employed for the analysis of the mixed methyl esters under the following operating conditions: column: DB-23 (0.32mmx 30m); temperatme programming: 150-230°C, 3.0°C/min; injector temperature: 230°C; detector: FID at 240°C; carrier gas: nitrogen at flow rate of 1.3ml/min and split ratio 100:1. Calibration was made using standard fatty acid methyl esters. The results were recorded by an electronic integrator as peak area per cent.

Triacylglycrol profile:

HPLC instrument (Toyo-Soda-CCPM) was employed for the determination of triacylglycerol (TAG) profile. A 10µl solution of oil in chloroform (300mg/ml) was injected into the column, ODS Capcel Pak, C_{18} (4.4x100mm). Gradient elution with acetonitrile: dichloromethane (starting from 90: 10 to 35:65 v/v) in 150-minute was conducted. FID detector (with moving band, Tracor 945) was attached to the instrument.

The carbon number assignment for the separated peaks was determined using HPLC chromatogram of soybean oil taken as reference containing 29 TAG starting with trilinolein and terminating with tristearin (EL-Mallah *et al.*, 1994 and 1999)

The elution sequence was the same as that reported by El-Hamdy and Perkins (1981). The following coding was used for fatty acyls: X = linolenic, L= linoleic, O= oleic, S= stearic, P=palmitic .The results were automatically printed as peak area percent by recording integrator.

Tocopherol Pattern:

Direct determination of tocopherols in oil was accomplished using Toyo - Soda - CCPM HPLC instrument. A sample of 10 µl oil in n-hexane (10% solution) was injected in HPLC column (silica, YMC-A-012, 6.0x 150 mm). Isocratic elution was conducted using n-hexane: isopropylalchol (100:0.5 v/v) at flow rate 2 ml/min Hitachi-650-10S fluorescence detector was used. Spectral absorption was set at excitation and emission wavelengths of 295 and 325 nm respectively (EL-Shami *et al.*, 1994 and EL-Mallah *et al.*, 1994).

Whole sterol profile:

The unsaponifiable portion of the oil prepared according to the A.O.C.S. (1978) was subjected to preparative TLC on silica gel G plates (0.5 mm thickness) to isolate pure sterols using chloroform/ diethylether/acetic acid (95/4/1 v/v/v) as a developing solvent. The sterols zone was located with the help of standard sterol. The scraped zone was extracted with diethylether and the solution was dried, distilled off and the sterols were converted into their trimethylsilyl sterol (TMS) derivatives (Christie 1973).

Hewlett Packard- HP 5890A gas chromatograph was employed for analysis using the following conditions: column DB-17 (1.0mmx15m, 0.53µ coating) at 250°C; detector, FID at 260°C; injection 250°C; carrier gas helium (8.6ml/min) and split ratio 35:1. Standard sterols mixture containing known weights of available standard sterols was used for identification and quantitation. The area under peak was measured using an electronic integrator and the percentage of each sterol was calculated.

RESULTS AND DISCUSSION

***Vitis vinifera* (red grape):**

From GLC analysis of fatty acid composition (Table 1), it can be observed that the oil of red grape seeds is rich in linoleic acid and amounting to 67.9%. Oleic acid (n-9 isomer) is present in a reasonable amount of 17.5% and n-7 isomer constitutes a very low amount. Palmitic and stearic acids are found at levels of 7.7 and 4.9%, respectively.

Table(1): Fatty Acid Composition in Grape, Lettuce and Soybean Seeds Oils

Oil sample	14:0	16:0	16:1	18:0	18:1 n-9	18:1 n-7	18:2	18:3	20:0	20:1 n-9	20:2	22:0	22:1 n-9
Red Grape	0.3	7.7	0.1	4.9	17.5	0.8	67.9	0.3	0.2	0.3	-	-	-
Oil lettuce	0.1	7.1	0.2	3.4	25.7	0.6	61.1	0.2	1.2	0.2	-	0.2	-
Soybean	-	11.0	-	2.0	31.2	0.5	49.0	6.0	-	-	0.2	0.1	-

With reference to the triacylglycerol profile (TAG), as determined by HPLC – FID, 16 TAG's ranging from ECN 42 to ECN 52 representing different critical pairs could be efficiently separated (Table 2).

Table (2): Triacylglycerol Profiles Composition in Grape, Lettuce and Soybean Seeds Oils

TAG	ECN	Oil sample		
		Grape	Lettuce	Soybean
XXX	36	-	-	0.1
XXL	38	-	-	1.1
XLL	40	-	0.4	6.3
XXO	40	-	-	0.5
XXP	40	-	-	0.3
LLL	42	32.7	23.3	15.8
XLO	42	-	0.4	4.6
XLP	42	-	-	3.1
LLO	44	22.8	20.7	15.4
XOO	44	-	-	0.9
LLP	44	13.5	10.0	14.0
XPP	44	-	-	0.6
Unknown 1	-	-	0.3	-
LOO	46	6.8	11.3	7.7
LLS	46	7.7	3.8	3.3
LOP	46	7.4	7.4	9.2
LPP	46	0.4	0.9	2.5
Unknown 2	-	0..2	0.3	-
OOO	48	1.2	4.6	2.8
Unknown 3	-	-	1.3	-
LOS	48	3.7	3.6	3.2
OOP	48	1.4	3.5	2.4
LSP	48	0.4	0.8	1.6
POP	48	0.3	0.5	0.8
PPP	48	0..1	0.4	0.4
Unknown 4	-	0.1	1.3	-
OOS	50	0.6	1.9	1.0
LSS	50	0.2	0.5	0.5
POS	50	0.2	0.5	0.6
SPP	50	-	0.1	0.2
SOS	50	0.1	0.3	0.2
Unknown 5	-	0.2	1.4	-
SSP	52	-	0.2	0.1
SSS	54	-	0.1	0.1
Unknown 6	-	-	0.2	0.7

TAG: Triacylglycerol
X: Linolenic L : Linoleic O : Oleic P : Palmitic S : Stearic
ECN: Equivalent carbon numbe r ECN: carbon number of TAG - 2X Number of double bounds.

The major TAG's in this oil are LLL, LLO and LLP amounting to 32.7, 22.8 and 13.5%, respectively. Other species, namely, LOO, LLS and LOP are present in moderate amounts at levels of 6.8, 7.7 and 7.4%, respectively. On the other side, TAG's, namely, OOO, LOS and OOP are present at comparatively lower amounts whereas, other constituent of TAG's are present as minor components.

Tocopherol composition, as determined directly by HPLC – fluorescence, showed that tocopherols are present together with tocotrienols (Table3). The total amount of tocopherols and tocotrienols constitute 485ppm of the weight of the oil. Alpha - and gamma -tocopherols are present in concentrations of 18.0 and 2.0% respectively, whereas alpha - tocotrienols, which is the major component, is present at a level of 54.0 % while gamma-tocotrienols constitutes 26.0% of the total tocopherol.

Table (3): Tocopherols and Tocotrienols Profiles in Grape, Lettuce and Soybean Seeds Oils

Oil Sample	Total Tocopherols (ppm)	Tocopherols %				Tocotrienols %	
		Alpha	Beta	Gamma	Delta	Alpha	Gamma
Red Grape	485	18.0	-	2.0	-	54.0	26.0
Oil Lettuce	300	100.0	-	-	-	-	-
Soybean	1080	8.3	1.4	64.4	25.9	-	-

Dealing with sterol composition of red grape seed oil, β-sitosterol is the major constituent and amounts to 75.6% as shown in Table 4. Campesterol and 5-stigmasterol are present at equal amounts of 10.5%, whereas isofucosterol and avenasterol were detected as minor components. 7-Stigmasterol was also found at a level of 1.6%. It was observed from the previous results that the lipid compositions of red grape seeds differed markedly from those of white grape seeds reported by (EL-Shami *et al.*, 1992).

Table (4): Whole Sterol Patterns in Grape, Lettuce and Soybean Seeds Oils

Oil Sample	Sterol Content %	Chole - sterol	Campesterol	5-stigma sterol	unknown sterol	B-sitosterol	Spinasterol	Isofucosterol	7-stigma sterol	avenasterol
Red Grape	0.7	0.4	10.5	10.5	-	75.6	-	0.8	1.6	0.6
Oil Lettuce	0.4	0.0	11.0	9.9	4.5	41.1	4.2	2.0	21.2	6.1
Soybean	0.3	trace	17.0	20.5	-	53.5	-	-	8.0	1.0

***Lactuca scaroila* (oil lettuce):**

Table (1) represents the fatty acid composition of oil lettuce. It shows the presence of linoleic acid as major component (61.1%) whereas, oleic acid (n-9 isomer) constitutes 25.7% and oleic acid n-7-isomer is present as minor component (0.6%). Palmitoleic and linolenic are present as minor components. Palmitic and stearic acid are detected and amount to 7.1 and 3.4%, respectively.

With reference to the TAG profile of the oil lettuce, it can be observed from Table 2, that about 28TAG molecular species were separated into

different species according to their equivalent ECN values. LLL and LLO are present as major components at levels of 23.3 and 20.7%, respectively. LLP, LOO and LOP are present at moderate levels, 10.0, 11.3 and 7.4%, respectively. TAG's, namely, LLS, OOO, LOS, and OOP are present at reasonable concentrations. Other TAG's are present as minor components.

The total tocopherols of oil lettuce is present in a somewhat lower amount and is present as only alpha-tocopherols which constitutes 100% of the total weight (Table3).With reference to the sterol patterns, determined as TMS derivatives by capillary GLC, it was found that β-sitosterol is present at comparatively high concentration. Campesterol constitutes 11.0%, whereas 5-stigmasterol forms 9.9% of the weight of the total sterols. Strikingly an unknown sterol, which may stand for ergosterol, was detected at a level of 4.5% whereas, 7- stigmasterol was detected at higher level of 21.1%. Isofucosterol and avenasterol were also detected at concentrations of 2.0 and 6.1%, respectively (Table4).

***Glycine max* (soybean):**

It is worthy to mention that soybean oil was selected as a standard for comparison due to the fact that it is a very common edible oil that contains a wide varieties of saturated and unsaturated fatty acids. Concerning fatty acid composition, the oil is characterized with the presence of a reasonable amount of linoleic acid (49.0%) accompanied with a lower amount of linolenic acid (6.0%) (Table1).

The TAG's profiles of this oil comprises about 30 molecular species of TAG's having ECN values ranging from 36 to 52. Thus, the major TAG's of the soybean oil are LLL, LLO and LLP, 15.8, 15.4 and 14.0%, respectively. Other TAG's, namely, LOO and LOP are present in moderate concentrations whereas, XLO, XLP, LLS, LPP, LOS, OOP and LSP are present at comparatively lower levels. The rest of the 30 TAG's are present as minor components (Table2).

Concerning, soybean oil tocopherols, the oil is recognized for its high total tocopherol content (Kanematsu, *et al.*, 1983). Thus, tocopherol pattern shows the presence of gamma- and delta-tocopherols amounting to 64.4 and 25.9% respectively, whereas alpha-and beta- tocopherols constitute 8.3% and 1.4% of the total tocopherols, respectively (Table 3).

With reference to the sterol composition of soybean seed oil, it is found that the oil is characterized by having a wide variety of sterol components (Table 4).

Thereafter, it was intended to carry out comparison between chemical compositions of soybean oil with those of red grape (from grape pomaces) and oil lettuce seed oils.

Thus, the following observations can be obtained when comparing

the different lipid profiles of red grape seed oil with those of soybean oil:

1- Linoleic acid is present at comparatively higher level than that present in soybean oil, whereas oleic acid is present at comparatively lower level than that of soybean oil.

2- Dealing with TAG's profile, it can be noticed that red grape seed oil contains comparatively higher amounts of LLL, LLO and LLS than soybean oil whereas, LLP is present in nearly similar amounts in both grape seed and soybean oil.
3- Total tocopherol in red grape seed oil is much lower than in soybean oil. Alpha-tocopherol is present at higher amount while gamma-tocopherol is present as minor amount in red grape seed oil. Alpha and gamma tocotrienols are only found in red grape seed oil.
4- Betasitosterol is detected at higher level, whereas campesterol and stigmasterol are comparatively lower in red grape seed oil.

Comparing the different lipid patterns of lettuce oil with those of soybean oil the following differences can be noticed:

1- The fatty acid composition in lettuce oil contains higher linoleic acid and lower oleic acid and very minute quantity of linolenic acid. Palmitic acid was found at lower level than that of soybean.
2- From theTAG's profile of lettuce oil, it can be seen that some TAG's containing linoleic acid moiety such as LLL, LLO, LOO are present at comparatively higher level in lettuce oil.
3- Total tocopherols in lettuce oil are much lower than that of soybean oil and the alpha-tocopherol is the only tocopherol constituent present in lettuce oil. In contrast, soybean oil contains gamma- and delta-tocopherols as major tocopherol constituents, whereas alpha-and beta-tocopherols are present as minor components.
4- Sterol composition in lettuce oil shows the presence of higher levels of 7-stigma and avenasterol whereas, campe-, 5-stigma- and β- sitosterol are markedly lower in lettuce oil than those found in soybean. Spinasterol as well as an unknown sterol (standing for ergosterol) are only present in lettuce oil.

It can be concluded that, seeds of grape pomaces as well as seeds of oil lettuce crop can be used as potential source for edible oil due to their high oil content on one hand and due to their chemical composition on the other. In addition, their properties comply with those of the common vegetable oils and suggests its potential use as special oils for health and nutrition.

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دراسات على تراكيب ليبيدات زيوت بذور كل من العنب و خس الزيت مقارنة بمثيلاتهما في زيت فول الصويا.

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تعتبر زيوت بذور العنب جناكليس الأحمر و التي تحتوى من ١٧-٢٠ % زيت (كمخلفات من عمليات عصر العنب) ومحصول خس الزيت من (٣٨-٤٠%) زيت من المصادر غير التقليدية للزيوت لذلك أجريت دراسات على مكونات هذه الزيوت باستخدام التحليل الكروماتوجرافي السائل ذات الضغط العالي (HPLC) في تحليل جزيئات الجلسريدات الثلاثية وكذلك التوكوفيرولات (مكونات فيتامين هـ) . كما استخدم التحليل الكروماتوجرافي الغازي على العمود (GLC) لتحليل مكونات الزيوت من أحماض دهنيه و كذلك مكوناته من الاستيرولات. وتم مقارنه مكونات الليبيدات المختلفة بمثيلاتها في زيت فول الصويا باعتباره زيت شائع الاستخدام في الأغراض الغذائية. وقد وجد أن زيت العنب يحتوى على ٩ ،٦٧% من حمض اللينوليك بينما زيت بذور الخس ٦١،١ % . أما الجلسريدات الثلاثية فان زيت العنب يحتوى على كميات كبيره من LLL , LLO بالمقارنة بالزيوت الأخرى . أما زيت العنب فيحتوى على نسبه عاليه من التوكوترينولات إضافة إلى كميات من التوكوفيرولات عكس زيت الخس الذي يحتوي فقط على ألفا-توكوفيرول.كمكون وحيد وبالنسبه الاستيرولات فان زيت الخس يحتوي على كميه كبيره من ٧- استجما استيرول. و يمكن استخدام بذور العنب المخلف و كذلك بذور خس الزيت كمصادر غير تقليديه للزيوت الغذائية و أيضا كزيوت صحية .