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Detailed studies on some lipids of *Silybum marianum*(L.) seed oil

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RESUMEN

Estudios detallados de algunos lípidos en aceite de semilla de *Silybum marianum L.*

La composición de ocho tipos de lípidos en aceite de semillas de *Silybum marianum* (L.) Gaertn, hasta ahora no reportados, han sido identificados en este laboratorio, usando GLC capilar y HPLC como herramientas principales de análisis. El aceite es rico en ácidos linoleico (53,3%) y oleico (21,3%). El ácido linoleico está contenido principalmente en cinco formas de triacilgliceroles: LLL, LLO, LLP, LOO y LOP, detectadas por HPLC, usando un detector FID, estos triacilgliceroles son también predominantes en los aceites de semillas de algodón o girasol, pero con diferentes proporciones. El contenido total de tocoferoles (260 ppm) fue determinado directamente en el aceite por HPLC. Se encontró que el aceite contenía alfa-tocoferol como principal constituyente (84,5%), recordando al aceite de girasol. El patrón completo de esteroles determinado por GLC como derivados de trimetilsilil incluyó campesterol, 5-estigmasterol, beta-sitosterol, 7-estigmasterol, avenasterol y spinasterol. Las cuatro clases de esteroles lipídicos, de libres y acilados esteroles y esterilglicósidos han sido determinados como sus derivados de 9- y 1- antroilnitrilo respectivamente por fluorescencia y UV- HPLC.

PALABRAS-CLAVE: Aceite – Lípidos – Semilla – Silybum marianum (L.)

SUMMARY

Detailed studies on some lipids of *Silybum marianum* (L.) seed oil.

Eight lipid patterns of *Silybum marianum* (L.) Gaertn seed oil, not hitherto reported, (20%) have been elucidated in this laboratory using capillary GLC and HPLC as main tools of analysis. The oil is rich in linoleic acid (53.3%) and oleic acid (21.3%). Five major triacylglycerols containing linoleic acyls namely LLL, LLO, LLP, LOO and LOP were detected by HPLC using FID detector, these triacylglycerols are also predominating in both cottonseed and sunflower oils but in different proportions. The total tocopherols content (260 ppm) was determined directly in the oil by HPLC. It was found that the oil contains alpha -tocopherol as a major constituent (84.5%) resembling sunflower oil. The whole sterols pattern, as determined as their trimethylsilyl derivatives by GLC, includes campesterol, 5-stigmasterol, beta-sitosterol, 7-stigmasterol, avenasterol and spinasterol. The four sterols lipid clases of free and acylated sterols and sterylglycosides have been determined as their 9- and 1- anthroylnitrile derivatives by fluorescence and UV- HPLC respectively.

KEY-WORDS: Lipids – Oil – Seed – Silybum marianum (L.).

1. INTRODUCTION

Silybum marianum (L.) (Sm) (Milk Thistle) belonging to Compositae is mainly used as a medical plant particularly for curing liver diseases due to the presence of active silymarin compounds.

Silybum marianum is a wild growing annually herb that is abundantly found in the Nile region (Delta) and Fayium region near water streams. The plant has been locally cultivated successfully in reclaimed lands of Salehiya for medical purposes and huge amounts of the oil-rich seeds, as secondary product, are produced. Flowering of Sm begins in spring and the plant is harvested at the beginning of summer. The seeds contain about 22% oil, which is similar to many vegetable oil seeds or even more.

Some authors have carried out chemical isolation of some amino acids (Varma et al., 1980) and some complex lipid (Basudeb et al., 1996). Seed lipids composition concerning sowing and harvesting of Sm seeds was also studied (Marquard and Voemel, 1982). Fatty acids composition of Sm oil was studied (Qifan et al., 1998), whereas the oil constituents (hydrocarbons, sterols, and fatty acids) of Sm fruits of the Egyptian wild and locally cultivated plants have been carried out (Hammouda et al., 1994). A process for the extraction of Sm Gaerten seed oil by squeezing was conducted followed by refining (Song et al. 1999). On the other side, the efficiency of high-pressure extraction in production of Sm oil with high content of vitamin E (alpha-tocopherol) was studied (Movca et al., 1999). Determination, oxidative and lipid peroxidation-inhibitory effects of some other oil antioxidants, namely, silymarin and silybin have been reported (Bondarenko 1980; Simova and Pangarova, 1980; Kurkin et al., 1996; Baek-Chen et al., 1997a; Baek-Chen et al., 1997b). Many publications on Sm as medical plant have been published (Mowrey, 1990; Flora et al., 1998; Luper, 1998; Skottova and Krecman, 1998; Skottova et al., 1999; Gupta et al., 2000), however, no detailed studies on the seed oil have been hitherto reported.

It was objective to elucidate the lipid patterns, namely, fatty acids, triacylglycerols molecular species, tocopherols, whole oil sterols and the four sterol lipid classes, namely, free and acylated sterols and sterylglycosides using capillary GC and HPLC analysis. The detailed lipids profiles of the Sm oil that has not been hitherto reported, were compared with the corresponding lipids patterns of some traditional seed oils previously studied in this laboratory (El-Mallah et al., 1999).

2. MATERIALS AND METHODS

2.1. Materials

A representative sample (500g) of Silybum marianum (Sm) seeds (season 2000, Salehiya) was extracted with chloroform- methanol (2:1 V/V) in a waring blender. The extract was dried over anhydrous sodium sulfate and the solvent was removed from the filtrate under reduced pressure at 45°C.

2.2. Methods

2.2.1. *Fatty Acids Pattern*

The oil is converted into methyl esters via transesterification with 5% methanolic hydrogen chloride (Christie, 1973). Transesterification reaction was monitored with the help of TLC using silica gel G plates and n-hexane: diethyl ether: acetic acid (80:20:1 by volume) as a developing solvent.

Hewlett Packard-HP 5890-A gas chromatograph was used for the analysis of the mixed methyl esters under the following operating conditions: column, DB-23 (0.32 mm x 30m); temperature programming, 150-230 °C, 3 °C/min; injector, 230 °C; detector, FID at 240 °C; carrier gas, Helium at flow rate of 1.3 ml/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 percent.

2.2.2. *Triacylglycerols Pattern*

TAG molecular species profile was elucidated using HPLC instrument (Toyo- Soda-CCPM). A 10 µl solution of oil in chloroform (300 mg/ml) was injected into the column, ODS capcel Pack, C_{18} (4.4x 100 mm). Gradient elution with acetonitrile: dichloromethane (starting from 90:10 to 35:65 v/v) in 150 minutes 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 for soybean oil taken as a reference, containing 29 TAGs starting with trilinolein and terminating with tristearin (El-Mallah et al., 1999; El-Mallah et al., 1994). The elution sequence was the same as that reported by El-Hamdy and Perkins (1981).

2.2.3. *Tocopherols Pattern*

HPLC analysis of tocopherols was carried out using Toyo- Soda-CCPM HPLC instrument. An oil sample of 10 gram was dissolved in n-hexane to make 10% solution and 10 µl was injected into the silica column (YMC-A-012, 6.0x150 mm). Isocratic elution was conducted using n-hexane: isopropyl alcohol (100:0.5, by volume) as mobile phase, at a flow rate of 1-2 ml/min. Hitachi-650-10S fluorescence detector was used. Spectral absorption was set at excitation and emission wavelengths of 295 and 325nm, respectively. The conditions were optimized to elute delta-T after 10 minute. The results were automatically recorded as peak area percentages by electronic integrator. From the peak area and the corresponding weight of each individual T in the standard mixture, the weight of each individual T in the oil (ppm) can be calculated (El-Mallah et al., 1994).

2.2.4. *Whole Sterols Pattern*

Whole sterols were isolated from the prepared unsaponifiable fraction (AOCS, 1987) by preparative TLC on silica gel G plates (0.5 mm thickness) using chloroform/ diethyl ether/ acetic acid (95/4/1 by volume) as developing solvent. The sterol zone was located with the help of standard beta-sitosterol applied alongside the sample prior to development. The scraped zone was thoroughly extracted with diethylether and the solvent was distilled off from the filtered solution. The sterol mixture was converted into trimethylsilyl derivatives (TMS).

Hewlett Packard-HP 5890-A gas chromatograph, was employed for analysis using the following operating conditions column, DB-17 (0.32 mm x 15m, 0.25 µm coating) at 250°C; detector, FID at 260°C; injection 250°C; carrier gas, Helium (8.6 ml/min) and split ratio, 35:1. Standard TMS sterols mixture containing known weights of sterols was used for identification. The area under each peak was measured using an electronic integrator and the percentage of each sterol was calculated.

2.2.5. *Patterns of Free and Acylated Sterols*

The isolation of free and acylated sterols (FSand AS), their derivatization into 9-anthroylnitrile (S-9-AN)) and their HPLC determinations were carried out according to El-Mallah et al. (1994 and 1999) and Wanaka and Murui (1992).

FS and AS were isolated from the oil by preparative TLC using n-hexane/diethyl ether /formic acid (70/30/1 v/v/v) as developing solvent. The FS and AS were scraped off the plate and extracted with chloroform. Only AS fraction was deacylated via mild alkaline hydrolysis (0.5N KOH in isopropyl alcohol). The obtained FS as well as the original FS were separately derivatized into their sterol S-9-AN derivatives according the same methods mentioned above.

HPLC of S-9-AN was conducted under the following conditions: reversed phase column,

ODS-1250Y, Senshu Pack (4.6 x250mm); detector, fluorescence; excitation and emission wave lengths set at 360 and 460 nm; isocratic elution using acetonitrile/ dichloromethane (75/25, v/v) at flow rate 1 ml/min.

2.2.6. *Sterylglycosides Profiles of Free and Acylated Sterols*

The procedure of separation from the oil, derivatization into sterylglycoside 1-anthroyl nitrile (SG-1 AN) and HPLC analysis were carried out according to El-Mallah et al. (1994 and 1999) and Murui et al. (1993).

The free sterylglycoside (FSG) and acylated sterylglycoside (ASG) were separated from the oil by injecting the oil solution into silica gel cartridge (Sep-Pak, Waters) and were eluted with chloroform: methanol (1:1 v/v). The mixed compounds were subsequently fractionated into pure FSG and ASG with the help of preparative TLC. Chloroform / methanol / formic acid mixture (90/15/1, v/v/v) was used as a developing solvent. Only ASG fraction was subjected to alkaline hydrolysis (0.5N KOH in isopropanol) to obtain FSG. The original and obtained FSG were derivatized separately, into SG-1-AN according to the same methods mentioned above.

HPLC instrument, equipped with UV detector (Toso, UV 8000) and ODS Wakosil-5, C_{18} (6.4 mm x 250 mm) was used for SG-1-AN analysis. Gradient elution with acetonitrile / dichloromethane (from 50/50 to 68/32 v/v) was used and absorption was measured at 254 nm. It is noteworthy to mention that this method is sensitive to 0.5 nanogram of SG.

All the methods were calibrated with standard substances and all the results recorded were the mean of two concordant replicates.

3. RESULTS AND DISCUSSION

3.1. Fatty Acids Pattern

The fatty acid composition of Sm seed oil is recorded in Table I. It was found that this oil is rich in unsaturated fatty acids, which constitute 75.1 % of the total fatty acids. Among the unsaturated fatty acids, linoleic acid is the major constituent and forms 53.3 % of the total composition followed by oleic acid (21.3 %). Cottonseed oil contains lower amounts of linoleic acid, 52.2 %, and oleic acid, 18.4 % (19). Whereas sunflower oil contains lower amount of linoleic acid, 42.0 %, but higher amount of oleic acid, 46.0 % (El-Mallah et al., 1999).

Saturated fatty acids, namely, palmitic acid, 9.4 %, and stearic acid, 6.6 % are the main saturated

Table I
Fatty Acids Pattern of Silybum marianum Seed Oil

Fatty Acid Composition (Wt. %)									
16:0	18:0	18:1 n-9	18:1 n-7	18:2	18:3	20:0	20:1	22:0	24:0
9.4	6.6	20.8	0.5	53.3	Trace	3.8	0.5	2.4	0.7

Table II
Pattern of Triacylglycerol Molecular Species of Silybum marianum Seed Oil

TAG Type	Ecn	Weight %	TAG Type	Ecn	Weight %
XLL	40	0.1	OOP	48	5.6
LLL	42	16.6	LSP	48	1.6
XLO	42	0.2	POP	48	2.1
LLO	44	17.4	PPP	48	0.3
LLP	44	12.3	OOS	50	1.8
LOO	46	11.3	LSS	50	0.5
LLS	46	2.7	POS	50	1.2
LOP	46	12.1	SPP	50	0.2
LPP	46	3.5	SOS	50	0.3
OOO	48	5.7	SSP	52	0.2
LOS	48	3.4	SOA	54	0.1

Two unknown TAG's (0,8 %); one unknown 0,35% (between POP and PPP) and the other, 0,45 % (between POS and SPP).

constituents in Sm oil. Cottonseed oil contains much higher amount of palmitic acid, 24.7 %, which nearly forms the saturated part of the fatty acid profile, whereas, in sunflower oil palmitic acid constitutes 6.2 % of the total fatty acids content.

3.2. Triacylglycerol Pattern

HPLC analysis of Sm seed oil TAG species shows the presence of 22 TAGs containing palmitic, stearic, oleic, linoleic and linolenic acyl groups designated as P, S,O,L and X respectively (Table II). From the results it can be noticed that LLL (16.6%), LLO (17.7%), LLP (12.3%), LOO (11.3%) and LOP (12.1%) are the major TAGs of Sm oil. These five TAGs are also found in cottonseed and sunflower oils but at different proportions. The oil of Sm contains LLS, OOO, LOS, OOP and POP in reasonable amounts similar to both of cottonseed and sunflower oils, but at different amounts. Triolein, OOO, is found at a level of 5.7 %. However it is present in sunflower oil at much higher level (12.9 %). On the other side, LLL and LOP of Sm oil are somewhat similar in quantity to those present in cottonseed oil. In addition, Sm seed oil contains 5.6 % OOP, which is higher than those present in cottonseed and sunflower oils. Generally it can be concluded that Sm seed oil has a characteristic TAG pattern as compared with those elucidated for both cottonseed and sunflower oils.

3.3. Tocopherols Pattern

The tocopherols composition of Sm seed oil is illustrated in Table III. It can be seen that the total tocopherols (T) content amounts to 260 ppm, which is very low in comparison with cottonseed oil (700 ppm) and sunflower oil (670 ppm.). The tocopherols profile of Sm seed oil shows that α-T (84.5 %) is the major component whereas ß-T, γ-T, and δ-T constitute 9.9 %, 5.4 % and 0.2 %, respectively. Therefore this profile is similar to that of sunflower oil (α-T, 95.8 %; ß-T, 3.3 % and γ-T, 0.9 %).

3.4. Whole Sterols Pattern

The sterol constituents of Sm seed oil were determined by GLC., as their TMS derivatives, and seven sterols were detected (Table IV). Beta -sitosterol, which is the major sterol of most vegetable oils, is also the major component of Sm seed oil and constitutes 57.4 % of the weight of the total sterols; however it constitutes 85.5 % and 62.0 % of the weight of the total sterols in cottonseed and sunflower oils respectively. Sm oil is characterized by its high content of 7-stigmasterol, which was found at a level of 20.4 % corresponding to 11.7% in sunflower oil.

3.5. Sterols Patterns of (FS) and (AS)

The composition of free sterols (FS) and acylated sterols (AS) of Sm seed oil are determined by HPLC as their 9-anthroylnitrile derivatives and the results are presented in Table V. Beta-sitosterol, campe+ stigma (unseperable pair) and 7-stigma sterols are the major sterols in both AS and FS fractions. Beta -sitosterol (50%) and campe+stigma sterols (9.7%) are less in AS fraction than in FS, reaching 58.2 % and 15.6 %, respectively. Meanwhile, 7-stigma is

Table III
Tocopherols Pattern of Silybum marianum Seed Oil

Tocopherol Content (ppm)	Tocopherol Composition %			
260	α-T	β -T	γ -T	δ -T
	84.5	9.9	5.4	0.2

Table IV
Whole Sterols Pattern of Silybum marianum Oil

Total Sterol Content (mg/100g)	Sterol Composition %							
	Chole-sterol	Campe-Sterol	Stigma-sterol	Sito-sterol	Isofuco-sterol	7Stigma-sterol	Avena-sterol	Spina-sterol
600	0.9	6.1	6.8	57.4	2.9	20.4	5.5	Trace

Table V
Free and Acylated Sterols

Sterol Fraction	Content (mg/100)	Sterol Composition %						
		Avena-sterol	Isofuco-sterol	Unknown	Spina-Sterol	Campe+ tigma-sterol	7 Stigma-sterol	β Sito-sterol
Free	320	2.9	1.7	3.7	3.0	15.6	14.9	58.2
Acylated	230	5.9	2.7	4.2	3.4	9.7	24.1	50.0

Table VI
Free and Acylated Sterylglycosides

Sterylglycoside Fraction	Content (ppm)	Sterylglycoside Composition %					
		Avena-sterol	Isofuco-Sterol	Unknown	Campe+ Stigma-sterol	7-Stigma sterol	β-Sito-Sterol
Free	760	2.0	3.5	5.8	9.0	6.8	72.9
Acylated	610	1.2	2.9	2.0	12.6	4.8	76.5

also much higher in AS fraction (24.1 %). Avena, isofuco, and spina sterols are present at higher levels in the As fraction than in FS. Comparing the sterol patterns of the FS and AS fractions of Sm seed oil with those of cottonseed and sunflower oils, it was found that they are significantly different. Thus avena, spina and 7-stigma sterols were detected in both fractions of Sm oil and not in cottonseed and sunflower oils.

3.6. Sterylglycosides Profiles of (FSG) and (ASG)

The sterylglycosides of Sm oil are present as nonesterified (FSG) and esterified (ASG) forms and they are determined by HPLC as their 1-anthroylnitrile derivatives (Table VI). ASG fraction of the oil is present at a level of 610 ppm, which is lower than that of the FSG fraction (760-ppm). It was found that beta-sitosterol is the major component in both ASG and FSG. It was found that Sm seed oil is rich in both sterylglycoside fractions in comparison with cottonseed and sunflower oils.

It is noteworthy to mention that advanced methods of analysis of HPLC and capillary GLC have helped determine comprehensive lipids profiles, not hitherto elucidated, of this new seed oil of the locally cultivated plant.

Generally, it can be concluded that Sm seed oil exhibit characteristic lipid profiles (fatty acids, TAGs, tocopherols, whole sterols, free and acylated sterols and sterylglycosides). On the other side, the oil shows some similarity with some traditional oils and can be used as new potential source of edible oils. Accordingly, it may help decrease the deficit between local oil production and consumption.

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