Determination of Tocopherols and Fatty Acids in Seeds of *Silybum marianum* (L.) Gaerth

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Abstract

Background: The dried fruits of *Silybum marianum* (L.) Gaertn (Compositae) contain silymarin, an isomeric mixture of flavonolignans. Silymarin acts as a strong anti -hepatotoxic. This fruits contains a high amount of oil.

Objective: The objective of this study was to determine total lipid content, fatty acid composition and content of different kind of tocopherols in seed oils of *S. marianum*.

Method: Fatty acid compositon and α-, γ- and δ-tocopherol (vitamin E) content from seeds of *Silybum maianum* were investigated by gas chromatography and high performance liquid chromatography methods.

Results and Conclusion: Total lipid percentage was approximately 25% and nine fatty acids including; palmitic acid (8.25 %), palmeotic acid (0.07 %), Stearic acid (6.67 %), oleic acid (31.58 %), Isomer oleic acid (0.53 %), linoleic acid (45.36 %), linolenic acid (0.87 %), arashidic acid (4.11 %), eicozantoic acid (0.088 %) and behenic acid (2.6 %) were determined. α-, γ- and δ -tocopherol content were around 563.157, 88.87 and 163.791 mg Kg $^{-1}$ DW respectively that could be a natural good sources of antioxidant.

Keywords: ***Silybum maianum*, Fatty acid, Tocopherol, GC, HPLC**

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Introduction

Silybium marianum is used traditionally as a hepaprotective agent and supportive treatment of liver disorders [1, 2, 3]. Fruits of *S. marianum* contain flavonolignan compounds, silymarin, which silybin is the main constitute [4, 5, 6]. This fruits contains a high amount of oil. The oil has to be removed from seeds prior extraction of silymarin. It is a by-product of silymarin production [7].

Vegetable oils nowadays are a great source of oil consumption in families and the saturated/unsaturated fatty acid ratio in the diet is very important and polyunsaturated fatty acids play a natural preventive role in different kinds of health problem [8]. Little research has been paid to the composition of fatty acids and tocopherols content of this species.

Tocopherols (vitamin E) are lipophilic antioxidant synthesized by all plants and some algaes and cyanobacteria [9]. Tocopherols act as antioxidants by trapping the hydroperoxide intermediates and stopping the autoxidation chain reaction [10]. Vitamin E is a collective term encompassing α- , β- , γ- and δ- tocopherol and the corresponding tocotrienols [11]. α-tocopherol is a liposoluble compound that is capable of fixing free radicals via its phenol group It plays an antioxidant role in biological membranes [12]. α-tocopherol affects human nutrition and health aspects, while γ- tocopherol shows a strong activity in the seed protecting compounds like fatty acids. γ- tocopherol has 10% of the activity of α-tocopherol [13] In addition, the seeds accumulate tocopherols contain the highest concentration of lipids. The occurrence of high levels of tocopherols is to limit nonenzymatic lipid oxidation during storage, germination and early seedling development [9].

In recent years, antioxidants have been subjected to many studies [6] that have related with the incidence of kind of diseases especially cancers. Recognized antioxidants are carotenoids, tocopherols, vitamin C, etc.

In the present study, high performance liquid chromatography (HPLC) with fluorescence detection method is applied for the determination of α-, γ- and δ- tocopherol in dried fruits of *S. marianum* [13]. Identification and determination of fatty acids in the seeds were also studied by gas chromatography (GC).

The objective of this study was to determine total lipid content, fatty acid composition and content of different kind of tocopherols in seed oils of *S. marianum*.

Materials and methods

Plant materials

The seeds of *S. marianum* were supplied by Institute of Medicinal Plant, Iranian Academic Center for Education, Culture and Research.

Extraction of seed oils

Three individual 3 g samples of dry seeds of *S. marianum* were refluxed with 250 ml of petroleum ether in a soxhlet apparatus [5, 14]. The oils were recovered by distilling the solvent in rotary evaporator at 40 °C. The total lipids were dissolved in petroleum ether and kept at -20 °C until analysis.

Fatty acid analysis

A transmethylation technique followed by GC-FID determination was used. Fatty acid methyl esters (FAME) were prepared pouring 0.5g of oil and 250 µl of methanolic 2 M KOH into a 15 ml vial and mixed with a vibration mixer for 60 S. After 15 min rest, 6 ml of n- hexane were added into the vial and the mixture was shaken in a rotary shaker for 10 min. The layers were allowed to separate

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and the n-hexane fraction was injected to GC for analysis. Fatty acid composition of the seeds of *S. marianum* was determined by GC, Shimadzo, 17A, Japan. GC analysis of fatty acids was achieved by CP-Sil 88 column and temperature program was from 170 °C in 5 min to 190 °C in 22 min.

α-, γ- and δ- tocopherol analysis

Total α-, γ- and δ - tocopherol were determined by HPLC method [13]. The separation of tocopherols were carried out using Knauer HPLC instrument consist of a K-1001 pump, a 20 µl injection loop, Eurosphere C_{18} 5 µm (250×4.6 mm) column, and a RF-10AXL fluorescence detector controlled by Eurochrom software (all from Knauer, Germany). The mobile phase was methanol- acetonitrile (50: 50 V/V) at a flow rate 1.0 ml/min and detection at excitation wavelength 288 nm, emission wavelength 329 nm, room temperature. The total run time was 15 min. All samples were analyzed in duplicate. The quantitative analysis is based on external standard.

Tocopherols were identified by comparing their retention times with appropriate standards (Sigma-Aldrich).

Statical analysis

The statical examination of data was performed using SAS software. Mean percentages of each fatty acid were compared by the analysis of variance (ANOVA). All the experiment was repeated at least twice and the average values are given here.

Results and Discussion

All plants contain oils or fats, mainly in their seeds. The world oilseed production is expected to be about 340 million tons for 2003 - 2004 and jumped by an average 13 million tons per year during the last 10 years. In most plants storage lipids are in the form of triglycerides. There are a very few examples of alternative forms of storage lipid in higher plants. The Seeds of *S. marianum* have a middle range amount of oil (about 25- 30 %) and is rich in unsaturated fatty acids especially diene (n-6) fatty acid, and monoene (n-9) fatty acids.

Fatty acid composition and tocopherols content were investigated by GC and HPLC methods, respectively (Fig. 1 and 2). There were nine fatty acids including, palmitic acid (8.25 %), palmeotic acid (0.07%), stearic acid (6.67%), oleic acid, W9 (31.58 %), linoleic acid, W6, (45.36 %), linolenic acid, W3 (0.87 %), isomer oleic acid (0.53 %), arashidic acid (4.11%), eicozantoic acid (0.88%) and behenic acid (2.6 %). Predominant fatty acids were linoleic and oleic acids that were higher than other fatty acids (Table 1).

The ratio of saturated/unsaturated fatty acid was 0.35 and saturated lipids were lower than unsaturated acids. Polyunsaturated fatty acids are very useful and we know that promote the reduction of both total and LDL cholesterol.

The unsaturated fatty acids represent almost 72% of total fatty acids. Palmitic acid sums up to 8 % of total fatty acids and is the highest content of saturated fatty acid found. Furthermore, stearic and arashidic acids sums up to 6.67 and 4.11 % of total fatty acid.

Linoleic and linolenic acids, being one of the nutrients needed by the body and is not naturally produced in the body. These essential fatty acids must be gained through consumption of food. Linoleic acid component of ceramides and is precursor of arashidic acid can produce prostaglandins thromboxanes, prostacydin and leukotrienes. Moreover, it should be better to prefer seed oils with low amount of saturated fats.

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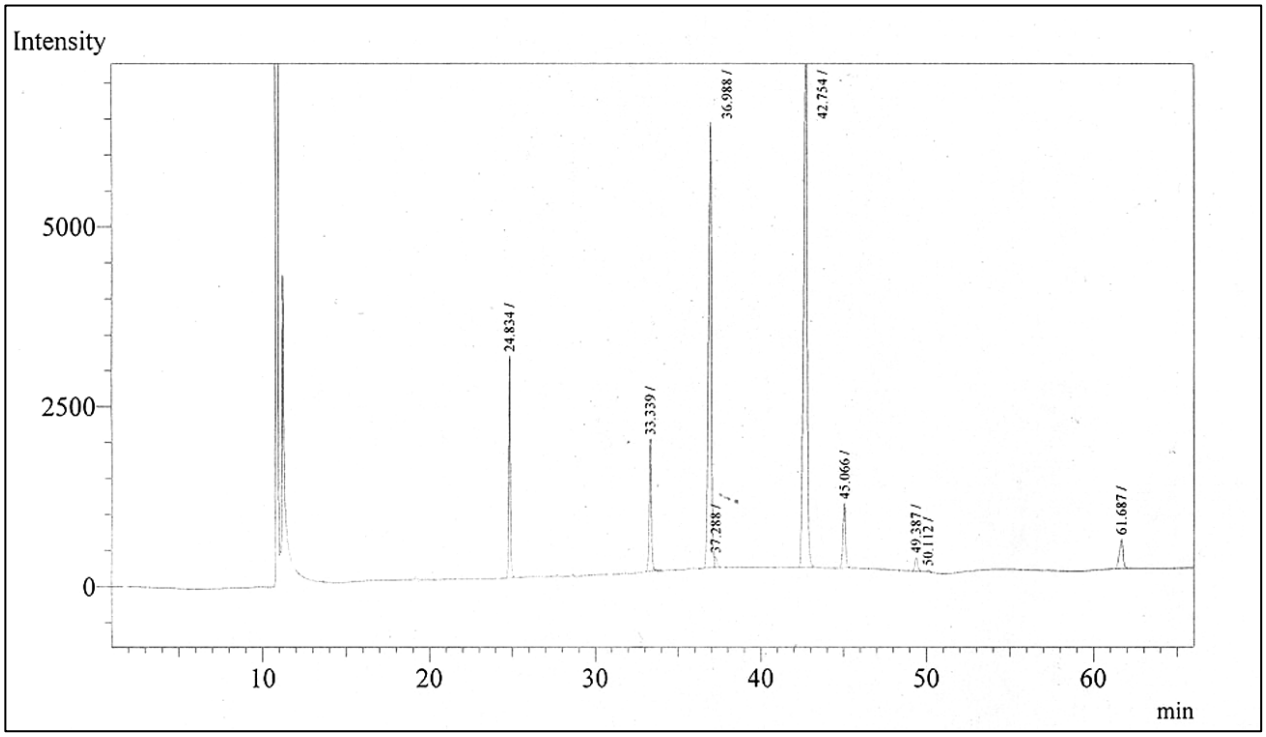

Fig. 1. Gas chromatogram of total lipid in seeds of *Silybum marianum* by GC

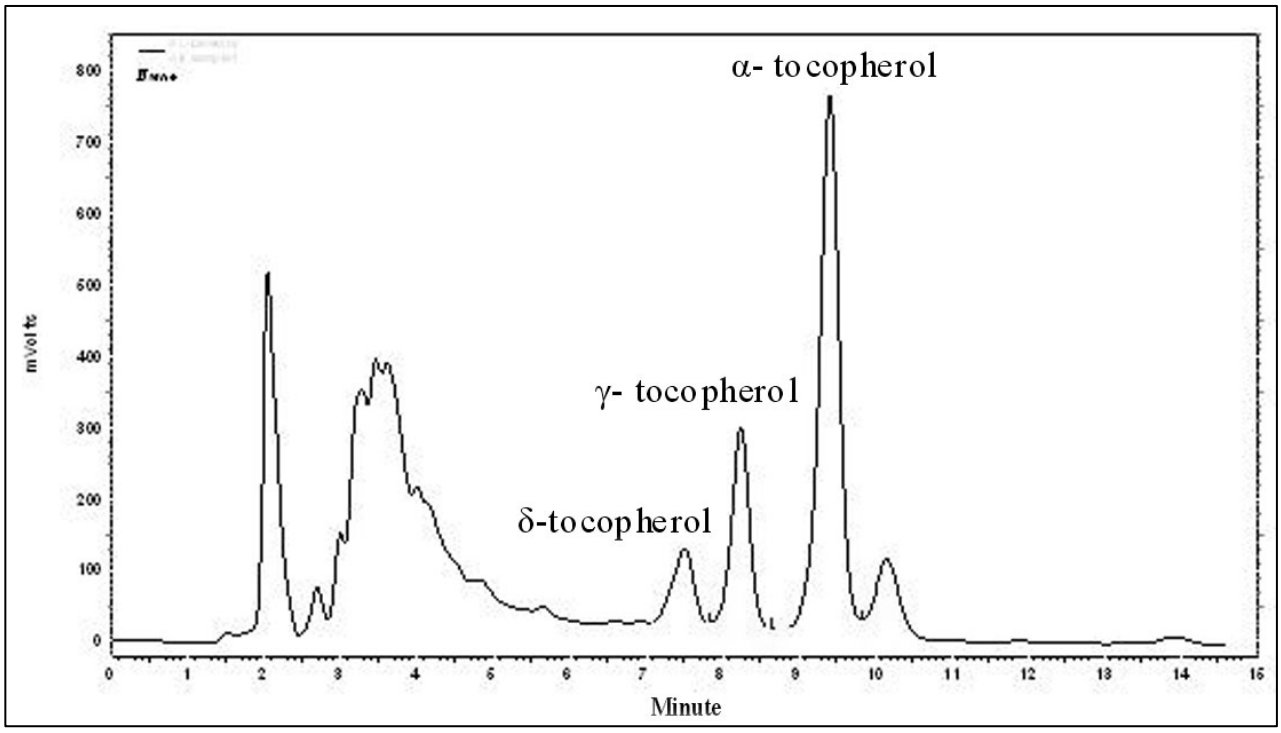

Fig. 2. HPLC chromatogram for α-, γ- and δ-tocopherol determination in seeds of *Silybum marianum*

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Table 1. Fatty acid composition (± SD) in seeds of *Silybum marianum*

Fatty acid (%)		Plant		
		S. marianum	**Soybean**	**Sunflower**
Palmitic acid	C16:0	8.25 ± 0.2	11.23± 0.2	6.5± 0.0
Palmeotic acid	C16:1n- 7	0.07± 0.1	0.05± 0.2	0.04±0.3
Stearic acid	C18:0	6.67± 0.1	4.7± 0.2	4.07± 0.2
Oleic acid	C18:1n - 9	31.58± 0.4	22.52± 0.1	31.27± 0.0
Isomer oleic acid	Iso-C18:1	0.53± 0.2	1.5± 0.3	0.75± 0.2
Linoleic acid	C18:2n - 6	45.36± 0.2	52.07± 0.2	56.04± 0.4
Linolenic acid	C18:3n - 3	0.87± 0.3	6.89± 0.5	0.27± 0.0
Arashidic acid	C20:0	4.11± 0.2	0.43± 0.2	0.31± 0.3
Eicozantoic acid	C20:1	0.088± 0.0	0.2±0.2	0.19± 0.4
Behenic acid	C22:0	2.6± 0.0	0.47± 0.0	0.75± 0.1

Fatty acid composition in this oil compared to other oilseeds such as sunflower and soybean (were studied in our lab). It is interesting that these seed oils have a similar fatty acids composition (Table 1.). While oleic, linoleic and linolenic acid in soybean is 22.52, 52.07 % and 6.89 % respectively and content of these fatty acids in sunflower are 31.27, 56.04 and 0.27 % respectively (Table 1.).

Content of arachidic and behinic acids in seedoils of *S. marianum* were 4.11 and 2.6 %, while in other seed oils such as soybean are 0.43 and 0.47 % and in sunflower are 0.31 and 0.75 % respectively.

Vitamin E comes from tocopherols, a family of fat-soluble antioxidants [15]. Sunflower oil is a good source of α-tocopherol (670 mg kg^{-1}) and Soybean oil has average 100 mg of tocopherols per 100 g of oil [16]. Our results showed that, the mean content of α-, γ- and δ- and total tocopherol in seeds of *S. marianum* were about 563, 88.87, 163.791 and 815.818 mg kg^{-1} dry weight respectively (Table 2.) while in other oilseeds such as soybean and sunflower are 1618.4 and 634.4 mg Kg^{-1} respectively. Therefore, it could be a natural good source of antioxidant and it is better absorbed in body tissues and is a powerful lipid-soluble antioxidant.

It has been reported that the retention of natural vitamin E is at least double that of the synthetic form. In recent years, the physiological functionality of foods has received much attention due to the increasing interest in human health. The antioxidative action is supposed to protect living organisms from oxidative damage [17]. Effective antioxidants are from natural source and may be safer for human. Accordingly, we think that the seeds of *S. marianum* are a strong source of antioxidants.

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Table 2. α-, γ- and δ-tocopherol content in seeds of *Silybum marianum*

Compound	Mean ± SD
α- tocopherol	563.157
γ- tocopherol	88.87
δ- tocopherol	163.791
Total	815.818

Results expressed as mg kg $^{-1}$ dry mass.

The ability of seeds to remain quiescent but viable during unfavorable environmental conditions and the rapidly germinate and become established as photoautotroph, when environmental conditions become favorable was clearly a key evolutionary advantage in the conquering of land by plants. Tocopherol synthesis was apparently a critical biochemical pathway for the evolution of seeds [9].

Sattler et al., (2004) were show vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination and primary function of tocopherols in plants is to limit nonenzymatic lipid oxidation.

The distribution of individual tocopherols and their total content in some of oil seeds were reported by Gliszezynska - Swiglo and Sikorska (2004). Their results showed that the concentration of δ- tocopherol in all oils with exception of soybean were not higher than 30 mg kg $^{-1}$, while the content of this tocopherol in seeds of S. marianum was about 163.791 mg kg $^{-1}$. α- tocopherol dominate in olive (160 mg kg $^{-1}$), grapeseed (100.55) and sunflower oils (591.25).

Conclusion

Based on our study, the seeds of *S. marianum* had similar fatty acid compositions compare with other seed oils such as sunflower and soybean.

Finally, these lipid contents of these seeds could be used for economic and food industrial exploitation.

Consequently, this study revealed that the seeds of *S. marianum* are rich source of unsaturated fatty acids and vitamin E. Our results showed that the oilseeds of *S. marianum* have good composition that can protect these seeds from antioxidation of oils during processing and storage that is the major responsible for quality deterioration [10].

These compounds are by-products of silymarin production which could be used in other industrial application. We propose an investigation to be carried out on the combined effect of vitamin E and silymarin.

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References

1. Kren V and Walterova D. Silybin and silymarin- New effects and application. *Biomed. papers* 2005; 149: 29 - 41.

2. Saller R, Meier R and Brignoli R. The use of silymarin in the treatment of liver diseases. *Drugs* 2001; 61, 2035 - 63.

[DOR: 20.1001.1.2717204.2008.7.25.24.0]

3. Wellington K and Jarvis B. Silymarin: a review of its clinical properties in the management of hepatic disorders. *Bio Drug*, 2001; 15: 465 - 89.

4. Quaglia MG, Bossu E, Donati E, Mazzanti G and Brandt A. Determination of silymarine in the extract from the dried *Silybum marianum* fruits by high performance liquid chromatography and capillary electrophoresis. *J. of Pharmaceutical and Biomedical Analysis* 1999; 19: 435 - 42.

5. Hasanloo T, Khavari-Nejad A, Majidi E, Ziai SA, Shams- Ardakani MR. Determination of flavonolignan of dried fruits of *Silybum marianum* L. Gaertn collected from different areas of Iran by spectrophotometer, TLC and HPLC. *J. of Medicinal Plants*. 2004; 4: 25 - 32.

6. Kvasnicka F, Bíba B, Sevcík R, Voldrich M and Kratka J. Analysis of the active components of silymarin. *J. of Chromatography A* 2003; 990: 239 – 45.

7. Hadolin M, Skerget M, Knez Z and Bauman D. High pressure extraction of vitamin E-rich oil from *Silybum marianum. Food Chemistry* 2001; 74: 355 - 64.

8. Fadavi A, Barzegar M and Azizi MH. Determination of fatty acids and total lipid content in oilseed of 25 pomegranates varieties grown in Iran. *J. Food composition and analysis* 2006; 19: 676 - 80.

9. Sattler SE, Gilliland LU, Magallanes-Lundback M, Pollard M and Dellapenna D. Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination. *The plant cell* 2004; 16: 1419 - 32.

10. Tuberoso CIG, Kowalczyk A, Sarritzu E and Cabras P. Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. *Food Chemistry* 2007; 103: 1494 - 501.

11. Schieber A, Marx M and Carle K. Simutaneous determination of carotenes and tocopherols in ATBC drinks by high-performance liquid chromatography. *Food Chemistry* 2002; 76: 357 - 62.

12. Aoun E, Rima J, Chidiac G and Hanna K. High performance liquid chromatographic and spectroflorometric determination of α-tocopherol in the natural plant: *Ferula hermonis. J. of food Composition and Analysis* 2005; 18, 607 - 15.

13. Sanchez-Machado DI, Lopez-Hernandez J and Paseiro-Losada P. High performance liquid chromatographic determination of α-tocopherol in macroalgae. *J. of Chromatography A* 2002; 976: 277 - 84.

14. Guil JL, Torija ME, Gimenez JJ, Rodriguez I. Identification of fatty acids in edible wild plants by gas chromatography. *J. of Chromatography A* 1996; 719: 229 - 35.

15. Britz SJ and Kremer DF. Warm temperatures or drought during seed maturation increase alpha-tocopherol in seeds of soybean (glycine max [l.] merr.). *J. of Agricultural and Food Chemistry* 2002; 50: 6058 - 63.

16. Van Eenennaam AL, Lincoln K and Durrett TP. Engineering vitamin E content; from arabidobsis mutant to soy oil. *The Plant Cell* 2003; 15: 007 - 3019.

17. Sanchez-Machado DI, Lopez-Hernandez J and Rios Vazquez NJ. High performance liquid chromatography method to measure α-tocopherol in leaves, flowers and fresh beans from *Moringa oleifera. J. of Chromatography A* 2006; 1105: 111 - 4.

[DOR: 20.1001.1.2717204.2008.7.25.24.0]

18. Gliszezynska-Swiglo A and Sikorska E. Simple reversed-phase liquid chromatography method for determination of tocopherols in edible plant oils. *J. of Chromatography A* 2004; 1048: 195 - 8.

[DOR: 20.1001.1.2717204.2008.7.25.24.0]