International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-3, Issue-2, February 2017 Pages 37-40

Physicochemical Properties of Silybum Marianum Seed Oil in Two Different Regions of Iran

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***Abstract*— In this experiment, Silybum marianum was collected from Kazeron and Lorestan in Iran. Hexane Soxhlet method were used and the oil was analyzed by gas chromatography. The fatty acid composition included C16:0, C18:0, C18:1, C18:2 were estimated. The oil refractive index was performed with Refractometr. Peroxide was measured with thiosulfate and peroxide value of the oil sample was 0.68 and 0.57 in Kazeron and Lorestan, respectively.**

***Index Terms*— Methyl esters, Peroxide,Silybum marianum, Soxhlet, Thiosulfate.**

I. INTRODUCTION

One of the important medicinal plant is milk thistle which contain nutritional and medicinal applications. Edible fats, which are suitable for certain food service andbakery purposes, need to be solid or semisolid at room temperature.Those commonly used are animal fats such as tallow,vegetable fats and blends containing palm oil, coconut oil,and partially hydrogenated soybean oil. It is shown that the oil extracted from leaves and seeds of *Silybum marianum*plant can prevention of liver diseases; and promote antioxidant and anti-cancer effects. The healthdisadvantage cause in high content of lauric, myristic,palmitic, or trans fatty acids whichhas been shown toraise the plasma low-density-lipoprotein (LDL) concentrationsin people[1 , 2]. In the last years, new analytical methods have been used to evaluate the processing and storage conditions of oil [3,4,5,6,7]. The quality of edible oils with regard to freshness, storability and toxicity can be evaluated by the determination of metals. Trace elements such as Fe, Cu, Ca, Mg, Co, Ni and Mn are known to increase the rate of oil oxidation compare to other elements such as Cr, Cd, and Pbwhich they are very important on account of toxicity and metabolic role. Thus, the development of rapid and accurate analytical methods for determination of trace elements in oil has been a challenge for quality control and food analysis [8]. Milk thistle or*Silybum marianum* is well known as medicinal plant thatis native to Mediterranean region of Europe and widely dispersed to many countries throughout the world [9,10].*Silybum Marianum* is a medicinal plant which has been used for centuries as herbal medicine treatment of some liver diseases and it is a biennial in nature or annual in cultivation medicinal plant that has been widely used in the European traditional medicine [11].It is widely prescribed by herbalists that we still didn't have information onits parallel effects. This plant is native inMediterranean and grows throughout Europe and NorthAmerica as well as grows in India, China, South America,Africa and Australia [12].*Silymarin*,as a flavonolignan complex that contain silibinin, was isolated from this plant in the 17th century and has been clinically used to treat various liver aliments for more than three decades [13] .

Seed oil chemical composition of wild growing *Silybum marianum* was studied for the first time in Bulgaria. In this research, physicochemical properties and fatty acid composition of extracted oil from milk thistle seeds were studied to identify the composition of fatty acids and measurement of the refractive index for determination of peroxide oilwhich collected from two different regions of Iran namely Lorestan (Khoramabad) (west ofIran) and Kazeroon (south of Iran) .

II. COLLECTION OF SAMPLES

Milk thistle seeds were collected in Kazeron (with 860 m height from sea) andLorestan(with 500 m height from sea) reigns(Figure 1) in July 2014. The plants were identified by Medicinal Plant Research Center, Shiraz University. The seeds were dried at room temperature ($25C^0$). For extraction of oil from the seeds,thistle and hexane soxhlet apparatus was used.

Figure 1 Geographical location of Kazeron and Lorestan

III. REAGENTS AND CHEMICALS

In this experiment, the chemicals were used including hexane, acetic acid, chloroform, potassium iodine, thiosulfate (Merck, Germany).

Oil extraction

The conventional oil extraction, a pilot-scalesoxhlet extraction unit was operated for 9 h using AW406,which is a commercial organic solvent applied to theextraction of crude edible oils. Based on the manufactorydata, the *n*-hexane in

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solvent was30% (with a total hexane content of 70%)[14] (Figure 2).

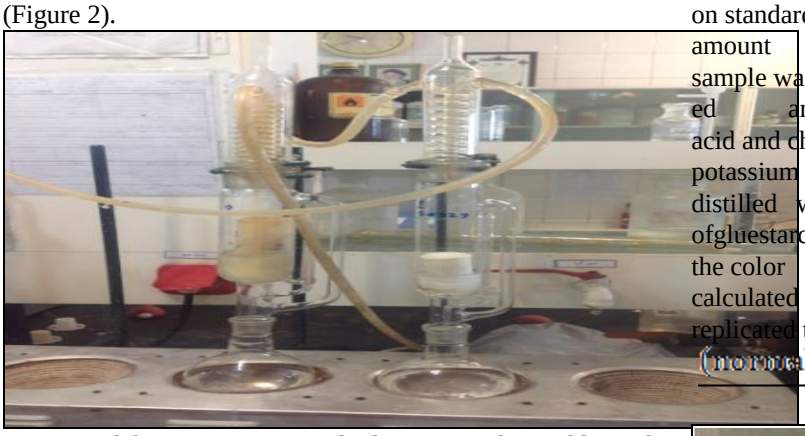

Figure 2*Silybum marianum* seed oil extraction by soxhlet and hexane

Analysis of fatty acids

The fatty acid composition was determined by conversionof oil to fatty acid methyl esters prepared (mixingof 950 µL of n-hexane and 50 mg of oil followed byadding 50 µL of sodium methoxide)[15].Fatty acids were transformed to their methyl esters(FAME), following method of He and Xia [16] anddetermined by using a gas chromatographytraceseries (PEG20 M) equipped with a flame ionizationdetector. The FAME sample (1.5 µL) was injected and GCseparation was carried out on a capillary column. The carrier gaswas nitrogen and the column flow rate was 0.8 mL/min.

The oven temperature was held initially at 180^{o} C for1 min, increased from 180 to 240^{o}C at a rate of 3^{o}C/minand then maintained at 240^{o} C for 10 min. The temperaturesof injection port and detector were 250 and260^{o} C, respectively. FAME were positively identified bymatching their retention time data and mass spectra withthose of the standards. The fatty acid composition wascalculated from the total identified fatty acid area [17].

Measuring the refractive index of oil

A drop of oil in Refractometr using refractive indexof oil compare to the refractive index of water is calculated at 40 ° C(Figure 3).

Figure 3Measurement of refractive index of oil

Determination of peroxide oil

The peroxide value estimated base on standard tests of food (AOAC) [18]. In this method, the amount of 5 g of sample was prepared in a 250 mL flask Maier sandingweighed and 30 mL of solvent (mixture of acetic acid and chloroform) added then about 5 ml of potassium iodine added and mixed for 1 minute. 30 ml of distilled water and solution was added then a few drops ofgluestarch applied usingsoluble thiosulfate 0.02%. When the color of a transparent cleared, peroxide values calculated by following formula [18]. Experiments were replicated three times for each samples (Figure 4).

$$\frac{(\text{normality} \times \text{Dose of titration volume} \times 1000)}{\text{Sample volumes}}$$

Figure 4 Measurement of oil peroxide

IV. RESULTS AND DISCUSSION

Lipids are considered one of the most elemental nutrients for humans and animals. Lipid metabolism generates many bioactive lipid molecules, which are fundamental mediators of multiple signaling pathways and they are also indispensable compounds of cell membranes.The total oil content of the seeds was 25.32% andthe fatty acids of*Silybum marianum* seed oil were identified by GC. The *Silybum marianum* seeds which was collected from two locations namely Kazeron and Lorestan, exhibited C16:0, C18:0, C18:1, C18:2(Table 1) with average of25.32% oil yield. This findings are according withthe previous reports on*Silybum marianum* seeds that reported variations in oilyield and content which may be due to the differences invariety of plant, cultivate on climate, ripening stage, theharvesting time of the seeds, location and the extractionmethod [19, 20].*Silybum marianum* seed oil also contained linoleic (54.71%)and oleic (30.42%) acids as the principal fatty acids [21,22].The linoleic acid was the main fatty acidfollowed by oleic 52.1% and 32.14%, respectively.For the oil extracted from anunspecified variety of *Silybum marianum* seed oil alsoreported that linoleic acid (52.78%) was a principle fatty acid [23,24].

Table 1 Fatty acid composition of *Silybum marianum* oil (%)

Fatty acid composition		Kazeron	Lorestan
C16:0	Palmitic acid	7.99	9.26
C16:1	Palmitoleic acid	-	-
C17:1	Margaric acid	-	-
C18:0	Stearic acid	5.607	5.01
C18:1	Oleic acid	28.54	30.42
C18:2	Linoleic acid	54.71	52.78
C18:3	α-Linolenic acid	3.13	2.51
C20:0	Arachidic acid	-	-
C22:0	dodecanoic acid	-	-

Theoil refractive of *Silybum marianum* was estimated with Refractometr. The results were shown in below (Table 2). The fatty acid double bond is less compare to the number of lower refractive index as well asthe refractive index is higher than the melting point with lower saturation. The physicochemical propertiesof oil that harvested in Kazeron and Lorestan were as follow: refractive index 1.4651 and 1.4656 (Table 2)and peroxides oil, 0.68 and 0.57 (Table 3) respectively.

Table 2. The refractive index of *Silybum marianum* seed oil

Location of collecting sample	Refractive index
Kazeron	1.4651
Lorestan	1.4656

The concentrations of peroxides and hydroperoxides were measured at the start of experiment. The peroxide value was estimated by taking expiration date of the product, normal oil index[25, 26]. The measurement of oil peroxides are shown in Table 3.

Table 3. Measuring the peroxide sample

Sample	peroxide (meq//kg)
Kazeron	0.68
Lorestan	0.57

The present study established similar compounds in *Silybum marianum* seed oil, some of them have valuable applications as a food and pharmaceuticals. We found that the main components in fatty acids were oleic andlinoleic acids.

V. ACKNOWLEDGEMENTS

The authors wish to express sincere thanks to the Faculty and Chairman (Head of the Department), Department of Animal Science, Darab branch, Islamic Azad University,Darab, Fars, IRAN and Herbal Medicine Research Center, Shiraz University, Iran for extending the laboratory facilities to carry out the research work.

ACKNOWLEDGMENT

The preferred spelling of the word “acknowledgment” in American English is without an “e” after the “g.” Use the singular heading even if you have many acknowledgments. Avoid expressions such as “One of us (S.B.A.) would like to thank ... .” Instead, write “F. A. Author thanks ... .” **Sponsor and financial support acknowledgments are placed in the unnumbered footnote on the first page**.

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