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Full Length Research Paper

The physicochemical properties and oil constituents of milk thistle (*Silybum marianum* Gaertn. cv. Budakalászi) under drought stress

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Milk thistle, *Silybum marianum,* is an annual or biennial plant. It has been reported that the extracted oil from milk thistle seed contains essential edible fatty acids. The aim of the present study was to determine the irrigation interval effects on different fatty acids content of milk thistle. Irrigation intervals consisted of D_1= 5, D_2= 10, D_3= 15 days and D_4= without irrigation. To show the water status of the investigated plants, relative water content of the leaves was measured. Milk thistle seed oil have been used for analysis of physicochemical properties such as oil content and composition using GC (FID). Our results proved that the irrigation intervals had significant effects on all of the studied parameters. The total oil content decreased by 4% due to increasing the water scarcity but the amount of the polyunsaturated fatty acids increased under the drought stress. The highest amount of the polyunsaturated fatty acids: linoleic acid (42.84%) and linolenic acid (0.65%) were measured in D_4 whereas the highest amount of the monounsaturated fatty acids; oleic acid (36.67%) and gadoleic acid (0.57%) have accumulated in D_1. The moisture content of the oil in D_4 decreased until 0.37% and the lowest chlorophyll content, pH, acid value and the highest refract index also were measured in D_4. It has proved that the drought stress increased the quality of milk thistle seed oil. Our results can justify the important value of milk thistle seed oil as an attractive candidate for use in food preparation.

Key words: Milk thistle, seed oil constituents, irrigation interval, essential fatty acids.

INTRODUCTION

Milk thistle, *Silybum marianum* Gaertn from *Asteraceae* plant family, is a well known medicinal plant, native to the Mediterranean region of Europe and widely dispersed to many countries throughout the world. Its medicinal effects are documented among the alternative medicines referred to as liver and bile-related diseases remedy (Fraschini et al., 2002; Kurkin, 2003). Hence milk thistle widely cultivated due to its striking medicinal values. It is important when known that for obtaining silymarin (the main important active compound), special difficulty is isolation of the considerable quantity of fatty oil that may reach to approximately 20 to 35% which is similar to many vegetable oil seeds (Ramasamy and Agarwal, 2008). The polyunsaturated fatty acids (PUFAs) namely α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6) are indispensable dietary components in human body (Huang and Brenna, 2001) and cannot be synthesized *in vivo* so, they must be ingested as part of the diet (Holman, 1969). Hence, milk thistle oil has been suggested as being suitable edible oil and a vitamin E reach source (El-Mallah et al., 2003).

Like many secondary metabolites, fatty acids and phenolic acid are known to be affected by biotic and abiotic stresses (Beckman, 2000). Among the different environmental constraints, drought is the most important

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abiotic factor limiting crop productivity but in some cases the change in plant metabolites could be an advantage of the production.

The present study aims to determine the fatty acids content and constituents under drought stress and evaluating the physicochemical properties of milk thistle seed oil grown in Tehran-Iran. In this paper for the first time we investigated some biochemical responses of this plant when submitted to water deficit.

MATERIALS AND METHODS

On field experimental setup

This part of the study was carried out at Tarbiat Modares University (TMU), in 2009 to 2010, situated in 16 km far from Tehran, Iran, with 1215 m altitude above sea level. The ecological situation of the experimental field was characterized as semi-dry climate with 242.7 mm annual precipitation.

The soil type was sandy loam (pH 7.68; EC 0.9 dSm^{-1}), containing total N (0.2%), total P (19.18 ppm), total K (328 ppm) and 0.92% of organic materials. The experimental design was a randomized complete block with three replications. Each experimental plot was 12 m^2 (3 x 4) and 2 m apart from each other. The seeds of *S. marianum* Gaertn., cultivar Budakalászi were directly sown in the plots in February 2009. In order to prevent the lateral spread of water, each plot was surrounded by metal dikes. Irrigation intervals consisted of D_1= 5, D_2= 10, D_3= 15 days and D_4= without irrigation considered after fourth leaves were developed. After the lapse of plant growth, the seeds was harvested and stored for the further studies.

Relative water content (RWC)

RWC was determined to show the water status of the investigated plants in full flowering stage. After determining fresh weight, leaves were immersed in distilled water for 6 h to estimate turgid weight, and then the disks were dried at 60°C for 24 h to measure dry weight (Weatherly, 1950).

Sterilization and oil extraction

In preparation for extraction, seeds were sterilized using 70% ethanol followed by 5% bleach / 1% sodium dodecyl sulfate (SDS) solution (Belitz and Sams, 2007). 100 g of each seed lots were dried and then, grounded to fine powder in a grinder. Then, 15 g of the powder were extracted with organic solvent -n-hexane- using a 250 ml capacity traditional Soxhlet apparatus for 8 h (60°C) in 3 replications (AOCS, 1989). The extract containing the oil were then separated and rotary-evaporated under reduced pressure at 35°C.

Physicochemical properties of oil

The oil content of seed lots was measured by weighting of oil per 100 g of seed. Moisture content of oil has been measured by AOCS methods (AOCS, 1993). The total chlorophyll content (mg of pheophytin A /kg oil) of oil was measured by spectrophotometer according to the method of Pokorny et al. (1995). The refractive index was measured by refractometer apparatus at 25°C. Acid value of the samples were measured and expressed by mg NaOH / g oil and the pH value of the oil samples also was measured (AOCS, 1993).

Fatty acid composition and GC analysis

Transesterification and preparation of fatty acid methyl esters (FAMEs)

FAMEs were produced to polarity reduction and measure the precise content of total fatty acids according to the (USP-NF, 2002). The residue was weighed and resuspended in 10.0 ml hexane for GC analysis.

GC analysis of FAMEs

Determination of the fatty acids was carried out by gas chromatographic measurement of the prepared samples. A Unicam 4600 GC instrument maid in Great Britain was equipped with a flame ionization detector and a split/splitless injector. A 30 m length with 0.22 mm internal diameter and 0.25 µm thickness fused-silica capillary column BPX70 (SGE, Melbourne, Australia) was used for analysis. Injector and detector temperatures were 230 and 250°C, respectively. Oven conditions were 180°C increased to 220°C at a rate of 2°C/min and maintained for 5 min. Helium was used as the carrier gas and nitrogen as the make-up gas at a flow rate of 30 ml/min. The quantification of FAMEs composition was realized by integration of the FID peak area and comparing their retention times with standards methyl esters to be expressed by percentage (El-Adawy et al., 1999). All the GC analyses were run in triplicate and the average values were reported in the work. All of chemical materials and solvents used in this study has prepared from Merck Company, Germany.

Statistical analysis

The data were statistically analyzed by (ANOVA) with SAS software. Results are expressed as the mean of three separate replicates. Means were compared by the Duncan's multiple range test (1955) at $P < 0.05$ significance level.

RESULTS

The highest RWC values (an average of 93%) were in the D_1, with 5 days irrigation interval whereas, in the D_4 treatment the RWC values decreased until 60%. This shows that in consequence of water scarcity of the soil the RWC of the leaves can decrease by almost 30 %. Results of the studied physicochemical characteristics of milk thistle seed oil under different irrigation intervals are presented in Table 1. The moisture content, refract index, pH and acid values positively decreased. The chlorophyll content increased under drought condition to 1.45 mg/kg which was still acceptable for the edible oils. As shown in Table 2, the milk thistle seed oil composition was determined having 8 detectable fatty acids: behenic acid (C22:0), arachidic acid (C20:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), gadoleic acid (C20:1), linoleic acid (C18:2) and linolenic acid (C18:3). The predominant fatty acid was the linoleic acid with 42.84% in D_4 followed by 32.87% oleic acid. The results of variance analysis of total oil content of milk thistle seed oil under drought stress are presented in Figure 1.

Total oil content, decreased by almost 5% as water deficit increased in D_4. The saturated fatty acids (SFAs),

Table 1. Physicochemical properties of milk thistle seeds under different irrigation intervals regime.

T	Moisture content %	Refract index	pH value	Chlorophyll scale	Acid value
D_1	0.61 a	1.55 a	6.78 a	1.25 c	0.48 a
D_2	0.61 a	1.52 a	6.70 a	1.26 c	0.48 a
D_3	0.41 b	1.27 b	6.56 b	1.36 b	0.44 b
D_4	0.37 c	0.55 c	6.54 b	1.45 a	0.34 c

Means in columns with different letters are significantly different at (P≤0.05).

Table 2. Fatty acids composition (%) in oil extracted from milk thistle seeds under different irrigation intervals regime.

T	Saturated fatty acids %				Monounsaturated fatty acids%		Polyunsaturated fatty acids %	
	Behenic Acid (C22:0)	Arachidic Acid (C20:0)	Palmitic Acid (C16:0)	Stearic Acid (C18:0)	Oleic Acid (C18:1)	Gadoleic Acid (C20:1)	Linoleic Acid (C18:2)	Linolenic Acid (C18:3)
D_1	2.48 c	3.61 c	10.22 a	6.86 c	36.67 a	0.57 a	39.7 d	0.45 d
D_2	2.56 b	3.86 b	9.44 b	7.38 b	36.17 b	0.56 a	39.82 c	0.50 c
D_3	2.56 b	3.88 b	9.43 b	7.53 a	34.08 c	0.53 b	41.21 b	0.53 b
D_4	2.63 a	4.06 a	9.17 c	7.57 a	32.78 d	0.50 c	42.84 a	0.65 a

Means in columns with different letters are significantly different at (P≤0.05).

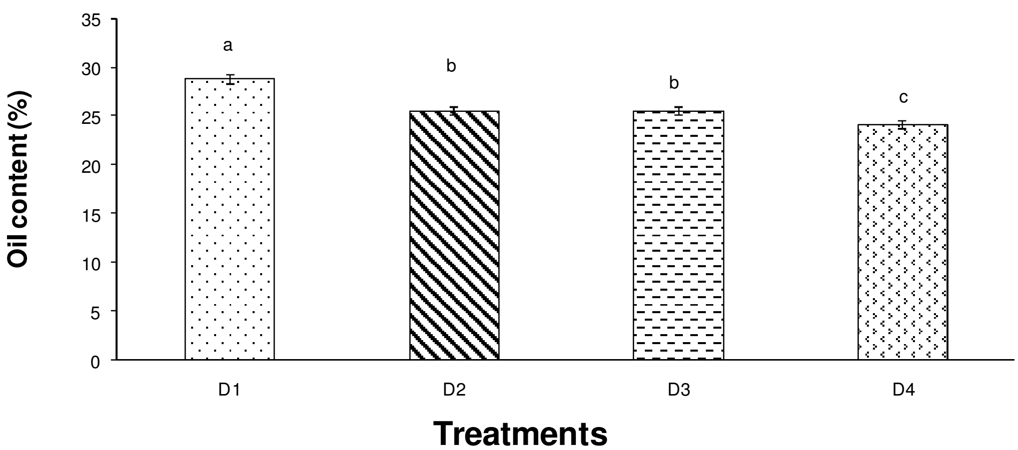

Figure 1. Total oil content of milk thistle seed oil under different irrigation intervals.

namely, behenic, arachidic and stearic acid increased with increasing water deficiency, whereas the palmetic acid which is also a SFA, decreased under the drought stress. Concerning the total SFAs content (23% of total oil content), less than 1% accursed while irrigation intervals increased. On the other hand, the variance of the UFAs with almost 75% of the total oil content, showed about 0.5% change amongst the treatments. The highest amount of MUFAs was measured in D_1 for oleic and gadoleic acid with 37.67 and 0.57%, respectively. Intriguingly, the amount of polyunsaturated fatty acids which are the most important constituents of the edible oils, showed about 5% increase according to the drought stress (Figure 2).

DISCUSSION

Annual global production and consumption of oils and fats is about 119 million tones and rising steadily at a rate of 2 to 6 million tons per year. This is required to meet the demand, which also grows at around this rate annually,

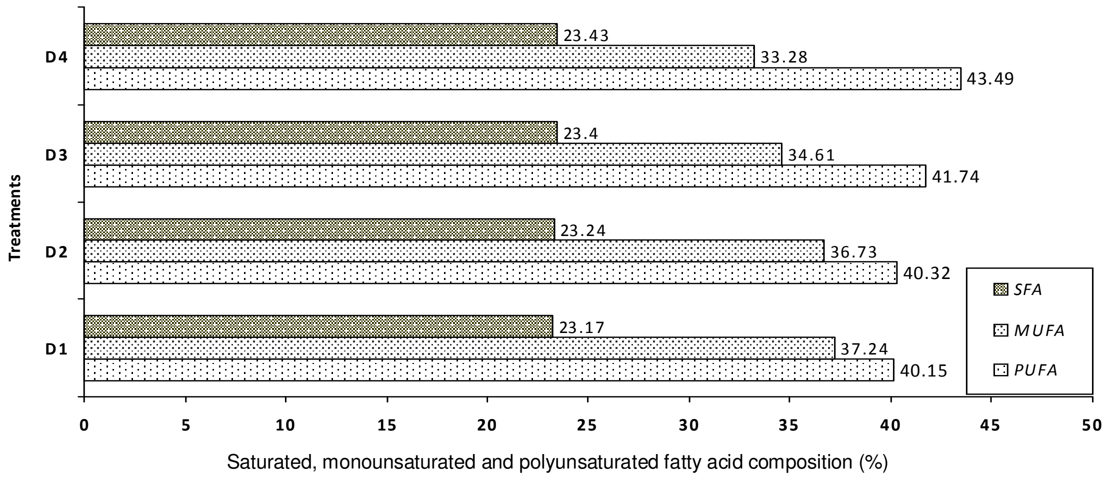

Figure 2. Effect of different Irrigation intervals on milk thistle fatty acid saturation levels.

especially in developing countries. Around 80% of current oil and fat production is used for human food like spreads, frying oil, salad oils, etc. (Gunstone, 2002).

According to the results of this study, milk thistle seed oil could be a rich source of polyunsaturated fatty acids which makes it an interesting candidate from a nutritional point of view. The drought stress can improve the quality of the oil significantly towards the increasing the ratio of polyunsaturated fatty acids up to 77% of the total oil content. 75.1% of UFAs have been reported by El-Mallah et al. (2003) from *S. marianum* (L.) seed oil extracted with chloroform- methanol (2:1 V/V). The fatty acid composition in our results was comparable with the statements of Fathi and Azadmard (2009). These seeds have relatively high oil content comparable with the other oilseeds like sunflower. For physicochemical properties of the oil the changes were also toward improvement except the chlorophyll content which is a critical compound in the development and metabolism of plants, and is always present to some extent in oilseeds. During oilseed processing chlorophyll is readily extracted by hexane into the crude oil. But even the highest level of chlorophyll content in milk thistle was less than the other vegetable oil seed like the typical Canadian canola that contains 13 to 30 ppm (Diosady, 2005). The extracted oil might be used as a salad oil, as cooking oil alone or mixed with other vegetable oils, especially mixed with saturated oils to improve their nutritional value. Even though, its stability during food preparation as well as during storage, still need more study.

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