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Direct measurement of fatty acid composition of sunflower and variation of the fatty acids distribution with different treatments of fertilizer using HPLC technique

MADEHA H. HUSSAIN[1] AND ALI S. HASSOON[2,*]

[1]*Department of Pharmacy*
Medical Institute Tech. Mansour, Middle Tech. University, Iraq
**(e-mail : alisalealtaie.2015@gmail.com)*

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ABSTRACT

The difference in the composition of fatty acid in the seed oil of sunflower using 11 doses of organic fertilizer treatments compared with control treatment has been studied. The organic fertilizer played an important role in elevating the percentage of unsaturated fatty acids, oil yield, which are the essential factors for determining oil quality of sunflower. The separation of saturated and unsaturated fatty acids occurred by using direct HPLC analysis method under the optimum separation conditions using Fast Liquid Chromatographic methods (FLC) applied, short and efficient HPLC (50 mm x 4.6 I.D.), C-18DB column with high surface area, 3 µm particle size packing. The analysis results showed that an increase in fertilizer resulted in steady increase in yield and contents of linoleic acid. The concentration and percentage of polyunsaturated fatty acid (PUFA) and linoleic acid have steady increased with fertilizer treatments (55.6-75.91%), whereas percentage of oleic acid responded negatively with elevated percentage of the fertilizer treatments compared with control treatment, consequently, the concentration and percentage of oleic acid slightly decreased in the range (33-19.06), however, the percentage of saturated SFA was in the range of 2.93-4.85% for palmatic acid and 2.2-5.13% for stearic acid. The results showed that the highest concentration of oil was in T_{12}. The results showed slight increase in specific gravity of oil with variation in organic fertilizing treatments. Oil yield was changed from 23% for control treatment to 43% through organic fertilizer treatments.

Key words : Fatty acid, fertilizer, HPLC, Iraq, sunflower

INTRODUCTION

Fatty acid is considered as one of the most important nutrients for humans. Large demands for sunflower oils for food and industrial applications have been widely increased. Sunflower oil originally belongs to sub-tropical and temperate zone (Demir *et al.,* 2006; Usman *et al.,* 2010). Most Asian countries countered severe shortage of sunflower oil due to domestic production highly below the rate of consumption level. Sunflower oil is widely used in European and East Asian countries for cooking as well as in margarine production due to absent of cholesterol (Ahmad *et al.,* 1992; Rabiei *et al.,* 2016). In sunflower seed oil, quality criteria are fatty acid composition of saturated (SAFA), palmatic acid, and stearic acid, while monounsaturated fatty acid (MUFA), oleic and polyunsaturated fatty acid (PUFA) linoleic and linolenic have important implications for product performance and population health (Ryland, 2003). Therefore, the nutrients play an important role in crop growth and development. Among these nutrients, nitrogen present in organic fertilizes is the major element which enhances the metabolic process consequently, leads to increase in vegetative and reproductive growth and yield of the crop (Koutroubas *et al.,* 2008; Esfahani *et al.,* 2016).

Attention is, therefore, focused on using various forms of organic matter as partial substitutions to mineral fertilizers. These practices have been recommended (Al-Taey and Majid, 2018). Sunflower oil was among the

[2]Department of Soil and Water Techniques, Al-Musaib Tech. College, Al-Furat Al-Awsat Tech. University, Iraq.

healthiest consumption oil, due to its high unsaturated contents of fatty acids, their metabolism generates several bioactive lipid molecules, which are fundamental mediators of multiple signalling pathways and they are also indispensable compounds of cell membranes. Any kind of changes in lipid metabolism can be related to modification of membrane composition and subsequently, changes in its permeability. Fatty acids could be associated with some pathological states, such as increase in cancer incidents, cardiovascular, neurodegenerative and metabolic diseases, and similarly with many inflammatory complications (Brenna, 2002; Gatek *et al.,* 2012). Lipids consist of fatty acids (FAs) classified mostly according to the presence or absence of double bonds as saturated (SFAs–without double bonds), monounsaturated (MUFAs-with one double bond) and polyunsaturated fatty acids (PUFAs-with two or up to six double bonds); the aim of this work was to evaluate the effect of different levels of mixed fertilizer application on yield and quality traits of sunflower in order to achieve the optimum use of resources.

MATERIALS AND METHODS

Equipment

The fatty acid constituents of sunflower oil were separated on reversed phase 3 µm particle size (50 x 2.0 mm I. D.) C-18DB column, separation occurred on liquid chromatography Shimadzu 10 AV-LC equipped with binary delivery pump model LC-10 A Shimadzu, the eluted peaks were monitored by Shimadzu SPD 10A vp Detector at 215 nm, the data were recorded on shimpack C-R8A integrator (Shimadzu, Koyota, Japan).

Mixed Fertilizer

The mixed fertilizers (Table 1) were applied at the same time of sowing. Remaining 2/3 of fertilizer were used into splits–first dose at first irrigation and second dose at the flowering stage of crop. Crop was irrigated as the crop required irrigations with any water stress. Sunflower seed oil obtained from each sample was analyzed to determine the relative composition of different fatty acids (oleic, linoleic, linolenic, palmitic and stearic acids) with a direct HPLC method, under the following conditions in Table 2. The optimum separation conditions were as follows :

Column : FLC (Fast Liquid Chromatographic) column, 3 µm particle size, (50 x 2.0 mm I. D.) C-8DB column mobile phase were : acetonitrile : tetrahydrofuran (THF) : 0.1% phosphoric acid in THF (50.4 : 11.6 : 38, V/V).

Detection : UV set at 215 nm, flow rate

Table 1. Treatments of fertilizer used through the study

Treatment	Type of fertilizer
T_0 (Control)	Without fertilizer
T_1	Organic fertilizer 2.5 t/ha
T_2	Organic fertilizer 5 t/ha
T_3	Foliar fertilizer 2 ml/l
T_4	Foliar fertilizer 4 ml/l
T_5	Foliar fertilizer 6 ml/l
T_6	Organic fertilizer 2.5 t/ha+foliar fertilizer 2 ml/l
T_7	Organic fertilizer 2.5 t/ha+foliar fertilizer 4 ml/l
T_8	Organic fertilizer 2.5 t/ha+foliar fertilizer 6 ml/l
T_9	Organic fertilizer 5 t/ha+foliar fertilizer 2 ml/l
T_{10}	Organic fertilizer 5 t/ha+foliar fertilizer 4 ml/l
T_{11}	Organic fertilizer 5 t/ha+foliar fertilizer 6 ml/l

Table 2. Retention time and the area of fatty acids

S. No.	Subjects	Retention time (min)	Area	Concentration (µg/ml)
1.	Palmetic C16 :1	1.94	68079	40
2.	Stearic acid C18 : 0	3.15	87275	40
3.	Myristic acid C14 : 0	3.88	76811	40
4.	Oleic C18 : 1 omega 9	5.13	71245	40
5.	Linolenic C18 : 2 omega 6	6.11	67910	4.0

1.5 ml/min, temp. 40°C. The sequences of the eluted fatty acids standard were as follows : each standard was 40 µg/ml.

Extraction Procedure

Aqueous extraction was conducted using 500 ml, blender crushed sunflowers (5 g) and 425 g of deionised water (pH close to 6.5 and corresponding to the optimal pH for the oil extraction) were lended for 5 min. The slurry was centrifuged (2000 × g, 10 min) to remove the insoluble phase. The extract was treated by the following procedure : 1 g potassium oxalate and 100 ml ethyl alcohol were placed in blender jar for homogenized for 3 min. The jar content was poured in a 250 ml centrifuged tube, to which 50 ml of diethyl ether and 50 ml petroleum ether were added. The contents were mixed for 1 min after each addition. The mixture was centrifuged at 1700 rpm for 7 min at room temperature. The lower phase was re-extracted two more times with 50 ml petroleum ether and diethyl ether 1:1 v/v. The combined organic phase was transferred to 1000 ml separating funnel containing 50 ml water and 30 ml saturated NaCl. The organic layer was washed with 100 ml distilled water. The emulsion was removed by adding 2-5 ml NaCl. The organic was allowed to stand for 5 min to settle down. The liquid extracted was evaporated to10 ml by rotary evaporator, and the clear oil was separated and the volume was measured for each sample. Then the density was measured for each sample to calculate the weight of oil for each sample by multiplying (the volume x density of oil and then the percentage of oil), then 20 ml were injected to HPLC for separation of different fatty acid according to previously fixed procedure. The baseline separation of main constituents of fatty acid in sunflower oil was done for T_{12} samples, after drying, the hulls (pericarp) were removed from the achenes and the seeds ground in a mortar and extracted with the fixed procedure for all the samples. The typical separation chromatograms gave excellent separation for all fatty acids composition as in typical chromatogram (Fig. 1). Relative amounts of the four major fatty acids of sunflower oil, palmitic, stearic, oleic and linoleic, were calculated by means of an electronic digital integrator. The standard deviation for individual fatty acid percentages was+0.1%.

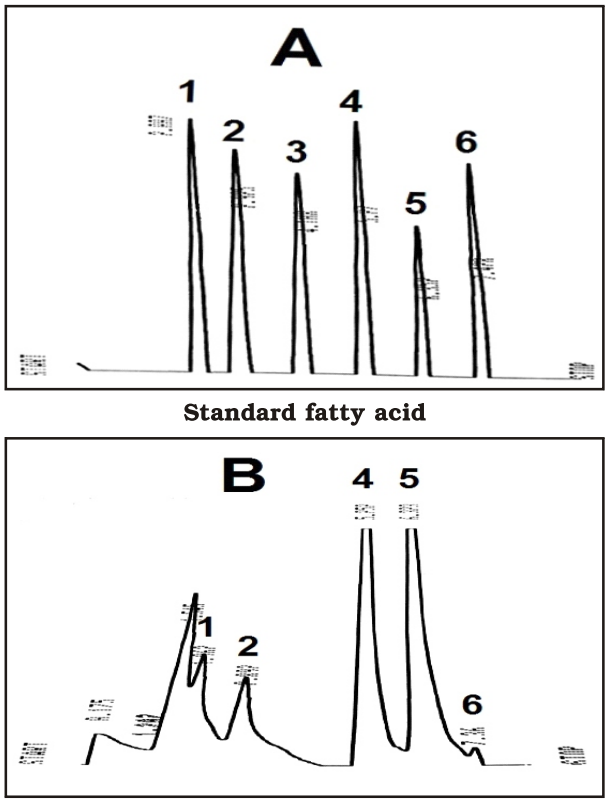

Fig. 1. Separation chromatogram of fatty acid under the optimum separation conditions as follows : Column : (50 x 4.6 mm I.D.) C-18DB column, 3 µm particle size, mobile phase were : acetonitrile : tetrahydrofuran (THF) : 0.1% phosphoric acid in THF (50.4 : 11.6 : 38, V/V), detection : UV set at 215 nm, flow rate 1.5 ml/min. 1-Palmatic acid, 2-Stearic acid, 3-Myristic acid, 4-Oleic acid, 5-Linoleic acid, 6-α- Linolenic acid. A : Standard fatty acid and B : Sunflower oil extract.

RESULTS AND DISCUSSION

Fast, accurate and direct analysis method for fatty acid in sunflower oil was obtained by using short reversed phase HPLC method directly without derivatization. The derivatization method took more time and probably occurred losing some amounts of sample in it. The HPLC separation profile revealed the presence of various chromatographic peaks in the studied seeds sample of sunflower extract. The assays of the separated compounds representing the major detected peaks and summarizing the obtained data for each of the detected chromatographic peak are discussed below. Quantitative determination of fatty acids was done by

Table 3. Concentration of fatty acid extracted from sunflower oils in µg/ml and % of each fatty acids in different samples, saturated fatty acids SFA (palmatic and stearic acid), mono unsaturated fatty acid (oleic acid) MUF, and polyunsaturated PUf (linoleic and linolenic fatty acid)

Treatment	Palmatic acid	Stearic acid	Myrestic acid	Oleic acid	Linoleic acid	α-linolenic acid	Total fatty acid
T_0 (Control)	38.50 (3.86%)	42.10 (4.91%)	-	280.83 (33.09%)	472.1 (55.62%)	15.10 (1.77%)	848.58
T_1	49.41 (4.85%)	50.3 (4.94%)	-	293.20 (28.84%)	617.23 (60.71%)	6.54 (.584%	1016.63
T_2	41.72 (3.30%)	38.71 (3.27%)	-	288.59 (22.86%)	803.28 (71.55%)	-	1182.3
T_3	50.0 (3.93%)	38.94 (2.99%)	-	304.18 (23.34%)	1064 (67.94%)	6.20 (0.47%	1302.69
T_4	38.25 (2.93%)	36.53 (2.64%)	-	365.0 (26.38%)	940.86 (68.0%)	2.91 (0.21%	1383.5
T_5	41.59 (2.99%)	37.12 (2.20%)	-	306.40 (25.07%)	995.86 (71.64%)	9.2 (0.66%	1390.0
T_6	47.32 (3.21%)	55.39 (3.76%)	-	368.6 (25.07%)	999.16 (67.9%)	-	1470.1
T_7	50.35 (3.199%)	80.82 (5.13%)	-	312.2 (19.84%)	1130.2 (71.82%)	-	1573.57
T_8	64.73 (3.90%)	52.95 (3.19%)	-	325.99 (19.66%)	1212 (73.12%)	1.88 (0.113%	1657.55
T_9	47.70 (3.03%)	103.34 (4.27%)	-	299.49 (19.06%)	1141.35 (72.67%)	6.84 (0.435%	1570.56
T_{10}	47.54 (3.85%)	10.34 (5.86%)	-	286.24 (16.23%)	1340.85 (75.60%)	-	1762.9
T_{11}	73.69 (4.07%)	82.23 (4.54%)	-	356.90 (19.72%)	1296.6 (71.65%)	-	1809.42

comparing the peak area of authentic standard with that of peak sample under the same optimum separation condition, by using the following equation :

$$\text{Concentration of sample (µg/ml)} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{Conc. of standard} \times \text{dilution factor}$$

Fatty Acid Composition

The total fatty acid concentrations were 848.58 µg/ml for control without fertilization, while the total fatty acids were increased with increasing the amount of fertilizer as shown in Table 3. The highest concentration was in treatment T_{11}, which reached 1809.42 µg/ml, as predicted in Table 3. Oleic and palmitic acid concentrations responded negatively to mixed fertilizer application as shown in Table 3. The control treatment T_0 gave the highest contents of oleic and palmitic acid which were 33.09 and 3.86%, respectively, followed by T_1-T_{11} having oleic acid concentrations decreased from 33.09 to 19.72% (T_{11}) treatment, and palmitic acid was in the range 2.64-5.13%. Likewise, T_{10} gave the lowest concentrations of oleic and palmitic acids which reached 16.23 and 3.85%, respectively. These results agree with those of Malik *et al.* (2001) and Munir *et al.* (2007) who observed decrease in the composition of this fatty acid with increased fertilizer application. There was significant difference between treatments regarding concentration of linoleic acid. The highest content of linoleic acid was in T_{11} (75.60%). T_0 recorded the lowest concentration of linoleic acid (55.62%). The treatment T_{10} gave the highest content of stearic acid (5.86%) compared to T_4 which gave the lowest content of stearic acid (2.64%). The results showed very low contents of α-linolenic acid in eight out of 12 samples in the range between 0.21-0.66% for samples treated with various fertilizers as shown in Table 3. The percentage of oil was measured with the help of following equation :

$$\text{Weight of oil} = \text{Density of oil} \times \text{Volume of oil}$$

$$\text{Percentage of oil} = \frac{\text{Weight of oil yield}}{\text{Weight of sample seed (5 g)}} \times 100$$

Oil Contents

The production of grain oil content (GOC) was significantly increased with increasing the amount of fertilizer treatments

used as shown in Table 4. The maximum grain oil content (GOC) was (43.1%) in treatment T_{11} followed by Hysun-38 (42.9%), while the minimum GOC (40.7%) was observed in Hysun-33 sunflower hybrid. The response to N fertilizer rates decreased with increasing application of N fertilizer. The maximum GOC (43.01%) was produced in N_{11} treatment, while the lowest GOC (23.41%) in control treatments. This result showed that an increase in the amount of fertilizer led to increase in the grain yield.

Table 4. Physical properties of sunflower oils (pale yellow oil)

No. of samples	Refractive index	Density (g/cm^3)	Volume of oil (in 5 g)	Percentage of oil
T_0 (Control)	1.4632	0.9143	1.28	23.40
T_1	1.4641	0.9142	1.38	25.23
T_2	1.4642	0.9162	1.45	26.56
T_3	1.4642	0.9140	1.57	28.69
T_4	1.4653	0.9177	1.61	29.54
T_5	1.4665	0.9180	1.67	30.66
T_6	1.4667	0.9183	1.75	32.14
T_7	1.4681	0.9184	1.82	33.29
T_8	1.4724	0.9185	1.91	35.08
T_9	1.4712	0.9186	2.10	38.58
T_{10}	1.4734	0.9191	2.21	40.62
T_{11}	1.4732	0.9192	2.34	43.01

CONCLUSION

It was concluded from this study that several treatments of fertilizer were applied to obtain the higher yield of oil and better quality oil contents. For maximum economic benefits, application of treatment T_{11} was better under well irrigated conditions compared to other different T rates evaluated. Further research may be required for sunflower crop to identify best agronomic management strategies under best agro-climatic conditions.

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