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Changes in Oil Fatty Acid Composition During Seed Development of Sunflower

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Abstract: Quality of vegetable oils is associated with fatty acid composition. A field study was conducted to determine accumulation of oil and fatty acids of traditional sunflower hybrid during seed development. Seeds were harvested at six times from R6 (reproductive growth stage) to over ripe of seeds. Oil content and thirteen fatty acids were identified as percentage of total fatty acids. As the results, the fatty acid composition and content of oil in crop species were monitored during seed development and affected by environmental conditions. Variations in percentage of miristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), arachidic (C20:0), behenic (C22:0), lignoceric (C24:0) and oil content in different stages of seed development were significant. Oil content had a significant linear increase from the beginning of seed filling to R8 (The back of the head is yellow but the bracts remain green), then showed a slowly increase, reaching a maximum value of 42.31% at fully ripe. Maximum linoleic acid percentage (61.29%) was found at fully ripe while R7 (The back of the head has started to turn a pale yellow) showed the greatest oleic acid accumulation (38.18%). Oil of sunflower seeds had maximum percentage of stearic and palmitic acids during the initial seed filling phase. The highest negative correlation coefficient was noted for oleic and linoleic acids (r:-0.97). The significance of the results lies in the demonstration of the feasibility of seed growth stages at which developing sunflower seed could be modified to change oil and fatty acid contents.

Key words: Correlation coefficient, fatty acids, oil content, seed development, temperature differences, traditional sunflower hybrid

INTRODUCTION

Sunflower (*Helianthus annuus* L.) oil is one of the world's major vegetable oils due to its excellent oil quality. Sunflower oil quality is determined mainly by fatty acid composition. It is a combination of monounsaturated and polyunsaturated fats with low saturated fat levels (Baydar and Erbas, 2005). Traditional sunflower oil rich in linoleic acid is used in the food industry and in various commercial products and it has been shown to have significant potential for biodiesel production. Linoleic acid is an essential fatty acid for humans and it is preferred by industries when oil hydrogenation is require (Izquierdo *et al*., 2002; Pereyra-Irujo and Aguirrezabal, 2007; Zlatanov *et al*., 2009).

Previous research has demonstrated that the fatty acid composition of sunflower oil depends on genetic and environmental conditions (Lajara *et al*., 1990; Santonoceto *et al*., 2002; Skoric *et al*., 2008; Seiler *et al*., 2010). It is well known that oil content and fatty acid composition of sunflower closely depends on the environmental conditions. Temperature, especially day/night temperature differences are main environmental factors driving seed oil percentage and oil chemical composition (Izquierdo *et al*., 2002, 2006; Rondanini *et al*., 2003, 2006; Qadir *et al*., 2006; Echarte *et al*., 2010). Oil fatty acid composition is also effected by solar radiation (Izquierdo *et al*., 2009; Echarte *et al*., 2010) and water regime-drought (Petcu *et al*., 2001; Baldini *et al*., 2002; Santonoceto *et al*., 2002; Ahmed *et al*., 2007).

Physical and chemical properties of vegetable oils and consequently their use, depend on the composition of fatty acids that accumulate in storage lipids during seed development. The biosynthetic pathways leading to the formation of oil fatty acids have been studied in oilseeds such as groundnut (Hassan *et al*., 2005), rapeseed (Bhardwaj and Hamama, 2003), safflower (Rahamatalla *et al*., 2001) and *Cuphea* (Berti and Johnson, 2008).

There is limited information on the processes that happen during seed development and maturation of sunflower (Izquierdo *et al*., 2002; Dong *et al*., 2007; Baud and Lepiniec, 2010). The main objective of this study was to investigate changes of oil content and fatty acid composition during seed development of a traditional sunflower hybrid.

MATERIALS AND METHODS

Site description and experimental management: A field study was conducted to determine accumulation of fatty

Table 1: Monthly climate data during sunflower growing season

Month	Rainfall (mm)	Rainy day No.	Relative humidity (%)	Temperature (°C) Mean	Max.	Min.
April	17.8	12	76.0	12.7	24.9	0.9
May	16.0	9	68.6	18.1	33.6	3.3
June	30.8	12	72.3	22.5	38.7	12.0
July	72.3	8	66.4	24.7	35.5	14.6
August	0.0	0	56.9	28.1	39.6	15.8
September	31.4	4	64.8	21.2	33.8	9.0

acids during seed development at a farmer field in Kesan, Edirne, Turkey (40^{0}51'N, 26^{0}38'E, elevation 185 m) on a silty clay loam soil. The region is under the influence of northern and northwestern air currents with rather cold and wet winter and hot summer conditions. It is surrounded by the Black sea, Marmara Sea, Aegean Sea and the Bosporus channel; Aegean, Black and Marmara Sea influence its climate. The climate is semi-arid and long term average of the annual total precipitation is 450-550 mm. Drought has become a recurring phenomenon in this region. In this region, only 6% of the total arable area has more than 2% soil organic matter (Onemli, 2004; Sen *et al*., 2012).

Seeds of a traditional hybrid "Pioneer 4223" were sown on April 20, 2010 with a 0.70 m spacing between rows and 0.30 m spacing between plants in row. Soil fertility was adjusted to 60 kg ha^{-1} of nitrogen and 60 kg ha^{-1} phosphorus. Samples were taken six times from R6 to over ripe of seeds about at one week intervals. The reproductive growth stages were R6, R7, R8, R9 (physiological ripe), fully ripe and over ripe. The experimental design was a randomized complete block with three replication. Mean monthly rainfall, humidity and temperature were recorded throughout the growing period. They are presented in Table 1.

Seed oil and fatty acids analysis: Seed oil and fatty acid analysis were conducted at the laboratory of Trakya Birlik, a Turkish Agricultural Cooperative. Seed oil content was determined with a pulsed NMR instrument (Bruker Minispec-Bruker Analytische Messtechnic, Karlsrube, Germany). Oil contents are expressed as a percentage of dry seed weight.

Gas Chromatography (GC) of Fatty Acid Methyl Esters (FAME) was performed with an Agilent 6890 N gas chromatography equipped with a Flame Ionization Detector (FID). Analyses were conducted on an Agilent capillary column with 100 m x 0.25 mm x 20 m according to ISO 5508. The column temperature was programmed from 120 to 230°C.; injector and detector temperature set at 250°C using helium, air and hydrogen. Thirteen fatty acids were identified as percentage of total fatty acids. These were miristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), margaric (C17:0), heptadecenoic (C17:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), eicosenoic (C20:1), behenic (C22:0), lignoceric (C24:0).

Statistical analysis: Statistical analyses were conducted by using Standard procedures for a randomized complete block design. The Least Significant Difference (LSD) at 5% probability was used to compare the factors. Collected data were analyzed using the SAS statistical computer package (SAS Institute, 1997).

RESULTS AND DISCUSSION

Significant differences in oil content and percentage of miristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0) in different stages of seed development were found.

Variations in oil content: The maximum oil content (42.31%) was observed at fully ripe, while R6 gave the minimum oil content (Table 2). Oil content had a significant linear increase until R8, about 21 days post anthesis, then showed a slowly increase to fully ripe. It was found a decrease in oil content after fully ripe. Similarly, authors indicate that the accumulation amount of oil in the developing sunflower seeds start at the beginning of seed development and increase rapidly after 15-20 days, finally reaching the highest levels at 30-35 days post anthesis, thereafter begin to decline (Goffner *et al*., 1988; Santonoceto *et al*., 2002; Rondanini *et al*., 2003; Dong *et al*., 2007).

Fatty acid composition of oil: The results of the fatty acid analysis of sunflower seed oil presented in Table 2. Examination of the variation in the content of thirteen fatty acids showed that the major fatty acids composition of the oil in the initial phases of seed formation differed substantially from that of the mature seeds. The accumulation patterns of saturated fatty acids were quite similar. Oil of sunflower seeds had maximum percentage of miristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0) behenic (C22:0) and lignoceric (C24:0) during the initial seed filling phase. Thereafter, they significantly declined with seed maturity. Generally, the saturated fatty acid concentration decreased with increasing time of development. These results are similar to previous studies (Izquierdo *et al*., 2002; Zlatanov *et al*., 2009; Baud and Lepiniec, 2010). However; a result of study by Santonoceto *et al*. (2002) showed that climate factors did not cause significant variations in the stearic acid

Table 2: Variations of oil and fatty acid contents during seed development in sunflower

	Reproductive stage				Seed stage		
Contents (%)	R6+	R7	R8	R9	Fully ripe	Over ripe	$LSD_{0.05}$
Seed oil content	20.70^{f}	27.90^{e}	39.10^{d}	40.88^{c}	42.31^{a}	41.65^{b}	0.060
Miristic (C14:0)	0.09^{a}	0.07^{b}	0.06^{c}	0.06^{c}	0.06^{c}	0.06^{c}	0.007
Palmitic (C16:0)	6.77^{a}	5.86^{cd}	5.77^{d}	5.89^{bcd}	6.01^{bc}	6.08^{b}	0.211
Palmitoleic (C16:1)	0.18^{a}	0.09^{d}	0.10^{cd}	0.10^{cd}	0.11^{bc}	0.11^{b}	0.015
Margaric (C17:0)	0.05	0.05	0.05	0.04	0.04	0.04	ns
Heptadecenoic (C17:1)	0.05	0.07	0.04	0.03	0.03	0.05	ns
Stearic (C18:0)	5.05^{a}	5.05^{a}	4.04^{b}	4.07^{b}	4.08^{b}	4.06^{b}	0.150
Oleic (C18:1)	29.69^{b}	38.18^{a}	29.79^{b}	27.90^{c}	27.01^{e}	27.21^{d}	0.161
Linoleic (C18:2)	55.75^{e}	48.64^{f}	58.63^{d}	60.46^{c}	61.29^{a}	60.99^{b}	0.285
Linolenic (C18:3)	0.10^{a}	0.06^{ab}	0.04^{b}	0.04^{b}	0.05^{b}	0.05^{b}	0.036
Arachidic (C20:0)	0.39^{a}	0.38^{a}	0.27^{b}	0.27^{b}	0.27^{b}	0.27^{b}	0.024
Eicosenoic (C20:1)	0.13	0.15	0.15	0.14	0.14	0.15	ns
Behenic (C22:0)	0.76^{b}	0.89^{a}	0.70^{b}	0.70^{b}	0.67^{b}	0.68^{b}	0.095
Lignoceric (C24:0)	0.37^{a}	0.37^{a}	0.27^{b}	0.25^{b}	0.25^{b}	0.25^{b}	0.052

Values within a row followed by same letters are not significantly different by the LSD test at $p<0.05$. ns: non significant, R-6: Flowering is complete and the ray flowers are wilting, R-7: The back of the head has started to turn a pale yellow, R-8: The back of the head is yellow but the bracts remain green, R-9: The bracts become yellow and brown and this stage is regarded as physiological maturity (Schneiter *et al*., 1998)

Table 3: Correlation coefficients of fatty acids and oil content in sunflower

	C14:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C24:0
C14:0	-0.95**	-0.66**	-0.62**	-0.44^{ns}	-0.41^{ns}	-0.95**	-0.57*	0.74**	-0.76**	-0.95**	0.50*	-0.63**	-0.88**
C16:0		0.76**	0.68**	0.32^{ns}	0.55*	0.85**	0.47*	-0.64**	0.73**	0.84**	-0.49*	0.41^{ns}	0.71**
C16:1			0.94**	-0.08^{ns}	0.25^{ns}	0.48*	-0.17^{ns}	-0.04^{ns}	0.68**	0.49*	-0.55*	-0.09^{ns}	0.32^{ns}
C17:0				-0.13^{ns}	0.05^{ns}	0.42^{ns}	-0.26^{ns}	0.04^{ns}	0.66**	0.45^{ns}	-0.49*	-0.07^{ns}	0.34^{ns}
C17:1					0.08^{ns}	0.51*	0.60**	-0.61**	0.33^{ns}	0.53*	-0.08^{ns}	0.55*	0.53*
C18:0						0.42^{ns}	0.51*	-0.54*	0.18^{ns}	0.38^{ns}	0.06^{ns}	0.17^{ns}	0.24^{ns}
C18:1							0.72**	-0.85**	0.68**	0.99**	-0.41^{ns}	0.80**	0.94**
C18:2								-0.97**	0.25^{ns}	0.69**	0.05^{ns}	0.84**	0.70**
C18:3									-0.42^{ns}	-0.82**	0.09^{ns}	-0.86**	-0.81**
C20:0										0.69**	-0.28^{ns}	0.29ns	0.61**
C20:1											-0.41^{ns}	0.80**	0.96**
C22:0												-0.12^{ns}	-0.36^{ns}
C24:0													0.89**

ns: Not significant, *,**Significant at $p<0.05$ and at $p<0.01$, respectively, C14:0: Miristic, C16:0: Palmitic, C16:1: Palmitoleic, C17:0: Margaric, C17:1: Heptadecenoic, C18:0: Stearic, C18:1: Oleic, C18:2: Linoleic, C18:3: Linolenic, C20:0: Arachidic, C20:1: Eicosenoic, C22:0: Behenic and C24:0: Lignoceric

concentration in the oil and it decline above 50% between beginning and full ripening of seed, although environmental factors determine significant variations in the palmitic acid concentration in the oil and this fatty acid, in general, progressively lowered during the initial stages of seed filling.

Oleic acid (C18:1) of sunflower oil ranged between 27.01 and 38.18%. The greatest oleic acid accumulation was recorded at R7 (sunflower reproductive stage). The minimum percentage of oleic acid was found at fully ripe of seed. Inversely, linoleic acid (C18:2) at R7 was minimum, while seed oil at fully ripe had maximum linoleic acid content. Linoleic acid content increased from 48.64 to 61.29% during seed filling. But it did not have a linear response to seed development from R6 to over ripe. Some decreases were observed in linoleic acid content during the beginning of seed filling and after fully ripe depend on climatic conditions, especially higher temperatures and lower relative humidity. The authors indicated that oleic and linoleic acid percentages of sunflower oil vary greatly, depending mainly upon the temperature during seed development and a high temperature during seed maturation results in oil with high oleic acid concentration and a low linoleic acid concentration (Izquierdo *et al*., 2002; Rondanini *et al*., 2003; Zlatanov *et al*., 2009; Baud and Lepiniec, 2010; Seiler *et al*., 2010).

The stage of seed filling did not have a significant effect on margaric (C17:0), heptadecanoic (C17:1), linolenic (C18:3) and eicosenoic (C20:1) acids.

Correlations among oil fatty acids: Analysis of the correlations of oil content and thirteen fatty acids during seed development pointed out significant associations (Table 3). The correlation between oil content and linoleic acid ($r = 0.74$) was significantly positive. Oil content showed highly negative correlations with saturated fatty acids and palmitoleic acid with the shorter carbon chain in unsaturated fatty acids. A negative correlation was also found between oil content of seed and oleic acid percentage of oil at 5% possibility.

The highest negative correlation coefficient was noted for oleic and linoleic acids (r = -0.97). Oleic acid had highly positive correlations with stearic, arachidic and behenic acids. Linoleic acid was negatively correlated with miristic, stearic, arachidic, behenic and lignoceric acids. It was also found a highly significant and positive correlation between arachidic and behenic.

Similarly, previous literatures reported a highly negative correlation between oleic acid and linoleic acid in sunflower (Santonoceto *et al*., 2002; Tahmasebi-Enferadi *et al*., 2004; Izquierdo *et al*., 2006). Correlations among only a few fatty acids were investigated in the most papers previously published in this area. Generally, these results are similar to previous studies. But, Santonoceto *et al*. (2002) reported a negative correlation between palmitic and stearic acids.

CONCLUSION

It is evident from the results that (1) synthesis of oil and all fatty acids are closely correlated with seed development in sunflower; (2) the modification of fatty acids in developing sunflower not only depends on seed development but also on environmental temperature. The accumulation of major fatty acids, oleic (C18:1) and linoleic (C18:2) of a traditional sunflower oil vary greatly, depending mainly upon the temperature during seed development and a high temperature during seed maturation results in oil with high oleic acid concentration and a low linoleic acid concentration. In addition, increase in temperature differences between day and night results with an increase of linoleic acid accumulation during seed development. Knowing better the combination effects of climatic fluctuations and seed development on fatty acid composition would be useful in designing management practices to obtain a specific oil quality and in improving the predictions of crop models.

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