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Influence of storage conditions on quality and viability of high and low oleic sunflower seeds

A. Ghasemnezhad[a], B. Honermeier[b]

[a]*Department of Horticulture, Faculty of Agriculture, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.*
[b]*Institute of plant production and plant breeding I, Faculty of Agriculture, Justus Leibig University of Giessen, Giessen, Germany.*
*corresponding author; Email: azim.ghasemnezhad@agarat.uni-giessen.de

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Abstract

Storage conditions of oil seeds before industrial extraction might influence the quality of the crude oil. The aim of present study was to clarify the storage able potency of high and low oleic cultivars of sunflower based on oil and fatty acid changes of seeds and their viability. The seed samples of four sunflower cultivars (two high oleic and two low oleic) were stored in three different temperature conditions (4-5 ºC, 21-22 ºC, and 35 ºC) in a period of four months. Results show that quality parameters of seeds such as oil content, fatty acid composition and protein content were significantly influenced by storage conditions in most cases. In all experimental cultivars oil content was significantly influenced by storage time. Indeed, the longer storage times the higher oil reduction and vice versa. In most cases the oleic acid reduced as storage time progressed. Free fatty acid content of crude oil increased in longer storage time and higher temperature. No clear effect of storage time and storage temperature was observed on seed germination of used cultivars. Generally it can be conclude that the storage life of sunflower seed can be decreased by longer storage time and higher storage temperature.

Keywords: free fatty acid; high oleic sunflower; linoleic acid; seed viability; storage conditions; storage temperature

Introduction

Storage conditions of oil seeds before industrial extraction might influence the quality of the crude oil (Martini and Anon, 2005). Storage is done to maintain harvesting quality of product not to improve it (Sisman and Delibas, 2004). During storage time product specially stored oils compositions can be influenced by several storage conditions. A fatty acid composition is the most important factor which determines oils susceptibility to oxidation. The types of fatty acids present in an oil, and in particular their number of double bounds, determine the type and extent of chemical reactions which occur during the storage

time (Morello et al., 2004). The unsaturated fatty acids are very important for the stability of oils because of the chemical reactions occurring at the double bonds. Oilseed plant ever industrial oilseed or oilseeds which used for dietetics, cosmetics or lipochemistry are very important in human life. In contrast to animal fats which are predominantly saturated and with low reaction potency with another chemicals especially oxygen, unsaturated vegetable oils are more reactive and instable (Naz et al., 2004). According to Sisman and Delibas (2004) and Sisman (2005) during storage period the oil content of sunflower (*Heliantus annus*) decreased progressively. They reported that increase in temperature and humidity caused to spoil of oil and increasing of free fatty acid. Neg and Anderson (2005) showed that storage time and storage temperature had significant effect on free fatty acid content in the Quinoa (*Chenopedium quinoa*) seed oil. Villiers et al., (1986) indicated that high storage temperature and humidity had significant effect on sunflower seed oil quality as well as quality. Martini and Anon (2005) reported that during storage of sunflower seed in different temperature the oil content did not influence. It seems temperature; moisture and the storage duration are the most important factors which influence on stored product quality and quantity (Anderson and Lingnert, 1998; Chen and Ahn, 1998). The aim of this investigation was to study the effect of storage conditions on quality and quantity of sunflower seed oil.

Material and Methods

The newly harvested seeds of low oleic sunflower cultivars (Jazzy and PRO64A54) and high oleic sunflower cultivars (Atomic and Olsavil) were obtained from the research station of Justus Leibig university in Gross-Gerau (Germany). The temperature chosen for this experiment were: 3-4 ºC, 21-22 ºC and 35 ºC for low, moderate and high temperature, respectively.

At the beginning of the experiment the relative air humidity's of 40-45%, 60-65% and 30-35% were recorded in room conditions (21-22 ºC), growth chamber (35 ºC) and refrigerator (4-5 ºC), respectively. The seed samples were stored under mentioned temperature conditions for a period of four months. There were four replications for each treatment. Regularly every month stored seed samples were examined their oil percentage, fatty acid composition, free fatty acid content, acid value and seed viability.

At the beginning of the experiment the experimental samples were analyzed and the results of the first measurements i.e., seed oil percentage, fatty acid composition, free fatty acid percentage and peroxide value were used as a control for remaining storage times.

Seed samples were finely ground in a coffee grinder (Bran) and extracted using n-hexane in a Soxhlet apparatus. The extraction lasted 8 hours at a constant temperature of 70 °C. Fatty acid composition of seed samples was determined as methyl esters after transesterification by GC according to a modified method of Court et al., (1993). Varian 3800 gas chromatograph with dual FID detector which was equipped with a Permabond (r) FFAP column (25m × 0.25 mm i.d., film thickness 0.25 µm) with CP-SIL 88 foe FAME stationary phase and a CS-FUSED-Silica precolumn was used in this experiment. For analysis one microliter injection was made with a Varian CX auto sampler. The column oven was temperature programmed from 200 to 240 at 10 °C/m and held at 220 °C for 1 min (120 °C to 200 °C at 3 °C/min recommended by Court et al., (1993).

The injector and detector temperature in the method of Court et al., (1993) was 183 °C and 200 °C, respectively. In our study both injector and detector the temperature was increased to 280 °C. The carrier gas (helium) flow rate was 2.0 ml/min. The free fatty acid percentage and peroxide value (PV) were determined according to IUPAC method (1987). The statistical analysis was performed using the SPSS software version 12.1 based on two ways ANOVA. Least significant differences (LSD) at α=5% were computed for measurements with F-value <0.05.

Results and Discussion

Oil content of seed

From the results of the four months of storage in which the oil analyses of the seed samples were carried out, it can be inferred that there is a significant effect of storage time on seed oil content of both high and low oleic cultivars (Table 1). The oil content of high oleic cultivars varied from maximum of 51.1% after one month of storage for Atomic and 55.5% for control samples in Olsavil to minimum (after 4 months of storage) of 49.3% and 53.9 % for Atomic and Olsavil cultivars, respectively. The changes in the oil content of low oleic cultivars Jazzy and PRO64A54 (PR) were relatively the same. In both cultivars the lowest oil percentage was recorded from the samples that stored longer. These results are in conformity with Sisman (2004), Sisman and Delibas (2005) who showed that during a period of three months of storage (independent from storage conditions) the percentage of seed oil gradually decreased with increasing the storage time. Same result was reported by Ghasemnezhad et al., (2007) in evening primrose. They showed that as the storage time increased, a rapid decrease of the oil percentage compared to the control samples was observed.

In vegetable oils, oxygen-dependent deterioration of lipids is known as rancidity. The development of rancidity has been recognized as the predominant cause of oil deterioration and reduction during storage (Ahmadkhan et al., 2000; Morello et al., 2004).

Due to the presence of double bonds in unsaturated fatty acids these fatty acids are susceptible to oxidation, which is a reaction between unsaturated fatty acids (regardless of whether they are in their free state or esterified in a triaglycerol molecule) and oxygen. It is well known that the rate of oxidation increases with the increase in oxygen concentration and the duration of exposure (the length of storage time). The oxidation of oil requires the presence of atmospheric oxygen. The longer the storage time the higher the oxygen availability and vice versa. This might be a reason why the percentage of oil of stored seeds tended to reduce during storage.

On the other hand, the metabolism of seeds during storage to provide energy for its physiological activities could be another reason of the seed oil reduction during storage. Enzymes such as acyl-CoA oxidase, malate synthase, citrate synthase, catalase and lipases are the main enzymes which are involved in oil and fatty acids metabolism of the seeds (Kindle, 1987). The activities of most of these enzymes are partially or wholly requiring the presence of oxygen. This can be another reason of observed reduction of oil content during storage. The activities of most of enzymes are partially or wholly requiring the presence of oxygen. Temperature as an important environmental factor of storage had significant

influence on the oil content of experimental cultivars except Atomic. The effect of temperature was not the same for all cultivars wherein Olsavil recorded the lowest oil percentage for seed samples which stored in low temperature conditions. Jazzy and PR cultivars recorded the lowest oil percentage at room conditions and high temperature conditions, respectively.

Table 1. Changes in seed composition of high oleic sunflower under different storage conditions.

ST T	OC		PA		SA		OA		LA		FFA		PC	
	AT	OL	AT	OL	AT	OL	AT	OL	AT	OL	AT	OL	AT	OL
Control	50.1	55.5	4.5	3.7	2.2	1.6	90.6	92.7	2.6	1.8	2.2	1.8	15.2	15.9
ST_1	51.0	54.8	3.8	3.0	2.4	1.7	90.7	92.3	2.7	2.4	2.7	2.2	15.2	15.5
ST_2	50.2	54.4	4.0	3.1	2.2	1.6	90.6	87.1	3.1	1.9	3.0	1.9	15.1	15.5
ST_3	50.7	53.9	4.0	3.2	2.2	1.6	90.6	92.9	3.1	1.9	3.5	1.9	15.2	16.7
ST_4	49.3	53.9	3.8	3.2	2.8	1.6	88.2	92.9	3.5	1.9	3.9	1.9	14.5	15.6
T_1	50.4	53.4	3.9	3.2	2.4	1.6	89.6	92.8	3.1	2.0	2.9	1.8	15.1	16.2
T_2	50.3	54.1	4.1	3.2	2.4	1.6	90.4	92.9	2.9	1.9	2.8	1.6	15.0	15.9
T_3	50.1	54.8	4.1	3.3	2.3	1.6	90.4	89.1	2.9	2.0	3.4	2.3	15.0	15.5
ST p-value	0.00	0.01	0.00	0.00	0.00	0.01	0.00	ns	0.00	0.00	0.010	0.00	0.000	0.00
T	ns	0.04	ns	ns	ns	ns	0.01	ns	ns	ns	0.000	0.00	ns	0.00
ST×T	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.00	0.010	ns	ns	ns
ST $LSD_{5\%}$	0.66	1.33	0.23	0.14	0.23	0.10	0.80	-	0.43	0.17	0.30	0.25	0.27	0.50
T	-	1.2	-	-	-	-	1.1	-	-	-	1.2	0.22	-	0.43
ST×T	-		-	-	-	-	-	-	-	0.44	1.2	-	-	-

AT: Atomic, OL: Olsavil, ST: storage time (month), T: storage temperature; T1: 4-5 °C, T2: 21-22 °C, T3: 35 °C, SO: seed oil, PA: palmetic acid, SA: stearic acid, OA: oleic acid, LA: linoleic acid, FFA: free fatty acid.

The observed conflict in oil content of different cultivars stored under different temperature conditions is not clear. Our results are in confirmation with Manti et al., (2005) in sunflower and Ghasemnezhad et al., (2007) in evening primrose who reported that there was no clear variation tendency in sunflower seed oil percentage with storage temperature.

The interaction of storage time and temperature on oil content of Olsavil (Figure 1) indicates that during first and second months of storage the samples stored in room temperature conditions had the lowest oil content. During third month of storage no significant difference was observed among different temperature conditions. As storage time increased the samples which were stored under high temperature conditions had the lowest oil percentage. Based on the observed results it can be concluded that a total of different parameters influence on seed oil content and its composition and the observed changes in a measured parameter mostly are not related to a single factor.

Protein content

The protein content of all experimental seed samples was significantly influenced by time of storage (Table 1 and 2). As presented in Table 1 and 2, although the protein content of seeds was influenced by storage time, no clear effect was observed. For instance in both Atomic and Olsavil cultivars (high oleic) the protein content of seed samples increased after third months of storage. On the other hand, the protein content of seed samples of PRO64A54 a low oleic cultivar gradually increased as storage progressed. The reason of protein content fluctuation during storage is not clear. It may be due to the negative correlation between seed oil and protein. The same result was observed by Ghasemnezhad et al., (2007) in evening primrose seed. They concluded that based on the negative relation between seed oil and protein content, the observed increased in protein percentage of stored seed could be due to oil content reduction of seeds during storage. The observed irregular changes of protein in sunflower seed samples can not be explain by direct relationship of oil and protein content.

Table 2. Changes in seed composition of low oleic sunflower under different storage conditions.

ST	T	OC		PA		SA		OA		LA		FFA		PC	
		JA	PR	JA	PR	JA	PR	JA	PR	JA	PR	JA	PR	JA	PR
0		51.8	50.4	6.1	6.6	3.3	4.1	39.9	21.9	49.8	66.3	0.3	1.7	14.4	15.9
1		50.6	50.2	6.4	6.1	3.2	4.37	41.1	22.7	50.2	65.8	1.8	2.1	15.4	14.7
2		51.9	49.3	6.6	6.5	2.8	4.26	40.5	22.3	50.2	66.5	1.9	2.6	15.4	14.2
3		52.3	49.2	6.9	6.5	2.8	4.29	39.8	23.5	50.2	66	2.2	4.4	15	15.5
4		52.1	49	5.8	6.1	3.4	4.49	39.7	21.9	49.5	65.5	2.3	4.5	15	15.5
	1	52.7	49.8	6.4	6.4	3.1	4.24	40.4	22.6	49.9	66	1.7	2.5	15.3	15.2
	2	50.2	49.7	6.4	6.4	3.1	4.32	40	22.5	50.2	66.3	1.3	2.7	15.4	15.3
	3	52.3	49.4	6.2	6.2	3	4.34	40.2	22.3	49.8	65.7	2.1	4	14.4	15
ST P-value		0.00	0.00	0.37	0.05	0.48	0.00	0.00	0.07	0.00	0.42	0.00	0.00	0.11	0.00
T		0.28	0.03	0.00	0.45	0.00	0.01	0.31	0.77	0.04	0.31	0.00	0.00	0.00	0.55
ST LSD		1.63	0.44	ns	0.40	ns	0.10	0.61	ns	0.42	ns	0.22	0.1	ns	0.69
T 5%		ns	0.34	0.37	ns	0.14	0.1	ns	ns	0.33	ns	0.19	0.1	0.71	ns

JA: Jazzy, PR: PRO64A54, ST: storage time (month), T: storage temperature; T1: 4-5 °C, T2: 21-22 °C, T3: 35 °C, SO: seed oil, PA: palmetic acid, SA: stearic acid, OA: oleic acid, LA: linoleic acid, FFA: free fatty acid.

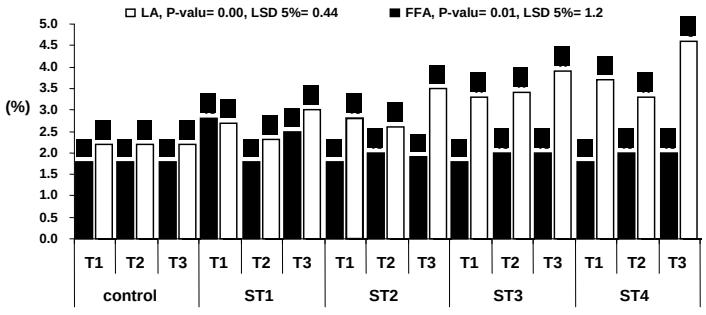

Figure 1. The interaction effect of storage time and temperature on linolenic acid percentage of Atomic and free fatty acid percentage of Olsavil (High oleic cultivars).

Fatty acid composition

Fatty acid composition and the proportions of different fatty acids of the seed oil during storage are dependent on the degradation rate of different fatty acids, which convert to each other (Ahmadkhan and Shahidi, 2000; Muangkaeo et al., 2005). In the present study the profile of fatty acids in both high and low oleic acid was significantly influenced by storage time (Table 1 and 2). As presented in Table 1, the content of palmetic acid and stearic acid of high oleic cultivars were significantly changed during storage but no clear direction was observed for this changes. Oleic acid as a predominant fatty acid in high oleic cultivar Atomic clearly decreased as storage time increased. Table 1 shows that after third month of storage of Atomic seeds a high reduction was observed (from 90.6% at the beginning of storage to 88.2% after four months of storage). This strong reduction in oleic acid could be related to the production of free fatty acid and the conversion to other fatty acid. Results indicate that contrary to oleic acid the content of free fatty acid gradually increased during storage. Fatty acids in free forms are more susceptible to oxidation. It can be concluded that increase of the free form of oleic acid and its oxidation during storage could be the reason why the content of this fatty acids decreased. Contrary to oleic acid a relative increase in the content of other measured fatty acids was observed during storage. It could be concluded that the conversion of oleic acid to other fatty acids like palmetic and linoleic acids is another possible reason of observed reduction in oleic acid. In low oleic cultivars although the content of linoleic acid as a predominant fatty acids significantly increased at the fourth month of storage, the reduction level was not high. Our finding about variation in the content of seed oil fatty acids was confirmed by Morello et al. (2004) in olive oil. In an investigation with olive oil they showed that after a certain period of storage a reduction in the content of linoleic and linolenic acid was observed. Contrary to that the content of oleic acid tended to increase. Figure 1 shows that in Olsavil cultivar after three months of storage a significant increase in the content of free fatty acids was observed in samples which were stored at high temperature conditions. Based on this finding it can be concluded that a combination of high temperature a long storage time can increase the oxidation of oil even in seed. The same result was observed by Ghasemnezhad et al. (2007) in evening primrose. It can be suggested that high FFA accumulation in experimental samples in a long storage period and high temperature could be related to higher activity of hydrolysis by lipases and other enzymes which are involved in fatty acid degradation. The results indicate that storage time and storage temperature had significant effect on lipid peroxide value (PV) of sunflower seed (since the PV of oil samples was measured only at the beginning and the end of experiment the results were not presented). Similar trends in the peroxide value of vegetables oil have been observed by Ghasemnezhad et al. (2007) in evening primrose, Abramovic and Abram (2005) in Camelina and Ullah et al. (2003) in sunflower and soybean. Ullah et al. (2003) demonstrated that the peroxide value of sunflower and soybean oil at room temperature and under different light conditions (fluorescent light, ambient light and dark conditions) sharply increased during a five-week period of storage.

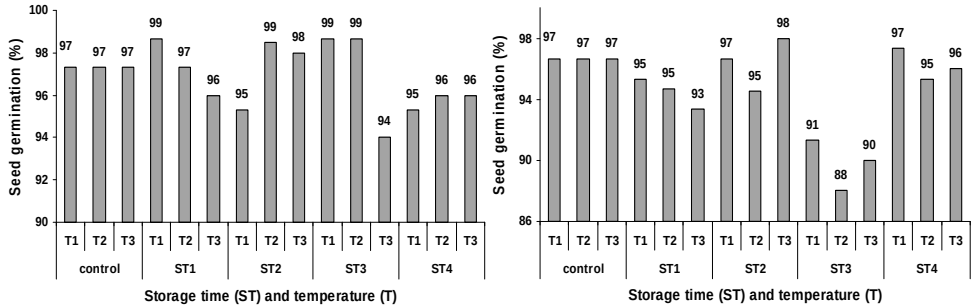

Figure 2. Seed germination percentage of Jazzy (left) and PR (Right) two high oleic cultivars under different storage temperatures and times.

Table 3. The interaction effect of storage time and temperature on oil content, fatty acid composition and protein content of Jazzy and PRO64A54 (Low oleic cultivars).

ST	T	OC		PA		SA		OA		LA		FFA		PC	
		JA	PR	JA	PR	JA	PR	JA	PR	JA	PR	JA	PR	JA	PR
C		51.8	50.4	6.1	6.6	3.3	4.1	39.9	21.9	49.8	66.3	0.3	1.5	14.4	15.9
ST_1	T_1	50.2	51	6.7	6.6	3.2	4.3	40.9	22.4	50.4	65.1	1.6	1.73	14.4	15.4
	T_2	51.9	49.7	6.9	6.6	3.2	4.5	41.1	22.6	50.7	67.1	1.6	1.63	16.3	14.2
	T_3	50.8	49.7	5.6	5	3.2	4.4	41.3	23.2	49.6	65.2	2.2	1.87	15.5	14.5
ST_2	T_1	51.8	49.4	6.5	6.4	2.7	4.2	41.3	22.7	49.6	66.1	1.8	1.9	16.1	14.3
	T_2	50.5	49.5	6.7	6.5	2.9	4.2	40.4	21.9	50.7	66.8	1.4	1.73	16.3	14.2
	T_3	53.5	49.1	6.6	6.6	2.7	4.3	39.8	22.2	50.3	66.4	2.5	1.97	13.7	14
ST_3	T_1	54.1	49.5	6.8	6.2	2.8	4.1	40	23.8	49.9	65.8	2.3	2.27	15.7	15.1
	T_2	50.7	49.6	7.1	6.3	2.8	4.3	39.4	24	50.7	65.6	1.5	1.87	15.1	16.1
	T_3	52.2	48.7	6.8	7	2.6	4.4	40	22.6	49.9	66.6	2.9	2.23	14.1	15.2
ST_4	T_1	51.7	48.7	6.1	6.2	3.6	4.5	39.8	22	49.8	66.7	2.5	2.5	15.7	15.2
	T_2	51.4	49.4	5.4	6.2	3.2	4.5	39.2	22.3	49.2	65.9	1.8	2.07	15.1	16.1
	T_3	51.2	48.9	6	6	3.4	4.5	40	21.4	49.6	63.9	2.6	2.5	14.1	15.2
ST×T		0.02	0.04	0.09	0	0.3	0.03	0.25	0.87	0.02	0.13	0	0.01	0.02	0.36
ST×T		2.36	0.76	-	0.34	-	0.17	-	-	0.72	-	0.37	0.11	1.42	-

JA: Jazzy, PR: PRO64A54, ST: storage time (month), T: storage temperature; T1: 4-5°C, T2: 21-22 °C, T3: 35 °C, SO: seed oil, PA: palmetic acid, SA: stearic acid, OA: oleic acid, LA: linoleic acid, FFA: free fatty acid.

Seed viability

Figure 2 shows that after first month of storage, the lowest seed germination both in Jazzy and PR cultivars was recorded in seed samples stored under 35 ºC. Two months after storage the germination behavior was quite different. In Jazzy the seeds which were stored in room temperature conditions had the lowest germination percentage. Contrary to that seeds of PR which were stored in low temperature conditions had the lowest germination percentage. Three months after storage a strong reduction in seed germination was observed in both cultivars specially when stored in high temperature conditions. On the other hand, in next month an increase in seed germination was observed again. No clear effect of storage conditions on seed germination percentage of Olsavil and Atomic (low oleic cultivars) was observed in present study. For example the highest reduction in seed

germination of Atomic cultivar was observed in the seed samples which were stored under room temperature conditions in a period of one month. In contrary, in Olsavil cultivar the highest reduction in seed germination was observed four months after storage at room temperature. Generally based on obtained results it is not easy to describe the effect of single parameters. But the results show that independent from temperature conditions of storage, progressive in storage time negatively influence on seed viability.

Conclusion

It was expected that sunflower oil is more stable during storage. The reason of observed low stability in seed oil is not clear. In this experiment the experimental design did not cover the record of some factors like moisture content of the seeds at the beginning and during storage, the relative humidity of the air of storage, the level of seed damage after threshing. This could be the reason why oil in the seeds did not seem so stable. On the other hand, the used cultivars could be another reason of why the observation is contrary to the expectations that the experimental cultivars, Olsavil, a high oleic cultivar, showed a strong increase in free fatty acid accumulation, when it was stored at high temperature conditions and a relative long storage time. Generally it can be conclude that the storage life of sunflower seed can be decreased by longer storage time and higher storage temperature.

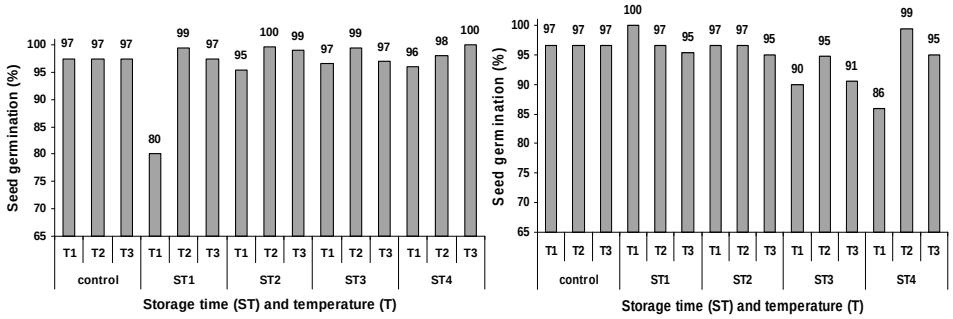

Figure 3. Seed germination percentage of Atomic (left) and Olsavil (Right) two low oleic cultivars under different storage temperatures and times.

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