Relation of Days after Flowering to Chemical Composition and Physiological Maturity of Sunflower Seed

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ABSTRACT

Hybrid sunflower seed (achene) were collected from plants at 7-day intervals after the initiation of flowering which occurred 58 days after planting. The seed were analyzed for moisture, total oil, free fatty acids, lipid classes, and fatty acid composition. Seed dry weight, oil and triglyceride contents were maximum 35 days after the initiation of flowering (DAF) when the seed moisture content was about 36%. This point was defined as "physiological maturity" for sunflowers. The fatty acid composition of the oil extracted from the seed was determined at each stage of maturity. Total saturated fatty acids were 27% at 7 DAF and then decreased to a constant 9% by 35 DAF. At 7 DAF, linolenic acid content was 10.7% then decreased to less than 0.1% by 28 DAF. Oleic acid was about 12% at 7 DAF, increased to 59.6% at 14 DAF, and then gradually decreased to 31.4% by 56 DAF. On the other hand, linoleic acid was about 48% at 7 DAF, decreased to 23% by 14 DAF, but then gradually increased to 59.2% by 56 DAF. An analysis of variance of linoleic and oleic acid contents from 21 DAF to 70 DAF showed a highly significant change in composition with maturation time. The changes in the composition of these fatty acids from 21 DAF to 70 DAF appeared to be related to the environmental temperature which gradually decreased until 56 DAF. Increase in free fatty acids after physiological maturity indicated that deterioration of seed oil was beginning to occur.

INTRODUCTION

The maturation of sunflowers is influenced by environmental factors such as temperature; however, maturity of the crop also involves seed moisture content which must be sufficiently low to permit harvesting of the crop (1,2). Physiological maturity is reached before seed moisture content is low enough to allow safe harvest and storage. Anderson (2) defined the point at which seed yield and oil content are maximum as "physiological" maturity for sunflowers.

Development of oil and fatty acids in sunflower seed during maturation has been studied by several scientists (2-6). Anderson (2) reported that in the field, achene dry weight became maximum when the achene moisture content was about 40% and the capitulum moisture content (head with seed removed) was about 70%. Both achene oil content and the linoleic acid content reached their maxima at about the same time as achene dry weight. Morozov and Shestakov (3), however, found that the optimum time for harvesting sunflowers was when the moisture of the seed was about 20% because at this time all the plants in the field had ceased oil filling. Hopkins and Chisholm (4) reported that oil formation began about 10 days after flowering and continued at a steady rate for 7 weeks. They also found that the individual fatty acids increased in weight fairly regularly to maturity with a large increase in linoleic acid. Linolenic acid was not detected. Gunstone and Padley (5) reported that lipid content in sunflower seed was low until 48 days after the onset of flowering. Lipid formed quickly during the next 18 days, and remained fairly constant thereafter. They found little change in the fatty acid composition after 48 days after flowering.

The above studies, however, were carried out in countries other than the United States; open pollinated sunflower varieties were used; and the studies generally were of limited scope. The present study was conducted with a sunflower hybrid that is representative of the sunflowers that are grown commercially in the U.S. and was designed to determine the relation of days after flowering to chemical composition and physiological maturity of sunflower seed.

MATERIALS AND METHODS

Sunflowers (*Helianthus annuus* L., Sun Gro 380 hybrid) were planted May 22, 1976, in the Piedmont region of Georgia (Winterville, GA). Day-night temperature data were obtained from NOAA National Weather Service station located about 5 km from the planting site. At the initiation of flowering about 200 plants were tagged. Initiation of flowering was defined as the first emergence of one to two yellow radii and anthers. Heads were hand-harvested weekly from 7 to 70 days after flowering (DAF). After physiological maturity, seed heads were covered with paper bags for protection against birds.

About 30 heads were required to yield enough seed for analyses at 7 DAF and about 10 heads at 42 DAF. Seed from the heads were removed by hand, cleaned, weighed, and moisture content determined (7). Then, seed were freeze-dried for about 24 hr to constant weight. Only those seed from 3-7 cm of the outer edge of each capitulum were sampled during the study. The term "seed" or achene as used in this study refers to whole seed which includes pericarp (hull) and kernel (8).

Total oil, free fatty acids, and peroxide values were determined on freeze-dried seed by AOCS methods Ai 3-75, Ca 5A-40, and Cd 8-53, respectively (7). Moisture content of receptacles was determined immediately after seed removal by drying at 100 C for 4 hr and at 130 C for 3 hr. Fatty acid methyl esters were prepared with BF_3-methanol reagent by the method of Metcalfe et al. (9). Gas chromatography was conducted with a Tracor MT 220 gas liquid chromatograph equipped with an Infotronics Model CRS 101 digital integrator (10). A 10 ft x 1/8 in. stainless steel column packed with 10% EGSS-X on 100/120 mesh Gas Chrom P was used for the analyses, and the oven was operated at 190 C.

Major lipid classes of crude sunflower oil were separated by silicic acid chromatography. Freeze-dried seed were ground in liquid nitrogen with a large mortar and pestle, and crude oil was extracted with chloroform-methanol (2:1) by a published procedure (11).

Samples of crude oil (300-1200 mg) were applied to a 1 x 25 cm silicic acid (Bio-Sil A, 100/200 mesh) column and eluted step-wise with 25 ml each of chloroform, acetone, and 1.0 N NH_4OH/methanol. Freshly packed columns of identical dimensions were used for each oil samples to insure elution reproducibility which is usually lost by column regeneration. Fractions were collected in preweighed flasks, and solvents were removed at 35 C with a

TABLE I

Effect of Stage of Maturity on Sunflower Seed Composition[a]

Days after flowering	Moisture (%)	Total oil (% dry basis)	Dry weight (100 seed)	Free fatty[c] acid (% as Oleic)	Peroxide[c] value (meq/kg)
7	83.0±1.32[b]	1.6±0.03[b]	1.3±0.03[b]	10.6 - - -	- - -
14	76.7±1.27	16.6±2.45	2.4±0.25	2.0±0.01[b]	3.2±1.20[b]
21	64.3±1.46	38.9±0.92	3.1±0.17	0.4±0.02	1.8±0.11
28	47.9±1.33	50.6±2.19	4.8±0.29	0.3±0.01	1.5±0.18
35	36,2±1.62	53.5±0.63	5.6±0.37	0.2±0.01	1.7±0.26
42	21.7±0.31	53.3±0.49	5.5±0.18	0.2±0.01	3.8±0.18
49	14.6±0.48	53.2±0.62	5.5±0.31	0.3±0.03	4.4±0.28
56	9.6±0.21	53.6±0.82	5.1±0.07	0.3±0.03	5.0±0.10
63	8.2±0.08	53.9±0.26	5.0±0.16	0.5±0.06	3.9±0.29
70	8.9±0.11	53.5±0.33	5.4±0.01	0.6±0.04	2.9±0.16

[a]Mean of three separate samples analyzed in duplicate.
[b]Standard deviations.
[c]Triplicate analyses.

TABLE II

Effect of Stage of Maturity on Fatty Acid Composition[a]

Days after flowering	Fatty acid composition (area percent) 16:0	16:1	18:0	18:1[c]	18:2[d]	18:3	20:0	20:1	22:0	Unknown
7	20.8	0.5	3.7	11.7	47.8	10.7	1.5	- - -	0.8	2.6
14	7.5	0.1	7.7	59.6	22.6	0.8	0.8	Tr[b]	0.9	Tr[b]
21	5.7	Tr[b]	5.0	51.7	36.1	0.1	0.4	0.1	0.9	- - -
28	5.4	Tr	3.7	43.3	46.4	Tr[b]	0.3	0.2	0.7	- - -
35	5.0	Tr	3.3	38.5	52.1	Tr	0.3	Tr	0.6	- - -
42	5.0	Tr	3.2	37.3	53.7	Tr	0.2	Tr	0.6	- - -
49	5.3	Tr	3.1	32.7	57.5	Tr	0.5	0.2	0.7	- - -
56	5.8	Tr	2.9	31.4	59.2	Tr	0.2	Tr	0.4	- - -
63	5.7	Tr	3.1	32.3	57.9	Tr	0.4	Tr	0.5	- - -
70	5.2	Tr	2.7	37.2	53.8	Tr	0.3	0.1	0.7	- - -

[a]Mean of three separate samples analyzed in duplicate.
[b]Trace, less than 0.1%.
[c]Average standard deviation was ± 0.92.
[d]Average standard deviation was ± 0.75.

rotary evaporator. Each column fraction was determined as a weight percentage of the total oil applied to the silicic acid column. The weight of each fraction per 100 seed was calculated by multiplying the oil percentage (chloroform-methanol extraction) by the dry weight of 100 seed, and then by the percentage of each lipid fraction in the oil. Each lipid class was identified by a three solvent system, two dimensional thin layer chromatographic technique (11).

RESULTS AND DISCUSSION

Sunflowers were planted somewhat late for this geographical location, but rainfall was adequate for good growth and normal maturity. Flowering was initiated 58 days after planting. Since the sunflowers were hybrids, flowering was uniform with 82% of the plants in full bloom within 4 days after initiation of flowering (DAF), and 100% were in full bloom and had dehised anthers within 10 days.

The effects of stage of maturity on sunflower seed composition are shown in Table I. Oil filling was rapid between 14 and 28 DAF, and seed (achene) oil percentage and dry weight were maximum (physiological maturity) about 35 DAF. Seed moisture content was about 36%, and receptable moisture content was about 85%. These data are similar to those of Anderson (2) who reported that physiological maturity occurred when seed moisture was about 40% and capitulum (receptable) 70% moisture. Our data, however, are contrary to those of Hopkins and Chisholm (4) who found that total oil and dry weight of seed continued to increase for 49 to 56 DAF and until seed moisture was about 20%. Their data for high free fatty acids and low seed moisture indicate that physiological maturity probably occurred about 42 DAF.

Our study was extended 35 days after physiological maturity to determine any change in oil quality when seed were left in the field. Total oil content remained unchanged, but dry weight decreased slightly at 46 and 63 DAF (Table I). In addition, the free fatty acid content increased with time in the field at 63 and 70 DAF which indicated that oil quality was beginning to deteriorate. The peroxide value increased slightly after physiological maturity and then decreased.

During the time between physiological maturity and harvest, temperatures ranged from 21 to 24 C. If seed had been planted at the normal time in mid-April, the average daily temperature during seed maturation would have ranged from 26 to 29 C; at these temperatures, free fatty acids of sunflower seed left in the field after physiological maturity possibly would have increased more rapidly.

Moisture content of the seed remained above 10%, an acceptable harvest moisture level (12), for 3 weeks after physiological maturity (56 DAF). Even then head moisture was still above 70%, which is too high for mechanical harvesting, and did not reach an acceptable level (25-30%) until about 63-70 DAF. The use of chemical desiccants immediately after physiological maturity (35-42 DAF) could speed up this moisture reduction and reduce risk of secondary fungal attack. In addition, oil quality deterioration would be retarded, and the time of susceptibility to bird and insect damage would be reduced (2).

The effect of stage of maturity on fatty acid composition is shown in Table II. At 7 DAF and low oil content (1.6%), linolenic acid (10.7%), palmitic acid (20.8%), and

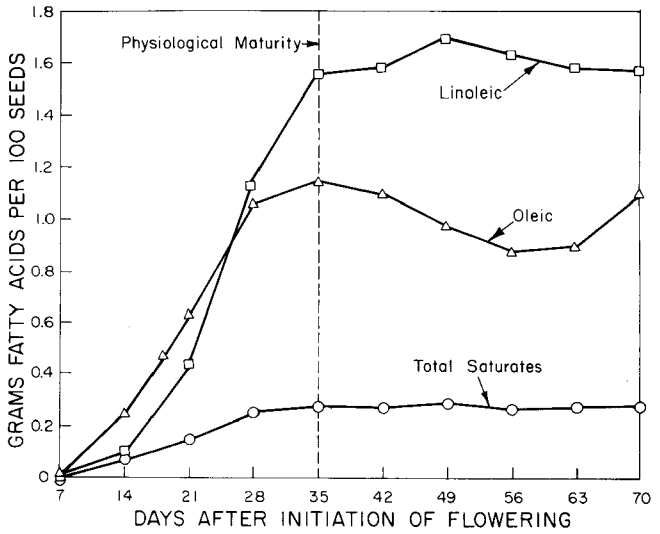

FIG. 1. Effect of stage of maturity on weight of sunflower seed oil fatty acids.

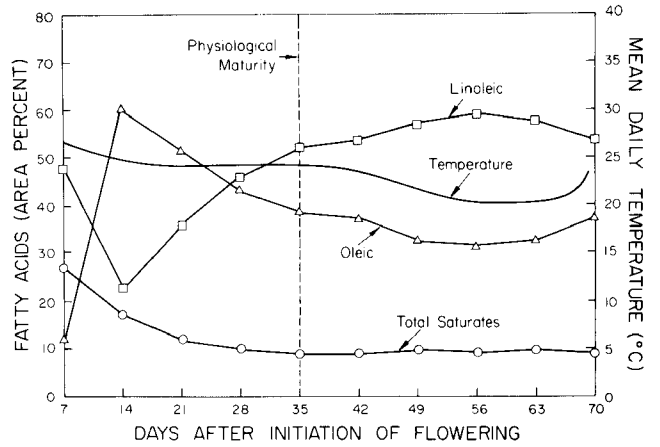

FIG. 2. Effect of temperature and stage of maturity on sunflower seed oil fatty acid composition. Temperature data smoothed by Kimball's cubic splines smoothing procedure (14).

arachidic acid (1.5%) contents were higher, and oleic acid (11.7%) content was lower than oil from mature seed. An unidentified fatty acid of about C20 chain length was present in substantial amount (2.6%). At 14 DAF, linolenic acid has decreased to 0.8% and by 28 DAF was less than 0.1%. Linoleic acid, on the other hand, was 47.8% at 7 DAF, decreased to 22.6% at 14 DAF, and then gradually increased to 59.2% at 56 DAF after which it decreased until seed was harvested at 70 DAF. Oleic acid increased to 59.6% at 14 DAF, and then gradually decreased to 31.4% at 56 DAF and then increased at 63 and 70 DAF. Analysis of variance showed that percent of linoleic acid increased and oleic acid decreased significantly ($P<.01$) until 56 DAF. In fact, a quadratic model fitted to the data explained 81% of the variability in oleic acid ($R^2 = .81$) and 83% in linoleic acid ($R^2 = .83$).

A plot of grams of fatty acids per 100 seed versus DAF (Fig. 1) shows that the saturated, oleic, and linoleic acids all increased until physiological maturity. Linolenic was the only fatty acid that actually decreased in weight with increase in seed weight. After physiological maturity, weight of oleic acid decreased until 56 DAF; whereas, linoleic acid continued to increase until 49 DAF before slightly decreasing. At about 28 DAF, the rate of formation of linoleic exceeded that of oleic acid. Hopkins and Chisholm (4) observed a similar inversion and concluded that formation of the characteristic seed oil of the species actually begins at that stage of development. The reduced rate of accumulation of oleic acid together with the rapid rise of linoleic acid appears to support the hypothesis that linoleate is formed by desaturation of oleate (13).

The effects of temperature and stage of maturity on fatty acid composition are shown in Figure 2. As indicated earlier, beginning at 14 DAF percent of linoleic increases and of oleic acid decreases until 56 DAF or 3 weeks beyond physiological maturity. These changes appear to be related to the mean daily environmental temperature which gradually decrease through the growing season until the last 2 weeks of the study when it increased slightly. Between 21 and 70 DAF, oleic acid content and temperature were significantly related ($P<.04$). For each 1 C decrease in temperature, oleic acid content decreased 0.87%. Linoleic acid increased 0.32% for each 1 C decrease in temperature; however, this relationship was not statistically significant.

The mode of control of the percents of oleic, linoleic, and linolenic acids in oilseeds differs among species. In safflower, the oleic and linoleic acid percentages are entirely under genetic control of the seed embryo; whereas, in soybeans the levels of oleic, linoleic, and linolenic acids are essentially controlled by the genotype of the maternal plant with minor contribution from the genotype of the embryo (15). For sunflower seed, very little is known about the genetic control of the fatty acids. Putt et al. (16) reported that oil quality was under genetic control and that breeding for different levels of oleic and linoleic acids is a practical objective. However, evidence indicates that the unsaturation of sunflower oil depends largely upon the environmental temperature during maturation of the seed (17-19). With constant growing temperatures of 10 C to 26.5 C, Canvin (17) showed graphically that linoleic acid decreased from about 75% at 10 C to about 30% at 26.5 C.

We reported (19) that the oil of sunflowers grown at locations in southern Georgia, Louisiana, and southwest Texas had a lower linoleic acid content (35.2%) than oil from sunflowers grown at cooler locations in South Carolina, northern Alabama, and central Georgia (linoleic acid,

TABLE III

Changes in Lipid Classes during Sunflower Maturation

Days after flowering	Triglycerides	Glycolipids (mg lipid/100 seeds)	Phospholipids
7	15.7	10.5	18.0
14	274.0	13.0	33.3
21	1142.9	13.9	47.0
28	2103.5	42.2	38.3
35	2628.9	17.1	61.0
38	2755.3	19.8	61.1
56	2688.8	17.9	63.9
70	2692.9	22.3	65.4

44.1%). Harris and James (20) concluded that an increase in the level of linoleic acid in sunflower seed at low temperatures was due to increased availability of 0_2 for desaturation. Experiments with different oxygen pressures to compensate for the effect of high temperatures on linoleate content did not conclusively support their hypothesis (21).

Lipid classes from silicic acid column chromatography were identified by thin layer chromatography. The chloroform fractions contained only triglycerides and other neutral lipids; the acetone fractions contained glycolipids and traces of phospholipids; and the 1.0N NH_4OH-methanol fractions contained only polar lipids, principally phospholipids.

Changes in the major lipid classes during sunflower maturation are shown in Table III. Triglycerides were synthesized rapidly between 14 and 35 DAF (112 mg/day), reached their maximum level at physiological maturity, and remained fairly constant thereafter. Maximum triglyceride formation occurred at the same time as maximum production of seed oil and dry weight (Table I). Results were similar for developing soybeans (22).

Glyco- and phospholipids were synthesized at much slower rates, but reached maximum levels at about physiological maturity. Glycolipids peaked one week earlier than phospholipids. After 35 DAF, the weight of all lipid classes remained virtually unchanged.

Anderson (2) reported that physiological maturity of sunflower corresponded to the accumulation of 1052 ± 61 C heat units after anthesis. Heat units were calculated as Σ_n^1 (daily maximum + minimum/2), where n is the number of days from first anthesis to physiological maturity. In our study, physiological maturity occurred after the accumulation of 902 C units.

ACKNOWLEDGMENTS

Technical assistance was provided by J.K. Thomas and L.F. Wimberly, and Kimball spline temperature smoothing by T.K. Woody III.

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[Received July 10, 1977]