Studies on changes in patterns of fatty acids, sterols and tocopherols of oil during seed maturation of oil crops. Part I. Sunflower seeds

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RESUMEN

Estudios sobre los cambios en perfiles de ácidos grasos, esteroles y tocoferoles de aceites durante la maduración de semillas de cuerpos oleaginosos. Parte I. Semillas de girasol.

Se ha investigado la variación de los constituyentes lipídicos en aceites de semillas de girasol en distintos estados de maduración, con el objetivo de determinar el tiempo de recolección apropiado así como la calidad del aceite. Se observaron variaciones apreciables en los ácidos grasos, esteroles y tocoferoles del aceite. La cromatografía gaseosa capilar y la cromatografía líquida de alta eficacia se usó en el análisis que permitió la determinación tanto de los componentes mayoritarios como minoritarios en el aceite. Además, se utilizó la cromatografía gaseosa acoplada a la espectrometría de masas para confirmar la estructura del esterol cicloartenol. Se han propuesto en el marco de la ruta biosintética principal postulados sobre los compuestos clave de ácidos grasos, esteroles y tocoferoles. Se encontró como tiempo de recolección apropiado el de 118 días después de la plantación, ya que el contenido de aceite fué elevado y los constituyentes fueron equilibrados.

PALABRAS-CLAVE: Aceite — Calidad — Composición lipídica — Girasol (semilla) — Maduración (efecto de).

SUMMARY

Studies on changes in patterns of fatty acids, sterols and tocopherols of oil during seed maturation of oil crops. Part I. Sunflower seeds.

The variation of lipid constituents in maturing sunflower oil seeds has been investigated with the aim of determination of the proper harvesting time as well as the oil quality. Marked variations in fatty acid, sterol and tocopherol constituents of the oil were observed. Capillary gas chromatography and high performance liquid chromatography were used in the analysis which enabled the determination of major as well as minor constituents in the oil. In addition, gas chromatography coupled to mass spectrometry was used to confirm the structure of cycloartenol sterol. Postulations on the key compounds of fatty acids, sterols, and tocopherols were proposed in the frame of the main biosynthetic pathways. The proper harvesting time was found to be 118 days after plantation since the oil content was high and the constituents of the oil were balanced.

KEY-WORDS: Lipid composition — Oil — Quality — Ripening (effect of) — Sunflower (seed).

1. INTRODUCTION

Oil seed plants have attracted the attention of many authors due to the fact that these plants can produce variety of lipids and related compounds which can be changed in composition during maturation and germination processes. These changes are of interest and can throw light on the conversions and interconversions of these components. Synthesis and transformation of unsaturated acids and biosynthesis of other lipid components have been reported (1-6). Many investigations have been carried out to study the compositional and quantitative changes in fatty acids, phospholipids, glycerides, sterols, triterpenes, hydrocarbons, tocopherols, gossypol, etc., during plant maturation and seed germination (6-16).

In the present work it was objective to try to propose mechanisms of the biochemical conversions and interconversions of three essential seed components namely, fatty acids, sterols and tocopherols. It was also objective to determine the appropriate harvesting time in the light of changes of the above mentioned three components.

2. EXPERIMENTAL

Sunflower oil seeds (*Helianthus annuus*, cv. Miak) were selected for this study. The seeds were cultivated in the National Research Centre Experimental Agricultural Station, Quanater El-Khirya (Season 1988). Sunflower heads were collected at different maturation stages of 77, 83, 90, 97, 104 and 118 days after plantation (DAP). Seeds from the collected heads were air-dried prior analysis.

2.1. Proximate seed analysis

Moisture, oil, unsaponifiable matter and phospholipid contents were determined according to the standard methods of AOCS (17). Whereas, the unsaturation character (iodine value) of the oil was measured according to Kaufmann method (18).

Oil was extracted with chloroform/methanol (2:1, v/v) to be used for the determination of fatty acid, sterol and tocopherol patterns (19).

2.2. Gas Liquid Chromatographic Analysis (GLC)

Packard HP 5890 A gas chromatograph was used for elucidation of fatty acid and sterol compositions of the oil.

For fatty acid analysis,the oil was firstly converted to methyl esters using methanol/HCl gas.

Gas chromatographic analysis of methyl esters was carried out under the following operating conditions: Column, 30mx0.32mm filled with DB-23 in a film thickness of 0.25 micron; column temperature 150ºC-230ºC

(3ºC/min.); injection temperature, 230ºC; detector, flame ionization; carrier gas, Helium at 1-3 ml/min and split ratio, 1:100.

For sterol analysis, preparative thin layer chromatography was used for isolation of pure sterol mixture from the unsaponifiable matter. Chloroform/diethyl ether/acetic acid (94:5:1,v/v/v) was used as developing system (20). The isolated sterol mixture was converted to TMS derivatives (21) before GLC analysis. GLC analysis was conducted under the following operating conditions: Column, 5m x 0.53mm coated with DB-1 phase in a film thickness of 1.0 micron; column temperature, 200ºC-280ºC (4ºC/min); injection and detector temperature, 290ºC and carrier gas, Helium at 8.0 ml/min.

2.3. Gas Chromatography - Mass Spectrometry (GC-MS)

Gas Chromatography-Mass Spectrometer, Hitachi M80 was used for confirming the structure of cycloartenol sterol under the same operating conditions of gas-chromatography. In addition, interface temperature of 280ºC with ionization voltage of 20 ev, electric ionization mode and ion source temperature of 200ºC were used.

2.4. High Performance Liquid Chromatography (HPLC)

Toyo Soda CCPM HPLC coupled with Hitachi 650-10S fluorescence detector (excitation and emmission wave lengths were 290 and 325 nm respectively) was used for determination of tocopherols directly in the oil. The following operating conditions were conducted: Column, 150 x 4.6mm, packed with silica gel YMC-A-012 SIL, particle size, 5 micron; solvent, isopropyl alcohol: hexane (0.5:100, v/v). The amounts of tocopherol components were estimated from the measured areas under the peaks in comparison with those of the standard tocopherol (22).

3. RESULTS

From Table I it can be seen that the weight of the seeds as well as the oil content increased by progressing in maturation until they reached their maximum values at full maturation.

The iodine value of the oil was 84.3 at the beginning of maturation and it increased by maturation until it reached a value of 106.9 at 118 DAP.

With reference to the phospholipid fraction of the oil it increased as maturation was progressing. However, the unsaponifiable matter was high at the beginning of maturation (7.4%) then decreased through out until it reached a minimum value of 2.1% at full maturation.

Concerning the compositional changes of fatty acids at different seed maturation stages (Table II) it can be noticed that a maximum amount of palmitic acid (25.0%) was obtained at 77 DAP. After that it decreased as the maturation was progressing till it reached a value of 7.6%

Table I. **Proximate seed analysis of sunflower cv. Miak during seed maturation**

Constituents	Days after planting					
	77	83	90	97	104	118
Seed moisture %	14.7	12.3	11.3	10.6	8.5	7.6
Oil content %	11.0	12.1	18.7	19.2	19.7	20.5
Weight of 100 seeds (g)	2.0	4.3	5.8	8.2	8.5	9.7
Weight of oil in 100 seeds	0.214	0.513	1.085	1.577	1.674	2.0
Unsaponifiable content in oil %	7.4	4.7	2.5	2.1	2.1	2.0
Weight of unsaponifiable in 100 seeds	0.016	0.024	0.027	0.033	0.035	0.041
Phospholipid in oil %	1.4	2.23	2.47	2.95	3.08	3.9
Weight of phospholipid in 100 seeds	0.002	0.011	0.027	0.047	0.052	0.078
Oil unsaturation	84.3	85.4	103.3	106.1	105.7	106.9

Table II. **Compositional changes of fatty acid of sunflower cv. Miak during seed maturation**

Days after plantation (DAP)	Saturated Fatty Acids %			Unsaturated Fatty Acids %			
	Palmitic 16:0	Stearic 18:0	Total	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Total
77	25.0	5.1	30.1	32.2	36.7	1.0	69.9
83	18.6	6.6	25.2	40.6	32.8	1.4	74.8
90	9.5	6.0	15.5	65.5	19.0	0.0	84.5
97	5.8	3.7	9.5	60.6	29.9	0.0	90.5
104	5.5	3.6	9.1	53.4	37.5	0.0	90.9
118	7.6	9.1	16.7	48.1	35.2	0.0	83.3

Table III. **Sterol composition of sunflower cv. Miak as determined by GLC of TMS sterols derivatives**

Days after plantation (DAP)	Sterol composition %						
	Cholesterol (a)	Campa-Sterol (b)	Stigma-Sterol (c)	Beta-Sito-Sterol (d)	7-Stigma-Sterol (e)	5-avena-Sterol (f)	Cyclo-artenol (g)
77	trace	10.0	20.0	65.5	3.0	1.5	trace
83	trace	11.0	13.9	69.2	4.0	1.9	trace
90	trace	10.0	13.9	70.0	2.0	4.1	trace
97	trace	10.0	13.3	71.7	1.0	4.0	trace
104	trace	10.3	8.9	75.8	1.0	4.0	trace
118	trace	16.0	9.5	67.5	0.5	2.0	trace

at full maturation. Stearic acid,on the other side, exceeded the level of palmitic acid at full maturation, however, it was formed in comparatively lower amounts during all other maturation periods.

With reference to unsaturated fatty acids, it was found that oleic and linoleic acids were the major constituents, however, linolenic acid was the minor constituent. Concerning oleic acid, it constituted 32.2% at the beginning of maturation then it increased by maturity until it reached its maximum value (65.5%) at 90 DAP after that it decreased to 48.1% at 118 DAP. However, linoleic acid behaved generally in an opposite manner to oleic acid. Linolenic acid was detected in minor amounts only at 77 DAP and 83 DAP and then disappeared at further maturation periods.

From the results recorded in Table III, it can be noticed that cholesterol and cycloartenol were detected in trace amounts at all maturation stages. The presence of cholesterol in sunflower oil was expected since it was reported to be among the sterol constituents of vegetable oils (23,24,25). Unexpectedly, cycloartenol was detected in trace amounts at all maturation stages, therefore it was necessary to confirm its structure with the help of GC-MS as indicated in the figure.

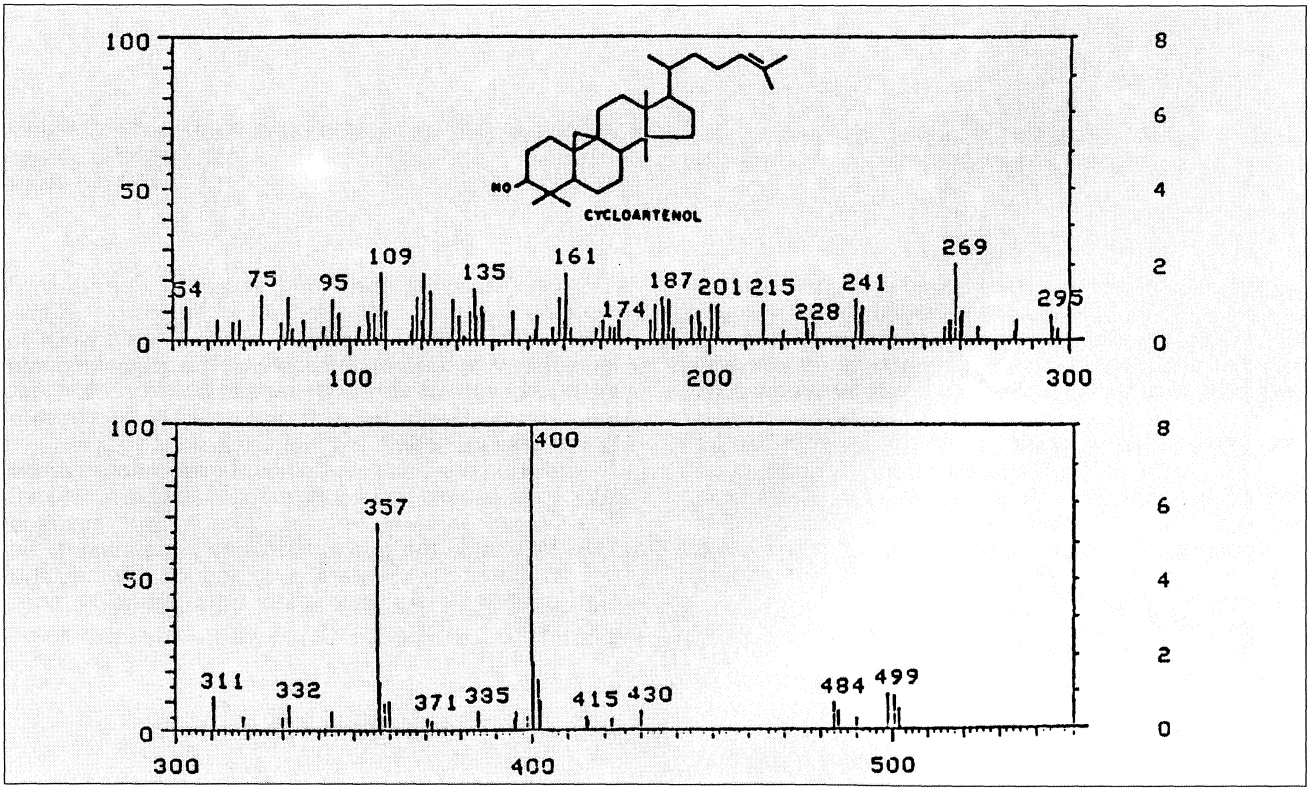

Figure
Mass Spectrum of TMS Sterol Taken At the Peak Corresponding to Cycloartenol on GLC Column.

The results obtained in Table III showed that campe- and 5-stigma-sterol were present in reasonable amounts at all maturation stages, whereas 7-stigma- and 5-avena-sterol were detected at all maturation periods but in comparatively lower amounts not exceeding 4.1%.

Beta-sitosterol was the predominant one at all maturation stages and constituted 65.5% of the total sterols at the beginning of maturation and then gradually increased to 69.2,70.0,71.7 and 75.8% at 83,90,97 and 104 DAP respectively. At full maturation,118 DAP, it decreased markedly to 67.5%.

HPLC analysis of tocopherols at different maturation periods showed a marked variation in its components (Table IV).

It was observed that the total tocopherol content was 410ppm at the beginning of maturation then it reached its maximum value of 710ppm at 83 DAP after which it decreased gradually to reach 440ppm at 118 DAP.

It can also be seen from Table IV that alpha-tocopherol was almost the predominating component at all maturation periods, it constituted 88.6% at the beginning of maturation then decreased to 38.2% at 83 DAP and rised to a maximum value of 93.9% at 97 DAP. Beta- and gamma-tocopherols were found to be the minor components at nearly all maturation stages, however, gamma-tocopherol was unexpectedly increased at 83 DAP to be the major tocopherol component instead of alpha-tocopherol. Therefore, gamma-tocopherol constituted 58.7% of the total tocopherols at 83 DAP corresponding to 38.2% alpha-tocopherol.

Delta-tocopherol was detected in trace amounts at most maturation periods.

4. DISCUSSION

From the changes in composition of fatty acids, it can be concluded that each maturation stage exhibits different fatty acid pattern which indicates that certain biochemical transformations can take place during seed maturation. It can be seen from the results of the fatty acids changes that the changes of total content of saturates coincide with that of linoleic acid but in an opposite manner with oleic acid. It can also be concluded that transformations of fatty acids can take place via conversions and interconversions processes (26). Therefore, it can be postulated that palmitic acid is firstly formed in an appreciable amount and then elongated to stearic acid ($C_{18:0}$) via C_2-unit addition, however, the stearic acid is converted at high rate via dehydrogenase enzyme, into oleic acid which is subsequently dehydrogenated into linoleic (26,27,28,29).

The marked variation in sterol pattern of the oil at different maturation stages showed that certain biosynthetic and transformation mechanisms can take place. Although lanosterol or cycloartenol was reported to be the key compound for sterols biosynthesis (30, 31), however, the present work support the view that cycloartenol is the key compound for all sterols biosynthesis. This view is in accord with that reported by Ehrhardt (32) and Benvensite (33).

Table IV. **Compositional changes of tocopherols of sunflower cv. Miak during seed maturation as determined by HPLC**

Days after plantation (DAP)	Total Tocopherol (ppm)	Tocopherol Composition							
		Weight in oil (ppm)				Tocopherol Composition %			
		Alpha-T	Beta-T	Gamma-T	Delta-T	Alpha-T	Beta-T	Gamma-T	Delta-T
77	410	363.1	11.6	35.3	trace	88.6	2.8	8.6	trace
83	710	271.2	7.1	416.8	14.9	38.2	1.0	58.7	2.1
90	690	604.4	41.4	39.3	4.8	87.6	6.0	5.7	0.7
97	510	478.8	26.0	5.1	trace	93.9	5.1	1.0	trace
104	470	430.1	33.8	6.1	trace	91.5	7.2	1.3	trace
118	440	410.0	25.0	5.0	trace	93.2	5.6	1.1	trace

After studying the compositional changes of tocopherols during maturation, it can be postulated that tocol is the parent component of all tocopherols which is formed from geranyl pyrophosphate. It is suggested that the first tocopherol to be synthesized from tocol is delta-tocopherol. The latter is transformed into different tocopherols via certain enzymatic reactions (30).

From the results of the compositional changes of the fatty acids, sterols and tocopherols, it can be concluded that the proper harvesting time of sunflower seeds is 118 DAP since the above mentioned components as well as the oil contents are good enough to produce a high quality oil. Agronomically, this period is more acceptable.

ACKNOWLEDGMENT

Dr. Mosaad Soliman and Dr. M. El-Gazzar of NRC for the facilities offered during the collection of the seeds under investigation.

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(Recibido: Diciembre 1992)