# A Study on the Lipidic Fraction Extracted from High Oleic Sunflower Seeds (*Helianthus annuus* L.) during the Ripening Process

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The paper discusses the chemical composition of the lipidic fraction extracted from a high oleic sunflower cultivar during ripening. The oil content, the fatty acids, and the composition of unsaponifiable matter were studied. During the ripening there is an increase in the oleic and a decrease in the palmitic, linoleic, and linolenic acid contents. At the beginning of ripening, the presence of some unknown acidic compounds (of which the mass spectra are reported) was observed which were not present at harvest. Practically all the components of the unsaponifiable matter decreased during ripening. In particular, it can be observed that in the sterols fraction the ratio between $\Delta^5$-avenasterol and $\Delta^7$-stigmastenol is reversed during the ripening. During the ripening there is also a marked decrease in the aliphatic alcohols fraction, whereas a rise in the triterpenic alcohols can be observed.

**Keywords:** *Sunflower oil; Helianthus annuus; ripening; oleic acid; unsaponifiable matter; sterols*

By using genetic engineering techniques, it has been possible to obtain some so-called "high oleic" varieties of sunflower (Fernández San Juan, 1993; Haumann, 1994; Menichincheri et al., 1995; Robertson and Green, 1981; Skillicorn, 1994; Soldatov, 1976; Sunseri et al., 1995), producing oil with a fatty acid composition very similar to that of olive oil. An oil with this kind of composition is desirable for its well-known resistance to oxidation (Capella and Lercker, 1992; Dobarganes et al., 1993; Frankel, 1985) as well as for its dietary properties (Berra and Rapelli, 1987), but it offers the possibility having a low cost product fraudulently mixed with olive oil. This kind of fraud is often difficult to detect with conventional analytical methods (Gigliotti et al., 1993; Mariani et al., 1990, 1991, 1995).

The aim of this paper is to widen the knowledge of the chemical composition of the oil extracted from high oleic sunflower cultivar during ripening, including oil content, fatty acid composition, and unsaponifiable matter composition.

## EXPERIMENTAL PROCEDURES

The study was conducted on cultivar VYP 70 (Agra, Gruppo Ferruzzi, Italy). The seeds were cultivated in 1997 in the rural estate of the University of Florence at Montepaldi (S. Casciano, Firenze, Italy). The average minimum and maximum temperatures of the area during development of the seeds were 18.4 °C and 31.0 °C, respectively. Flowering occurred between 06/15 and 06/20. Samples were collected weekly between 06/26 and 08/02 and at harvesting (09/10). After harvest the seeds were cleaned and stored at 4 °C until use.

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**Table 1. Experimental Conditions Used in Gas Chromatographic Determinations**

| analytical parameters | fatty acids | fatty acids GC−MS EI and CI | unsaponifiable matter |
|---|---|---|---|
| column stationary phase | SP 2330[a] | Supelcowax 10[a] | CP-TAP CB[b] |
| column length (m) | 60 | 60 | 25 |
| internal diameter (mm) | 0.25 | 0.25 | 0.32 |
| film thickness ($\mu$m) | 0.25 | 0.12 | 0.1 |
| sample injection system | splitter | splitter | splitter |
| detection system | F.I.D. | ion trap | F.I.D. |
| carrier | He | He | He |
| column flow (mL/min) | 1.3 | 1.0 | 1.5 |
| split ratio | 1/60 | 1/60 | 1/80 |
| oven temperature initial (°C) | 130 (1 min) | 130 (1 min) | 200 (1 min) |
| oven temperature final (°C) | 240 (10 min) | 240 (10 min) | 300 (10 min) |
| temperature rate (°C/min) | 3 | 3 | 3 |
| injector temperature (°C) | 250 | 250 | 340 |
| detector temperature (°C) | 250 | 250 | 340 |
| reagent gas[c] | | methane | |
| pressure in the trap[c] (Pa) | | $2.6 \times 10^{-3}$ | |

[a] Supelco, Bellefonte, CA. [b] Chrompack, Middleburg, The Netherlands. [c] Only for chemical ionization analysis.

**Oil Extraction.** The seeds were oven-dried at 40 °C for 4 h in order to eliminate most of the moisture; they were then ground with a Janke & Kunkel (IKA Labortechnik, Staufen, Germany) water-cooled mill (at a temperature below 35 °C). The meal was extracted with *n*-hexane for 8 h in a Soxhlet-type extractor, after mixing with sodium sulfate anhydrous (3:1 w/w) to retain the residual moisture. Hexane was evaporated by distillation at reduced pressure (15 Torr) in a rotary evaporator, at a temperature below 40 °C, until a steady weight was reached. Crude extracts were then weighed.

**Analysis of Total Fatty Acids and Unsaponifiable Matter.** An aliquot of each oil sample was added with a 10%

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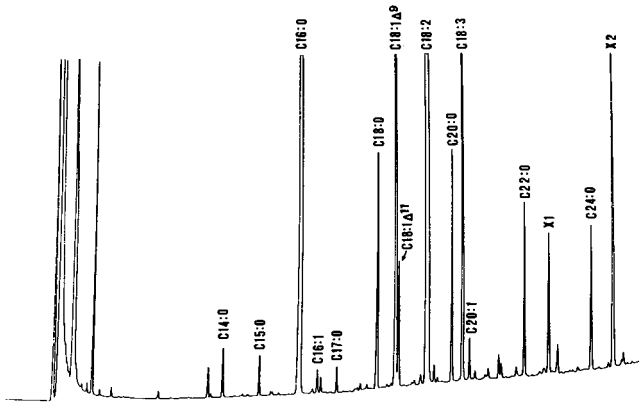


**Figure 1.** Gas chromatographic trace of the fatty acids methyl esters of the 06/26 sample. Peak identification is in Table 2.

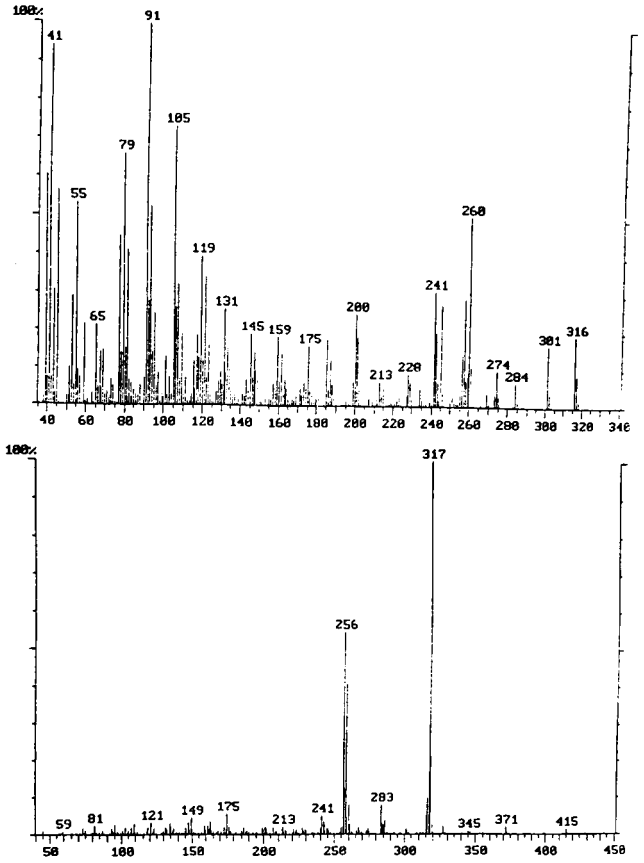


**Figure 2.** Mass spectra by electron impact (upper part) and chemical ionization (lower part) of component X1.

solution of squalane ($C_{30}H_{62}$, internal standard) in benzene (50 $\mu$L/g of oil) and then saponified according to the procedures detailed in Norme Grassi e Derivati (Stazione Sperimentale per le Industrie degli Olii e dei Grassi, 1976). The fatty acids were methylated by treatment with an ethereal solution of diazomethane ($CH_2N_2$) (prepared according to Fieser and Fieser, 1967) and analyzed by using a Mega 5160 gas chromatograph (Carlo Erba, Rodano, Italy) interfaced to a Mega 2 computing integrator, using the analytical conditions reported in Table 1. The fatty acids were purified by thin-layer chromatography (TLC) with silica gel G (Stratochrom SI, Carlo Erba), using *n*-hexane/diethyl ether 60/40 (v/v) as eluent. The analysis of total fatty acids was also performed by a GC−MS instrument (Finnigan Magnum, San Josè, CA). The instrumental parameters are described in Table 1. The whole unsaponifiable fraction, after treatment with diazomethane to transform any residual free fatty acids into methyl esters, was treated with a silanizing mixture to transform the hydroxyl

**Table 2. Oil Yield[a] (Percent of Dry Seeds) and Fatty Acid[b] Percentage[a] Composition of Lipidic Fraction Extracted from Starting Seeds and during the Ripening Process**

| sample | oil yield | 14:0 | 15:0 | 16:0 | 16:1 | 17:0 | 18:0 | 18:1$\Delta^9$ | 18:1$\Delta^{11}$ | 18:2 | 18:3 | 20:0 | 20:1 | 22:0 | X1 | 24:0 | X2 | other peaks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| seeds | | tr[c] | tr | 4.4 ± 0.1 | tr | tr | 3.8 ± 0.5 | 76.1 ± 0.7 | 0.2 ± 0.1 | 13.5 ± 0.2 | 0.1 ± 0.1 | 0.3 ± 0.1 | 0.2 ± 0.2 | 0.9 ± 0.2 | tr | 0.5 ± 0.1 | tr | tr |
| 06/26 | 1.9 ± 0.3 | 0.3 ± 0.1 | 0.3 ± 0.1 | 30.9 ± 1.1 | 0.2 ± 0.1 | 0.2 ± 0.0 | 2.0 ± 0.1 | 6.6 ± 0.2 | 1.2 ± 0.1 | 42.8 ± 0.6 | 5.5 ± 0.2 | 1.6 ± 0.1 | 0.3 ± 0.2 | 1.2 ± 0.1 | 1.2 ± 0.1 | 1.4 ± 0.1 | 3.2 ± 0.2 | 1.2 ± 0.1 |
| 07/05 | 1.7 ± 0.3 | 0.2 ± 0.1 | 0.2 ± 0.1 | 25.9 ± 0.3 | 0.1 ± 0.0 | 0.2 ± 0.1 | 3.9 ± 0.2 | 12.3 ± 0.2 | 1.2 ± 0.1 | 43.6 ± 0.6 | 5.5 ± 0.1 | 2.4 ± 0.2 | 0.4 ± 0.2 | 1.2 ± 0.1 | 0.3 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 | 0.8 ± 0.1 |
| 07/12 | 5.1 ± 0.4 | tr | 0.1 ± 0.1 | 13.4 ± 0.2 | 0.2 ± 0.1 | tr | 6.7 ± 0.1 | 48.9 ± 0.2 | 0.7 ± 0.1 | 23.3 ± 0.3 | 2.6 ± 0.1 | 1.5 ± 0.1 | 0.2 ± 0.1 | 1.0 ± 0.1 | 0.1 ± 0.1 | 0.7 ± 0.1 | 0.4 ± 0.1 | 0.2 ± 0.1 |
| 07/19 | 26.2 ± 1.1 | 0.1 ± 0.1 | tr | 6.5 ± 0.2 | 0.1 ± 0.0 | tr | 6.7 ± 0.1 | 55.8 ± 0.3 | 0.6 ± 0.1 | 28.0 ± 0.3 | 0.1 ± 0.1 | 0.6 ± 0.1 | 0.2 ± 0.2 | 0.8 ± 0.1 | 0.1 ± 0.0 | 0.3 ± 0.1 | 0.1 ± 0.1 | tr |
| 07/27 | 30.6 ± 1.6 | tr | tr | 4.7 ± 0.2 | tr | tr | 5.2 ± 0.1 | 70.2 ± 0.4 | 0.5 ± 0.1 | 17.6 ± 0.2 | 0.1 ± 0.0 | 0.4 ± 0.1 | 0.1 ± 0.1 | 0.8 ± 0.1 | tr | 0.4 ± 0.1 | tr | tr |
| 08/02 | 37.6 ± 1.0 | tr | tr | 5.0 ± 0.2 | tr | tr | 4.9 ± 0.1 | 74.4 ± 0.2 | 0.6 ± 0.1 | 13.4 ± 0.1 | tr | 0.4 ± 0.0 | 0.1 ± 0.1 | 0.8 ± 0.1 | tr | 0.3 ± 0.1 | tr | tr |
| 09/10 | 45.1 ± 1.6 | tr | tr | 4.3 ± 0.1 | tr | tr | 3.1 ± 0.1 | 76.4 ± 0.4 | 0.4 ± 0.1 | 13.6 ± 0.2 | 0.1 ± 0.1 | 0.2 ± 0.0 | 0.2 ± 0.2 | 0.8 ± 0.1 | tr | 0.8 ± 0.1 | tr | tr |

[a] Mean values and standard deviations from three determinations. [b] As methyl esters. 14:0 = myristic acid; 16:0 = palmitic acid; 16:1 = palmitoleic acid; 17:0 = heptadecanoic acid; 18:0 = stearic acid; 18:1$\Delta^9$ = oleic acid; 18:1$\Delta^{11}$ = *cis*-vaccenic (asclepic) acid; 18:2 = linoleic acid; 18:3 = linolenic acid; 20:0 = arachidic acid; 22:0 = behenic acid; 24:0 = lignoceric acid; X1, X2 = unknown. [c] tr < 0.1.

**Table 3. Unsaponifiable Content and Composition of the Unsaponifiable Fraction of High Oleic Sunflower Cultivar during Ripening Process**

| sample | fractions[a] (mg/100 g of oil[b]) 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | unsap. content (g/100 g oil[b]) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 06/26 | 308 ± 7 | 397 ± 6 | 276 ± 6 | 53 ± 2 | 82 ± 2 | 37 ± 2 | 410 ± 11 | 1197 ± 17 | 3437 ± 38 | 123 ± 3 | 445 ± 5 | 63 ± 2 | 55 ± 2 | 42 ± 3 | tr[c] | 21.0 ± 2.0 |
| 07/05 | 257 ± 5 | 548 ± 8 | 395 ± 12 | 63 ± 2 | 132 ± 3 | 62 ± 3 | 470 ± 15 | 1039 ± 15 | 3380 ± 41 | 154 ± 5 | 452 ± 7 | 88 ± 3 | 82 ± 3 | 83 ± 3 | tr | 23.3 ± 2.0 |
| 07/12 | 118 ± 3 | 260 ± 4 | 171 ± 4 | 60 ± 2 | 64 ± 2 | 63 ± 3 | 85 ± 3 | 190 ± 4 | 766 ± 21 | 78 ± 3 | 106 ± 2 | 77 ± 3 | 35 ± 2 | 39 ± 2 | 42 ± 3 | 4.9 ± 0.4 |
| 07/19 | 14 ± 2 | 31 ± 2 | 29 ± 2 | 58 ± 2 | 12 ± 1 | 58 ± 2 | 28 ± 1 | 67 ± 2 | 259 ± 8 | 57 ± 2 | 15 ± 1 | 35 ± 2 | 25 ± 2 | 17 ± 2 | 22 ± 2 | 2.0 ± 0.2 |
| 07/27 | 10 ± 1 | 19 ± 1 | 18 ± 2 | 54 ± 2 | 8 ± 1 | 55 ± 2 | 27 ± 1 | 48 ± 2 | 214 ± 9 | 36 ± 2 | 11 ± 1 | 32 ± 2 | 17 ± 2 | 17 ± 2 | 15 ± 2 | 1.4 ± 0.2 |
| 08/02 | 4 ± 1 | 14 ± 1 | 12 ± 1 | 40 ± 2 | 8 ± 1 | 42 ± 2 | 27 ± 1 | 46 ± 2 | 247 ± 10 | 42 ± 2 | 11 ± 1 | 48 ± 2 | 24 ± 2 | 25 ± 2 | 27 ± 3 | 1.5 ± 0.2 |
| 09/10 | 2 ± 1 | 9 ± 1 | 10 ± 1 | 17 ± 2 | 6 ± 1 | 41 ± 2 | 26 ± 1 | 33 ± 2 | 240 ± 7 | 34 ± 2 | 8 ± 1 | 49 ± 3 | 29 ± 3 | 27 ± 2 | 35 ± 3 | 1.3 ± 0.2 |

[a] Fractions: 2, docosanol; 3, tetracosanol; 4, hexacosanol; 5, squalene; 6, octacosanol; 7, α-tocopherol; 8, campesterol; 9, stigmasterol; 10, β-sitosterol; 11, unidentified; 12, $\Delta^5$-avenasterol; 13, $\Delta^7$-stigmastenol; 14, cycloartenol; 15, 24-methylencycloartanol; 16, citrostadienol. [b] Mean values and standard deviations from three determinations. [c] tr < 0.1

**Table 4. Percentage Composition[a] of the Sterol[b] Fractions of Oil Extracted from High Oleic Seeds during the Ripening**

| sample | 8 | 9 | 10 | 12 | 13 |
|---|---|---|---|---|---|
| 06/26 | 7.4 ± 0.1 | 21.6 ± 0.2 | 61.9 ± 0.2 | 8.0 ± 0.1 | 1.1 ± 0.1 |
| 07/05 | 8.7 ± 0.2 | 19.1 ± 0.1 | 62.3 ± 0.2 | 8.3 ± 0.1 | 1.6 ± 0.1 |
| 07/12 | 7.0 ± 0.1 | 15.5 ± 0.2 | 62.5 ± 0.2 | 8.7 ± 0.1 | 6.3 ± 0.1 |
| 07/19 | 7.0 ± 0.1 | 16.6 ± 0.2 | 64.2 ± 0.3 | 3.7 ± 0.1 | 8.6 ± 0.2 |
| 07/27 | 8.2 ± 0.1 | 14.4 ± 0.1 | 64.6 ± 0.3 | 3.2 ± 0.1 | 9.6 ± 0.2 |
| 08/02 | 7.1 ± 0.1 | 12.1 ± 0.1 | 65.2 ± 0.2 | 2.9 ± 0.1 | 12.7 ± 0.1 |
| 09/10 | 7.3 ± 0.1 | 9.2 ± 0.1 | 67.5 ± 0.5 | 2.3 ± 0.2 | 13.7 ± 0.3 |

[a] Mean values and standard deviations from three determinations. [b] As TMS derivatives. Peak identification is in Table 3.

groups into trimethylsilyl derivatives, according to Sweeley et al. (1963), and analyzed by gas chromatography with the instrument as previously described and with the conditions listed in Table 1. Peak identification was carried out by comparison of relative retention times with those of pure standards (supplied by Sigma Chemical Co., St. Louis, MO, and Supelco Inc., Bellefonte, CA) and by comparison with the results published in the literature (Frega et al., 1992).

## RESULTS AND DISCUSSION

In Tables 2 and 3 are reported the oil accumulation and the percentage content of the unsaponifiable matter of the oil extracted from sunflower seeds during ripening. The highest rate of oil deposition occurs in the period between 07/12 and 08/02. The percentage content of the unsaponifiable fraction of the oil decreases during ripening, particularly in the period of the maximum increase in oil. Afterward, from 08/02 to 09/10 the increase in oil content is more moderate.

The fatty acids composition varies widely during ripening (Table 2). Although the major changes were in the first stages of ripening, only at harvest is the fatty acid composition of the oil similar to that of the original seeds. Particularly, the main fatty acids, palmitic, linoleic, and linolenic decrease during ripening while oleic acid increases. Stearic acid shows a different behavior, with a maximum in the sample of 07/19. As reported in previous works (Garcés and Mancha, 1989; Garcés et al., 1989), one can observe that only when oil synthesis starts (~3 weeks after flowering) the oleic acid percentage markedly increases despite the linoleic acid percentage: in the early stages of ripening, the seeds have a fatty acid composition more similar to normal than high oleic varieties.

It is interesting to notice that the compounds indicated by X1 and X2, decrease during ripening and are practically undetectable at harvest. Figure 1 shows a gas chromatographic trace of the fatty acid methyl esters of the 06/26 sample. The compounds marked with "X1" and "X2" are methyl esters of acids without any other functional group; in fact, in preparative TLC they show the same retention factor ($R_f$) of fatty acids. Figures 2 and 3 show the mass spectra (electron impact and chemical ionization) of peak X1 and X2, respectively. GC−MS electron impact analysis shows that the two compounds have similar structures; in fact, the fragmentation patterns show the same type of ions differing mainly on the relative amounts. By comparing the GC−MS electron impact and chemical ionization spectra one may suppose that X1 and X2 compounds have a molecular weight of 316. Such compounds are esters of cyclic carboxylic acids, in fact they generate well evident molecular ions, with a loss of the carboxylic group (fragment mass = 257) and show the base peak at mass 91 (tropylium ion), characteristic of cyclic or aromatic compounds. Ions with mass 241, 213, and 185/187 are generated by the partial or total loss of the side chains of the molecules. At the current stage of the research it is difficult to establish the exact structure of the molecules; one may suppose that such compounds are isomers and have a structure similar to abietic {[1*R*-(1α,4a,β,4bα,10aα,)]-1,2,3,4,4a,4b,5,6,10,10a-decahydro-1,4a-dimethyl-7-(1-methylethyl)-1-phenanthrenecarboxylic acid) and pimaric {7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,4a,7-trimethyl-1-phenantrenecarboxylic acid} acids, typical components of rosin (Lange et al., 1994). In fact the GC−MS analysis of the methyl ester of pure abietic acid (supplied by Sigma Chemical Co., St. Louis, MO) shows a slightly different retention time and a similar fragmentation pattern. The compounds X1 and X2, present principally in the early

**Table 5. Percentage Composition[a] of the Alcohol[b] Fractions of Oil Extracted from High Oleic Seeds during the Ripening**

| sample | 2 | 3 | 4 | 6 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|
| 06/26 | 26.6 ± 0.2 | 34.2 ± 0.2 | 23.8 ± 0.2 | 7.1 ± 0.1 | 4.8 ± 0.1 | 3.6 ± 0.1 | tr |
| 07/05 | 17.2 ± 0.2 | 36.6 ± 0.2 | 26.4 ± 0.2 | 8.8 ± 0.2 | 5.5 ± 0.1 | 5.5 ± 0.1 | tr |
| 07/12 | 16.2 ± 0.2 | 35.7 ± 0.2 | 23.5 ± 0.2 | 8.8 ± 0.1 | 4.8 ± 0.1 | 5.3 ± 0.1 | 5.7 ± 0.1 |
| 07/19 | 9.1 ± 0.1 | 20.5 ± 0.2 | 19.4 ± 0.1 | 8.0 ± 0.2 | 16.8 ± 0.2 | 11.4 ± 0.1 | 14.8 ± 0.1 |
| 07/27 | 9.3 ± 0.1 | 18.5 ± 0.2 | 17.4 ± 0.1 | 7.7 ± 0.1 | 16.1 ± 0.2 | 16.4 ± 0.2 | 14.5 ± 0.1 |
| 08/02 | 3.8 ± 0.1 | 12.5 ± 0.1 | 10.4 ± 0.1 | 6.7 ± 0.1 | 21.3 ± 0.2 | 21.7 ± 0.2 | 23.7 ± 0.2 |
| 09/10 | 2.0 ± 0.2 | 7.7 ± 0.2 | 8.0 ± 0.1 | 5.3 ± 0.2 | 24.7 ± 0.3 | 23.1 ± 0.2 | 29.1 ± 0.3 |

[a] Mean values and standard deviations from three determinations. [b] As TMS derivatives. Peak identification is in Table 3.

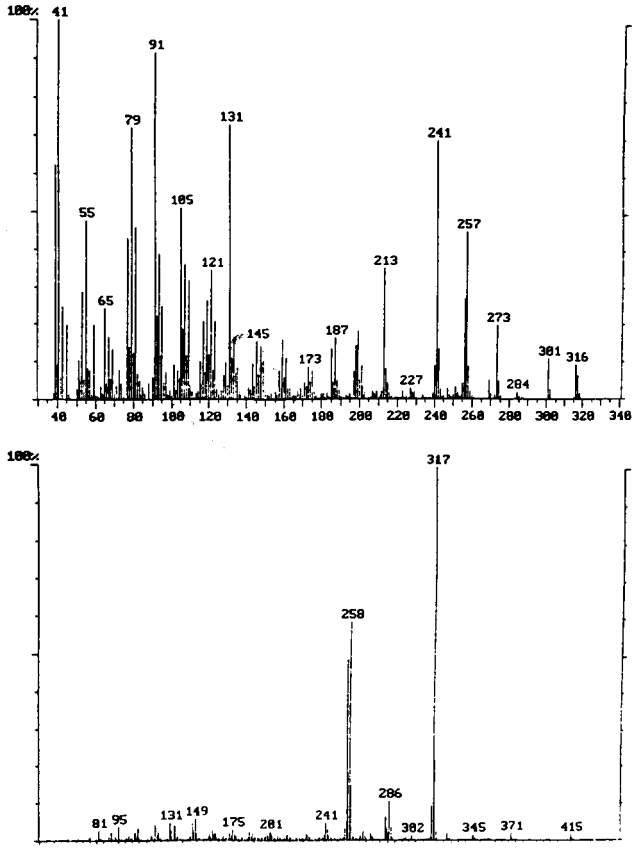


**Figure 3.** Mass spectra by electron impact (upper part) and chemical ionization (lower part) of component X2.

samples, when the seed is still in formation, probably originate from the integument which encloses the seed, which it is difficult to separate at this stage.

Table 3 shows the unsaponifiable matter composition during ripening. Citrostadienol is present at trace levels in the first and second samples. The principal variations are quantitative: all the components diminish as the seeds ripen. The percentage composition of sterols and alcohols fractions are more interesting. In the sterols fraction (Table 4) the main modification is the reversal of the ratio between $\Delta^5$-avenasterol and $\Delta^7$-stigmastenol. One can observe also a decrease in the stigmasterol content, while campesterol and $\beta$-sitosterol percentages are nearly steady. At harvest the composition of the sterols fraction is similar to high oleic sunflower data reported by other authors (Fernández San Juan, 1993; Purdy, 1986), and it is comparable to the one for normal sunflower oil, especially with regard to the $\Delta^7$-stigmastenol percentage. In fact genetic manipulation does not seem to be completely uninfluential on the sterolic fraction composition, usually considered the "fingerprint" of an oil. Conte et al. (1984) have found an inverse correlation between the percentages of oleic acid and $\Delta^7$-stigmastenol. A similar correlation was reported in the literature for another composite, safflower (Conte et al., 1983). Considering these data, even if they refer to different cultivars, we find the behavior of $\Delta^7$-stigmastenol during the maturation process rather singular.

Table 5 reports the percentage composition of the alcohols fraction of the unsaponifiable matter. During the ripening there is a marked decrease in the aliphatic alcohols fraction, whereas a rise in the triterpenic ones can be observed at the same time.

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