

# Wax Composition of Sunflower Seed Oils

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**ABSTRACT:** Waxes are natural components of sunflower oils, consisting mainly of esters of FA with fatty alcohols, that are partially removed in the winterization process during oil refining. The wax composition of sunflower seed as well as the influence of processing on the oil wax concentration was studied using capillary GLC. Sunflower oils obtained by solvent extraction from whole seed, dehulled seed, and seed hulls were analyzed and compared with commercial crude and refined oils. The main components of crude sunflower oil waxes were esters having carbon atom numbers between 36 and 48, with a high concentration in the  $C_{40}$ – $C_{42}$  fraction. Extracted oils showed higher concentrations of waxes than those obtained by pressing, especially in the higher m.w. fraction, but the wax content was not affected significantly by water degumming. The hull contribution to the sunflower oil wax content was higher than 40 wt%, resulting in a 75 wt % in the crystallized fraction. The oil wax content could be reduced appreciably by hexane washing or partial dehulling of the seed. Waxes in dewaxed and refined sunflower oils were mainly constituted by esters containing fewer than 42 carbon atoms, indicating that these were mostly soluble and remained in the oil after processing.

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**KEY WORDS:** Dewaxing, gas chromatography, sunflower oil, sunflower seed, waxes.

The quality and stability of vegetable oils such as sunflower oil are influenced by the presence of minor constituents such as waxes. Waxes are mainly esters of FA with fatty alcohols, having 36 to 50 carbon atoms. Waxes tend to crystallize and cause turbidity when the oil is cooled, interfering with oil processing and marketing. They are partially removed during refining in the winterization or dewaxing process, which is carried out in order to obtain a completely clear oil that is not affected by low storage temperatures.

Waxes are mainly located in the hull of sunflower seeds, in concentrations up to 3% depending on the hybrid and origin of the seed (1–3). Waxes are extracted with the oil in amounts that depend on the extent of dehulling and the extraction method, be it pressing or solvent extraction. As a result, the content of waxes in oil can change according to the seed variety, its origin and conservation, the percentage of

hull removed, and the temperature and technology used in the processing steps through which the oil was obtained (2–5). Therefore, the total wax content can vary between 0.02 and 0.35 wt% for crude sunflower oils and can be as high as 0.06 wt% for refined sunflower oils (1–7).

Waxes in sunflower oil are difficult to measure because of their low concentrations, the lack of uniformity in crystal formation, and the presence of other mucilaginous materials that tend to inhibit crystallization (2). There are different methods to measure total wax in oil and to evaluate the effectiveness of dewaxing process. However, techniques commonly used by the industry are inadequate and do not provide information on wax composition. Hence, there is a need for more sensitive and reproducible methods.

Early techniques, such as the cold test for refined oils, and the gravimetric method require long analytical times (8,9). Turbidimetric methods, which use the turbidity of cold oil as a measurement of the wax content, were developed and applied to crude and refined sunflower oils (1,6,7). Recently, a new method based on crystallization and optical measurement has been proposed to determine wax concentration in refined oil (10).

Other recent methods use column chromatography for the separation of the less polar fraction that contains the wax esters, followed by high-resolution GC. The chromatographic method has been used to analyze different vegetable oils (3) and has been adopted as the official method for olive oil by the European Economic Community (EEC) (11). An improvement of this EEC method simplifies the procedure by using a commercially available silica gel column and a chromatographic column with a different stationary phase (12). Lately, column chromatography has been replaced by liquid chromatography (LC) and a coupled LC–GC system has been used for simultaneous analysis of several minor components including waxes in vegetable oils (13,14).

## EXPERIMENTAL PROCEDURES

**Oil samples.** Two different seed lots were used to obtain two sets of laboratory sunflower oils. These oils were derived by hot hexane extraction of whole seed, of totally dehulled seed, and of seed hull. In addition, oil samples were extracted from both unwashed and hexane-washed seed in a Soxhlet apparatus with an extraction time of 4 h. Commercial crude sunflower oils from the different processing stages, and from the

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same seed lot—hot-pressed oil and hexane-extracted oil, both before and after degumming with water—were also analyzed. In addition, a laboratory-dewaxed oil and refined sunflower oils, purchased in a local supermarket, were used to study the effect of the refining process. A degummed sunflower oil was also used for wax composition analyses.

**Materials.** All reagents were analytical-reagent grade, except *n*-hexane, *n*-heptane and ethyl ether, which were chromatographic grade (J.T. Baker Inc., Phillipsburg, NJ). Silica gel 60, particle size 0.063–0.200 mm, 70–230 mesh (Merck, Darmstadt, Germany) was dried at 500°C for 4 h, hydrated with 2% of water, and stabilized for 12 h, prior to use in column chromatography. The following wax standards of almost 99% purity (Sigma Chemical Co., St. Louis, MO) were used for chromatographic analysis: C32 = lauric acid arachidyl ester ( $C_{32}H_{64}O_2$ ), C36 = stearic acid stearyl ester ( $C_{36}H_{72}O_2$ ), C38 = arachidic acid oleoyl ester ( $C_{38}H_{74}O_2$ ), C40 = arachidic acid arachidyl ester ( $C_{40}H_{80}O_2$ ), C42 = arachidic acid behenyl ester ( $C_{42}H_{84}O_2$ ), and C44 = behenic acid behenyl ester ( $C_{44}H_{88}O_2$ ).

Standards of FAME and fatty alcohols with a purity of almost 99% over a wide range of carbon atom number ( $C_{14}$ – $C_{30}$ ), were acquired from Sigma Chemical Co. A standard solution of pentacosanoic acid methyl ester in hexane (1 mg/mL) was used for FA quantification by the internal standard method. A standard solution of heneicosanol in hexane (1 mg/mL) was also employed in fatty alcohol analysis.

**Wax analyses.** The technique utilized is based on the ECC official method for olive oil (11) and consists of (i) heating the oil to 80°C, adding an internal standard ( $C_{32}$ ), and fractionating by chromatography on a hydrated silica gel column; (ii) recovering the first fraction eluted, whose polarity is lower than that of the TG, evaporating the solvent, and adding 500  $\mu$ L of *n*-heptane; (iii) performing an analysis by capillary GLC with an on-column injection system and FID.

The column chromatography was performed in a glass column (i.d. = 15 mm, length = 400 mm) with hydrated silica gel (15 g, 2% water content) as a solid stationary phase. Around 600 mg of oil, weighed exactly, 500  $\mu$ L of standard internal solution (0.02% of  $C_{32}$  in *n*-hexane), and a drop of a 1% solution of the dye sudan I in *n*-hexane were loaded onto the column with the aid of two 2-mL portions of *n*-hexane. Sudan I is used to indicate the end of the elution because it has a retention time between those of waxes and TG (15). The waxes were eluted with *n*-hexane/ethyl ether (8.5:1.5, vol/vol) at a flow rate of 3 mL/min.

A Varian 3700 gas chromatograph equipped with an FID detector and a temperature-programmable on-column injector was used for the final analysis. The capillary column was an HP5 (5% diphenyl and 95% dimethyl-polysiloxane), fused-silica 11 m length  $\times$  0.32 mm i.d., 0.52  $\mu$ m film thickness (Hewlett-Packard, Palo Alto, CA). The operating conditions were: hydrogen at 3 mL/min and a pressure 8 psig as carrier gas; oven temperature programming: initial temperature, 80°C, increase at 30°C/min to 200°C, hold for 1 min, increase at 3°C/min to 340°C, hold for 20 min; on-column in-

jector programmed from 80 to 320°C at 40°C/min and injection volume of 3  $\mu$ L; FID at 350°C and attenuation  $2 \times 10^{-12}$ . A recorder-integrator Millennium 2010 (Millipore Corporation, Milford, MA) was used for quantitation.

**FA and fatty alcohols separation.** The determination of the constituents of sunflower oil waxes required the saponification of the wax oil fraction, extraction of both saponifiable and unsaponifiable matter, and separation of alcohols in the unsaponifiable matter by TLC, followed by the analysis of the FA and separated alcohols by GC. The separation technique was based on the EEC method (16) with some modifications to secure a complete saponification and a good recovery of the analytes. Briefly, the wax oil fraction obtained by column chromatography was saponified with 4 mL of 2 N KOH for 6 h, followed by three washings with 4 mL of ethyl ether, and a subsequent washing of these joint fractions with three 5-mL portions of ethyl alcohol (50%). The aqueous alcohol fraction contains the FA and the ethyl ether fraction contains the fatty alcohols. In this step the corresponding internal standards were added. FA were converted to methyl esters by acid-catalyzed esterification according to IUPAC standard method 2.301 (9). Fatty alcohols were separated from other unsaponifiable matter by TLC chromatography as described in the EEC method (16). By applying this procedure to wax standards, recoveries higher than 90% were obtained for the different compounds.

**GC of FA:** FA composition was determined by GC analysis according to IUPAC standard method 2.302 (9). The FAME were separated on a SP-2380 [stabilized poly (90% bis(cyanopropyl)/10% cyanopropylphenylsiloxane)] fused-silica capillary column, 30 m length  $\times$  0.25 mm i.d., 0.25  $\mu$ m film thickness (Supelco, Inc., Bellefonte, PA) maintained at 170°C for 15 min and then increased at 4°C/min to 240°C (held for 10 min), using hydrogen as the carrier gas.

**GC of fatty alcohols.** Standard solutions of alcohols and alcohol samples were converted into trimethylsilyl ethers and analyzed according to EEC method (16). Fatty alcohols analysis was carried out on a SE-54 fused-silica capillary column (30 m  $\times$  0.25 mm i.d.) of film thickness 0.25  $\mu$ m (Supelco), increasing the temperature at 7°C/min from 170 to 300°C (held for 15 min), using hydrogen as gas carrier.

## RESULTS AND DISCUSSION

Figure 1 represents a typical chromatogram from crude sunflower oil wax. Adequate separation and resolution have been obtained for waxes up to  $C_{48}$  under the chromatographic conditions employed. The wax fraction in sunflower oil was found to range between 36 and 48 carbon atoms, in agreement with literature data (3). Peaks with retention times higher than  $C_{50}$  usually correspond to other compounds such as sterols, methylsterols, and terpenic alcohol esters (3,11,14).

Table 1 presents the averages for wax distributions of three replicates for two sets of sunflower oils obtained from whole and totally dehulled seed and seed hull. The results are expressed in relative percentages in the wax, and the total wax

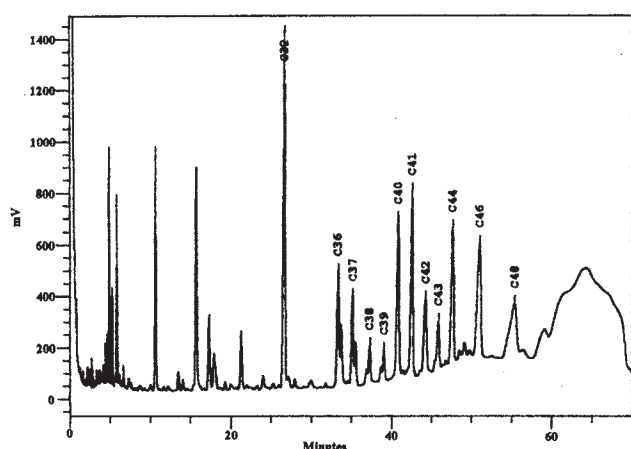


FIG. 1. Chromatogram of crude sunflower oil waxes.

content is given in ppm (mg of waxes per kg of oil) with its corresponding relative standard deviation (RSD). As the RSD are relatively low, the difference between sets could be due to the heterogeneity in seed composition. The wax contents of hull oils were higher than those obtained from dehulled seeds, and those extracted from the whole seed showed intermediate values. The total wax content of hull oils was around 23–26 times greater than that obtained from dehulled seed; the hull oils contained a higher concentration of the C44–C48 fraction. From a mass balance, the hull contribution to the wax content in sunflower oil proved to be higher than 40 wt%, reaching around 80 wt% when only the C44–C48 fraction was considered.

Figure 2 shows the GC wax profile in oils obtained from unwashed seed and hexane-washed seed under different operating conditions (contact time: 10 or 20 s., solvent temperature: 20 or 40°C). The washing procedure preferentially removed

the C<sub>44</sub>–C<sub>48</sub> fraction in a quantity that increased with time and temperature. After 20 s, approximately 30% of the total wax content had been extracted, reaching values between 65 and 75% with respect to the C<sub>44</sub>–C<sub>48</sub> fraction. The differences from a previous work (12), which reported more than 90% removal of crystallized waxes after washing with boiling hexane, can be attributed to the different seed and treatment conditions. The washing solvent was also analyzed, and the material removed was observed to consist mainly of waxes from C<sub>44</sub> to C<sub>48</sub>, with relatively low amounts of C<sub>40</sub> and C<sub>42</sub>.

Table 2 presents the results for one set of crude industrial sunflower oils obtained by either hot pressing or hexane extraction, both with and without water degumming, which are compared with the cold pressed oil obtained in the laboratory from the same seed lot. The total wax content in industrial oils was in the range of 950–1090 ppm. The higher percentages of waxes corresponded to the C<sub>40</sub>–C<sub>41</sub> and C<sub>36</sub>–C<sub>37</sub> fractions, followed by the C<sub>48</sub> and C<sub>46</sub> esters. Significant differences in the total wax content of oils obtained by extraction and by pressing have been reported, with a higher concentration of high M.W. waxes in extracted oils (2,3,14). In this work, the extracted oils contained nearly 13% more waxes than the pressed oils, but the differences in the wax profiles were slight except for the higher concentration of C<sub>48</sub> in hexane-extracted oils. These results are in agreement with those reported in a previous work (4), in which the water degumming also had little effect on wax content, the differences between crude and degummed oils being relatively low. On the other hand, the wax content decreased significantly (30%) in the case of laboratory cold-pressed oil. The effect, which could be attributed to the extraction temperature, was particularly important with respect to components with carbon atom numbers higher than 42, in which a reduction of up to 70% was recorded.

TABLE 1  
Wax Distribution in Sunflower Seed Oils (wt%)

Wax (carbon number)	Set 1 Hull	Set 2 Whole seed	Dehulled seed	Hull	Whole seed	Dehulled seed
36	2.7	8.5	14.5	1.5	11.6	16.1
37	1.7	7.0	11.1	0.6	8.5	11.2
38	1.9	2.9	4.6	0.7	3.4	4.9
39	0.8	2.6	4.2	0.2	3.0	4.2
40	3.6	10.0	16.5	2.3	12.5	18.0
41	2.2	11.5	19.4	0.8	13.7	18.8
42	10.0	7.1	6.6	7.7	7.0	6.3
43	1.9	3.5	5.3	1.1	4.3	4.9
44	24.4	14.1	3.4	26.6	10.9	1.9
45	2.5	2.3	1.8	2.2	2.0	1.7
46	24.4	15.9	4.6	30.0	11.9	3.1
48	23.9	14.6	8.0	26.3	11.2	8.9
Wax content <sup>a</sup> (mg/kg)	17,250	1,254	759	14,955	1,008	580
RSD <sup>a</sup> (%)	1.2	1.8	1.2	1.3	6.6	1.2

<sup>a</sup>Average wax content and relative SD ( $n = 3$ ).

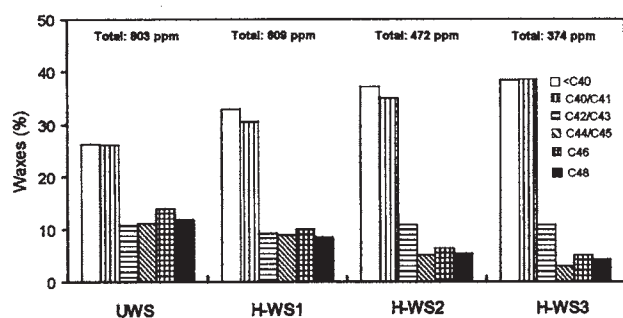


FIG. 2. Effect of the hexane washing of sunflower seed on the oil-wax content. Abbreviations: UWS = unwashed seed; H-WS1 = hexane-washed seed (10 s at 20°C); H-WS2 = hexane-washed seed (20 s at 20°C); H-WS3 = hexane-washed seed (20 s at 40°C).

The wax contents for three commercial refined oils are given in Table 3. The total wax content varied between 360 and 620 ppm, indicating a strong dependence on variety and condition of the seed and refining process. There is a high contribution of  $C_{40}$  and  $C_{41}$ , the waxes with 36 and 37 carbon atoms also being present in significant amounts. In all cases, the percentages for waxes with 44 or more carbon atoms are lower than 17%. Table 3 also compares the wax relative percentages of wax from a crude oil with its laboratory-dewaxed oil obtained by storage at 6°C over a week. Dewaxed oil exhibits a relatively high wax content, the main fraction corresponding to  $C_{40}$  and  $C_{41}$ . Most waxes with fewer than 44 carbon atoms are soluble and remain in the oil after processing.

A study of the wax constituents of crude sunflower oil was also carried out. Table 4 is a profile of waxes. FA and fatty alcohols from crude sunflower oil. FA were in the range of 14–30 carbon atoms with linoleic acid ( $C_{18:2}$ ), oleic acid ( $C_{18:1}$ ), behenic acid ( $C_{22:0}$ ) and palmitic acid ( $C_{16:0}$ ) being most prevalent. Fatty alcohol distribution was in the range of 16–32 carbon atoms, dominated by octadecanol

( $C_{18}$ ), nonadecanol ( $C_{19}$ ), and tetracosanol ( $C_{24}$ ) content. The relatively high quantity of nonadecanol and small quantities of other odd-carbon compounds can probably explain the presence of odd-carbon wax esters in sunflower oil. Several previous studies indicated that sunflower oil wax esters are composed mainly of FA in the range of 16–30 carbon atoms, especially  $C_{20}$  and  $C_{22}$ , with fatty alcohols between 20 and 32 carbon atoms, principally  $C_{24}$  and  $C_{26}$  alcohols (2,3,18).

This apparent discrepancy between our results and literature data on the FA and alcohol distributions can be explained in terms of the material analyzed. Many previous studies have been based on the precipitation or crystallization of wax fractions, which are composed mainly of even-carbon high M.W. esters. In this study, quantitative analyses of the total wax content in oil gave the following results: wax esters = 995 mg/kg, FA = 855 mg/kg, and fatty alcohols = 596 mg/kg. The relatively high quantity of FA could arise as a contribution from sterols, methylsterols, and terpenic alcohol esters. The average MW of wax constituents, calculated from compositions given in Table 4, were 288 and 321 kg/mol for acids and alcohols, respectively. This allowed the prediction of an average MW for the resulting waxes of 591 kg/mol, in close agreement with that estimated from experimental composition (approximately 609 kg/mol).

In conclusion, the described method allowed us to perform the qualitative and quantitative analysis of sunflower oil waxes, which are of use for oil characterization and quality control during oil processing. Sunflower oil waxes have carbon atom numbers between 36 and 48 and there is a significant contribution from odd-carbon atom waxes. Large quantities of waxes are present on the surface of the hull, and they can be partially removed by dehulling or solvent washing. Extracted oils have higher concentrations of waxes than those obtained by pressing, especially in the higher M.W. fraction. The extractability of waxes depends strongly on the extraction temperature. The wax content in crude sunflower oil is

TABLE 2  
Influence of Extraction and Degumming on Wax Content<sup>a</sup> in Sunflower Oils (wt %)

Wax (carbon number)	Pressed Lab – Cold	Hot pressed		Hexane extracted	
		—	Degummed	—	Degummed
36	12.2	9.8	10.0	9.1	8.8
37	14.0	11.3	11.3	10.2	10.4
38	3.9	2.9	3.1	3.1	3.1
39	3.9	3.9	3.8	3.6	3.3
40	15.3	11.8	11.6	10.7	10.9
41	23.6	18.7	18.6	16.6	16.3
42	8.0	7.4	7.1	6.5	7.3
43	6.0	5.5	5.3	4.9	5.2
44	3.3	7.3	7.7	8.7	8.7
45	0.6	0.9	1.4	1.1	1.0
46	3.6	10.1	10.1	11.2	11.9
48	5.7	10.2	10.0	14.2	13.1
Wax content (mg/kg)	771	947	955	1,073	1,088

<sup>a</sup>Average of two replicates.



**TABLE 3**  
**Comparison of Wax Content in Crude, Laboratory Dewaxed, and Commercial Refined Sunflower Oils (wt%)**

Wax (carbon number)	Crude	Laboratory dewaxed	Refined oils		
			1	2	3
36	13.0	15.8	10.5	9.7	13.1
37	9.2	12.4	12.1	9.9	20.0
38	4.2	5.0	3.7	1.9	4.6
39	3.4	4.3	4.8	2.1	2.2
40	11.5	15.4	14.3	22.9	13.7
41	14.3	18.0	24.9	35.3	28.1
42	7.5	7.5	6.4	4.3	4.1
43	4.8	5.9	7.2	3.1	3.6
44	7.0	2.7	0.8	1.1	1.9
45	—	—	1.8	1.2	1.6
46	11.2	5.3	4.2	2.1	3.0
48	13.9	7.7	9.3	6.4	4.1
Wax content (mg/kg)	995	735	624	516	366

**TABLE 4**  
**Composition of the Wax Fraction and Its FA and Fatty Alcohol Constituents in Crude Sunflower Oil**

Wax	(wt %)	FA	(wt%)	Fatty alcohol	(wt%)
C36	13.0	C14:0	1.4	C16	4.3
C37	9.2	C16:0	9.8	C18	23.1
C38	4.2	C16:1	1.2	C19	18.4
C39	3.4	C18:0	4.9	C20	2.0
C40	11.5	C18:1	18.6	C22	7.5
C41	14.3	C18:2	44.0	C23	0.6
C42	7.5	C18:3	1.3	C24	11.9
C43	4.8	C20:0	4.3	C25	1.8
C44	7.0	C20:1	1.0	C26	9.3
C46	11.2	C21:0	0.2	C27	0.5
C48	13.9	C22:0	9.7	C28	8.3
		C22:1	0.7	C29	0.6
		C24:0	0.1	C30	7.9
		C26:0	0.9	C32	3.8
		C27:0	0.2		
		C28:0	1.0		
		C29:0	0.3		
		C30:0	0.4		

not significantly affected by water degumming. The wax fraction of dewaxed and refined sunflower oils is composed mainly of esters containing less than 42 carbon atoms, indicating that these are retained in the oil.

## ACKNOWLEDGMENTS

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Notes to Authors

Re: J10150

1. In the byline, we would appreciate it if you will spell out the acronym PLAPIQUI.
2. Page 7 line 22 [of the manuscript]. Verify changes to “The **aqueous alcohol** fraction contains the FA and the ethyl **ether** fraction contains the fatty alcohols.”
3. Page 9, lines 15-17. Verify changes, especially “was around **23-26** times greater. . . .”
4. Table 2. Verify change of straddle head to “Hot pressed”.
5. Page 10, lines 11-14. This sentence is unclear. With respect to the crude industrial sunflower oils, are these oils that you yourselves prepared, using industrial procedures, in your laboratory? And then you took another portion of seed from the same seed lot and prepared cold pressed oil? Otherwise, how did you ensure that everything had been made using the same seed lot?
6. Page 12, lines 20-22. I do not understand “. . . analysis of sunflower oil waxes, which are of use for oil characterization . . . .” Perhaps do you mean “. . . sunflower oil wax qualitative and quantitative analyses, which are of use for oil characterization . . . .”? That is, the **analyses [= results of analyses]** are used for characterization, not the waxes themselves.
7. Reference 16. Verify addition of the word **of**.

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