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Sunflower-Oil Wax Reduction by Seed Solvent Washing

Erica R. Baümler · Guillermo H. Crapiste · Amalia A. Carelli

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Abstract Wax distribution in sunflower seeds was determined by capillary-gas chromatography, as well as both the wax composition in sunflower oils obtained from washed seeds and the wax composition in the solvent extracts. The dehulling efficiency was evaluated by using a laboratory centrifugal process. The washing effect on hull morphology and on wax distribution was observed by scanning-electron microscopy. Washing preferentially removed the crystallized fraction, hexane being the most effective solvent. Short contact times (20 s) at 25–40 °C were sufficient to extract the insoluble waxes by hexane washing. The extracted material consisted of C40–C54 waxes with higher percentages of extracted C44, C46 and C48. These are superficially in the hull of sunflower seed presenting a non-uniform distribution as observed by microscopy. Solvent washing with pre-heating of the seeds caused a decrease in sample moisture content, which reduced dehulling ability. Ethanol-washed seeds were the easiest to dehull, but higher production of fines was also observed. Solvent washing improves both the dehulling-seed ability increment and the recovery of sunflower waxes as a by-product for commercial use.

Keywords Dehulling ability · Dewaxing · Gas chromatography · Microscopy · Solvent washing · Sunflower oil · Sunflower seed · Waxes

E. R. Baümler · G. H. Crapiste · A. A. Carelli (✉)
Planta Piloto de Ingeniería Química (PLAPIQUI),
Universidad Nacional del Sur-Consejo Nacional de Investigaciones Científicas y Técnicas (UNS-CONICET),
Camino La Carrindanga km. 7, CC 717,
8000 Bahía Blanca, Argentina
e-mail: acarelli@plapiqui.edu.ar

Introduction

The cultivated sunflower (*Helianthus annuus* L.) is among the most important oil crops all over the world. The oil has excellent nutritional properties being high in linoleic acid [1]. Its quality and stability are affected by the presence of minor constituents, such as waxes. Waxes are mainly esters of fatty acids and fatty alcohols, having 36–50 carbon atoms [2]. Waxes tend to crystallize and cause turbidity when the oil is cooled, interfering with oil processing and marketing. Also, today, the trend is to replace the use of synthetic waxes with natural ones in a great variety of applications such as cosmetics, food and paper products, pharmaceutical uses, candles, polishes, matches, inks and pyrotechnics. This makes wax recovery promising [3].

Waxes are mainly located on the hull surface of sunflower seeds in concentrations up to 3% depending on the hybrid and the origin of the seed [2, 4]. The hull of oilseed types of sunflower comprises 21–30% of the total weight of the achene, with some hybrids having hull contents as low as 10%. There is an inverse correlation between the hull content and the wax content of the hull. Waxes probably prevent seed desiccation. The degree that the kernel is held close to the hull is increased in hybrid seeds [5]. As a result, hull removal is less efficient and a larger percentage of the hull remains attached to the kernel resulting in greater wax content in the oil.

Sunflower seeds may be dehulled before extraction, depending upon the design of the crushing plant. Dehulling reduces the movement of an unnecessary mass through the system. In addition, it reduces the oil wax content, the meal fiber content, and wear in screw presses [6]. In contrast, hulls obtained by mechanical decortication can contain more than 30 g kg^{-1} oil. Most of it probably results from

kernel adsorption or incomplete separation of the kernels from the hulls [6].

Waxes are extracted with the oil in amounts that depend on the extent of dehulling and the extraction method, i.e., by pressing or solvent extraction. As a result, the wax content in the oil can change according to the seed variety, its origin, the percentage of hull removed, and the temperature and technology used in the processing steps through which the oil was obtained [2, 7, 8].

The winterization of sunflower seed oil is quite difficult because of the low percentage of waxes in the oil and the presence of gums that coat the waxes and retard filtration. In Argentina, waxes are eliminated simultaneously with the phospholipids in the degumming process, but the wax is not suitable for sale. Alternatives to the classical winterization process would include more efficient hull removal or washing the seed with warm hexanes before crushing and extraction [9].

Extraction would alleviate the need to remove the hulls prior to oil extraction in order to reduce the wax content of the oil. If a high-protein, low-fiber meal however, were desired, hulls need to be removed. Solvent washing would also allow one to obtain a commercially useful by-product, called ''sunflower waxes''.

The aim of the present work was to investigate the effect of washing sunflower seed with a solvent on both the extracted-oil wax content and the ability to dehull the seed.

Experimental Procedures

Plant Material

The sunflower seeds, which were provided by a local crusher, cleaned and selected. Empty, immature or underdeveloped seeds were rejected. Their initial moisture content was determined by using the vacuum oven technique according to AOAC standard method 14003 [10].

Materials

All reagents were analytical-reagent grade, except for the *n*-hexane, *n*-heptane and ethyl ether used in wax analyses, 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 ($C_{44}H_{88}O_2$).

Waxes Extraction Assay

The seeds were superficially washed with analytical-reagent grade hexane at 25 and 55 °C for 40 s, and at 40 °C for 20, 40 and 60 s. For comparison, ethanol was also used as the washing solvent, but only at 40 °C for 40 s. The assays were carried out by preheating the seeds and solvent to the desired temperature. One assay (55 °C and 40 s.) was carried out by preheating the solvent alone to evaluate the effect of the seed temperature on the elimination of waxes. After evaporating the solvent, the extracted materials were stored for further analysis. All the treatments were performed in triplicate.

The washed seeds were ground and their oil extracted with *n*-hexane (bp 68–72 °C) in a Soxhlet apparatus following the IUPAC standard method 1.122 [11]. The solvent contained in the extracted oils was removed by a nitrogen stream. The oil was weighed to calculate the extraction yields and stored at 5 °C under nitrogen atmosphere for further analysis.

Wax Analyses

Wax composition of extracted oils was determined by separating on a silica gel chromatographic column and analysis by GC [2]. Briefly, the method consisted of (1) heating the oil to 80 °C, adding an internal standard (C32), and fractionating by chromatography on hydrated silica gel column; (2) recovering the first fraction eluted, whose polarity was lower than that of the triglycerides, evaporating the solvent, and adding *n*-heptane, (3) 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, L = 400 mm) with hydrated silica gel (15 g, 2% water content) as a solid stationary phase. About 600 mg of oil, 500 μL volume of standard internal solution (0.02% of C32 in *n*-hexane), and a drop of a 1% solution of the coloring Sudan I in *n*-hexane were loaded onto the column with the aid of two 2 mL portions of *n*-hexane. The waxes were eluted with *n*-hexane:ethyl ether (98.5:1.5 *v*/*v*) at a 3 mL min^{-1} flow rate.

For removing material from washed seed, column chromatography was omitted. The material was directly dissolved in *n*-heptane, filtered through an organic-solvent 0.5-μm filter to remove sand particles and analyzed by GC.

A Varian 3700 gas chromatograph, equipped with a FID and a temperature-programmable on-column injector (Varian Associates Inc., Palo Alto, CA), was used for the final analysis. The capillary column was an HP5

fused-silica 11 m length × 0.32 mm i.d., 0.52 μm film thickness (Hewlett Packard, Palo Alto, CA). The operating conditions were: 3 mL min^{-1} hydrogen flow rate and 8 psig pressure as carrier gas; oven temperature programming of 80 °C initial temperature 30 °C min^{-1} to 200 °C heating, 1 min holding, increasing at 3 °C min^{-1} to 340 °C, and holding for 20 min; on-column injector programmed from 80 to 320 °C at 40 °C min^{-1} and injection volume of 3 μL; FID at 350 °C and attenuation 2×10^{-12}. A recorder-integrator Millennium 2010 (Millipore Corporation, Milford, MA) was used for quantification.

Dehulling Ability

Pilot-plant equipment based on a centrifugal process with a rotation speed of 3,300 rpm was employed. The dehulling ability expressed as percentage hull removed mechanically to the total hull weight percentage in the seed. The total hull content was determined by manually dehulling 10 g seeds, while the hull content removed mechanically was also measured from 10 g of clean seed. The results are the means of three replications and are expressed in a dry basis (d.b.). In addition, the total quantity of fine meals was determined by sieving the dehulled samples to separate particles with <2 mm diameter.

Microscopy

The whole seeds were adhered to a cover slip, coated with a gold thin film in a sputter coater (Pelco 91000) and observed in a JEOL 35 CF scanning electron microscope at 5 kV using 100×–8,000× magnification.

Results and Discussion

The sunflower seeds contained 24.7% (d.b.) hulls, yielded 49.2% (d.b.) oil by extraction with hexane and contained 8.8% initial moisture. The hull and kernel oil yields were 2 (d.b.) and 64.8% (d.b.), respectively.

Table 1 presents the mean wax distribution of sunflower oils obtained from whole and totally dehulled seed and seed hull. The results are expressed in ppm (mg of waxes per kg of oil) with their corresponding relative standard deviation (RSD). The wax fraction in sunflower oil ranged between 36 and 48 carbon atoms, in agreement with literature data [2]. This fraction consisted mainly of fatty acid esters and fatty alcohols, being waxes with 36 and 40 carbon atoms. The waxes with 42 carbon atoms are the partially soluble fraction, and the waxes with more than 44 carbon atoms constitute the crystallized fraction. Compounds with retention times assignable to odd-carbon waxes, especially C41 were present. The relatively high quantities of these compounds can probably be explained by the identification of nonadecanol (C19) and other odd-carbon alcohols as constituents of sunflower oil waxes [2]. The total wax contents of hull oils were 58 and 31 times greater than those obtained from dehulled seed and whole seed, respectively. The whole seed oil contained 37% of the crystallized fraction, while the hull oil and dehulled seed oil contained 78 and 12% of this fraction, respectively. In accordance with previous studies, the hull contribution to the wax content in sunflower oil proved to be higher than 37%, reaching around 80% when only the C44–C48 fraction was considered [2]. Relatively low RSDs were obtained in the oil analysis from the whole seeds and dehulled seeds. In contrast, the wax content in the hull oil showed higher RSD that was attributed to differences in the percentages of kernel attached with the hull.

Table 1 Wax distribution in sunflower seed oils (wt%)

Wax (carbon number)	Whole seed	Dehulled seed	Hull
34	0.4	0.8	1.1
36	9.8	16.5	1.9
37	5.2	8.9	0.4
38	2.6	4.6	1.1
39	1.8	3.2	0.3
40	14.2	23.3	2.9
41	12.1	20.2	0.5
42	7.4	5.9	12.3
43	2.8	4.2	1.0
44	14.0	1.9	29.1
45	1.7	1.3	2.1
46	17.3	3.4	30.0
47	0.8	0.2	1.3
48	9.9	5.6	16.0
Wax content[a] (mg kg^{-1})	1,119	593	34,662
RSD[a] (%)	7.3	3.9	19.6

[a] Average wax content and relative SD (n = 3)

The oil content of seeds after solvent washing was around 49.2%, the value being similar to the initial oil seed content. This indicated that little oil was removed with the solvent. Figure 1 shows wax chromatograms of sunflower oil obtained from hexane-washed seeds and from the respective solvent extract. When the wax fraction from the seed oil was analyzed, waxes with more than 48 carbon atoms could not be detected because sterols, methylsterols, and terpenic alcohol esters peaks were also present. In contrast, the material removed by solvent washing did not have these compounds, allowing the identification of waxes up to C54. Due to the purity of this material, it could be analyzed directly by gas chromatography. It consisted

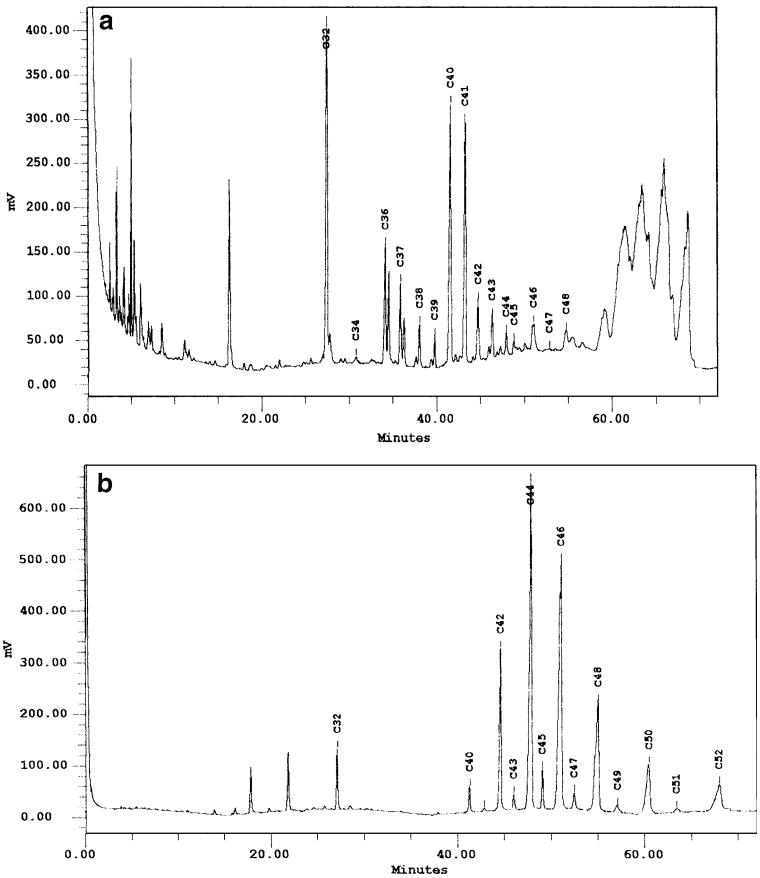

Fig. 1 **a** Chromatogram of crude sunflower oil waxes from hexane-washed seed (40 °C–40 s), **b** chromatogram of removal waxes from hexane-washed seed (40 °C–40 s)

mainly of even-numbered waxes in the range of 40–54 carbon atoms. The quality presented opportunities for their use. Table 2 presents the wax distribution in the removal material. When hexane was the solvent, the residues consisted mainly of crystallized waxes, corresponding to the higher percentages to C44, C46 and C48. A more uniform composition with around 30% of soluble and partially soluble waxes was obtained with ethanol. This residue

Table 2 Wax distribution in the removal materials from solvent washing of sunflower seed (wt%)

Waxes (carbon number)	Hexane[a]					Ethanol[a]	Hexane[b]
	25 °C–40 s	40 °C–20 s	40 °C–40 s	40 °C–60 s	55 °C–40 s	40 °C–40 s	55 °C–40 s
<40	0	0	0	0	0	9.2	0
40	1.9	1.3	1.1	1	1.1	6.9	1.6
41	0.2	0.3	0.2	0.2	0	0	0
42	10.0	9.3	7.4	8.5	7.6	13.4	8.8
43	1.1	1.2	0.9	1	1	0.8	0.9
44	26.5	25.6	22.7	25.9	24.3	22.1	22.0
45	2.1	2.6	2.4	2.4	2.6	1.5	2
46	25.7	25.6	26.1	27.5	26.1	18.5	22.4
47	1.4	1.8	1.8	1.6	1.8	0.9	1.5
48	13.9	13.5	15.3	14.4	14.4	11.9	14.2
49	0.8	1.1	1.2	1	1.1	0	1.1
50	8.1	7.6	9.2	7.6	8.4	12.5	10.5
51	0.7	0.9	1	0.8	0.9	0	0.9
52	4.5	5.6	6.7	5.1	5.8	2.2	6.5
53	0.4	0.6	0.7	0.5	0.6	0	0.7
54	2.7	3	3.3	2.5	4.3	0	6.9

[a] With seed preheating

[b] Without seed preheating

Table 3 Effect of the solvent washing of sunflower seed on the oil-wax content (wt%)

Waxes (carbon number)	Hexane[a]					Ethanol[a]	Hexane[b]
	25 °C–40 s	40 °C–20 s	40 °C–40 s	40 °C–60 s	55 °C–40 s	40 °C–40 s	55 °C–40 s
34	0.6	0.7	0.8	0.7	0.5	0.6	0.9
36	14.5	15.2	15.2	15.3	13.4	11.6	13.2
37	9.7	8.9	9.1	9.6	9.5	6.7	12.1
38	4.3	4.5	4.3	5.1	4.2	3.4	4.3
39	3.5	3.4	3.1	3.3	3.7	2.4	4.4
40	18.7	22.8	22.1	21.6	18.8	16.6	15.5
41	19.1	19.0	20.6	19.4	19.1	14.3	22.6
42	6.1	8.3	6.0	6.6	6.4	7.6	6.6
43	4.8	4.5	4.3	4.3	4.9	3.4	6.3
44	3.9	2.3	2.8	2.7	4.1	10.0	2.3
45	1.5	1.5	1.5	2.0	1.9	1.5	1.2
46	5.6	3.5	4.2	2.7	5.0	11.8	3.4
47	0.3	0.1	0.3	0.2	0.5	0.6	0.0
48	7.4	5.3	5.7	6.5	8.0	9.5	7.2
Wax content[c] (mg kg^{-1})	622	684	670	602	739	887	653
RSD[c] (%)	12.3	4.4	3.2	9.8	11.4	4.9	7.4

[a] With seed preheating

[b] Without seed preheating

[c] Average wax content and relative SD (n = 3)

consisted in C34–C52 waxes, being waxes with 42, 44, 46, 48 and 50 carbon atoms in percentages higher than 10%.

The solvent-washing effect on the wax composition of the seed oil is shown in Table 3. The washing procedure preferentially removed the crystallized fraction, with hexane being the most effective solvent. Hexane washing extracted approximately 40% of the total wax content, while ethanol washing removed only 21%. When the C44–C48 fraction was only considered, these values increased to 80 and 40%, respectively. Furthermore, it should be taken into account that the recovery of the alcohols for reuse demands more energy, as their latent heats of vaporization are much higher than those of hydrocarbons. About 39% of the total wax content was extracted by hexane-washing at 40 °C after 20 s, reaching values of 40 and 46% when the contact time increased to 40 and 60 s, respectively. When the crystallized fraction was only taken into account, the percentage of extracted waxes reached values of 80%. This finding indicated that 20 s was sufficient to efficiently extract the insoluble waxes.

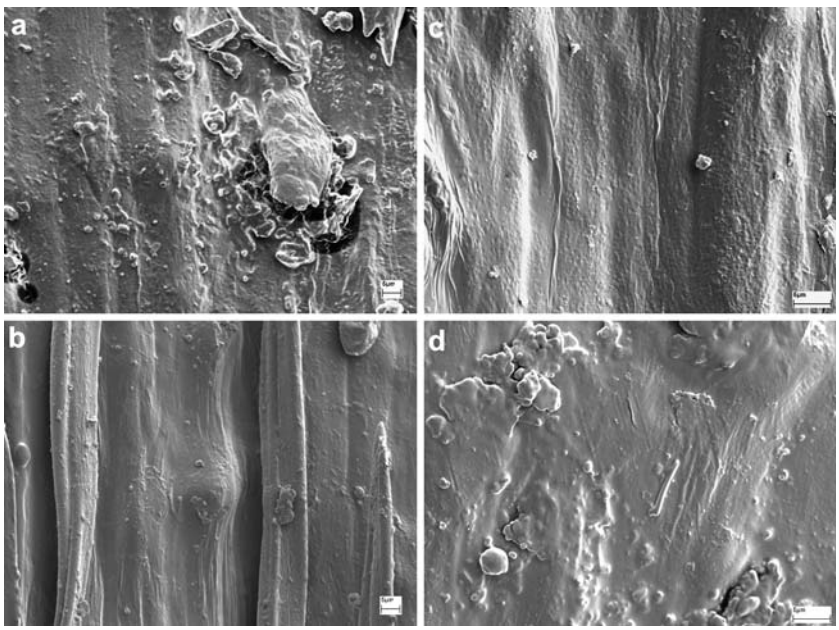

Fig. 2 Scanning-electron microscopy of sunflower seed. **a** Unwashed seed (2,500×), **b** heated seed to 55 °C (2,500×), **c** hexane-washed seed to 25 °C (5,000×), **d** hexane-washed seed to 55 °C (5,000×)

We observed the major waxes were removed at low temperatures (25 and 40 °C) being on the order of 80% of crystallized waxes. The yield declined to 71% when both seeds and hexane were heated to 55 °C before contact. In contrast, the crystallized-fraction extraction yield gave 81% when only the solvent was preheated to 55 °C, the total wax fraction removed was around 42%.

This behavior could be explained through the microphotography analysis. By scanning-electron microscopy (Fig. 2), the wax distribution and the washing effect on hull morphology was observed. Figure 2a shows an unwashed seed with a non-uniform wax distribution located superficially on the hull with a granular appearance. Figure 2b belongs to seeds heated at 55 °C presenting a uniform layer of waxes, which may be attributed to structural changes suffered by the amorphous area of the waxes with the increment of temperature [12]. X-ray diffraction studies suggested that the hydrocarbon chains in the crystalline parts of cuticular wax are assembled in an orthorhombic crystal lattice at low temperatures and in an hexagonal one at high temperatures, just below the melting point. Components of cuticular waxes unincorporated into the orthorhombic crystal lattice constitute another amorphous zone. This zone may consist of solid and mobile (liquid) amorphous fractions, depending on chemical composition and temperature. The phase transition from amorphous solid to amorphous liquid may be induced by the action of certain compounds. These considerations indicated that the amorphous fraction of a wax is a conglomerate with locally varying solubilities and mobilities [12]. Figure 2c and d show the washing effect on hull morphology at 25 and 55 °C, larger amounts of waxes can be observed in the corresponding to 55 °C. This result was attributed to a change of solubility that the amorphous waxes present owing to variations of temperature [12].

Unwashed seed presented 30% hull removal. Solvent washing with seed preheating caused around 20–30% decrease in seed moisture content. This moisture reduction increased dehulling ability to values in the range of 41–52%. In contrast, the initial seed moisture content did not change for the test performed at 55 °C and 40 s without seed preheating. However, in this case, the dehulling ability increased to 38%, indicating that the content of superficial waxes could also affect this property.

The highest value of dehulling ability (55%) was obtained in seeds washed with ethanol, but with more fines production (3.6%). In seeds washed with hexane at the same conditions, these values were 46% dehulling ability and 2% fines production.

In conclusion, a previous seed washing with hexane for short times reduces the wax oil content and improves seed dehulling efficiency. This practice may reduce processing losses and refining costs. Moreover, this practice would allow the recovery of sunflower waxes as a by-product.

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