Open Pollinated and Hybrid Sunflower Seed Structures That May Affect Processing for Oil

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ABSTRACT

Oil and wax contents of confectionery (non-oil) and oilseed, open-pollinated and hybrid sunflower seed and their hulls, testae, and kernels were determined. Wax content inversely correlated with hull content, and the degree to which the seeds were compressed correlated with hull thickness. Light and electron microscopy showed increased connective tissue in the hybrid seed. This combination of higher wax content in the dehulled hybrids and increased connective tissue between hull and kernel could explain the reports of high wax content of the oil from newly developed hybrid seed.

INTRODUCTION

Hybrid sunflowers that are replacing the open-pollinated varieties have improved plant uniformity, yields and disease resistance (1). Breeding studies have been directed toward increasing oil yield by reducing the hull content of the seed (2,3). The wax content should also be reduced because the principal source of wax is the hull. However, the wax content of the oil depends on several factors: the efficiency of decortication, the percentage of waxy material in the pericarp, and the method of obtaining the oil (4).

There have been occasional reports of increased wax content in oil from hybrid seed as compared to oil from the open-pollinated varieties. We report the results of both physical and chemical studies on open-pollinated and hybrid, oil-type confectionery (non-oil) sunflower seed and the waxes associated with them.

MATERIALS AND METHODS

Two open-pollinated, high-oil varieties (Peredovik and Sputnik), grown in Watertown, SD, 2 high-oil hybrids (Interstate 903 and 3113) and one confectionery (non-oil) hybrid (Interstate 923) grown in Fargo, ND, were tested. Seed were hand-separated into hull, testa (the thin material or seed coat which covers the kernel), and testa-free kernel. Oil was obtained from each fraction and whole seed by grinding with Hyflo Super Cel in a Krups 75 grinder and extracting for 24 hr with petroleum ether in a Soxhlet apparatus. Wax content was determined by a gas liquid chromatographic (GLC) procedure which measures the amount of long chain alcohols produced upon hydrolysis of the wax esters (5). Lignin and acid detergent fiber (6) were also determined. Cracking force was measured with an Instron TM-SM equipped with an automatic integrator and a 100-kg compression load cell was used to compress samples for objective evaluation. The Instron was set at 20-kg full-scale load, crosshead speed at 10 cm/min, and chart speed of 50 cm/min. The seed were placed in the Instron so that the force was applied to opposite edges of the seed. The force needed to crack the seed and the degree of compressibility for 10 seeds were averaged. Compressibility was calculated by dividing the width of the seed at the instant of cracking by width of the seed before cracking.

For microscopic examination of the hull, cross sections and longitudinal sections of the whole seed were sliced with a razor blade. While the in situ relationship of hull to kernel was maintained, these sections were mounted on a light microscope slide, stained for lignin with acid phloroglucinol (AP) (7), and examined with a stereoscopic microscope. Thin sections for examination of the hull were excised as 3- to 4-mm squares from the center of the seed. From this area, freehand sections were sliced with a new razor blade that had been cleaned with acetone. Cross sections to the longitudinal plane of the hull were prepared for light microscopy by staining for lignin with acid phloroglucinol or chlorine-sulfite (7). Stained cross sections from each of 10 seeds/variety (5 representative sites/seed) were measured with a calibrated eye-piece micrometer for width of hull and AP layer. Longitudinal and cross sections of 903 and 3113 seeds were also prepared by scanning electron microscopy (SEM). Sections were adhered to SEM stubs with glue, coated with gold-palladium (60:40) alloy, and observed in a JEOL, JSM-35 scanning electron microscope at 15 kV.

RESULTS AND DISCUSSION

The compositions of the seed are shown in Table I. As expected, the confectionery hybrid, Interstate 923, had the

TABLE I

Composition of Sunflower Seed

Hybrid or variety	Physical structure[a]			Oil (%)			Wax (%)		
	Hull (%)	Testa (%)	Kernel (%)	Whole seed	Hull	Testa	Hull	Oil (actual)[b]	Oil (expected)[c]
Interstate 923	41.8	2.4	55.8	28.4	0.5	6.3	0.021	0.015	0.031
Peredovik	21.6	2.4	76.0	47.6	1.2	11.3	0.097	0.030	0.049
Sputnik	20.1	2.2	77.7	48.9	0.9	16.8	0.099	0.027	0.040
Interstate 903	22.3	2.4	75.3	42.7	2.7	11.7	0.092	0.026	0.048
Interstate 3113	17.1	3.2	79.8	48.0	1.4	13.9	0.147	0.035	0.052

[a]Percentage dry weight basis of whole seed.

[b]Wax content of oil extracted from the whole seed.

[c]Calculated wax content of the oil from wax content of the hull.

largest percentage of hull and the smallest amount of kernel and oil. Peredovik, Sputnik, and Interstate 903 contained about the same amount of hull and kernel, with Sputnik containing the most oil. Interstate 3113 contained the least amount of hull and the largest amount of kernel. The amounts of testa were about the same for all samples, except for 3113 which contained 3.2% compared to 2.2-2.4% for the other samples.

No wax was found in the testa or kernel. The principal source of wax is the hull. Because of its low hull content, 3113 would be expected to contain oil with the least amount of wax (Table I). However, 3113 had the largest percentage of wax in the oil as well as in the hull. Interstate 923 had the lowest percentage of wax. The amount of wax in the extracted oil was always less than the calculated amount based on the wax content of the hull, probably because of incomplete extraction of the waxes from the whole seed. There was a high negative correlation ($r^2 = .95$) between the amount of wax in the hull and the log of the hull content of the seed. This suggests that seed with a lower percentage of hull, and thus, less fibrous tissue to protect the kernel from dehydration, produce increased amounts of wax.

Micrographs of longitudinal and cross sections of seeds showed the expected results (8) in that confectionery hybrid 923 had a loose kernel within a thick hull (Fig. 1) whereas both open-pollinated and hybrid, oil-type varieties had thinner hulls that more tightly surrounded the kernels (Figs. 2-5). Furthermore, 923 had a larger air cavity than the high-oil varieties and had little of the fibrous material connecting kernel and hull compared to the oil-type varieties. The resolution and depth of field of SEM shows these papery layers of the testa in 903 and 3113 and the connection of kernel to hull (Figs. 6 and 7).

Seeds of the oil-type varieties sectioned longitudinally through the top, i.e., the scar of disk flower attachment, showed fibers that gave a strongly positive reaction for lignin when tested with acid phloroglucinol (Figs. 2-5). Lignin is a polymer of phenylpropanoid units that gives stability and rigidity to plant cell walls and the connective fibers within these seed coats. In 3113, these fibers appeared to bind the kernel and hull. Scanning electron microscopy shows typical arrangements of the kernel at the top of the seed pericarp for 903 and 3113 (Figs. 6 and 7). In 3113, the kernel is tightly associated with the acid phloroglucinol-positive connective fibers of the top of the seed, whereas the kernel of 903 is separated from this section and the connective fibers. Indeed, upon hand crushing, kernels of 3113 remained associated with their hulls and the region of those connective fibers, but the hulls of 903 tended to separate more cleanly.

Seeds of all hybrids and varieties had a region of thick wall cells just underneath the epidermis that were thicker

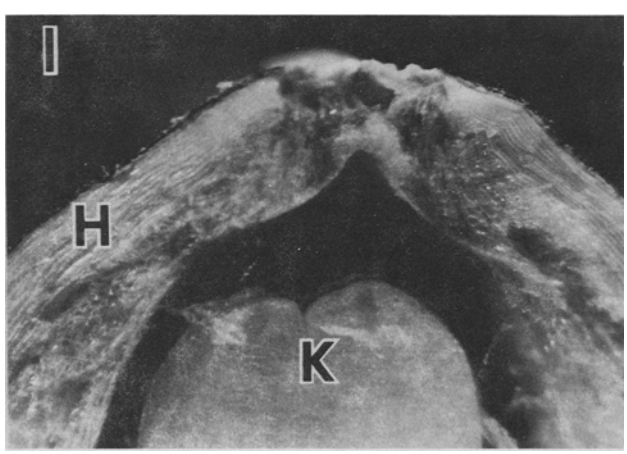

FIG. 1. Light micrograph of confection-type 923 seed, longitudinal section. The thick hull (H) encloses a detached kernel (K) with little connective tissue between hull and kernel (× 51).

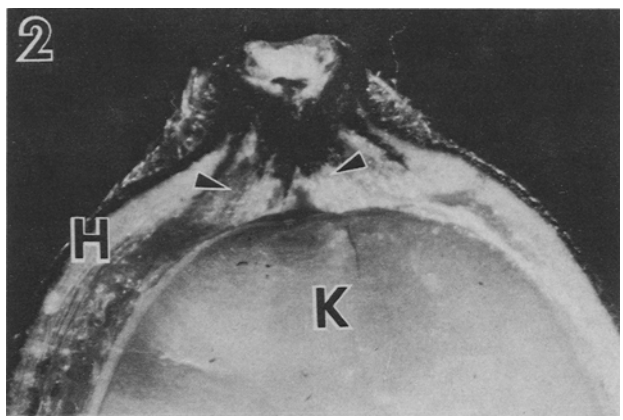

FIG. 2. Light micrograph of open-pollinated, oil-type Peredovik seed. Hull (H) and other tissue tightly surrounds the kernel (K). Connective-type fibers (arrows) in the hilum extend to the kernel (× 51).

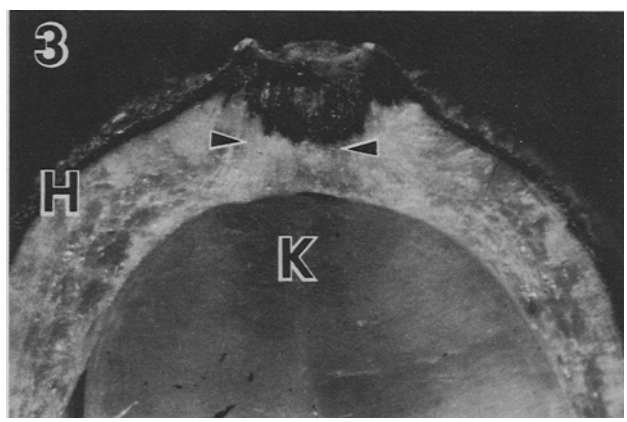

FIG. 3. Light micrograph of open-pollinated, oil-type Sputnik seeds. Hull (H) and other tissues tightly surround the kernel (K). Connective-type fibers (arrows) in the top extend to the kernel (× 51).

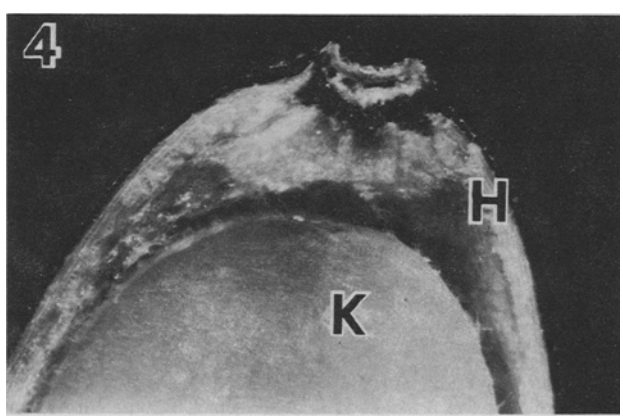

FIG. 4. Light micrograph of hybrid, oil-type 903 seed. Hull (H) is not tightly associated with the kernel (K) at the top of the seed (× 51).

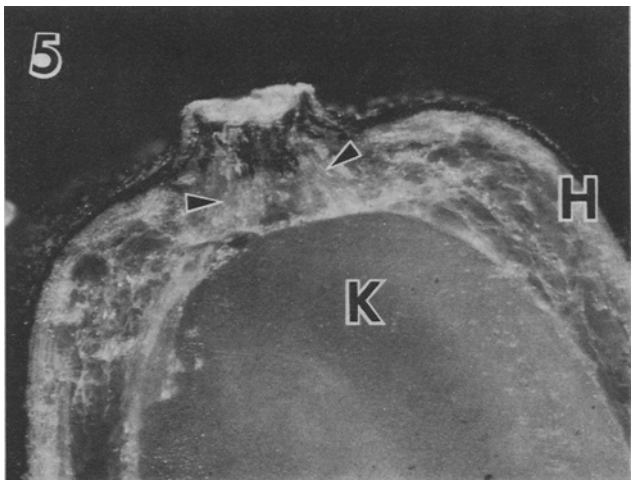

FIG. 5. Light micrograph of hybrid, oil-type Interstate 3113 seed. Hull (H) and other tissue is tightly associated with the kernel. Connective fibers (arrows) in the top extend to the kernel (X 51).

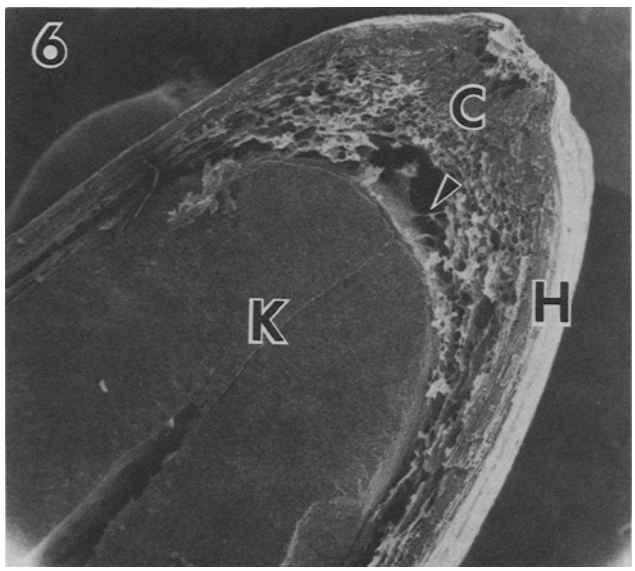

FIG. 6. Scanning electron micrograph of Interstate 903. Connective-type fibers (C) in the hilum are present, but the kernel and hull are not attached at this point. Papery layers of cells are present between the kernel (K) and hull (H) and are associated with kernel (arrow) beneath the plane of the section (X 20).

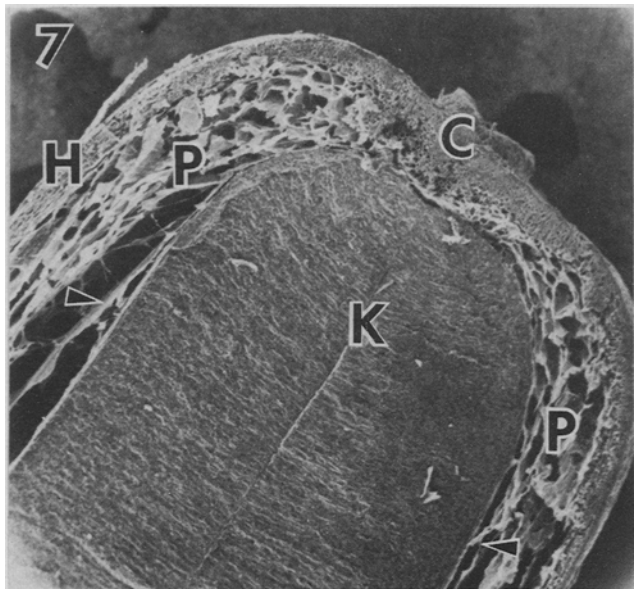

FIG. 7. Scanning electron micrograph of Interstate 3113. Connective fibers (C) are seen at the top of seed and are associated with the papery layers of cells and the testa. Extensive amounts of those papery (P) layers are present between the kernel (K) and hull (H) and are associated with the kernel (arrows) (X 20).

walled and more highly lignified than other cells of the hull. Table II shows the average thickness of the hulls of 10 different seeds of each type under investigation as well as the thickness of the lignified portion (AP layer) of the hull. Peredovik, Sputnik, and 923 had acid-phloroglucinol-stained, lignified layers of sclerenchyma nearly twice as thick as those of the 2 hybrid varieties, 903 and 3113, even though the overall thickness for all but 923 were about the same. The amount of lignin in these hulls is about the same for all varieties. However, 3113 had slightly less lignin and considerably less acid detergent fiber than did the other varieties (Table II). Because the lignified layer is much thinner in 903 and 3113 than in the other seed, it might be expected that seed of these hybrids would not be as rigid and thus be more difficult to crack because of the seeds' resiliencies.

In tests of force required to crack sunflower seed and the percentage compression before cracking (Table II), 903 and 3113 needed slightly less force and showed more compressibility than the open pollinated varieties and confectionery seed. The hybrid oil seed appeared more flexible

TABLE II

Physical and Chemical Evaluations of Sunflower Seeds and Hulls

Hybrid or variety	Thickness (mm) of hull and lignified layer (AP-positive)		Fiber composition		Cracking force (kg)	% Seed compression[b]
	Total hull	AP layer[a]	% Acid detergent fiber	% Lignin		
Interstate 923	647 ± 108	98 ± 17	65.7	19.4	4.28	13.1[a,c]
Peredovik	320 ± 56	105 ± 25	62.2	20.4	4.23	20.9[b,c]
Sputnik	283 ± 92	110 ± 47	61.1	21.2	4.68	16.8[b,c]
Interstate 903	201 ± 29	53 ± 9	62.8	21.3	4.08	23.6[b]
Interstate 3113	253 ± 42	57 ± 19	57.8	18.3	3.37	25.6[b]

[a]Acid phloroglucinol positive.

[b]Those percentages with different superscripts showed significant differences at the 5% level.

than did the open-pollinated or confectionery seed having a compressibility of about 24% as compared to 13% for the confectionery seed. Regression analysis with a simple log expression showed that compressibility correlated negatively (r^2 = 0.69) with hull thickness. Although the varietal characteristics that influence dehulling appear complex, our data suggest that the adherence of the hull to the kernel, the width of the hull, and thickness of the lignified layer of the hull could affect the dehulling process.

Complete separation of hull and kernel is neither practical nor advisable if the seed is to be prepressed and solvent-extracted. The presence of some hull improves extraction. The increased wax content in the hull of the hybrid seed, along with possibly more of the hull fragments being held to the kernel by fibrous material, probably accounts for the increased wax content of the oil from newly introduced hybrids.

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Fatty Acids of Canola *Brassica campestris* var Candle Seed and Oils at Various Stages of Refining

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ABSTRACT

Fatty acids of oil of a current variety of canola *Brassica campestris* var Candle, at 3 stages of commercial production and refining, were compared with authentic seed oils, and with the oil of *B. napus* var Tower. The proportion of *cis*-9, *cis*-12, *trans*-15 and *trans*-9, *cis*-12, *cis*-15-octadecatrienoates relative to the all-*cis* isomer was lower than that previously observed in processed oils. The minor C_{14}, C_{15}, C_{17} fatty acids previously documented for Tower were also found in the same proportions in the Candle oil. The proportion of 22:1ω7 isomer (1.1% of a total 1.2% 22:1) was intermediate to that of a high erucic variety (0.9% out of 23% 22:1) and the very low 22:1 Tower (2.3% out of 0.1% 22:1). Thus the proportion of ω7 isomers is governed by total 22:1 present.

The exceptionally rapid development of new varieties of canola (the registered name for low glucosinolate, low erucic acid varieties of *Brassica campestris* and *Brassica napus*) means that, in the near future, established varieties such as the *B. campestris* var Candle (of turnip or Polish rape origin) will be replaced by higher yielding varieties (1). Numerous feeding trials have been conducted with meal from this variety (2), many based on comparisons (3) with the *B. napus* var Tower (of Argentine rape origin). Crude fat is about 4% of candle meal and the oil content of the whole seed sometimes used in feed studies is about 41% (4,5). The fatty acid composition of the oil is therefore of interest to animal nutritionists. The oil has also been readily accepted for salad use or margarine stock (6), and canola oil now amounts to over 45% of production of deodorized vegetable oils in Canada (1). However the effects of refining on fatty acids have not, to our knowledge, been published for candle oil.

For the benefit of future researchers who are interested in making comparisons between Tower and Candle seed, oils, or meal, we report (Table I) a study of Candle oil fatty acids, including minor fatty acids such as we reported for Tower oil (7).

EXPERIMENTAL

Candle seed was obtained from Agriculture Canada in Saskatoon and from CSP Foods in Nipawin, Saskatchewan. Commercial oils from the CSP Foods were supplied in crude, degummed and refined form. The seeds were crushed and extracted by refluxing twice with hexane to yield an oil for comparison with the commercial oils. Fatty acid analysis of methyl esters of oils followed procedures outlined earlier (7), including combined gas liquid chromatography, $AgNO_3$ thin layer chromatography, and oxidative fission for determination of proportions of monoethylenic isomers.

RESULTS AND DISCUSSION

The effect of refining on the fatty acid composition of the Candle oil was negligible (Table I). The two mono-*trans* geometric isomers (8) of the dominant *cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid (18:3ω3) were apparent in the gas liquid chromatograms of the crude commercial oil, as well as in the degummed and refined oils. The levels were considerably below those found (ca. 0.5%) in retail soybean oils (8). The *cis*-9, *cis*-12, *trans*-15 isomer was also found in the laboratory-extracted oil, but at a low level in one lot of seed and at levels similar to those of the processed oils in the other lots of seed (Table I). As percentages of the *cis*-9, *cis*-12, *cis*-15 isomer, these isomers were very much less (ca. 1%) than the 25% of 18:3ω3 originally observed (8). This reduction probably reflects the modification of seed and oil processing, especially milder deodorizer conditions, possible with recent canola varieties (9).

A variety of shorter-chain (C_{14}, C_{15} and C_{17}) minor fatty acids were found in the Candle oil, in proportions similar to those previously found in the Tower oil (7). Clearly, these are not qualitatively or quantitatively different in *B. campestris* and *B. napus* varieties of canola.