DENSITY GRADIENT ISOLATION AND FRACTIONATION OF HAZE-CAUSING SOLIDS OF CANOLA OIL

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ABSTRACT

The packaging of retail canola (*Brassica* sp.) oil in clear containers occasionally shows a fine precipitate or haze. This can be centrifuged down in the laboratory at 2°C and the bulk of the oil cleanly removed by flotation with an aqueous methanol solution of appropriate density at 2°C. The dried solids can then be extracted, also with hexane at 2°C yielding from some oil samples a clean hexane-insoluble fraction of C_{42}-C_{63} normal and branched-chain wax esters. The hexane-soluble fraction includes about 1% total of three steryl esters which could be part of the haze problem in some oils. These can be purified by thin-layer chromatography. Otherwise this fraction is mostly triacylglycerols with the fatty acid composition of the parent oil.

INTRODUCTION

Waxes are an inescapable fact of life in some oilseed industries. In canola oil they are occasional problems and generally suspected of causing "haze" in retail salad oils. In our research we have used a density gradient fractionation approach (Ong *et al.*, 1983) to isolate a hexane-insoluble precipitate (HIP), primarily waxes, and a more complex hexane soluble precipitate (HSP).

EXPERIMENTAL

Samples of about 15 g of canola oil from crushers in western Canada were weighed into a 40 ml centrifuge tube and chilled to 2°C. When formation of solids appeared to be complete the tube was centrifuged at 2°C to produce a compact mass and a clear oil. A water:methanol medium (for refined oils 44:56, v:v) at 2°C was carefully applied to the top of the oil. Centrifugation at 800 rpm for 10 min equilibrated this between the bottom solids and the clear oil. The upper layer of oil was removed and the glass walls washed with five rinses of cold hexane (10 ml) prior to careful removal of the bulk of the water : methanol medium. Freeze drying removed the rest of the medium and the solids were extracted three times with cold hexane (1.5 ml) with mechanical agitation of the solids followed by vortex mixing and centrifugation steps. The remaining solids were the HIP fraction and the material in the 4.5 ml of hexane was termed the HSP fraction.

RESULTS AND DISCUSSION

The HIP fraction of certain oil samples consisted of very long chain linear wax esters (WE) although both IR and NMR spectra showed a few ethylenic bonds and some methyl branching. Comparison of GLC area and Iatroscan TLC-FID areas with an internal standard WE confirmed that only WE ranging from C_{42} to C_{63} were present (Table 1). The fatty acids of the HIP fraction determined by GLC ranged from 14:0 to 32:0 and were all linear with a small amount of unsaturated components (Table 2). The fatty alcohols ranged from 15:1 to 33:0 and included low levels of the iso and anteiso branched-chain structures already mentioned (Table 2).

TABLE 1. Chain-length compositions (w/w%) of HIP wax esters from a hazy oil.

Chain length	%	Chain length	%	Chain length	%
C42	0.35	C50	15.68	C58	2.04
C43	0.11	C51	2.52	C59	0.79
C44	5.07	C52	8.36	C60	1.26
C45	1.89	C53	2.37	C61	0.31
C46	13.78	C54	5.18	C62	0.74
C47	5.30	C55	2.59	C63	0.10
C48	18.93	C56	5.91	unknown	0.54
C49	4.22	C57	1.93	-	-

TABLE 2. Quantitatively important (w/w% of total) fatty acids and of fatty alcohols of WE from HIP similar to that of Table 1.

	acid	alcohol		acid	alcohol
16:0	1.85	-	28:0	17.04	17.30
18:0	2.19	0.07	29:0	2.40	3.18
18:1n-9	4.19	-	30:0	7.58	12.46
20:0	6.68	0.25	32:0	1.16	4.00
22:0	8.80	2.85	33:0	Trace	1.88
24:0	12.45	9.26	Σ Saturated	91.25	93.85[c]
25:0	1.36	1.58	Σ Monoethylenic	7.59[a]	6.15[d]
26:0	26.14	19.40	Σ Diethylenic	1.21[b]	nil
27:0	1.88	2.69			

[a] Includes minor amounts of 20:1, 22:1, 24:1, 26:1 and 18:1.
[b] Includes 0.95% 18:2n-6.
[c] Includes 6.83% iso alcohols (4.34% is tentatively identified as iso-25:0) and 9.64% anteiso alcohols.
[d] Includes 3.83% tentatively identified 31:1.

The HSP fractions always contained (Table 3) a high proportion of triacylglycerides and included a TLC peak (Iatroscan) corresponding to wax and/or steryl esters. Planar TLC followed by GLC showed that this material was mostly steryl esters with some shorter chain WE. The proportions of minor lipid classes were in fact almost identical to those in the parent oil winterized at 2 °C (Table 3). The triacylglyceride in the HSP did not differ to any important degree in fatty acid composition from that of the winterized oil. Other groups in Canada have reached some of the same conclusions (Hu *et al.*, 1993; Lui *et al.*, 1993a; 1993b; Przybylski *et al.*, 1993) with different isolation technologies.

TABLE 3. Lipid classes in a "hazy" sample of refined and bleached canola oil winterized at 2 °C with removal of 1.8 mg/100g of HIP and of 340 mg/100g of HSP material, and of those in the HSP fraction.

Lipid class	Winterized oil	HSP fraction
Wax / steryl ester	1.05	1.28
Triacylglyceride	98.04	97.20
Free fatty acid	ND	ND
Free sterol	0.81	0.83
Diacylglyceride	1.14	0.70
Phospholipid	ND	Trace

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