Proportions of C18:1n-7 and C18:1n-9 Fatty Acids in Canola Seedcoat Surface and Internal Lipids

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Lipids of canola seedcoats (*Brassica napus* L. and *B. rapa* L.) were prepared by surface washing and by complete extraction of seed coats with toluene. The major fatty acyl-containing triacylglycerols, wax esters and free fatty acids were separated by thin-layer chromatography prior to transesterification and analysis by gas–liquid chromatography. The proportion of C18:1n-7 to C18:1n-9 was higher in the extracted lipids than in the surface-washed lipids for all three classes.

KEY WORDS: *Brassica napus* L., *B. rapa* L., canola, *cis*-vaccenic acid, C18:1n-7 fatty acid, free fatty acid, seed coat, surface lipids, triacylglycerol, wax ester.

It has been generally agreed that C18:1n-7 (*cis*-vaccenic) fatty acid (FA) results from Δ9 desaturation of C16:0 fatty acid and subsequent elongation (1) although its biosynthetic pathway has not yet been confirmed in higher plants (2). It is also well-established that many common vegetable oils contain small amounts (1 to 15%) of C18:1n-7 (3). In canola oil, the C18:1n-7 has been noted as a minor constituent, but it is a major constituent in the triacylglycerols (TAG) of canola hulls (4). Structural compartmentalization of FA types may be of interest to plant physiologists studying the role of unusual FAs in the function of plant cells.

Lipids from the seed surface of canola have received considerable attention recently because they are associated with haze formation in oils (5,6). As a result of this interest, we have undertaken a study of the composition of lipids in the seedcoat of canola. The objective of this investigation was to determine the distribution of C18:1n-7 fatty acid between the surface and interior lipids of canola seedcoats by measuring the proportion of n-7 and n-9 C18:1 fatty acid methyl esters (FAMEs) in free fatty acids (FFA), TAG and wax esters (WE) prepared by extraction and surface washing.

MATERIALS AND METHODS

Certified *Brassica napus* cv. Westar and *B. rapa* cv. Tobin seeds were soaked in water overnight at 4°C before being peeled by hand. The seedcoats were rinsed with water to remove the small residual pieces of meats, and then they were air-dried.

For extraction of neutral lipids, approximately 1 g seedcoats was refluxed with toluene for 24 h. Toluene was chosen as the extraction solvent because refluxing with relatively high-boiling aromatic solvents had previously been found to give better recoveries of WE than lower-boiling aliphatic solvents. The extract was filtered, and the toluene was removed by evaporation under vacuum below 45°C. For surface-washing, approximately 100 g seeds was washed with 500 mL hot (*ca.* 70°C) toluene in a filter paper-lined funnel, followed by removal of toluene as above. The lipids were redissolved in toluene and were then separated by thin-layer chromatography on 1000 μm Whatman silica gel LK6 plates (Maidstone, England) with toluene/chloroform (7:3). After being sprayed with chloroform by soaking them in chloroform overnight at 4°C, FA and TAG were converted to methyl esters by acid-catalyzed esterification (7) or by base-catalyzed methanolysis (8), respectively; WE were first hydrolyzed by base-catalyzed saponification (7) to fatty alcohols and FAs and the FAMEs were prepared by acid-catalyzed esterification.

Gas–liquid chromatography analyses were carried out on a 30 M × 0.25 mm fused-silica column coated with 0.25 μm (Supelcowax 10; Supelco, Bellefonte, PA); carrier gas, hydrogen (35/cm/s); injection temperature, 250°C; temperature program, starting at 150°C for 1 min, then to 220°C at 2°C/min, followed by 4°C/min to 260°C and hold for 14 min.

RESULTS AND DISCUSSION

The proportion of C18:1n-7 to C18:1n-9 was higher in the lipid classes from seedcoats refluxed with toluene than in the surface-washed lipids (Table 1). The amount of C18:1n-7 was also higher in the WE fraction than in TAG and FFA. Toluene refluxing, which removed about 12% of material from the seedcoats, would be expected to give good extraction of both surface and internal lipids, whereas the surface washing, which removed about 0.3% of the

TABLE 1

Area% of C18:1 Fatty Acids in Different Lipid Classes[a]

		Reflux extraction			Surface washing		
Cultivar	Lipid	n-7	n-9	n-7:n-9	n-7	n-9	n-7:n-9
Westar	FFA	18.6	33.2	1:2	4.73	55.8	1:12
	TAG	26.7	26.3	1:1	5.17	56.5	1:11
	WE	17.1	19.5	1:1.2	2.74	19.8	1:7
Tobin	FFA	20.3	28.6	1:1.4	5.32	46.7	1:9
	TAG	20.9	30.1	1:1.5	2.92	57.4	1:18
	WE	12.4	12.0	1:1	2.71	9.48	1:3.5

[a]Abbreviations: FFA, free fatty acids; TAG, triacylglycerols; WE, wax esters.

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seedcoat material, might be expected to remove primarily lipids from the outer surface of the seedcoat. The ratio of n-7:n-9 FA in the lipids extracted by toluene reflux was similar to that reported by Åppelqvist for seedcoat lipids (4), but the ratio of n-7:n-9 in the surface-washed lipids was closer to that reported for cotyledons or hypocotyl. The results obtained suggest that the C18:1n-7 FA is more closely associated with the internal, possibly structural lipids than with the surface lipids.

ACKNOWLEDGMENT

C.T. Thorsteinson of the grain Research Laboratory assisted with the gas-chromatographic analysis. Paper No. 703 of the Grain Research Laboratory and No. 956 of the University of Manitoba Department of Plant Science.

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[Received April 23, 1993; accepted November 3, 1993]