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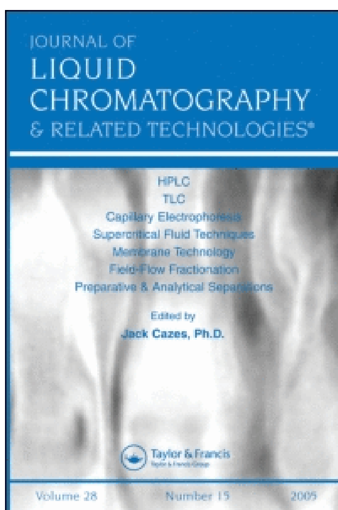

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James F. Lawrence[a]; Rajinder K. Chadha[a]; Henry B. S. Conacher[a]
[a] Food Research Division Food Directorate Health Protection Branch, Ottawa, Ontario, Canada

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CHARACTERIZATION OF BROMINATED VEGETABLE OILS BY NORMAL AND REVERSED PHASE LIQUID CHROMATOGRAPHY

James F. Lawrence, Rajinder K. Chadha, and Henry B. S. Conacher
Food Research Division
Food Directorate
Health Protection Branch
Ottawa, Ontario, Canada K1A OL2

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ABSTRACT

Brominated vegetable oils are characterized by normal and reversed phase chromatography using UV absorbance detection at 220 nm. A series of seven brominated vegetable oil preparations are transesterified using acid methanolysis and the resulting dibromo-, tetrabromo- and hexabromostearate components quantitated by HPLC. The identification of the oils compare favorably with results obtained by gas chromatography.

INTRODUCTION

Brominated vegetable oils (BVOs) are permitted for use in citrus beverages as dispersing agents for flavoring oils. A variety of methods have been studied

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for their determination including x-ray fluorescence (1), specific ion electrodes (2), titration (3) and gas chromatography (GC) (4, 5). The last mentioned is the only technique able to differentiate the various BVOs. Acid methanolysis (4) is preferred to the sodium methoxide method (5) for reasons described elsewhere (6). However, the resulting hexabromostearate, a component specific to certain BVOs is not easily quantitated by packed-column GC without great care and a well conditioned chromatography system (6) although it has been shown to chromatograph well by capillary GC (7). In the present work we report on the application of high-pressure liquid chromatography (HPLC) to the separation and quantitation of the methyl esters of dibromostearate (DBS), tetrabromostearate (TBS) and hexabromostearate (HBS) by both normal and reversed phase chromatography and apply the technique to the characterization of different commercial and laboratory prepared BVOs.

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MATERIALS AND METHODS

Reagents

All solvents were glass distilled or HPLC grade materials. The brominated standards, DBS, TBS and HBS were prepared exactly as described earlier (4). During preparation of HBS, crystals of one HBS isomer precipitated from solution. These were filtered, washed with

ether, dried and weighed for preparation of the analytical standard. The second isomer remained in the supernatant liquid and was isolated separately. (The identity of the isomers was confirmed by mass spectrometry.) The isomer that precipitated eluted first in the HPLC system.

The brominated vegetable oils studied were olive, sesame, corn, cottonseed, and soybean. These were either obtained commercially (Abbott) or prepared from the unbrominated oils in the same manner as described earlier (8). A partially hydrogenated soybean oil sample was also brominated in the same manner. A commercial preparation of brominated partially hydrogenated soybean oil, Akwilox 133, was also studied.

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Before analysis all standards and oil samples were transesterified using acid catalysed methanolysis exactly as described earlier (6). Briefly, 6 mg of each standard or oil were refluxed in 25 mL acid-methanol solution for 1 hour. The mixture was cooled, 40 mL water added and then it was extracted with hexane. The organic phase was removed and evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in 5 mL of acetonitrile-tetrahydrofuran (8 + 2) for reversed-phase chromatography and in 5 mL of hexane for normal phase chromatography.

Apparatus

The HPLC system consisted of a Beckman 112 solvent delivery module and 420 controller along with a Micromeritics 788 dual variable wavelength detector and a Spectra-Physics 4270 integrator. The mobile phase flow rate was 1 mL/min. Detection was made at 220 nm. The injection volume was 20 μL.

For reversed phase chromatography an Ultrasphere ODS (Altex) 25 cm x 4.6 mm, 5 μ column and a mobile phase consisting of acetonitrile-water (95 + 5) were selected. For normal phase chromatography an Ultrasphere Si (Altex) 25 cm x 4.6 mm, 5 μ column and a mobile phase consisting of hexane-isopropanol (500 + 1) were employed.

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RESULTS AND DISCUSSION

Figure 1 shows typical results obtained for standards and two of the brominated oils analysed. As expected, the elution order for DBS, TBS and HBS was reversed in the two HPLC systems. Two isomer peaks were observed for TBS by both normal phase and reversed phase chromatography. The two isomers of HBS were separated by reversed phase chromatography while by normal phase chromatography they eluted as a single peak. DBS produced a single peak in both systems. By gas chromatography all three compounds appear as predominantly single peaks, perhaps indicating that the

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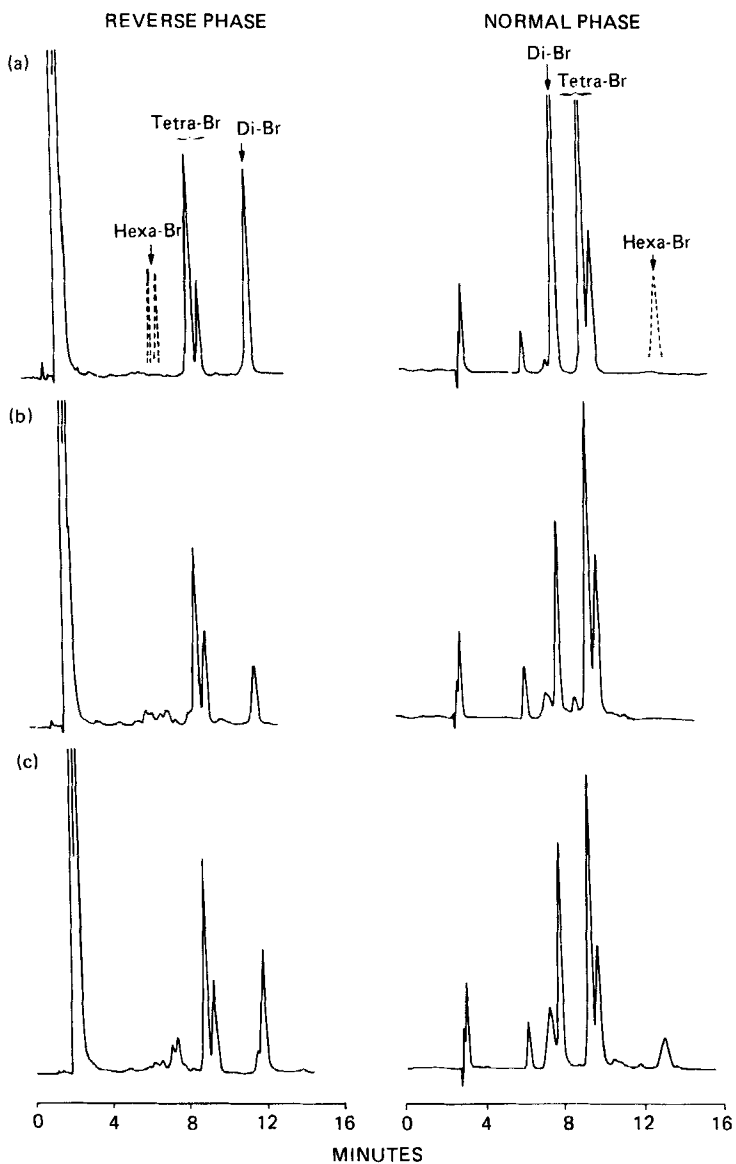

Figure 1: Reversed and normal phase chromatograms of: A. HBS, TBS and DBS standards; B. brominated cottonseed oil; C. brominated partially hydrogenated soybean oil. Conditions as described in the text.

isomers are not separated under those conditions either with a packed (6) or a capillary column (7).

It was found that in the normal phase system capacity factors for the peaks tended to increase slightly during the day. This was due to the very low quantity of isopropanol in the mobile phase, and perhaps the accumulation of sample coextractives on the column. However this was not a problem since standards were always run throughout the day.

The major peaks in the chromatograms of the BVO samples were those arising from DBS, TBS and HBS. This was in contrast to results by gas chromatography where other peaks due to unbrominated constituents were observed (6). Figure 2 shows a reversed phase chromatogram of a mixture of the standards with the methyl esters of unsaturated oleic, linoleic and linolenic acids, as well as methyl heptadecanoate. These substances were all separable except for linoleic acid and the major peak of TBS. These two have been separated using the same mobile phase with a Spherisorb ODS-2 column. Other detector wavelengths were evaluated (from 205-270 nm) but 220 nm was selected as optimum in terms of sensitivity and selectivity for the brominated oils. It can be seen in Figure 1 that the chosen HPLC conditions enabled a rather selective characterization of the two BVO samples without interferences from other non-brominated constituents.

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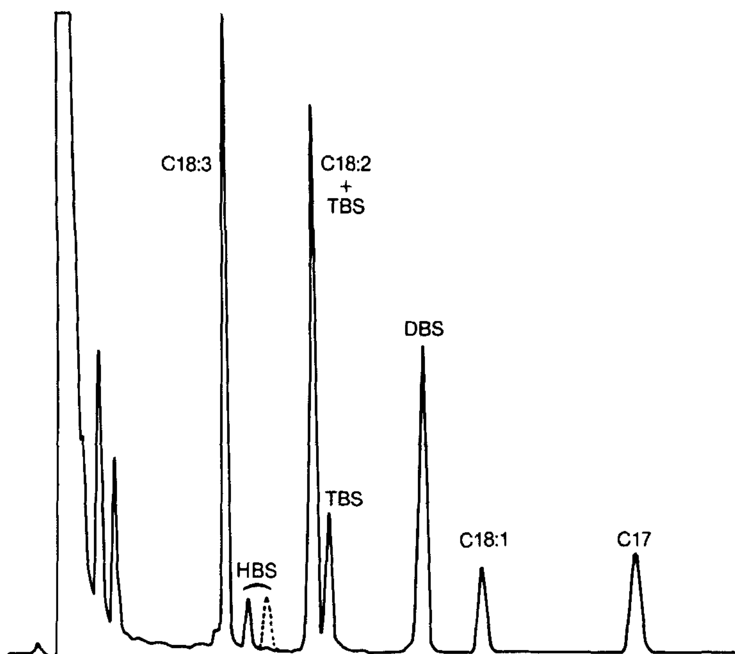

Figure 2: Reversed phase chromatogram of a mixture of HBS, TBS and DBS with linolenic (C_{18}:3), linoleic (C_{18}:2), oleic (C_{18}:1) acid methyl esters and methyl heptadecanoate (C_{17}).

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Quantitation of HBS in the oil samples by reversed-phase chromatography was done using only the first eluting isomer which represented 51% of total HBS. In some samples with low HBS concentrations, the second peak was distorted by the presence of unknowns which interfered in the quantitation of that peak. Total HBS was calculated from the first isomer by multiplying by 1.95.

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Table 1

Brominated Fatty Acid Composition of BVOs

Brominated Oil	Fatty Acid (%)[a]									Ratio		
	DBS			TBS			HBS			TBS/DBS		
	HPLC		GC	HPLC		GC	HPLC		GC	HPLC		GC
	RP	NP		RP	NP		RP	NP		RP	NP	
Olive	66	70	64	11	7	7	-[b]	-	-	0.17	0.11	0.11
Sesame	32	40	33	39	40	37	-	-	-	1.2	1.0	1.1
Corn	21	21	21	64	67	69	-	-	-	3.1	3.1	3.3
Cottonseed	15	20	16	50	53	52	-	-	-	3.4	2.7	3.3
Soybean	19	20	19	56	58	56	8.8[c]	10	-	2.9	2.9	2.9
Soybean (partially hydrogenated)	27	24	NA	46	45	NA	5.7[c]	4.2	NA	1.7	1.9	NA
Akwilox 133	32	32	37	31	32	31	-	-	-	1.0	1.0	0.9

a = percent by weight, average of duplicates. b = not detected. c = calculated from first eluting isomer. NA = not analysed. RP = reversed phase. NP = normal phase.

The ratio of TBS to DBS provides useful information as to the identity of a brominated oil. Also, HBS is particularly useful in identifying brominated canbra and brominated soybean oils since these are the only two that contain detectable levels of HBS. Table 1 presents results obtained for the various oils studied. The two HPLC methods generally agree very well. For comparison purposes the same samples were analyzed by gas chromatography as described earlier (6) and the results are in good agreement with the HPLC methods. The gas chromatographic method however was not able to detect the low percentages of HBS in the soybean samples.

With the exception of corn and cottonseed oil, the BVOs can be differentiated by their TBS/DBS ratio. The soybean samples analysed produced different results which were related to hydrogenation. Laboratory brominated soybean oil produced the largest HBS response and gave a TBS/DBS ratio of 2.9. The laboratory brominated partially hydrogenated soybean oil gave an average TBS/DBS ratio of 1.8 and a lower percent of HBS as expected. In the commercial sample, Akwilox 133, the ratio was only 1.0 and no HBS was detected. This indicates that perhaps the sample was hydrogenated to a greater extent than that above.

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CONCLUSION

HPLC has been shown to offer potential for characterizing brominated vegetable oils. Both normal and reversed phase systems were useful in determining the DBS, TBS and HBS content of several oils after transesterification. In a comparison to a packed-column gas chromatographic method the HPLC techniques were equal for determining DBS and TBS but superior for detecting HBS.

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