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Note

Gas chromatographic patterns of some apple surface wax constituents

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In earlier work, we had characterized some commercial waxes by high-temperature gas chromatography[1] and determined paraffin wax on a variety of fruits and vegetables[2]. Commercial food grade waxes such as shellac wax and carnauba wax are permitted for use in apple-coating preparations. Attempts at identifying such waxes on apples available to the consumer have not been reported although such methodology would be useful for regulatory purposes. In our efforts to develop methodology for shellac and carnauba wax, we used high-temperature gas chromatography to determine the wax components of both commercially coated and uncoated apples. The findings reported herein include the chromatographic patterns of natural surface wax constituents of apples and the effect of commercial coating on these patterns.

EXPERIMENTAL

Gas chromatography

A Varian Model 2700 gas chromatograph with flame ionization detector was employed for the analyses. The column was a 1 m × 2 mm I.D. stainless steel, packed with 80–100 mesh, acid washed and silanized Chromosorb W coated with 1.5% Dexil 300. Helium carrier gas flow-rate was 60 ml/min and the oven was temperature programmed from 150 to 360°C at 4°C/min.

Mass spectrometry

A VG ZAB-2F high-resolution mass spectrometer interfaced to a Varian 3700 gas chromatograph was used to characterize the wax components. A 2 m × 2 mm I.D. 1.5% Dexsil 300 column, temperature programmed from 200°C to 300°C at 5°C/min and splitless injection at 300°C was employed. The mass spectrometer conditions were: chemical ionization (methane); resolution, 1000 (10% valley); mass ranges, m/z 50–550; electron energy, 100 eV; ion source temperature, 150°C; emission current, 0.5 mA.

Extraction of apples

Each apple was weighed and the waxes removed by immersion in a beaker of analytical quality chloroform for 10 sec[3]. The immersion was repeated successively with three additional beakers of chloroform. The pooled extracts were evaporated

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to dryness by rotary vacuum evaporation at 30°C then the residue was dissolved in 5 ml of fresh chloroform and either analysed directly or treated with diazomethane or acetic anhydride as described below.

Esterification

Methyl esters were formed using diazomethane[4]. Freshly prepared diazomethane in diethyl ether was added until the yellow color persisted and the mixture refrigerated overnight. The solution was evaporated to dryness, then dissolved in 5 ml of chloroform for chromatographic analysis or the residue acetylated as described below.

Acetylation

A volume of 1 ml of acetic anhydride followed by 2.0 ml of anhydrous pyridine were added to the residue. The contents were allowed to react at room temperature overnight, then the solvents removed by rotary evaporation. The residue was dissolved in 5 ml of chloroform for analysis.

RESULTS AND DISCUSSION

Fig. 1 shows a gas chromatography–mass spectrometry total ion chromatogram obtained after esterification and acetylation of a Granny Smith apple extract. Peak 1 in the chromatogram represents C_{29} hydrocarbon and was found to be the predominant hydrocarbon peak in all samples of apples analysed. When the extracts were analysed directly, this constituent was the only significant peak in the chromatograms. Peak 2 is C_{28} alcohol (as the acetate) and was observed only after acetylation of the extracts. Its content varied significantly with the type of apple. Peak 4 appears to be ursolic acid (3β-hydroxyurs-12-ene-28-oic acid) (methylated, acetylated) a triterpene constituent known to be present in the natural surface wax of apples[5,6] since it exhibited a fragmentation pattern with major fragments at *m/e* 513

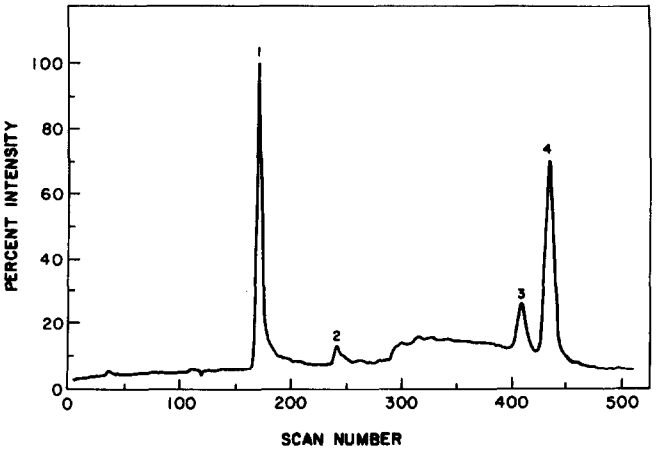

Fig. 1. Total ion chromatogram of a Granny Smith apple wax extract obtained after esterification and acetylation treatments. Peak 1 = C_{29} hydrocarbon, peak 2 = C_{28} alcohol, peaks 3 and 4 = triterpenes.

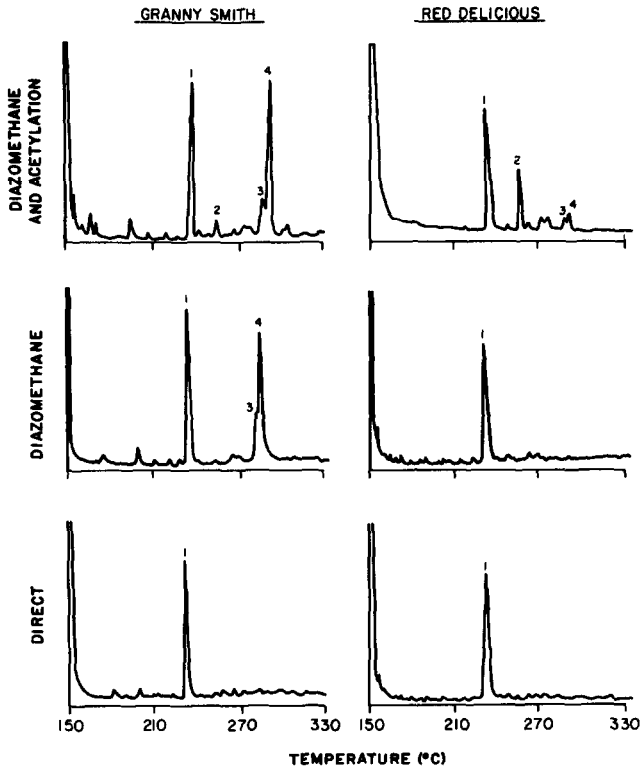

Fig. 2. Chromatograms obtained by direct analysis, after esterification with diazomethane and after diazomethane plus acetylation treatments. Peak numbers as in Fig. 1.

(molecular ion +H), 452, 262 and 203. The relative fragmentation pattern although shifted because of the presence of the acetate group compares well with that of the ursolic acid methyl ester electron impact spectrum obtained from the EPA/NIH library. Also, the spectrum is virtually identical to the spectrum of acetylated, methylated oleanolic acid, an isomer of ursolic acid that is not found in apples although has been reported in grapes[7] and cranberries[8]. Peak 3 appears to be an analogue of ursolic/oleanolic acid since it had a very similar spectrum but with an additional peak at $m/e = 511$.

The wide variation in triterpene content (peaks 3 and 4) with type of apple can be seen in Fig. 2. It also is noted that triterpenes do chromatograph as the methyl esters with the hydroxyl groups free although some tailing is observed. At very low levels such as in the Red Delicious apple, the methyl ester peaks could not be observed although the isomers are observed as the methyl ester acetates. Fig. 3 illustrates chromatograms obtained from a variety of commercially purchased apples after the extracts were subjected to esterification and acetylation treatments. The variation in constituent peaks is readily observed. In two cases namely, Ida Red and Mackintosh apples, one triterpene (peak 4) represented the largest peak in the chromatograms. Upon resampling Mackintosh apples from a different supplier, peaks 1 and 2 remained reasonably consistent, however the triterpene peaks were very small. Since

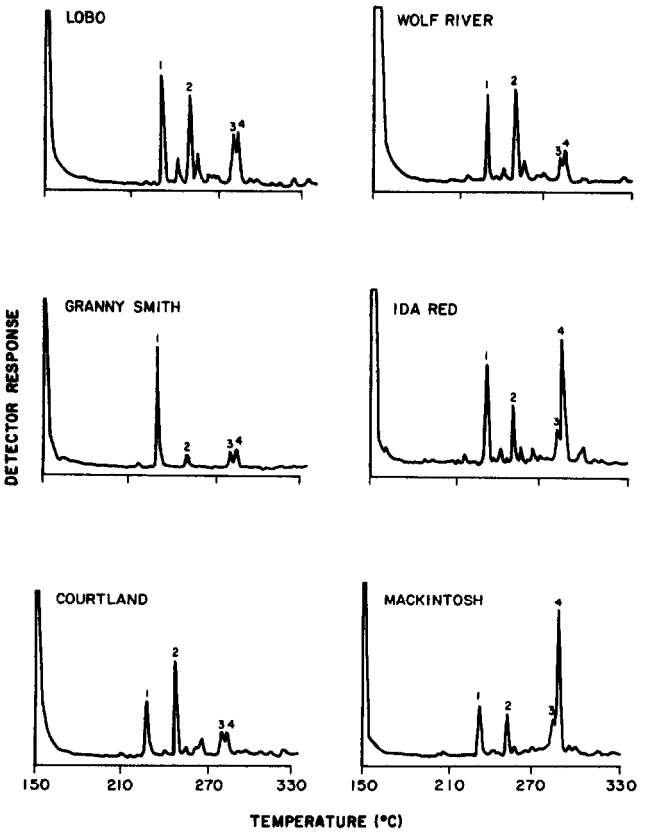

Fig. 3. Chromatograms (after esterification and acetylation) of apple surface waxes obtained on different types of apples purchased locally. Peak numbers as in Fig. 1.

apples sold in Canada may or may not be coated with food grade shellac or other waxes, we examined the effect of apple coatings on triterpene presence in the chromatograms. Coated and uncoated apples were obtained from commercial operations in the provinces of Ontario and British Columbia. Apples were collected immediately before and after the coating process and then shipped to our laboratories for analysis. Fig. 4 compares results of before and after coating for three types of apples. It can be seen in all cases that the triterpene content in the coated apples is dramatically increased, with peak 4 predominating.

The reason for the increased content of triterpene in the apples extracts is unclear at present. It is possible that coating the apple affects the biological processes involved in producing these compounds (at least two weeks elapsed from the time of coating to analysis of the samples studied herein, perhaps allowing for triterpene formation). Another possible explanation is that the wax coating solutions all contained morpholine which being basic could facilitate the extraction of the weakly acidic triterpene into the chloroform via ion pair formation. Whatever the reason, it is possible that the different results for the triterpene content of the extracts analysed in Figs. 2 and 3 could be due to, at least in part, the presence of commercially

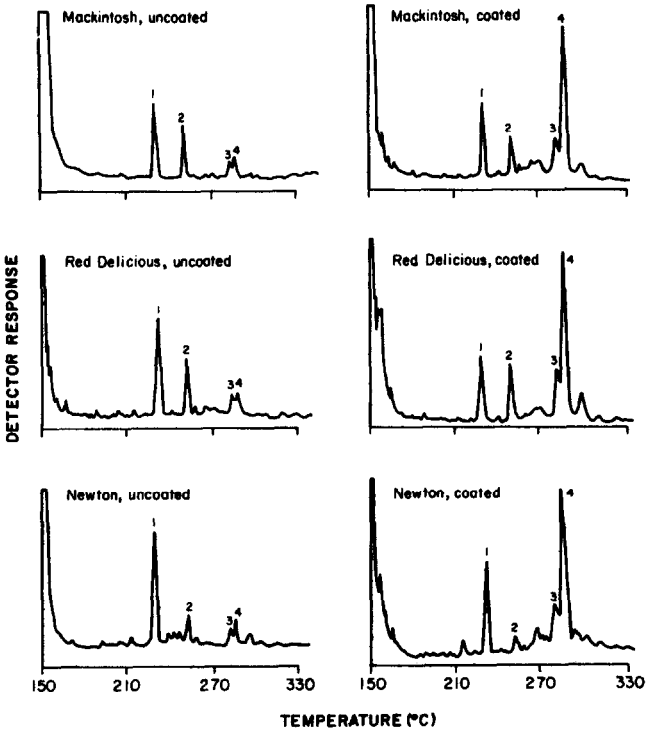

Fig. 4. Chromatograms (after esterification and acetylation) obtained from apples before and after coating. Peak numbers as in Fig. 1.

applied wax coatings. Gas chromatographic identification of the commercial waxes on coated apples was not successful even with the known coated apples in Fig. 3, although derivatized pure shellac wax yielded alcohol acetates[1] which appeared between peaks 2 and 3.

REFERENCES

1 J. F. Lawrence, J. R. Iyengar, B. D. Page and H. B. S. Conacher, *J. Chromatogr.*, 236 (1982) 403.
2 J. F. Lawrence and J. R. Iyengar, *J. Food Safety*, 5 (1983) 119.
3 A. M. Silva-Fernandes, E. A. Baker and J. T. Martin, *Ann. Appl. Biol.*, 53 (1964) 43.
4 A. P. Tulloch, *J. Am. Oil Chem. Soc.*, 50 (1973) 367.
5 C. E. Sando, *J. Biol. Chem.*, 56 (1923) 457.
6 M. Windholz (Editor), *Merck Index*, 10th ed., E. Merck, Rahway, NJ, 1983, p. 9697.
7 F. Radler and D. H. S. Horn, *Aust. J. Chem.*, 18 (1965) 1054.
8 R. Croteau and I. S. Fagerson, *Phytochemistry*, 10 (1971) 3239.