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Identification of steroidal hydrocarbons in refined confectionery fats by gas chromatography–mass spectrometry

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Abstract

Steroidal hydrocarbon degradation products (steradienes) of sterols formed during the bleaching of confectionery fats were isolated by a new rapid silica column technique. The steradienes were separated by gas chromatography with mass spectrometric detection. Refined mango butter contained predominantly a sterene which was unrelated to the major desmethyl sterols. Dehydration products of triterpene alcohols were isolated from stearin fractions of refined shea butter which is used in the manufacture of chocolate.

Keywords: Cocoa butter equivalents; Fats; Food analysis; Steradienes; Sterols

1. Introduction

Vegetable fats derived from a variety of tropical plants are used in the formulation of supplements (cocoa butter equivalents) for cocoa butter in the manufacture of chocolate [1]. The fats used for this purpose are bleached and fractionated before use. Acid earth bleaching leads to the formation of olefinic degradation products of the plant sterols [2]. The principal degradation products have been identified as steradienes resulting from the dehydration of the major plant sterols β-sitosterol, campesterol and stigmasterol, as shown in Fig. 1. Analysis of these products can be used to detect the addition of cocoa butter equivalent fats to cocoa butter, which is generally not bleached [3]. Shea butter, however, contains very low levels of these sterols but high levels of triterpene alcohols such as α-amyrin, β-amyrin, butyrospermol and lupeol [4]. Triterpene alcohols have been reported to undergo isomerisation rather than dehydration on bleaching [4]. Typical reaction products formed by bleaching are shown in Fig. 1.

The non-polar fraction of oils has previously been isolated by column chromatography using silica gel with hexane elution [5]. The elution flow rate has been carefully controlled and a particular narrow fraction cut to avoid the presence of squalene and other undesirable hydrocarbons. We have applied a new rapid separation technique to isolate the non-polar fraction of a series of fats used for cocoa butter equivalent manufacture and made an initial examina-

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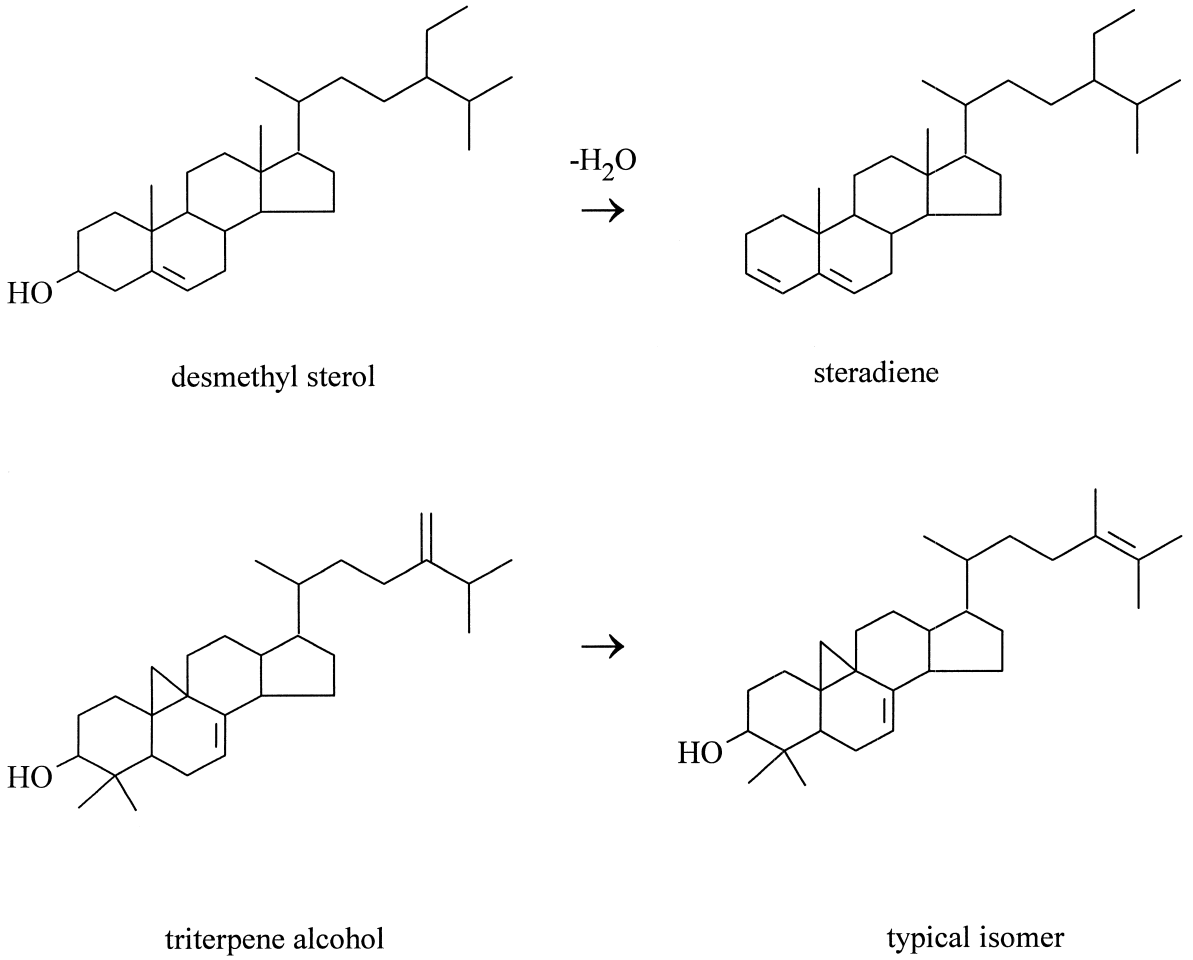

Fig. 1. Effect of bleaching conditions on a normal sterol and a triterpene alcohol.

tion of steradiene content. The fats were stearins of palm, illipe, kokum, mango, sal and shea.

2. Experimental

2.1. Materials

Pre-packed disposable 10-g silica columns were obtained from Jones Chromatography (Hengoed, UK). Bleaching earth (Fulmont XMP3) was obtained from Laporte Absorbents (Cheshire, UK). Cholesta-3,5-diene was obtained from Sigma (Poole, UK).

2.2. Samples

Fats used were stearins of palm, illipe, kokum, sal and shea, provided by Britannia Food Ingredients (Goole, UK).

2.3. Gas chromatography–mass spectrometry

A 30 m×0.32-mm J&W DBWax column with a 0.25-μm film (Jones Chromatography) connected to a ThermoSep MD800 benchtop quadrupole mass spectrometer. The gas chromatograph column oven was programmed from 80 to 250°C at a rate of 10°C/min. The injector and transfer line temperatures were 280°C, split injections (20:1) were made.

The mass spectrometer was operated in full scan mode over the mass range 40 to 600 u at a scan rate of 1 scan/s with an interscan delay of 0.7 s. Selected ion monitoring was used to measure the response at the molecular ion (m/z 408) and fragment ions (m/z 189, 203 and 218) of triterpene sterenes.

2.4. Isolation of steradienes

Fat (0.5 g) was dissolved in hexane (1 ml) and after the addition of internal standard (cholesta-3,5-diene, 1 μg) was applied to a pre-packed disposable silica columns (IST Isolute) which had been pre-wetted with hexane. The column was fitted with a 23-g hypodermic needle to reduce the flow rate and the non-polar fraction eluted with 50 ml hexane. The extract was evaporated to dryness under vacuum and the residue dissolved in 0.2 ml hexane. The components of the non-polar fraction were separated by capillary gas chromatography using a polar stationary phase (J&W DBWax 30 m×0.32 mm) and identified by mass spectrometry.

3. Results

The non-polar fraction of the refined fats was composed mainly of steradienes. For palm, illipe, kokum, and sal the major steradienes were stigmasta-3,5-diene, campesta-3,5-diene and stigmasta-3,5,22-triene. A typical total ion current chromatogram is shown in Fig. 2 where the principal steradienes are indicated.

The major steradiene of mango eluted before the steradienes of the major sterols (Fig. 3). It had a molecular mass of 378 and an unsaturated steroid ring structure evidenced by the ion at m/z 253 (Fig. 3). It was thought that this compound might result

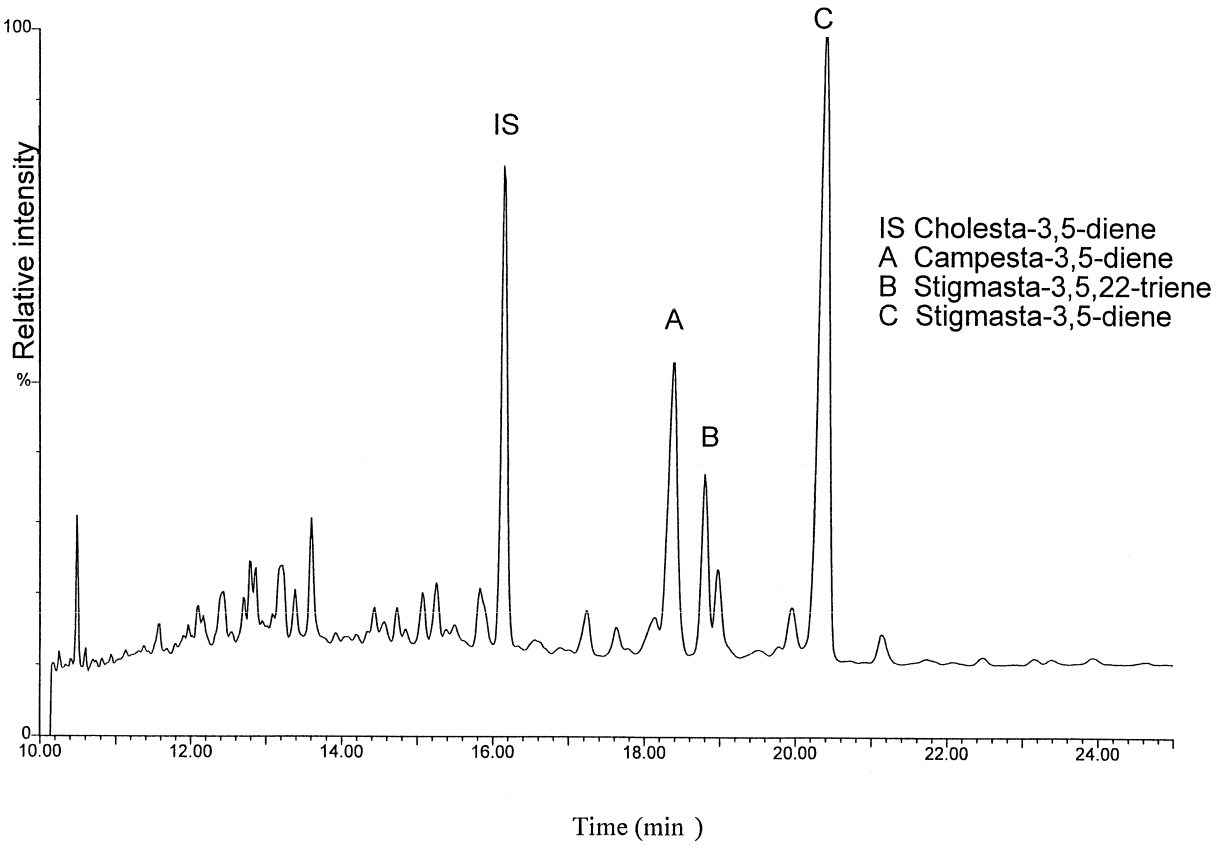

Fig. 2. A typical total ion current chromatogram showing the principal steradienes.

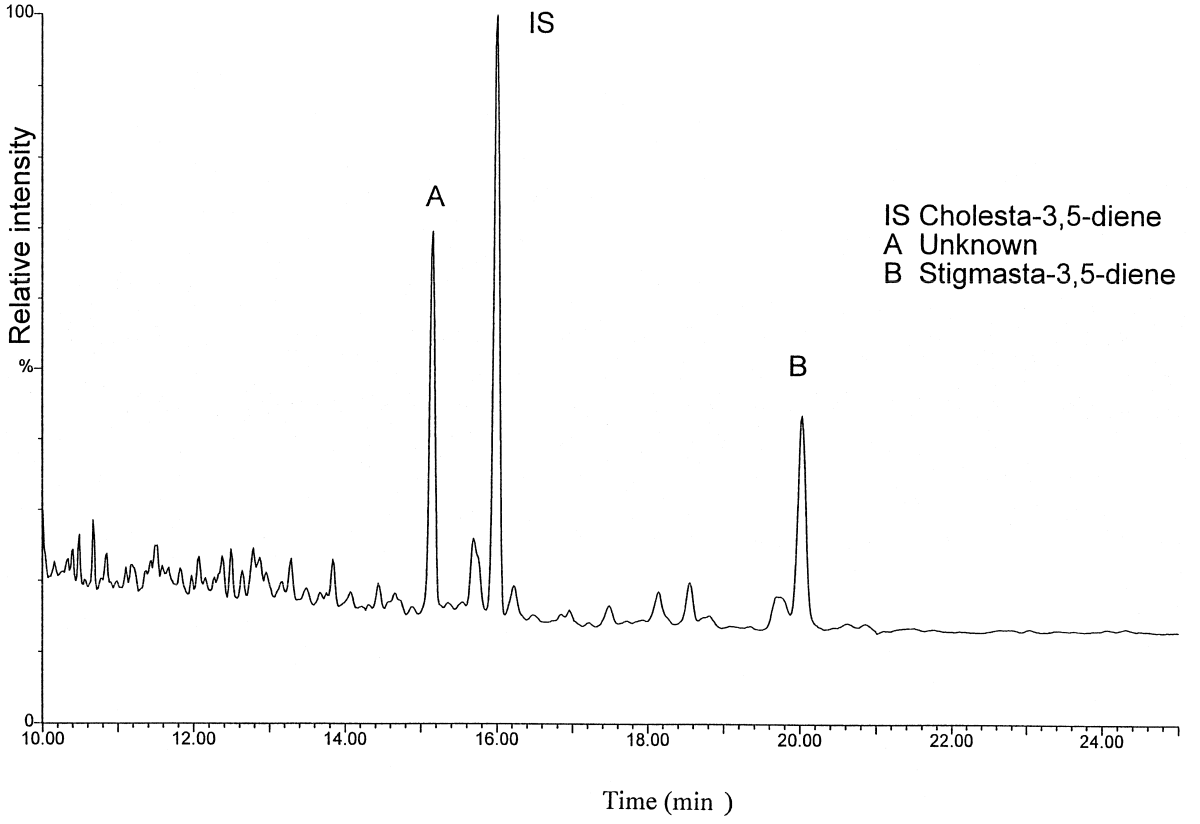

Fig. 3. Total ion current chromatogram of mango stearin steradienes.

from the dehydration of ergosterol present as fungal contamination of the kernel, but laboratory dehydration of ergosterol with bleaching earth gave many products, each of which had similar spectra but different chromatographic retention times to the unknown compound. The compound has been reported previously as a minor component of the steradiene fraction of refined vegetable oils [2] (Fig. 4).

The total ion current chromatogram of refined shea contained numerous non-polar compounds (Fig. 5, top), virtually all of which had mass spectra identifiable as triterpene-derived hydrocarbons by virtue of the molecular ion at m/z 408 and characteristic triterpene fragments at m/z 189, 203 and 218 (Fig. 6).

Many of these triterpenes were absent from the stearin fraction used in confectionery (Fig. 5, bottom) but sufficient remained to indicate the presence of shea butter in cocoa butter equivalents and in chocolate by selected ion monitoring of the molecular ion and the characteristic fragment ions. Fig. 7 shows the selected ion chromatogram at m/z 408 for chocolates with and without added vegetable fat.

Model dehydration experiments conducted with bleaching earth show that α- and β-amyrins and lupeol are dehydrated in substantial yield to steroidal hydrocarbons, but that butyrospermol is not. Dehydration products of the amyrins could be detected in shea butter but were not the major triterpene steradienes present.

4. Discussion

This work has shown that steradiene analysis can indicate the presence of refined fats in vegetable fats. The steradiene profiles matched those of the major

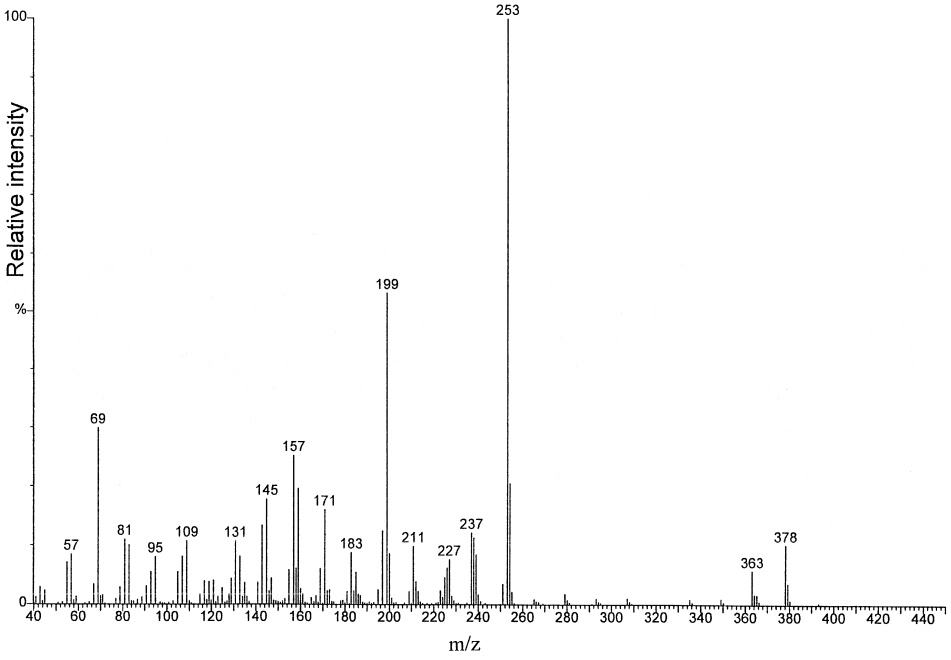

Fig. 4. Mass spectrum of the major steradiene in mango stearin.

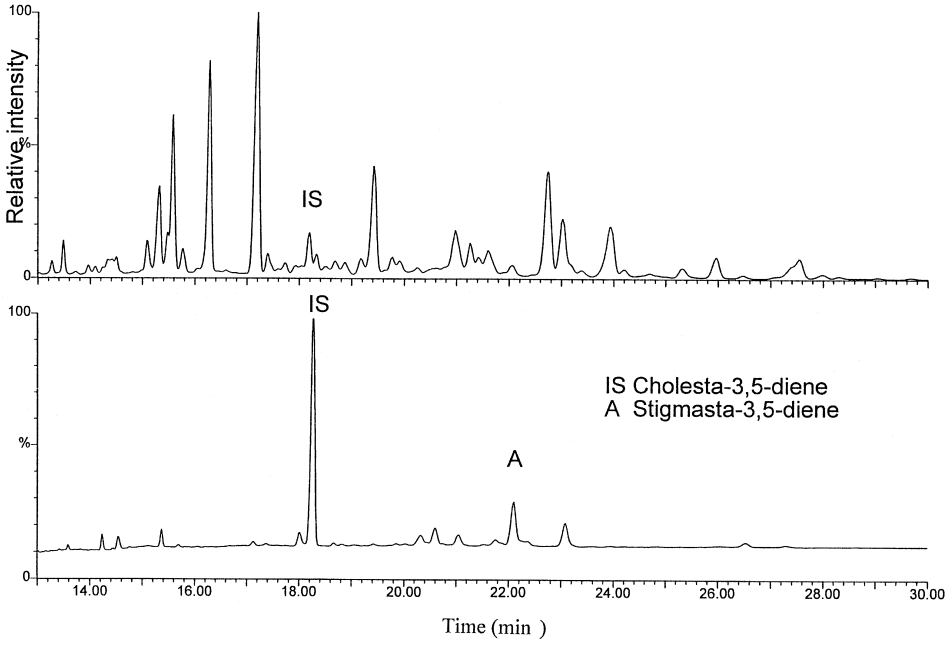

Fig. 5. Total ion current chromatogram of refined shea butter (top) and shea stearin fraction (bottom).

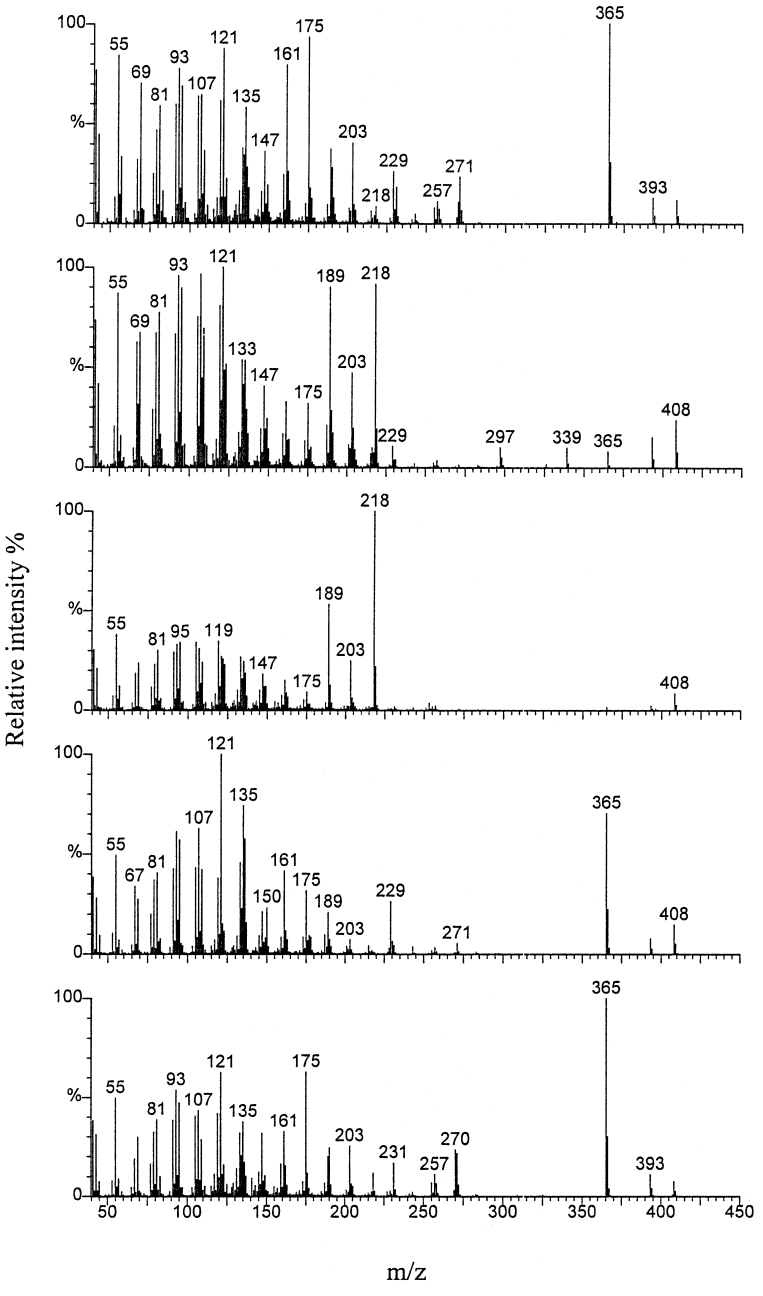

Fig. 6. Mass spectra of shea butter steradienes.

plant sterols. In the case of shea butter the major sterols in this fat (triterpene alcohols) gave a large number of distinctive steradienes.

Although these shea triterpene steradienes have not been identified, a comparison of their relative proportions can be used with existing methods [6] to help to identify and quantify cocoa butter supplements in chocolate.

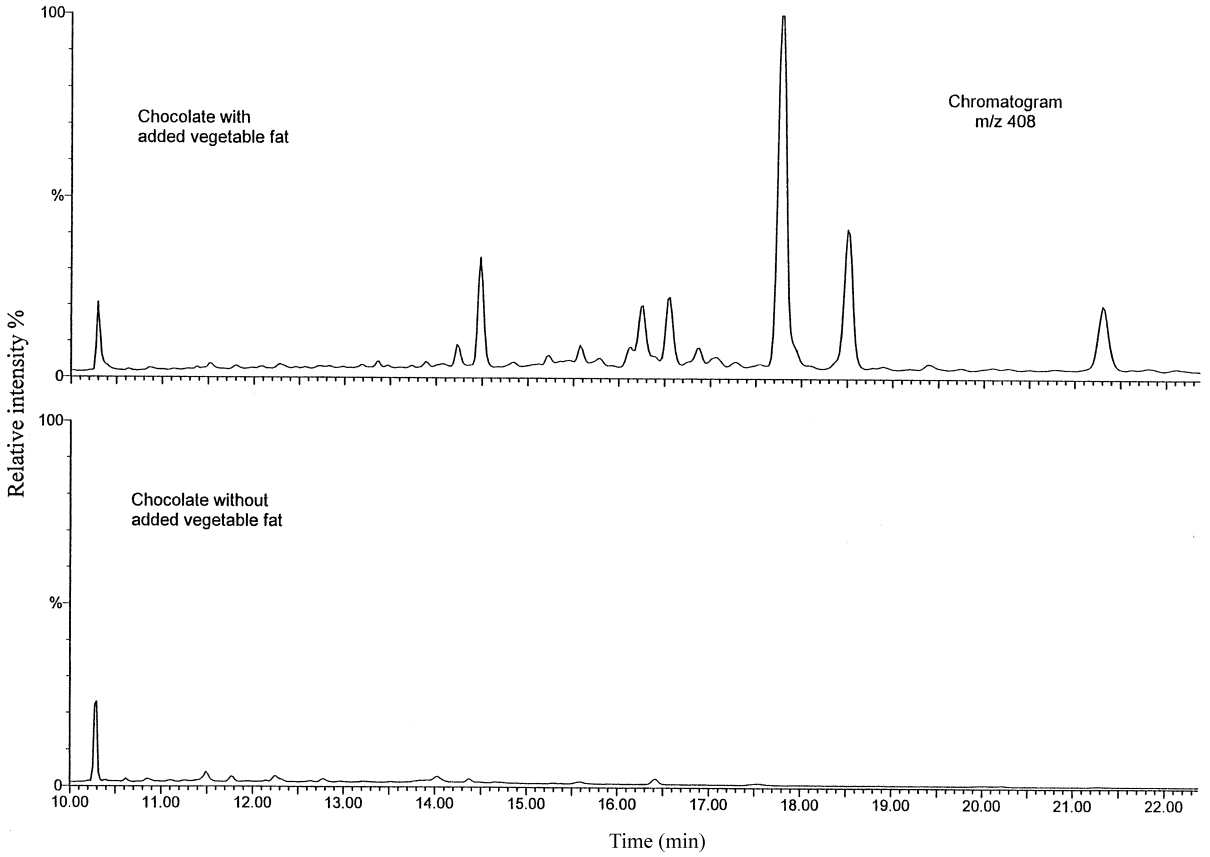

Fig. 7. Selected ion chromatogram of the triterpene sterene molecular ion m/z 408 for extracts of chocolates with and without added vegetable fat.

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