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Gas chromatographic characterization of soapstocks from vegetable oil refining

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Abstract

Gas chromatography was used after trimethylsilyl derivatization to determine the composition of soapstocks from corn germ and peanut oil refining. Soap fatty acids, polyalcohols, small carbohydrates, sterols, steryl glycosides, mono-, di- and triglycerides were measured using a hexamethyldisilazane/pyridine preparation and a high-temperature capillary column. Additional peaks associated with the fatty acids appeared in the chromatograms when pyridine was incorporated into the sample preparation. Limiting the concentration of soapstock in the sample or replacing pyridine with a chlorinated solvent reduced the formation of these artifacts. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Vegetable oils; Soapstock; Fatty acids; Polyalcohols; Carbohydrates; Sterols; Glycerides; Glycosides

1. Introduction

The main co-product of the vegetable oil refining industry is soapstock. Sodium soaps are formed during the initial refining of the crude oil by reaction of extracted free fatty acids with sodium hydroxide. The soap and most entrapped non-oil material are separated from the oil by centrifugation. This material is called soapstock or foots.

Soapstock is a complex heterogeneous material that can be difficult to handle and analyze. At room temperature, sample consistency varies from watery or oily to pasty or firm. Careful handling of the material is required to ensure that compositional changes do not occur when transferring or storing samples. When exposed to air, soapstocks lose moisture and residual solvent rapidly, and they can be unstable at elevated temperatures because of the presence of residual alkali. While some alkali catalyzed reactions are probably unavoidable because soapstock usually exits from the process centrifuge at an elevated temperature, samples must be stored frozen to limit additional changes. Individual samples can vary greatly in composition. The material typically contains sodium fatty acid soap, glycerides, phosphoglycerides, sterols, organic phosphates, polyalcohols, carbohydrates and proteinaceous material. Because of variations in processing conditions and market needs, soapstocks can also be further processed. The material can be neutralized to improve its stability or acidified to recover a fatty acid rich oil.

Because of its complex nature, analysis of soapstock is a challenging problem. Standard American Oil Chemist's Society (AOCS) methods are available to measure total fatty acids, neutral oil, and volatiles that include moisture and residual solvent [1]. Metals can be determined by atomic absorption spectroscopy [2], and the overall distribution of fatty acids can be determined by methylation and gas chroma-

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tography (GC) [3]. Little information, however, is available on the distribution of the fatty acids among the soap, glycerides and phosphoglycerides contained in the material.

Few analytical techniques are available to provide a detailed analysis of soapstock. The lack of suitable solvent systems and the small stage efficiencies of liquid chromatographic columns limit the usefulness of liquid chromatography. Selective quantitative extraction of individual components is difficult because soapstocks tend to form emulsions. When phase separation is possible, non-quantitative extractions are achieved. Even the standard AOCS method for measuring neutral oil in soapstock, which extracts the oil into an ether phase, substantially overestimates the concentration of this component [2].

GC is a potentially useful technique for characterizing soapstocks. Trimethylsilyl (TMS) derivatization has been widely used to detect and measure many soapstock components, including fatty acids, monoglycerides, diglycerides, carbohydrates, polyalcohols and sterols [1,4]. Silylation has also been reported to displace soap counterions to produce chromatographic peaks identical to those of the corresponding derivatized free fatty acids [5,6]. In addition, new high-temperature gas capillary columns elute triglycerides, which eliminates the need to extract these components prior to analysis.

In this report, chemical methods and GC are used to characterize soapstocks from the standard refining of corn germ and peanut crude oils. The results are compared with the previous analysis of miscella-refined cottonseed soapstocks, and limitations of the chromatographic techniques are discussed.

2. Experimental

2.1. Sample preparation and chemical analyses

Peanut and corn soapstocks were donated by Lou Ana Foods (Opelousas, LA, USA) and AC Humko (Champaign, IL, USA), respectively. Both samples were stored frozen at −20°C until use. Moisture and residual solvent were determined by drying in a forced-draft oven at 105°C for 24 h. Phosphorus and sodium were measured by nitric acid digestion and inductively coupled plasma emission spectroscopy with a Leeman Laboratories Plasma-Spec 1 (40 MHz) spectroscope (Lowell, MA, USA). The samples were prepared according to US Environmental Protection Agency (EPA) digestion procedures for measuring metals in sludge [7]. Phosphorus was detected at 213.618 nm, and sodium was measured at a secondary absorbance of 589.592 nm. Total fatty acids and neutral oil were determined by AOCS methods G 3-53 and G 5-40, respectively [1], and nitrogen was measured by AOCS method Ba 4e-93 with a LECO nitrogen analyzer (Model FP-428, St. Joseph, MI, USA). At least two determinations were made for each test.

2.2. Gas chromatography

TMS derivatization was used to increase the volatility of soapstock components. Samples (~10 mg or ~100 mg) were prepared by adding solvent (2 ml), hexamethyldisilazane (2 ml) and trifluoroacetic acid (0.15 ml). Silylation reagents were from Pierce (Rockford, IL, USA). The solvent was either pyridine or chloroform and contained cholesterol methyl ether as an internal standard. Samples were heated for 45 min at 70°C and were cooled before chromatography. All samples were analyzed within 12 h of preparation. The gas chromatograph (Hewlett-Packard 5890 Series 2 Plus) was fitted with a fused-silica column (15 m×25 μm I.D.) coated with DB-5 active phase (0.1 μm film thickness) from J&W Scientific (Folsom, CA, USA). Helium was used as the carrier gas. The instrument was operated in constant flow mode (~1 ml/min) with a split injector (340°C, 1:50) and flame ionization detector (340°C). The oven temperature program was 100°C for 3 min, 10°C/min to 150°C, 5°C/min to 250°C, 10°C/min to 360°C, which was held for 15 min.

Soapstock components were identified by comparing the retention times of unknown peaks with the retention times of known standards and by mass spectroscopy (MS) (discussed below). Quantitative analysis was performed by internal standardization.

2.3. Mass spectroscopy

Derivatized samples were analyzed with a Finnagan TSQ700 GC–MS system (San Jose, CA,

USA). The chromatographic column and conditions were similar to those described above, except that the injection was splitless, a constant head pressure was maintained, and the column flow exited into a vacuum. MS was operated in electron ionization mode and spectra were collected from m/z 50 to 1000.

3. Results and discussion

The combination of hexamethyldisilazane and solvent was most suitable for derivatizing the sample. Because soapstock contains several classes of compounds, it was difficult to identify an ideal solvent for preparing the sample. Uniform solutions were not formed with dimethylformamide, dimethylsulfoxide, acetylnitrile, acetone or methyl ethyl ketone. Preparations with pyridine, chloroform and methylene chloride, however, did form a single liquid phase. With pyridine, samples were initially uniform and clear, but with time a clear gummy material accumulated on the sides of the reaction vial. Dissolution of this material initially did not affect the resulting chromatograms, but if the samples were left for several days, the fatty acid and glyceride peaks decreased, presumably because of partitioning of fatty components into this second phase. To minimize this partitioning, samples prepared by this method were analyzed within hours after completion of the derivatization chemistry. Several extraneous peaks were also detected when using pyridine as solvent. These peaks were not present when either chloroform or methylene chloride replaced pyridine in the sample preparation. With the chlorinated solvents, however, some particulate material remained after reaction. Filtration or centrifugation removed this material, which also formed upon mixing the derivation reagents alone. No further dissolution occurred with time. The presence of this particulate material did not appear to influence the chromatography and similar results were obtained with either pyridine or chloroform.

The chemical assays indicated that some composition differences existed among the corn, peanut and cottonseed soapstocks (Table 1). Volatile components were considerably different between the corn and peanut samples, but they were within the wide range of values reported for cottonseed soapstocks. The total fatty acid concentration of both samples was significantly greater than the mean total fatty acid concentration of the cottonseed samples. In contrast, phosphorus was considerably lower in both the corn and peanut foots than in the average cottonseed foots [2]. An inverse relationship was previously reported between phosphorus and total fatty acids among cottonseed soapstocks [2], and this trend appears to exist among soapstocks in general. Similarly, the concentration of phospholipids in oil-bearing materials has been correlated with crude oil refining loss [8]. These relationships indicate that seed phospholipids promote extraction of significant amounts of non-oil components into the miscella. Both components are then concentrated in the foots during refining. Neutral oil measured by the AOCS method was greater for the peanut sample than for either the corn or average cottonseed sample. The sodium concentration of the peanut soapstock was

Table 1
Chemical analysis (%) of corn and peanut soapstocks[a]

Component	Corn	Peanut	Cottonseed[b] (range, 39 samples)
Moisture and residual solvent	37.9 (0.1)	56.7 (0.2)	32.1–67.1
Total fatty acids	67.8 (0.6)	71.4 (1.1)	39.9–73.8
Neutral oil	23.9 (0.1)	33.5 (1.1)	5.6–55.7
Phosphorus	0.898 (0.018)	0.552 (0.009)	0.464–1.74
Sodium	4.56 (0.19)	9.86 (0.08)	3.4 (0.7)[c]
Nitrogen	0.587 (0.009)	0.184 (0.023)	0.293–1.38

[a] Except for moisture and residual solvent, all values are reported on a dry basis. Parentheses indicate standard deviations. All tests were conducted in triplicate, except for the sodium and phosphorus analyses, which were conducted in duplicate.
[b] Ref. [2].
[c] Mean value of 39 samples calculated from the fatty acid profile. Sodium from residual sodium hydroxide was not included in this value.

also considerably greater than the sodium concentration of the corn soapstock or the reported estimated average of the cottonseed soapstocks [2]. This reported average value for cottonseed soapstock, however, is likely to be low, because it was calculated from the soap-forming fatty acid profile [2] and excluded any residual unreacted sodium hydroxide present. Comparison of this estimation method with measured values on a few cottonseed samples indicated that the calculated values were on average lower by ~20%. The high concentration of sodium in the peanut soapstock is likely the result of a difficult to refine starting material or a stringent refining specification required for the final oil product.

The main fatty acids detected by GC (Fig. 1) included palmitic, stearic, oleic and linoleic (Table 2). The distribution of these acids in the corn soapstock was 48.9% linoleic, 25.7% oleic, 23.7% palmitic and 1.4% stearic, while in the peanut sample this distribution was 46.1% oleic, 31.5% linoleic, 20.6% palmitic and 1.9% stearic. Myristic and arachidic acids were also detected in small concentrations in both samples, and trace concentrations of the longer chain fatty acids (C_{22}–C_{26}) were present in the peanut soapstock but were less concentrated than expected based on the reported fatty acid distribution of peanut oil [9].

For both soapstocks, the percentage of palmitic acid among the soap fatty acids was higher than is typically found for the total fatty acid distributions of the refined oils. A similar effect was reported for cottonseed soapstock [2]. In this regard, a greater percentage of saturated fatty acids was also reported among the total fatty acids in corn, cottonseed, sunflower, soybean and safflower soapstocks [3,10]. Several factors likely contribute to these effects. Saturated fatty acids of all of these vegetable oils are concentrated at the s*n*-1 and s*n*-3 positions of the triglyceride backbone [9,11,12]. Because chemical and enzymatic glyceride hydrolyses occur preferentially at these positions [13], limited triglyceride degradation within the kernel or crude oil would lead to the release of a higher proportion of saturated free fatty acids. These free acids would separate with the foots. Limited triglyceride hydrolysis within the soapstock itself (discussed below), while not affecting the overall fatty acid distribution, would contribute to the increase in soap saturated fatty acids. The partitioning of polar lipids into soapstock also enhances the overall proportion of saturated fatty acids, because both corn germ and peanut phosphoglycerides contain a significantly higher percentage of palmitic acid than their triglycerides do [12,14].

Linolenic and linoleic TMS esters co-eluted on the non-polar stationary phase used in this work. Although linolenic acid is only a minor component of the glycerides in corn and peanut oils (<1%), the linoleic acid concentrations reported in Table 2 include any linolenic acid that is present. Separation of these fatty acids requires a more polar stationary phase. Unfortunately, these column phases are not stable at the high temperatures needed to elute triglycerides.

Measurable quantities of 1-palmitin, 1-oleitin and 1-linoleitin were found in both soapstocks, and 1-steritin and 2-palmitin were present in trace levels. Diglycerides with 34 and 36 acyl carbon atoms were separated in both samples. Because the diglycerides would not be expected to preferentially separate into the soap phase, a higher diglyceride-to-triglyceride ratio in the soapstock than in the crude or refined oil is an indication of alkali-catalyzed hydrolysis. Some glycerolysis does appear to have occurred in these samples, based on the reported diglyceride-to-triglyceride ratio of refined peanut and corn oils [15]. However, this ratio did not change during the several month course of this study, indicating that the samples were stable when stored at −20°C.

With non-polar stationary chromatographic phases, triglycerides separate primarily by carbon number with some minor discrimination by degree of saturation. Response factors for these components decreased sharply with increasing acyl carbon number. To calculate the concentration of the individual triglyceride peaks, response factors were determined for triglycerides with increasing acyl carbon numbers from tripalmitin through triarachidin. These values were fitted as a function of elution time with a second-degree polynomial (correlation of determination >0.999), and interpolation of this function gave response factors for the individual triglyceride peaks. Both samples contained primarily triglycerides with 52 and 54 combined carbon atom acyl chains consistent with the triglyceride profiles of these oils [15]. The peanut sample also exhibited

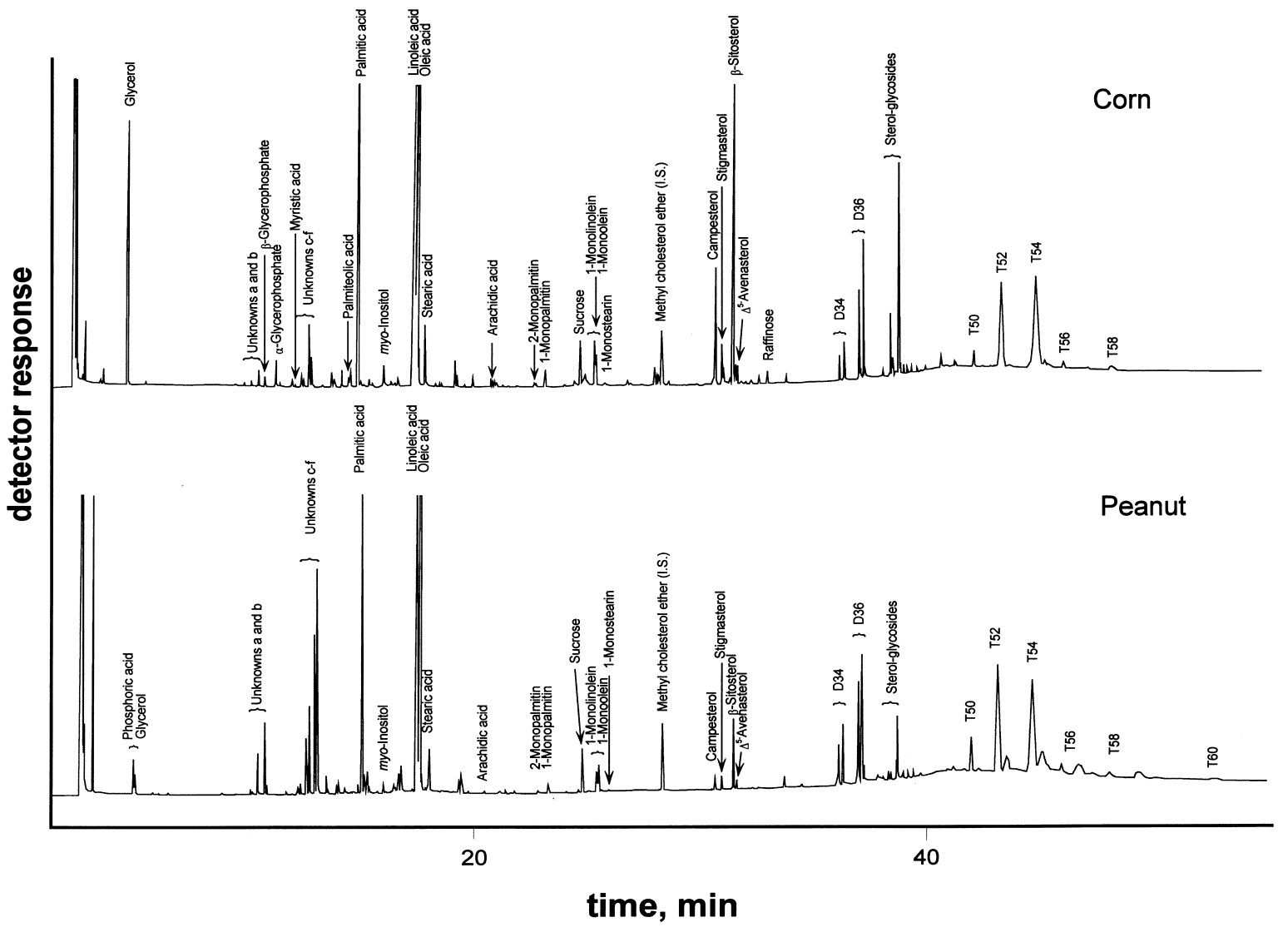

Fig. 1. Chromatograph of trimethylsilyl-derivatized corn and peanut soapstocks.

Table 2
Components of soapstocks detected by trimethylsilylation and gas chromatography

Component	Concentration[a]		
	Corn	Peanut	Cottonseed[b] (range, $n=39$)
Phosphates			
Phosphoric acid	tr	0.177 (0.028)	tr
β-Glycerophosphate	0.083 (0.029)	nd	0.025–0.453
α-Glycerophosphate	0.176 (0.018)	tr	0.079–0.622
Fatty acids			
Myristic acid	0.031 (0.003)	0.066 (0.008)	0.101–0.409
Palmitoleic acid	tr	tr	0.021–0.387
Palmitic acid	8.62 (0.53)	4.89 (0.18)	4.14–15.1
Linoleic acid	17.8 (1.0)	7.75 (0.20)	8.41–25.2
Oleic acid	9.36 (0.28)	11.6 (0.42)	3.50–10.1
Stearic acid	0.507 (0.043)	0.458 (0.040)	0.447–1.39
Arachidic acid	0.076 (0.002)	tr	tr–0.131
Monoglycerides			
1-Monopalmitin	0.160 (0.008)	0.107 (0.018)	nd–0.771
1-Monolinolein	0.277 (0.014)	0.211 (0.005)	nd–2.054
1-Monoolein	0.653 (0.037)	0.267 (0.002)	nd–0.730
Diglycerides (by acyl carbon number)			
D32	nd	nd	nd–0.227
D34	0.440 (0.013)	0.806 (0.123)	nd–2.33
D36	1.81 (0.07)	2.01 (0.23)	nd–3.21
Triglycerides (by acyl carbon number)			
T48	tr	tr	nd–0.388
T50	0.312 (0.018)	0.608 (0.044)	nd–3.65
T52	3.02 (0.20)	4.64 (0.053)	nd–9.51
T54	6.80 (1.41)	6.00 (1.60)	nd–8.60
T56	0.297 (0.040)	1.09 (0.06)	nd–0.547
T58	0.655 (0.065)	3.17 (.64)	nd–0.784
T60	nd	tr	nd
Sterols			
Campesterol	1.27 (0.02)	0.126 (.004)	nd–0.215
Stigmasterol	0.290 (0.004)	0.117 (0.005)	nd–0.211
β-Sitosterol	3.30 (0.048)	0.563 (0.019)	0.55–2.80
Δ^5-Avenasterol	0.129 (0.003)	0.075 (0.002)	nr
Campesterol glycoside	0.414 (0.050)	0.057 (0.012)	tr
Stigmasterol glycoside	0.127 (0.022)	0.050 (0.024)	tr
β-Sitosterol glycoside	1.34 (0.15)	1.34 (0.15)	0.889–2.84
Polyalcohol and carbohydrates			
Glycerol	1.014 (0.083)	0.078 (0.015)	0.310–5.06
myo-Inositol	0.162 (0.068)	0.026 (0.006)	0.143–0.472
Sucrose	0.538 (0.052)	0.358 (0.047)	0.027–0.503
Raffinose	0.106 (0.017)	tr	0.025–1.069
Stachyose	nd	nd	tr–0.163

[a] All values are reported in percent on a dry basis. Parentheses indicate standard deviations ($n=3$).
tr=Trace; nd=not detected; nr=not reported.
[b] Ref. [2].

peaks with acyl chains totaling up to 60 carbon atoms.

By chromatography, the concentration of triglycerides was higher in the peanut and corn soapstocks than the average value reported for cottonseed soapstocks [2]. This difference is attributed to the different refining techniques used for these oils. During the hexane extraction of most vegetable oils (soybean, peanut, corn germ, etc.), the solvent is recovered directly after extraction and the oil is marketed as a crude product. These oils are later refined in dedicated refining facilities. In cottonseed processing, the presence of pigments in the kernel can result in the crude oil becoming ''color-set'' and difficult to refine. Consequently, cottonseed crude oils are refined immediately after extraction to reduce this potential for discoloration. Because extraction and refining occur in the same facility, cottonseed mills can begin refining prior to solvent recovery, a process referred to as miscella refining. One advantage of this modified processing scheme is that the lower density of the miscella improves the centrifugal separation of the soap and oil/solvent phases and results in a lower refining loss. Consequently, miscella refined soapstocks tend to have a lower concentration of triglycerides and total fatty acids than crude oil refined soapstocks.

As was previously reported [2], the total concentration of neutral lipids (triglycerides, diglycerides and sterols) calculated by GC was significantly lower than neutral oil concentration measured by the AOCS method.

Sucrose was the most concentrated sugar in both samples (Table 2). Raffinose was measurable in the corn sample and detectable in the peanut sample. Other galactosugars were not significant. Compared with cottonseed soapstock, the sucrose concentration was higher and the raffinose concentration lower in these soapstocks. Waste water (acid water) produced by corn or peanut soapstock acidification contained relatively low levels of both of these sugars compared with waste water produced from acidification of cottonseed soapstock [16].

β-Sitosterol, campesterol, stigmasterol and Δ^5-avenasterol were the main sterols in both samples. The assignment of Δ^5-avenasterol is tentative. This identification was based on the elution patterns of TMS-sterol ethers on an OV-1 packed column [17], the correspondence of a sterol peak from caster oil, which contains a relatively high concentration of this sterol [17,18], and the reported concentration of Δ^5-avenasterol relative to the concentrations of stigmasterol and campesterol in corn, peanut and castor oils [17,18]. Small concentrations of other sterols were also indicated. Glycosides of the three principal sterols were identified by MS. These components existed in the same relative concentrations as the free sterols. In crude cottonseed oil, the principal sterol is β-sitosterol [9] and the sugar moiety of the steryl glycosides fraction has been isolated and identified as glucose [19]. Because the main compound of this class from cottonseed soapstock has a retention time identical to that of the β-sitosterol glycoside from the corn and peanut samples, this suggests that the carbohydrate of these compounds is also glucose. Unequivocal identification of these sugar moieties, however, cannot be made at present because of the similarity of the mass spectroscopic fragmentation patterns of pyranosyl monosaccharides [20]. Steryl esters and steryl glycosides have been reported as components of whole corn kernels [21,22], with the concentration of the steryl esters being much greater than the concentration of steryl glycosides. Steryl esters were not pronounced components of these soapstocks, although small unidentified peaks eluting at times similar to the steryl glycosides may represent these compounds. The relatively high concentration of the glycosides is likely due to preferential partitioning of these more polar components into the soapstock phase.

A series of unknown compounds (labeled a–f, Figs. 1 and 2) eluted early in the chromatograms of sample preparations that incorporated pyridine. MS did not identify these components because the splitless injection greatly reduced these peaks (discussed below). Indirect evidence suggests that these components were related to the principal fatty acids within the sample and resulted from chemical changes that occurred in the high temperature chromatograph inlet. For each sample, the principal six unknown peaks appeared as two sets of three; each set with peak areas in the same relative order as the peak areas of the sample's TMS-esters of palmitic, oleic and linoleic acids (Fig. 2). Among the different samples, the size of these peaks correlated with the sodium content of the soapstocks. While the

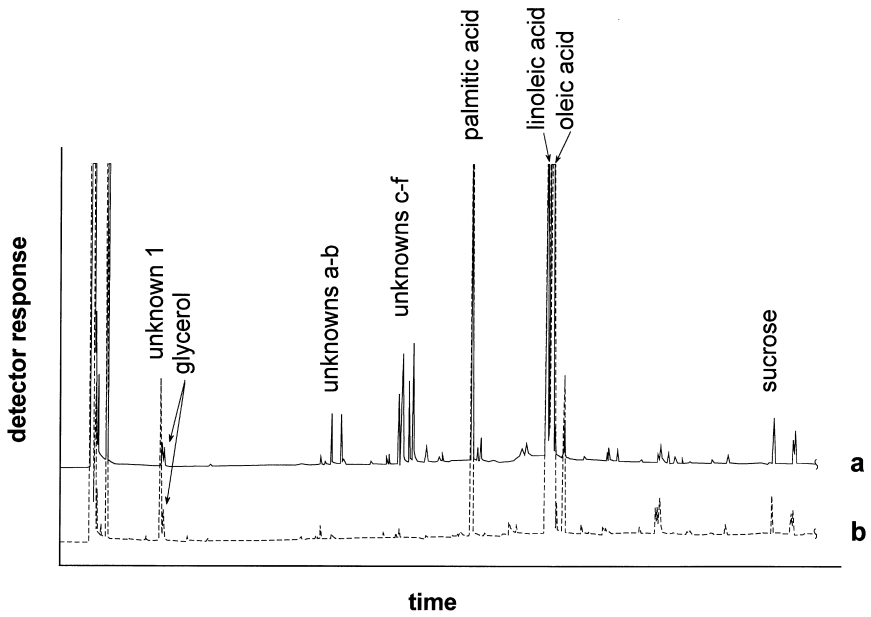

Fig. 2. Partial chromatograms of trimethylsilyl-derivatized peanut soapstock incorporating pyridine (**a**) and chloroform (**b**) as solvents. Several additional peaks (unknown peaks a–e) were detected when pyridine is included in the sample preparation. A single unknown peak is formed when chloroform was used as solvent (unknown peak 1).

formation of these artifacts was relatively minor for cottonseed soapstocks, their occurrence was problematic in the peanut sample, which contained a very high concentration of sodium.

In attempting to overcome this problem, several methods of sample injection were tried. While these approaches reduced or eliminated the peaks, they resulted in other chromatographic difficulties. Splitless injection with a reduced concentration of soapstock in the preparation did not produce these peaks but yielded very complicated chromatograms resulting from solvent impurities, derivatization byproducts, minor constituents, etc. Cool on-column injection exhibited similar complicated chromatograms, and a wide band of severe column bleed occurred in the middle of the chromatogram. The introduction of sodium directly to the column may be responsible for this latter effect, as the deposited metal may promote phase degradation during column heating.

Because the formation of these compounds appeared to be dependent on the concentrations of fatty acids and sodium, reducing the amount of soapstock in the preparation greatly reduced the formation of the unknown artifacts. Because the fatty acids are the most concentrated components in the samples, quantification of these compounds could be achieved at lower soapstock concentrations, which minimized the formation of the unknown peaks. This approach was used to calculate the reported palmitic, oleic and linoleic concentrations in Table 2.

Alternatively, these peaks did not occur when chloroform (or methylene chloride) was used as a substitute solvent (Fig. 2), although a single peak did occur that co-eluted with the TMS-derivative of phosphoric acid (Fig. 2). This peak was not associated with the soapstock because it is also found if the derivatization chemicals were mixed alone. Disadvantages of this derivatization method include the removal of the sediment formed in the reaction vial and the added care needed for safe handling of chlorinated solvents.

4. Conclusions

GC after TMS derivatization is a useful tool for analyzing vegetable oil soapstocks. The method has

now been tested on sodium soapstocks from both conventional and miscella refining processes. Limitations include the inability to separate TMS-esters of linoleic and linolenic acid (with high-temperature capillary columns of low polarity) and some degradation of the sample fatty acids (that is believed to be due to chemical rearrangements involving pyridine and sodium at high temperature) in the gas chromatographic inlet. Reducing the concentration of soapstock injected into the chromatograph minimized the second problem. Removing pyridine from the sample preparation also eliminated these artifacts.

Soapstocks from corn germ and peanut oil refining contained fatty acids (as soap), mono-, di- and triglycerides, sterols, sterol-glycosides, carbohydrates and polyalcohols. Compared with the corn or average cottonseed sample, the peanut soapstock contained a high concentration of sodium and a low concentration of phosphorus. The corn soapstock was characterized by having a high concentration of sterols and sterol glycosides.

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