Compositional Characterization of Cottonseed Soapstocks

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ABSTRACT: Cottonseed soapstock samples, collected during the 1993–1994 crushing season from oilseed extraction mills throughout the United States Cotton Belt, were analyzed by chemical and chromatographic methods. Volatiles averaged 48.7 ± 10.6% (mean ± SD, $n = 39$). On a dry basis, the samples averaged 33.3 ± 7.3% fatty acids, 26.3 ± 6.9% phospholipids, 8.4 ± 6.4% triglycerides, and 7.5 ± 3.0% gossypol. The analytical techniques accounted for 93.3 ± 8.6% of the dry soapstock matter. The AOCS method for total fatty acids in soapstock yielded values in agreement with the chromatographic and phosphorus analyses. In contrast, the AOCS method for neutral oil in soapstock gave values that were significantly higher than those obtained by chromatography. The amount of nonlipid material in the samples correlated with the phosphorus content. Total gossypol and nitrogen levels were also related.
JAOCS 73, 1287–1295 (1996).

KEY WORDS: Cottonseed soapstock, gas chromatography, gossypol, silylation, soapstock, trimethylsilylation.

Soapstock is formed by treating (refining) crude vegetable oils with alkali to produce a sodium soap, which is separated from the oil by centrifugation. Typically, soapstock will account for 5 to 10% of the crude oil mass, although higher values are possible if the crude oil is "color set" or has a high concentration of free fatty acids. In the past, soapstock from oilseed refining has been used as a source of fatty acids and glycerol. These processes are no longer cost-effective. Consequently, in cottonseed oil extraction facilities, soapstock is added to extracted marc (meal) to increase the energy content, reduce dust, and improve pelleting of feed products. In oil-refining operations where no feed material is produced, the material is often acidified, and the acid oil sold to supplement animal feeds.

Crude cottonseed oil is rich in nonoil material (1). Hence, soapstock from cottonseed oil is a mixture of soap, neutral lipids, phospholipids, and nonlipid components. General compositional information has been published on raw and acidulated cottonseed soapstocks (2,3), and some information can be inferred from the compositions of crude and refined oils. A detailed compositional study has not, however, been reported.

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As an initial step of a project to find alternative uses for soapstock, a compositional study was conducted on samples of raw cottonseed soapstock that were collected by the National Cottonseed Products Association (NCPA) and the author. The collection, developed during the 1993–1994 crushing year, included samples from plants throughout the United States Cotton Belt, from three types of oil-extraction processes, and from soapstocks produced in the early fall through late spring.

Gas chromatography (GC) has not been used to study soapstock. Trimethylsilyl derivatization has been reported to displace soap counterions to form products that are easily detected by GC (4). Although sodium soaps were not considered in that work, sodium ions are also readily displaced. Silylation has also been used to study sterols, polyalcohols, sugars, and partially esterified glycerides (5–7), all compounds that have been reported in crude cottonseed oil or soapstock. Finally, new capillary columns with extended temperature ranges elute triglycerides, eliminating the need to pre-extract these compounds. These features make it feasible to develop techniques to study soapstock by GC. Combined with standard chemical methods, a detailed analysis is possible.

EXPERIMENTAL PROCEDURES

Sample collection. NCPA soapstock samples were collected by plant personnel and shipped to a collection point at the Texas A&M Agriculture Experiment Station (San Angelo, TX). Delivery typically took three to four days. On arrival, all samples were stored frozen in the original containers. Subsamples were taken immediately after thawing, refrozen, shipped on ice to the Southern Regional Research Center (SRRC), and stored frozen (−20°C) until use. Additional samples, collected by the author, were frozen immediately after collection. A total of 39 samples were analyzed. Most of the samples were from expander-solvent processes, although direct-solvent and prepress solvent processes were represented. For statistical purposes, the samples were classified by region as East, Delta, Texas, or West, and by processing season as Fall, Winter, or Spring. Seventeen mills were represented in the sampling, ten mills shipped samples during the three processing seasons.

Chemical analyses. Dry matter was determined from the weight difference of an initial 2-g sample after 24 h in a

forced-draft oven at 105°C. Phosphorus was measured by nitric acid digestion (8) and inductively coupled plasma emission spectroscopy with a Leeham Labs 40 Mhz Model Plasmaspec 1 spectroscope (Lowell, MA). The instrument was operated for phosphorus at a wavelength of 213.618 nm with a detection limit of 0.5 ppm. Official AOCS methods (7) were used to analyze for total fatty acids (G 3-53), neutral oil (G 5-40), nitrogen (Ba 4e-93), and total gossypol (Ba 8-78). Woodsen-Tenent Laboratories, Inc. (Little Rock, AR) conducted the total fatty acid and neutral oil tests. Values reported from tests conducted at SRRC are the means of at least two determinations. To reduce costs, tests performed by commercial labs were conducted only once. If not explicitly mentioned in the text, values are reported on a dry mass basis.

GC. Silylation reagents were from Pierce (Rockford, IL), and standards were from the Sigma Chemical Co. (St. Louis, MO). Approximately 120 mg of raw soapstock was weighed into a reaction vial. Pyridine (2 mL), hexamethyldisilazane (2 mL), and trifluoroacetic acid (150 μL) were added. Cholesterol was used as an internal standard. The solution was sealed, shaken for 30 s, and heated in a dry bath at 60°C for 1 h. This procedure produced a transparent uniform sample that was stable for several hours. With time, a clear gummy material, believed to be derived from the phospholipid component of the sample, accumulated on the sides of the vial. Initially, dissolution of this material had no effect on the chromatographic results. On standing several days, however, the concentrations of fatty materials decreased, presumably due to partitioning into this second phase. To avoid this complication, all samples were analyzed within 12 h of preparation.

A Hewlett-Packard (Palo Alto, CA) 5890 Series 2 Plus GC with a Series 7673 autoinjector was used for chromatography. The instrument was fitted with a J&W Scientific (Folsom, CA) DB-5ht capillary column (12.5 m × 0.25 μm i.d. × 0.1 μm film thickness). The carrier gas was helium, and the instrument operated in constant flow mode with an initial head pressure of 1.48 atm (7.1 psig), corresponding to a volumetric flow rate of ~1 mL/min. A split injector, fitted with a straight flow-through inlet plugged with silylated glass wool, was operated at 330°C with a split ratio of 1:50. Injected volumes were 2 μL. The oven temperature program was 100°C for 3 min, 10°C/min to 150°C, 5°C/min to 250°C, and 10°C/min to 360°C, which was maintained for 10 min. Detection was by flame-ionization at 350°C. All samples were prepared and analyzed in triplicate.

Mass spectroscopy. A Hewlett-Packard Model 5890 Series II GC coupled to a Model 5988A mass spectrometer was used for electron ionization mass spectroscopy. The chromatograph was operated as above, except that the injector was not pressure-controlled and the mass detector required a vacuum. Only four representative samples were analyzed to help identify unknown peaks.

Statistics. The SAS statistical analysis package (Cary, NC) was used to analyze for statistical differences. Linear regression was used to search for correlations among measured components, and analysis of variance was used to test for seasonal and processing effects.

RESULTS AND DISCUSSION

Means, SD, and ranges from the chemical analyses are given in Table 1. Volatiles, mainly moisture and residual solvent, accounted for 48.7 ± 10.6% (mean ± SD) of the total sample. Because soapstocks lose moisture and solvent readily, part of the variation may be associated with the different containers used and times needed to ship samples to the collection point. The organic matter also varied considerably. Total fatty acids averaged 60.5 ± 7.2% on a dry basis (db). On average, neutral oil accounted for 25.1 ± 11.1% (db) or 12.9 ± 6.0% of the whole sample (wb). Jamieson (9) reported a value of 18.7% (wb). This value, however, is for settled soapstock, and the continuous centrifugation process used today achieves better recovery of the neutral oil (10,11). The average phosphorus and nitrogen contents were 1.20 ± 0.29% (db) and 0.72 ± 0.28% (db), respectively. Total gossypol averaged 7.5 ± 3.0% (db).

Trends existed among many of the chemical constituents. Figure 1 shows a plot of total fatty acids vs. phosphorus, where a negative correlation was found ($R^2 = 0.60$, $P < 0.0001$). As most of the phosphorus is associated with phospholipids, a positive trend might have been anticipated. The contrasting result suggests that extraction of oilseed phospholipids dramatically affects extraction of nonlipid material. This effect may contribute to the high refining losses that are characteristic of oilseeds containing high levels of phospholipids (12).

A correlation between nitrogen and phosphorus was also apparent ($R^2 = 0.54$, $P < 0.0001$) (Fig. 2). Because three of the four major classes of phospholipids contain nitrogen, some correlation between the components was expected. The distribution of phospholipid classes in solvent-extracted crude cottonseed oil was reported by Cherry (13). From this distribution, the expected nitrogen/phosphorus ratio is 1:3.3 and is shown as a dashed line in Figure 2. The data indicate that more nitrogen exists in soapstock than is accounted for by phospholipids. This additional nitrogen is probably associated with protein fragments, which have been reported as a

TABLE 1
Composition of Cottonseed Soapstocks[a]

Component	Mean[b] (%)	Range (%)
Volatiles[c]	48.7 (10.6)[d]	32.1–67.1
Total fatty acids	60.5 (7.2)	39.9–73.8
Neutral oil	25.1 (11.1)	5.6–55.7
Phosphorus	1.198 (0.292)	0.644–1.74
Nitrogen	0.723 (0.276)	0.293–1.382
Total gossypol	7.49 (3.04)	0.33–13.05

[a]Values on given on a dry-matter basis except where noted.
[b]$n = 39$.
[c]Volatiles were determined on a whole-sample basis.
[d]Parenthesis indicate SD.

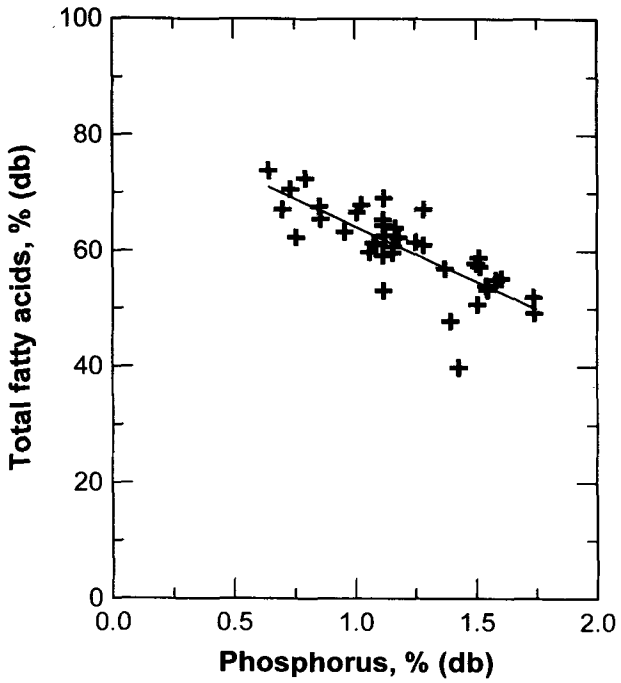

FIG. 1. Association between total fatty acids and elemental phosphorus in cottonseed soapstocks. The solid line represents the linear regression model ($R^2 = 0.60$, $P < 0.0001$). db, Dry basis.

minor component of crude cottonseed oil (14). From the difference in the total and phospholipid nitrogen concentrations, protein content (6.25 × nitrogen) was estimated to be 2.3 ± 1.4% of the dry soapstock mass after accounting for the nonlipid phosphate detected by GC (see below). Because the slope of the data regression line was greater than the slope derived from the expected phospholipid nitrogen/phosphorus ratio, proteinaceous nitrogen increases with phosphorus content, an additional indication that phospholipids enhance the entrapment of nonoil materials during extraction.

Figure 3 shows that nitrogen and total gossypol concentrations were also correlated ($R^2 = 0.39$, $P < 0.0001$). Because the aldehyde moieties of gossypol can react with amines, this correlation suggests that a considerable fraction of soapstock gossypol is in the bound form (see below). The heat and moisture used in preparing cottonseed for oil extraction facilitates the covalent binding of gossypol to proteins and phospholipids (15).

A typical chromatogram of trimethylsilylated cottonseed soapstock is shown in Figure 4. Nearly all peaks were identified. Response factors were calculated by internal standardization for determining the concentrations of individual compounds. Coefficients of determination from the response factor regressions were always >0.999 and were generally >0.9997. Coefficients of variation from the triplicate determinations were typically 3–5% for major components and 10–15% for minor components. A summary of retention times and concentrations is given in Table 2.

On a db, the soap-forming fatty acids averaged 33.3 ± 7.2%. The distribution of these acids was 49.9 ± 3.0% linoleic, 27.6 ± 3.4% palmitic, 18.3 ± 1.5% oleic, 2.9 ± 0.6% stearic, 0.72 ± 0.20% myristic, 0.54 ± 0.26% palmitoleic, and 0.25 ± 0.13% arachidic. These values are typical of refined

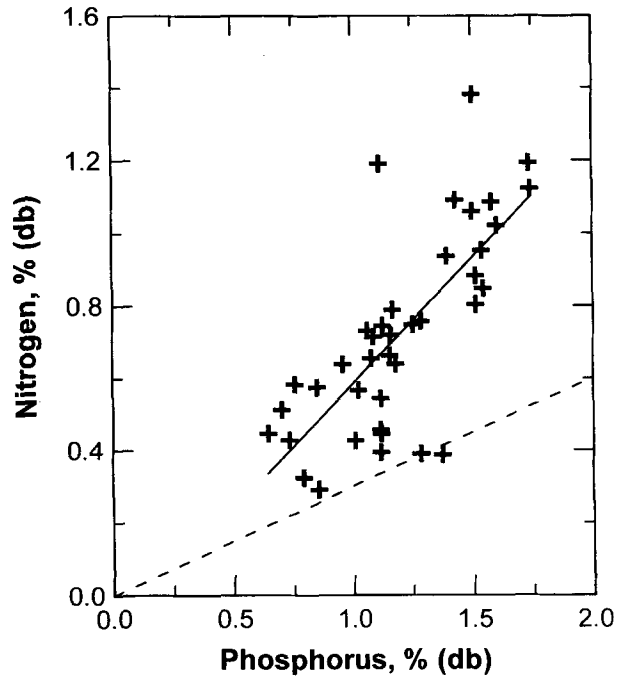

FIG. 2. Association between nitrogen and phosphorus content in cottonseed soapstock samples. The dashed line represents the expected nitrogen level if all phosphorus is attributed to phospholipids. The solid line represents the linear regression model ($R^2 = 0.54$, $P < 0.0001$). See Figure 1 for abbreviation.

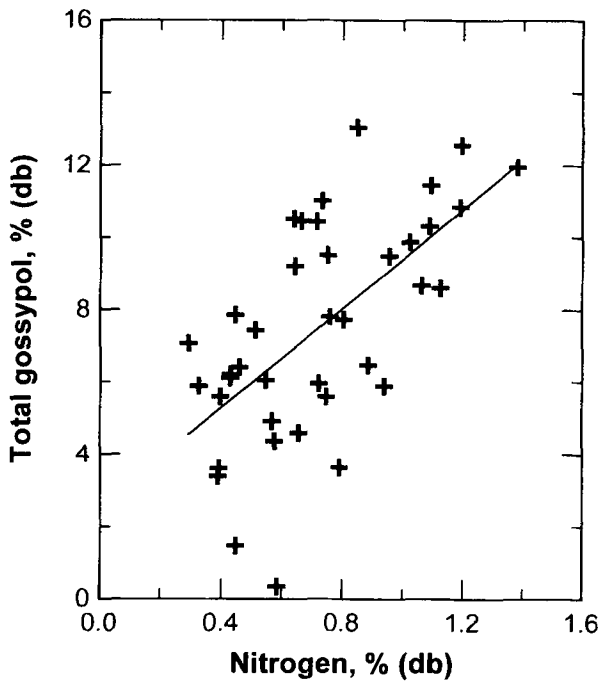

FIG. 3. Association between total gossypol and nitrogen content in cottonseed soapstock samples. The solid line represents the linear regression model ($R^2 = 0.39$, $P < 0.0001$). See Figure 1 for abbreviation.

TABLE 2
Retention Time, Average, and Range Concentrations of Important Components of Trimethylsilyl-Derivatized Cottonseed Soapstocks

Compound	Relative retention time	Concentration, % (db)	
		Mean[a]	Range[b]
Organic phosphates[c]			
β-glycerophosphate	0.322	0.18 (0.11)	0.025–0.453
α-glycerophosphate	0.338	0.33 (0.13)	0.079–0.622
Free fatty acids			
Myristic acid	0.361	0.24 (0.09)	0.10–0.41
Palmitoleic acid	0.448	0.18 (0.09)	0.02–0.39
Palmitic acid	0.466	9.31 (2.61)	4.14–15.1
Stearic acid	0.571	0.96 (0.19)	0.45–1.39
Oleic acid	0.558	6.07 (1.26)	3.50–10.1
Linoleic acid	0.554	16.50 (3.56)	8.40–25.2
Arachidic acid	0.580	0.077 (0.035)	trace–0.13
Total:		33.34 (7.26)	18.81–50.88
Monoglycerides			
1-Monopalmitin	0.713	0.287 (0.16)	0.00–0.77
1-Monolinolein	0.846	0.676 (0.42)	0.00–2.05
1-Monoolein	0.848	0.260 (0.18)	0.00–0.73
Total:		1.22 (0.70)	0.00–3.08
Diglycerides (grouped by acyl carbon number)			
D32	1.232-1.238	0.082 (0.059)	0.00–0.23
D34	1.265-1.272	1.12 (0.60)	0.00–2.33
D36	1.297-1.303	1.42 (0.74)	0.00–3.21
Total:		2.62 (1.36)	0.00–5.76
Triglycerides (by acyl carbon number)			
T48	1.445	0.15 (0.11)	0.00–0.39
T50	1.473	1.36 (1.01)	0.00–3.63
T52	1.507	3.55 (2.70)	0.00–9.51
T54	1.547	2.92 (2.36)	0.00–8.60
T56[d]	1.604	0.17 (0.17)	0.00–0.55
T58[d]	1.633	0.25 (0.26)	0.00–0.78
Total:		8.41 (6.36)	0.00–22.52
Sterols (free and esterified)			
Campesterol[e]	1.043	0.088 (0.043)	trace–0.22
Stigmasterol	1.057	0.086 (0.063)	trace–0.21
β-Sitosterol[e]	1.079	1.16 (0.49)	0.55–2.80
β-Sitosterol–glucoside[f]	1.360	1.71 (0.50)	0.89–2.84
Polyalcohols			
Glycerol	0.089	2.09 (1.28)	0.31–5.06
Myo-inositol	0.504	0.48 (0.14)	0.14–0.47
Carbohydrates			
Sucrose	0.822	0.24 (0.11)	0.027–0.50
Raffinose	1.145	0.56 (0.25)	0.025–1.07
Stachyose	1.324	0.087 (0.039)	trace–0.16
Miscellaneous			
Phosphoric acid	0.086	nr[g]	trace–0.39
Gossypol (1)	1.221	0.62 (0.64)	0.00–2.81
Gossypol (2)	1.255	0.11 (0.14)	0.00–0.43
Unknown a[h]	0.376	0.14 (0.18)	0.00–0.90
Unknown b[h]	0.380	0.081 (0.10)	0.00–0.51
Unknown c[h]	0.388	0.26 (0.31)	0.00–1.26
Unknown d[h]	0.392	0.11 (0.15)	0.00–0.62
Unknown e[i]	1.117	0.28 (0.17)	0.00–0.93
Cholesterol (IS)[j]	1.000		

[a]n = 39. [b]Trace levels. [c]Excludes two samples with high levels of glycerophosphates (4.20 and 3.92%). [d]Measured as T54. [e]Measured as stigmasterol. [f]Measured as cholesteroyl–myristate ester. [g]nr = Not reported. Value is not thought to be representative of an average sample. [h]Measured as myristic acid. [i]Measured as raffinose. [j]Retention time for cholesterol was 26.77 min.

cottonseed oils (1,16), although a slight concentration of the saturated fatty acids cannot be ruled out, as has been previously reported (3,17). Traces of lauric, myristoleic, behenic, and lignoceric acids also appeared, as did traces of heptadecanoic and nonadecanoic acids.

Raffinose was the most prevalent of the carbohydrates, followed by sucrose and stachyose. The same relative distribution of galactosugars has been reported for cottonseed kernels (18) and cottonseed meal (19). Verbascose was not detected; nor were monosaccharides significant, although trace levels of pentoses and hexoses were indicated by mass spectroscopy. On average, free carbohydrates composed 0.89 ± 0.37% of the dry sample. The concentration of carbohydrates also increased with phosphorus levels ($R^2 = 0.18$, $P < 0.0068$).

Glycerol and *myo*-inositol were the principal polyalcohols with concentrations of 2.1 ± 1.3 and 0.48 ± 0.14% (db), respectively. Trace levels of sorbitol and mannitol were also detected.

Phosphates not associated with phospholipids included phosphoric acid and α- and β-glycerophosphate. Most samples contained trace levels of phosphoric acid, but only seven samples (from four mills) contained measurable levels. Phosphoric acid is occasionally added to crude oils to reduce the amount of caustic needed for refining by converting phospholipids into a hydratable form (20). This processing treatment may explain the elevated phosphoric acid levels in a few samples. The average concentration of the glycerophosphates was 0.69 ± 0.84% but dropped to 0.51 ± 0.24% if two samples with high levels (4.20 and 3.92%) were excluded. The phosphorus contribution from these components represented less than 10% of the total phosphorus, except in the two anomalous samples. Subtracting nonlipid organic phosphate from the total phosphate did not affect the significance of the phosphate-based correlation with total fatty acids ($R^2 = 0.36$, $P < 0.0001$) or nitrogen ($R^2 = 0.41$, $P < 0.0001$), although the coefficients of determination decreased. The correlation with carbohydrates improved ($R^2 = 0.47$, $P < 0.0001$). It is not clear why two samples (from the same oil mill) contained high levels of glycerophosphates. Severe degradation of lipids due to excess alkali was not implied, as the triglyceride levels of these samples were above average. Both samples contained measurable levels of phosphoric acid.

Trimethylsilylated gossypol is detectable by GC but has a complicated chromatogram (21,22). At the conditions employed in this work, gossypol–acetic acid standards yielded a single broad nonsymmetrical peak, followed by a second much smaller broad peak. Both peaks were found in most of the soapstock chromatograms. Mass spectroscopy of the first peak yielded a fragmentation pattern identical to that reported by Abou-Donia *et al.* (23) with a molecular ion of *m/e* 950, which indicates complete silylation. No useful spectrum could be obtained for the second peak. Incomplete silylation may account for this extra peak, although derivatizing longer and at a higher temperature did not remove this peak, nor did derivatizing with N,O-*bis*(trimethylsilyl)acetamide, a stronger silylation reagent. Raju and Cater (21) noticed a similar second peak in the chromatographic analysis of cottonseed meals, although they did not report a similar peak in their gossypol standards. They suggested that this second peak was the gossypol derivative gossyverdurin, which has been isolated from pigment glands (24), although many other derivatives have been identified (25) that could also account for this peak. The lack of symmetry and broadness of the principal peak indicated the presence of multiple compounds. These compounds may be different tautomers, as has been postulated by Raju and Cater (21). McClure (22), using different chromatographic conditions, showed that multiple peaks result from incomplete silylation; yet, complete silylation still yielded a nonsymmetrical peak. Upon silylation, a skewed tautomeric distribution is possibly due to slower reaction of the aldehyde tautomer, which contains sterically hindered C-1 and C-1′ hydroxyl groups, compared with the hemiacetyl (lactol) tautomer, which lacks these hindered groups. In addition, different tautomeric forms have been detected for gossypol–amine complexes (26,27). These points suggest that a population of silylated tautomers is present. Even with these nonideal chromatographic conditions, a linear response factor was obtained with $R^2 > 0.999$ for the two combined peak areas.

The aldehyde moieties of gossypol react readily with amines to form anils (25). Chromatography of several trimethylsilylated gossypol–amine complexes did not yield a chromatographic peak with the same retention time as hexatrimethylsilyl–gossypol (Dowd, M.K., unpublished results). Hence, the derivatization chemistry did not convert bound gossypol to unbound gossypol, and the soapstock gossypol detected by GC must be unbound. The average dry mass concentration of gossypol detected by chromatography was 0.74 ± 0.74%. Because this value was only ~10% of the total, most gossypol in the cottonseed soapstock was covalently bound. Although there is no official procedure for measuring "free gossypol" in soapstock, aqueous acetone extracts most of the gossypol from soapstock (28). Hence, chromatographically measured gossypol is not equivalent to "free gossypol." Bound gossypol (calculated by subtracting the chromatographic value from the total) correlated with nitrogen with a higher coefficient of determination ($R^2 = 0.47$, $P < 0.0001$).

In cottonseed meal, gossypol is bound primarily to the ε-amine of lysine (29). The lysine content of cottonseed flours (~60% protein) is 2.46 g/100 g flour (30). From this lysine value, the average protein concentration calculated above (2.3% db), and the assumption that all lysine ε-amine moieties are bound to gossypol, protein-bound gossypol can be estimated to be 0.36% (db) of the soapstock mass. Because this is considerable less than the estimated value of ~7%, the majority of bound gossypol must be bound to nonproteinaceous components, presumably phospholipids. Pons *et al.* (31) reached a similar conclusion regarding the form of gossypol in cottonseed gums.

The principal free sterol in cottonseed soapstock was β-sitosterol (1.2 ± 0.5%). Measurable levels of campesterol and stigmasterol were also present. A sterol-glycoside, previously

isolated from crude cottonseed oil (13,32), was also a significant component (1.7 ± 0.5%). Mass spectroscopic analysis identified this compound as β-sitosterol esterified to a carbohydrate, which has been identified as glucose (33). A possible fourth sterol eluted between stigmasterol and β-sitosterol.

Monoglycerides, containing palmitic, oleic, and linoleic acids, accounted for 1.2 ± 0.7% of the dry soapstock mass. Diglycerides with acyl chains that contained 32, 34, and 36 total carbon atoms accounted for 2.6 ± 1.3%, and triglycerides with acyl chains from 48 to 58 total carbon atoms totaled 8.4 ± 6.4% of the dry matter.

Despite repeated efforts, the identities of a few chromatographic peaks remained uncertain. Four peaks (unknowns a–d, Fig. 4) were found in several samples and appeared to correspond to two degradation or rearrangement products of trimethylsilyl-derivatized oleic and linoleic acids. Mass spectroscopy gave a series of fragments similar to those for unsaturated acyl chains. Molecular ions were not deducible from the fragmentation patterns, and no *m/e* 73 fragments, characteristic of the trimethylsilyl moiety, were apparent. In addition, the retention times did not correspond to methyl or ethyl esters of oleic or linoleic acids. These peaks may have been artifacts, resulting from either the derivatization procedure or the high-temperature chromatographic conditions. Regardless of origin, the concentration of these components was relatively small (total ~0.5%) and should not have affected the overall characterization. An additional unknown, eluting before raffinose, appeared to be of carbohydrate origin, based on mass fragments of *m/e* 204 and 217 that are characteristic of trimethylsilyl-derivatized pyranosyl rings. The concentration of this compound correlated with the concentration of galactosugars ($R^2 = 0.43$, $P < 0.0001$).

The concentration of total fatty acids determined by the extraction procedure (60.5 ± 7.2%) agreed with the concentration derived by chromatography and phosphorus analyses (65.5 ± 7.1%) after accounting for the nonlipid components of glycerides and phospholipids (see below). In contrast, the average dry mass concentration of triglycerides determined by GC (8.4 ± 6.4%) was substantially lower than the average neutral oil content measured by extraction (25.1 ± 11.1%). Including the free sterols (1.3%), which are also extracted in the chemical procedure, the difference was reduced, but still suggested that the chemical analysis over-estimated soapstock neutral oil. A correlation existed between the two analytical procedures ($R^2 = 0.29$, $P < 0.0005$) (Fig. 5). The regression line had a slope of 0.88 and a standard error of 0.23. Although the slope was not significantly different from unity, the positive intercept was significant ($P < 0.0001$). Chromatography

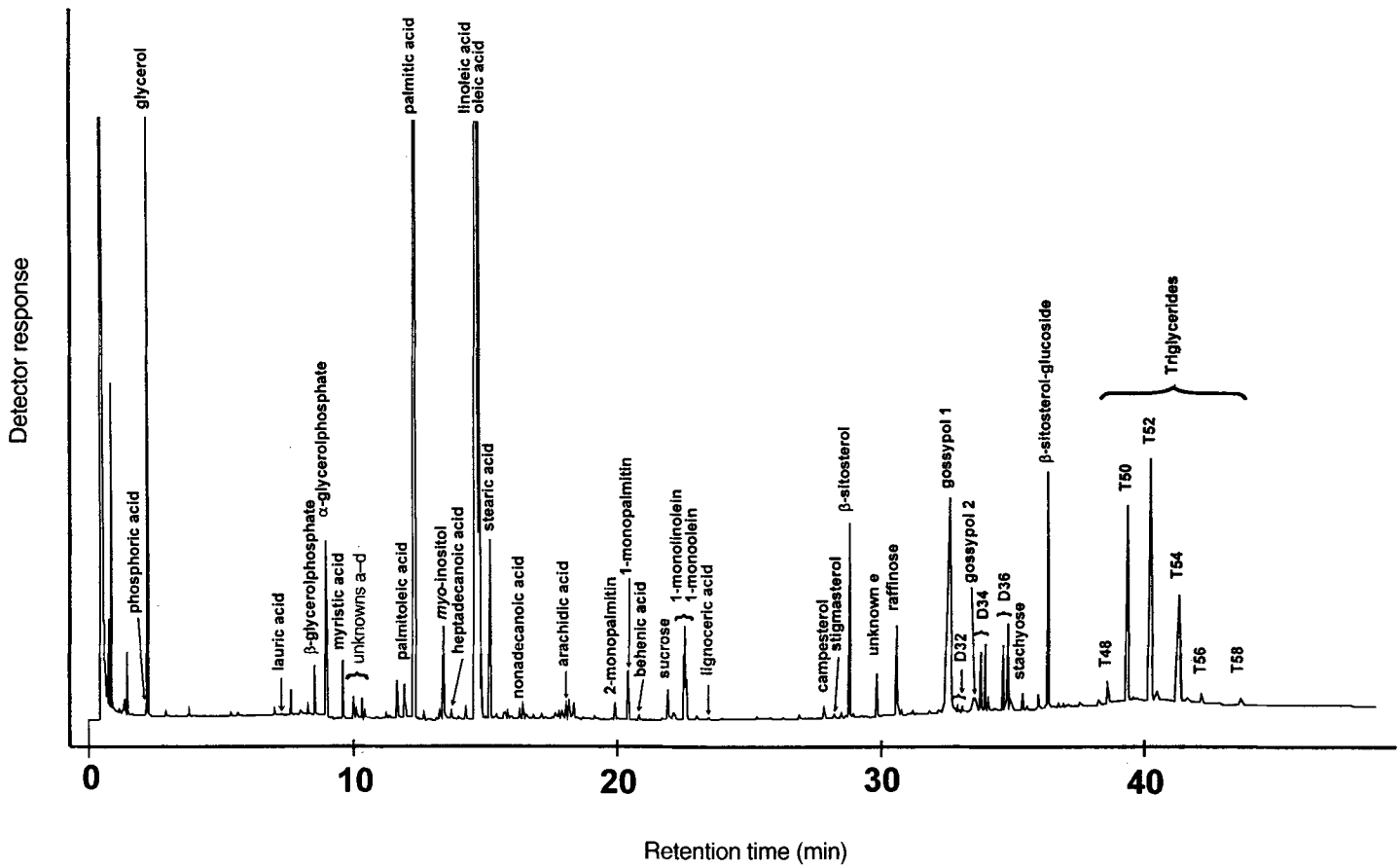

FIG. 4. Gas chromatogram of trimethylsilyl-derivatized cottonseed soapstock.

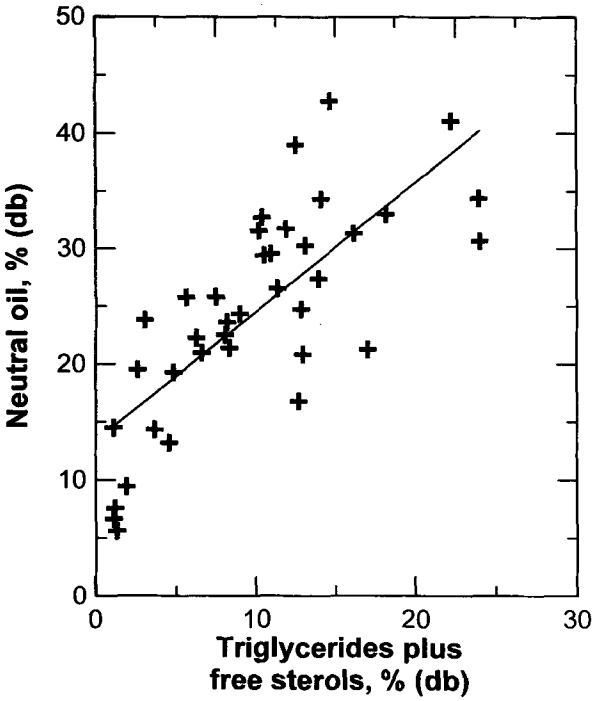

FIG. 5. Associaton between neutral oil and gas-chromatographically determined total triglycerides plus free sterols in cottonseed soapstocks. The line represents the linear regression model ($R^2 = 0.29$, $P < 0.0001$). See Figure 1 for abbreviation.

of the oil residue from the neutral oil test showed, in addition to the expected triglycerides and free sterols, high levels of diglycerides and ethyl fatty acid esters (Fig. 6). The ratio of triglycerides to diglycerides in the extracted oil was comparable to that of the whole soapstock, suggesting that most of the diglycerides were also extracted by the procedure. However, diglycerides accounted for only ~20% of the discrepancy between the two analytical techniques. The presence of small amounts of ethyl esters indicated that some esterification or transesterification occurred during the procedure, even when limiting the time of contact of the ether and alkali phases as recommended.

From Cherry's (13) estimate of the distribution of phospholipid classes in crude cottonseed oil, a conversion factor of 24.5 can be calculated to relate phosphorus and phospholipid concentrations. Applying this factor, phospholipids were estimated to be 26.3 ± 6.9% (db) of the sample after the nonlipid phosphates detected by chromatography were subtracted from the elemental phosphorus. A comparable range of phospholipid values can be derived from the literature. In the late 1930s, Thurman (34,35) reported an approximate phospholipid concentration of 10% (wb), which corresponds to ~20% (db). As this value is for batch-settled soapstock, phospholipid concentrations from the continuous centrifugation processes used today would be slightly greater. More recently, El-Shattory (2) reported values of 0.41 to 0.84% (wb), which appear to be expressed in terms of percent phosphorus. These values are in good agreement with those found here after converting to phospholipids on a dry mass basis (19.0 to 36.5%).

Variation in the composition of individual samples is the result of intrinsic seed differences and processing effects. Detailed processing information and seed history are needed to separate these two factors. Unfortunately, this information was not obtained as part of the sample collection. Because some of the soapstock variation appeared to be greater than the expected variation in seed composition, processing effects appear to be an important factor.

Part of the large variability in glyceride and gossypol concentrations appeared to be related to the use of excess NaOH during refining. Additional caustic improves refined oil color but increases refining losses. Five of the samples (from three oil mills) contained no measurable levels of mono-, di-, or triglycerides, and low levels of gossypol, but contained above-average levels of fatty acids. This pattern is consistent with extensive alkali degradation of the glycerides and gossypol. Most of this degradation is believed to have occurred during processing or in transit to the central collection point, although some degradation during storage cannot be ruled out. For samples that contained measurable levels of triglycerides, little degradation was observed during storage over the several months of this study.

Because most of the collection consisted of expander-processed soapstocks, only this subset (33 samples) was tested for regional or seasonal processing effects. No significant differences were found among the fall-, winter-, or spring-processed samples. Nor were seasonal differences apparent when analyzed by individual region. Regional differences were apparent (Table 3). The Texas region had the most pronounced differences, with higher phosphorus, nitrogen, and gossypol, and lower total fatty acids than the other regions. No differences were observed in the neutral oil concentrations among regions, and a similar result is reflected in the chromatographic values for triglycerides plus sterols, except for the West region, where all samples contained low levels. The West region also had low levels of gossypol. As dis-

TABLE 3
Regional Variations of Expander-Processed Cottonseed Soapstocks[a]

	Region[b]				
Component	East (3)	Delta (11)	Texas (16)	West (3)	LSD[c]
Phorphorus	0.762[a]	1.089[b]	1.402[c]	0.856[a,b]	0.279
Lipid phosphorus[d]	0.724[a]	0.984[a,b]	1.233[b]	0.792[a]	0.320
Nitrogen	0.412[a]	0.608[a]	0.965[b]	0.607[a]	0.235
Total fatty acids	67.7[a]	64.1[a]	54.7[b]	66.7[a]	6.9
Neutral oil	19.3[a]	24.6[a]	27.4[a]	25.9[a]	13.8
Triglycerides and sterols	9.9[a]	10.9[a]	10.9[a]	1.1[b]	7.4
Total gossypol	6.90[a]	6.79[a]	9.71[b]	1.81[c]	2.76
Carbohydrates	0.574[a]	0.878[a,b]	1.053[b]	0.683[a,b]	0.469

[a]Values represent sample means from each region expressed as percent of dry mass. Identical superscripts indicate regions where means are not significantly different ($\alpha = 0.05$).
[b]Numbers in parentheses are number of samples per region.
[c]LSD = Least significant difference.
[d]Total phosphorus minus phosphorus attributable to phosphoric acid and glycerophosphates.

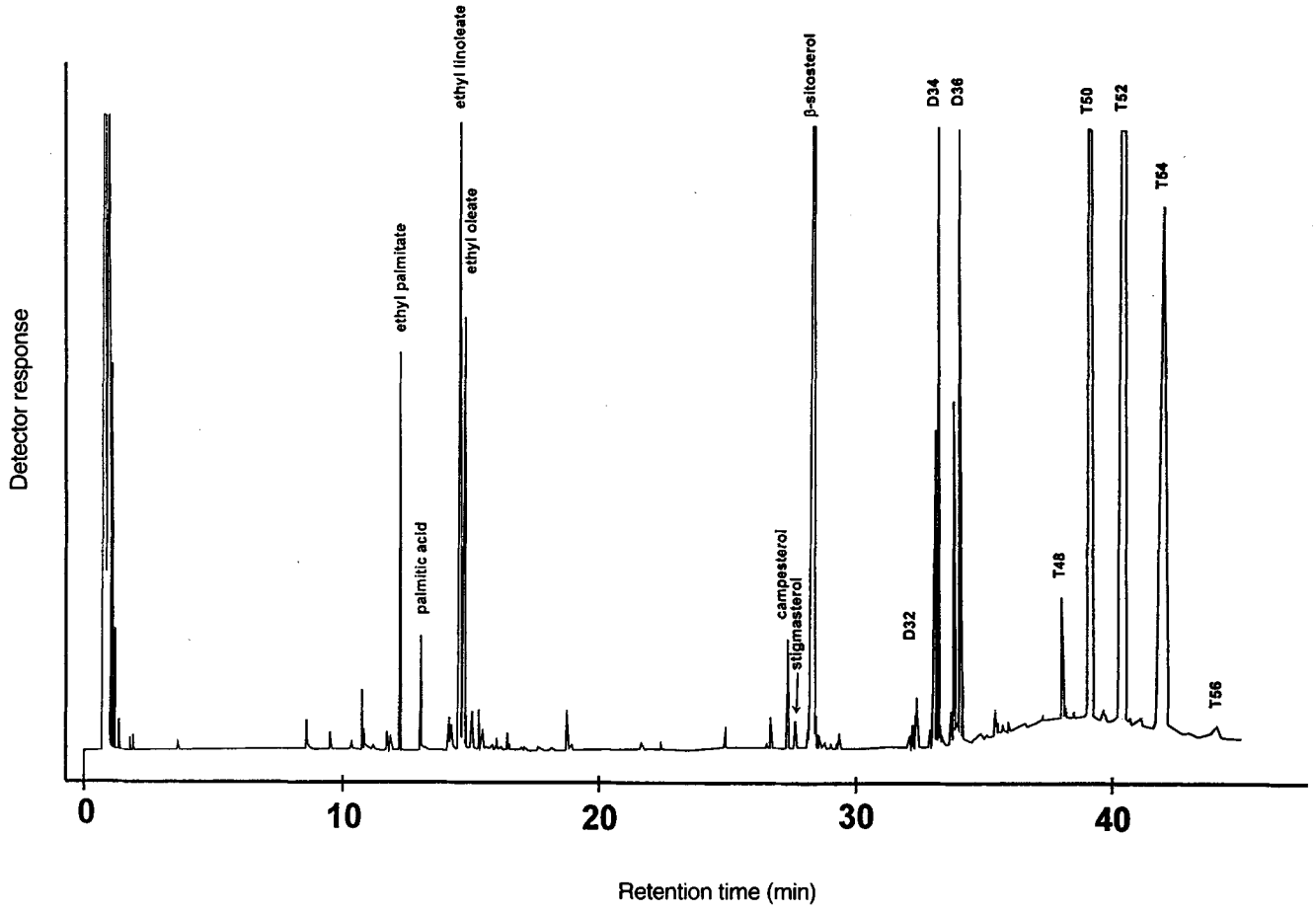

FIG. 6. Gas chromatograph of the trimethylsilyl-derivatized neutral oil residue extracted from cottonseed soapstock.

cussed above, five samples from three oil mills had low glyceride and gossypol levels, possibly due to alkali degradation. All three samples from the West region were in this condition. These samples illustrate that the chemical and chromatographic determinations of oil and triglycerides, respectively, can yield conflicting results. Similar regional effects were apparent within each processing season.

Finally, a material balance was possible by addition of the results from the various analyses. The components from Table 2 yielded 54.3 ± 7.6% of the dry soapstock matter. Phospholipids and protein, as discussed above, were estimated as 26.3 ± 6.9 and 2.3 ± 1.4%, respectively, and bound gossypol was 6.8 ± 2.9%. From the fatty acid profile, sodium from soap was 3.4 ± 0.7%. Summation of the individual components yielded 93.3 ± 8.6% of the dry matter, indicating that most of the soapstock dry matter was accounted for by the combined analyses.

ACKNOWLEDGMENTS

The author thanks M.C. Calhoun (Texas A&M) and L.A. Forster, Jr. (NCPA) for soapstock samples; C. Grimm for mass spectroscopy; L. Wartelle, R.J. Hron, Sr., and J.L. Landry for help with some of the chemical analyses; and B. Vinyard for help with the statistics. The author also thanks P.J. Wan, R.J. Hron, Sr., E.J. Conkerton, and M.S. Kuk for useful discussions.

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[Received February 5, 1996; accepted July 6, 1996]