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# COMPOSITION OF COTTONSEED SOAPSTOCK FATS

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The compositions of cottonseed soapstocks from first-grade and low-grade cotton seeds have been studied by the methods of CC on polyamide and on silica gel, TLC, GLC, ESR, and UV, IR, and mass spectrometry. It has been found that saturated, and also oxygenated, fatty acids, nonpolar acylglycerols, glycolipids, sterols, arylalkanes, gossypol pigments, and ions of metals of variable valence are concentrated in the acid fat of the soapstock from low-grade seeds. The soapstock contains a very small amount of tocopherols and phospholipids, mainly phosphatidylinositols. The combined gossypol pigments of the soapstocks include stabilized gossypol radical ions.

Cottonseed oil for food purposes is freed from undesirable impurities by treatment with aqueous solutions of sodium hydroxide. The waste formed in this process – soapstock – consists of a complex mixture of nonfatty substances (mucilages, traces of metals, alkali) and fatty, lipophilic, substances (soap, neutral fat, phospholipids, pigments, and other unsaponifiable substances).

Soapstock fats are a traditional raw material for soap boiling, although the use of cottonseed soapstocks for these purposes is limited because of their dark-brown coloration. Other methods of utilizing soapstocks are possible [1], but the most rational use is being hindered by the inadequacy of information about their chemical composition.

This paper gives the results of the analysis of samples of cottonseed soapstocks obtained in the processing of high-grade seeds (soapstock-1) and seeds of mixed grades (soapstock-2) in the course of the batch refining of the oil in the Tashkent Oil and Fats Combine.

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The total compositions of the samples were determined by known methods [15]:

| Index | Soapstock-1 | Soapstock-2 |
|---|---|---|
| Content, % on the total weight of the fat, including: | 79.6 | 64.4 |
| neutral fats | 65.2 | 25.1 |
| acid fat | 13.1 | 33.1 |
| amorphous residue | 1.3 | 6.2 |
| water and nonfat substances | 20.4 | 35.6 |
| Color at 35 yellow units in a 1-cm layer of the | | |
| total fat | 64.0 | Not considered |
| neutral fat | 3.0 | 64.0 |
| acid fat | Not considered | Not considered |
| Amounts, mg-% in the total fat of | | |
| unsaponifiable substances | 2.9 | 6.8 |
| tocopherols | 4.0 | 8.9 |
| free gossypol | 0.29 | 0.21 |

The soapstock, previously freed from neutral fat (NF), was decomposed by treatment with 15% $H_2SO_4$. The acid fat (AF) was isolated by extraction, during which an amorphous dark-brown deposit formed at the phase separation boundary. In the separation of soapstock previously subjected to supplementary saponification no deposit is formed. The amount of deposit was determined after the separation of the organic phase, its evaporation, and the elimination of moisture from the residue in the form of an azeotropic mixture with benzene in a rotary evaporator. It can be seen from the facts given above that the total yield of soapstock depends on the composition of the crude oil [2] and, consequently, on the quality of the seeds.

In the refining of oil obtained in the course of the processing of freshly gathered (unstored) high-grade seeds almost three times as much NF was entrained in the soapstock (soapstock-1) and it contained less moisture, nonfat, and unsaponifiable lipophilic substances than in the soapstock from oil of low-grade seeds (soapstock-2). At a comparable level of free gossypol in the total fat of the two samples, the coloration of the NF of soapstock-1 (0.3% of free gossypol) differed little from the color of unbleached refined oil [2].

For the analysis of the saponified and unsaponified fractions of the soapstocks, the NF and the AF of two samples were separated by column chromatography (CC). Polyamide and silica gel were used as sorbents successively. From the polyamide, hexane–ether (1:1) eluted weakly polar lipids, and methanol eluted a complex of polar lipids and gossypol pigments. Then, by CC on silica gel, the weakly polar lipids were separated into coarse fractions to estimate the ratio of free and bound FAs (% by weight):

| Fraction | Soapstock-1 | | Soapstock-2 | |
|---|---|---|---|---|
| | NF | AF | NF | AF |
| Polyamide | | | | |
| I. Polar lipids, pigments | 0.6 | 11.6 | 2.6 | 8.4 |
| II. Weakly polar lipids | 99.4 | 88.4 | 97.4 | 91.6 |
| Silica Gel | | | | |
| II-1. Alkanes, esters | 0.8 | 2.9 | 0.2 | 3.2 |
| II-2. Triacylglycerols | 80.1 | 2.7 | 44.4 | 11.2 |
| II-3. Fatty acids | 13.7 | 74.2 | 45.2 | 66.2 |
| II-4. Oxylipids, sterols, nonpolar acylglycerols | 4.8 | 8.6 | 7.6 | 10.8 |

A substantial difference can be seen in the compositions of the NFs of two samples: The NF from soapstock-1 consisted mainly of triacylglycerols (TAGs), while that of soapstock-2 consisted of equal amounts of TAGs and FAs. Oxidized and incomplete acylglycerols were concentrated in the AFs, together with the unsaponifiable components, pigments, and phospholipids.

By separating the total fat on polyamide we obtained fraction (I) in an amount of 3.7% from soapstock-1 and 6.2% from soapstock-2. The further separation of the total fat from soapstock-1 was carried out by CC on silica gel only, and that of the total fat from soapstock-2 in a similar manner to the NF and AF.

After the elimination of the neutral lipids from the amide, the glycolipids, with pigment impurities, were eluted by acetone, and the phospholipids, with the bulk of the pigments, were eluted by methanol. The mixed fractions were purified by preparative rechromatography in a thin layer. The amount of glycolipids was calculated after the saponification of the fraction from the weight of the FAs liberated in the light of their mean molecular weight and the molar fraction in the main component of the glycolipids – monogalactosyldiacylglycerols (MGDGs). The level of phospholipids was estimated from the results of preparative two-dimensional TLC.

The compositions of the total fats of the soapstocks are given below (%):

| Class of Lipids | Soapstock-1 | Soapstock-2 |
|---|---|---|
| Paraffins, arylalkanes | 1.4 | 4.5 |
| Triacylglycerols | 65.6 | 20.2 |
| Tocopherols | Traces | Traces |
| Epoxyacyldiacylglycerols | 0.1 | – |
| Oxoacyldiacylglycerols | 0.1 | – |
| Fatty acids | 23.6 | 58.3 |
| Fatty alcohols | 0.2 | Traces |
| Epoxy acids | Traces | 0.4 |
| Oxo acids | Traces | 0.3 |
| Diacylglycerols | 1.1 | 3.7 |
| Hydroxyacyldiacylglycerols | 0.3 | 0.7 |
| Triterpenols | 0.3 | 0.4 |
| 4-Monomethylsterols | 0.3 | 0.3 |
| Sterols | 2.2 | 2.5 |
| Hydroxy acids | Traces | 1.0 |
| Dihydroxyacyldiacylglycerols | 1.0 | 0.6 |
| Monoacylglycerols | 0.4 | 0.6 |
| Dihydroxy acids | Traces | 0.3 |
| Glycolipids | 0.8 | 1.8 |
| Phospholipids | 0.3 | 0.2 |
| Pigments | 2.3 | 4.2 |

The total fat of soapstock-1 was enriched with triacylglycerols, and that of soapstock-2 with free fatty acids (FFAs). In soapstock-2 the level of paraffins and arylalkanes, diacylglycerols, glycolipids, and pigments was high. A comparison of the figures given above with the composition of the crude oil from the identical technological stream [2] showed that, together with the soap, not only unsaponificables (paraffinic and aromatic hydrocarbons and sterols) but also oxidized lipids (epoxy-, hydroxy-, and dihydroxyacyldiacylglycerols) and glycolipids had been eliminated, due to their pronounced surface-active properties.

In soapstock-1, the oxylipids were present mainly in unhydrolyzed form, while in soapstock-2 they were present in the form of oxidized acids.

The compositions of the total fatty acids of the soapstock lipids determined by GLC are given in Table 1. The soap fraction of soapstock-2, as compared with that of soapstock-1, was half composed of the Na salts of more saturated acids. It must be mentioned that the degree of saturation of the acid fat of the soapstocks affects the choice of the conditions for its further processing in soapboiling [1].

A comparison of the fatty-acid compositions of the TAGs of soapstock-1 and the crude oil [2] shows that a considerable amount of palmitoyl-containing species of TAGs had been entrained in the soapstock. This fact was confirmed by the results of an analysis of the structure of the TAGs of soapstock-1 by enzymatic hydrolysis. The main TAG species were as follows (mole %): POP – 2.7; PLP – 11.1; POO – 2.2; POL – 6.5; PLO – 8.5; PLL – 25.8; OLL – 2.6; OLO – 1.6; SLL – 1.6; LOL – 3.8; OLL – 9.9; LLL – 15.0, where P represents the 12:0, 14:0, and 16:0, S the 18:0, O the 18:1, and L the 18:2 acids.

In their qualitative compositions and structures, the epoxy, oxo, and hydroxy acids of the soapstocks were similar to the oxygenated acids of the crude oil [2].

The soapstock fats concentrated sterols (2.8, 3.2%). To determine their composition, the sterol fraction of soapstock-2 after CC on silica gel was separated by preparative TLC

TABLE 1. Composition of the Fatty Acids of the Cottonseed Soapstock Lipids

| Sample, lipids | Fatty acids, % on the oil | | | | | | |
|---|---|---|---|---|---|---|---|
| | 12:0 | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 |
| Soapstock-1 | | | | | | | |
| Total fat* | Tr. | 0,8 | 24,7 | 1,3 | 2,5 | 19,2 | 51,5 |
| Na salts of fatty acids | Tr. | 1,3 | 32 6 | 1,6 | 2,9 | 15 6 | 46,0 |
| Free fatty acids* | 0,3 | 0,7 | 19,7 | 1,3 | 2 4 | 18,8 | 55,0 |
| Triacylglycerols | Tr. | 0,3 | 23,8 | 1,3 | 1,7 | 16,5 | 55,9 |
| Epoxyacyldiacylglycerols | — | 1,2 | 55,5 | 1,3 | 11,4 | 28,8 | Tr. |
| Hydroxyacyldiacylglycerols<br>Diacylglycerols | Tr. | 0,6 | 24,0 | 1,9 | 3,5 | 45,2 | 24,8 |
| Dihydroxyacyldiacylglycerols | — | 1,3 | 32,5 | 2,8 | 6,8 | 40,0 | 16,6 |
| Monoacylglycerols | — | 0,5 | 22,1 | 1,2 | 1,9 | 28,2 | 46,1 |
| Soapstock-2 | | | | | | | |
| Total fat* | Tr. | 0,7 | 23,9 | 1,0 | 2,3 | 23,5 | 48,6 |
| Na salts of fatty acids | 0,4 | 1,0 | 45,0 | 1,3 | 5,2 | 27,2 | 19,9 |
| Free fatty acids | — | 0,6 | 20,4 | 0,8 | 2,2 | 22,5 | 53,5 |
| Triacylglycerols | — | 0,4 | 18,8 | 0,7 | 2,6 | 25,7 | 51,6 |
| Hydroxyacyldiacylglycerols | 1,5 | 1,3 | 23,5 | Tr. | 1,1 | 25,3 | 47,3 |
| Diacylglycerols | Tr. | 0,7 | 20,3 | Tr. | 4,0 | 24,9 | 50,1 |
| Dihydroxyacyldiacylglycerols | Tr. | 0,8 | 11,4 | 1,4 | 1,3 | 24,5 | 60,6 |
| Monoacylglycerols | Tr. | 0.5 | 8,5 | 1,3 | 1,1 | 30,6 | 58,0 |
| Glycolipids† | — | 1,2 | 33,8 | Tr. | 4,0 | 15,5 | 40,6 |
| Phospholipids | 7,7 | 7,0 | 28,4 | 5,9 | 10,7 | 20,6 | 19,2 |

*In addition, there were trace amounts (total fat) and 2.5% (FFAs) of an unidentified compound. In all classes of both samples there were traces of the 17:0 acid.
†In addition, 0.5% of the 13:0, 0.9% of the 15:0, 1.4% of the 17:0, 0.1% of the 18:3, and 2.0% of the 20:0 acids.

on Silufol in system 1 into 4-demethylsterols, 4-monomethylsterols, and 4,4-dimethylsterols (triterpenols). Each group of compounds was investigated by the methods of GLC and mass spectrometry and identification was made by comparison with authentic compounds and with literature information [3]. Their compositions are given below (%, GLC):

| Sterols | % on weight of fraction | Sterols | % on weight of fraction |
|---|---|---|---|
| 4-Demethylsterols | | 4,4-Dimethylsterols | |
| campesterol | 2.8 | a $\Delta^8$-sterol | 16.8 |
| stigmasterol | 1.6 | β-amyrin | |
| β-sitosterol | 95.6 | α-amyrin | 50.0 |
| $\Delta^5$-avenasterol | | cycloartenol | |
| 4-Monomethylsterols | | 24-methylene-lanostenol | |
| obtusifoliol | 8.6 | 24-methylene-cycloartanol | 31.5 |
| gramisterol | 27.0 | cyclobranol | 1.7 |
| cycloeucalenol | | | |
| citrostadienol | 62.0 | | |
| ethyllophenol | | | |
| a $\Delta^8$-sterol | | | |
| friedelanol | 2.4 | | |

The predominating sterols in the total are shown by underlining.

It is known that phytosterols possess a hypocholesteremic [4] and 4-monomethylsterols an antioxidant action [5], and on this is based their use in dietetic foods. The composition of the glycolipids of soapstocks is identical with that of the glycolipids of unrefined cottonseed oil [2]. Plant glycolipids have been recommended as additives in the bakery industry, improving the shape stability and other properties of bread [6], and as components of drugs with an adjuvant activity [7].

In the development of effective methods for fractionating soapstock fats, it is desirable to isolate the sterols and glycolipids as independent products.

It has been reported that in cottonseed soapstock the amount of phospholipids (PLs) ranges from 0.4 to 0.84%, while phosphatidylinositols, phosphatidylserines, phosphatidylethanolamines, and lysophosphatidylcholines have been found in them [8]. In the PLs of soapstock-2, by two-dimensional TLC in systems 2 (direction I) and 3 (direction II) we detected in very small amounts phosphatidylinositols, lysophosphatidylcholines, and an unidentified PL. The bulk of the gossypol pigments accompanying the phospholipids migrated as a band in the ammoniacal system 2 and a smaller fraction in the acidic system 3. No phosphorus-containing compounds were detected in the pigment zones. The high degree of saturation of the total FAs of the phospholipids is explained by the predominance of phosphatidylinositols in them (Table 1) [9].

In order to determine the rate of hydrolysis of the PLs under refining conditions, model experiments were performed which showed that 3% NaOH hydrolyzes the PLs of cottonseed oil completely after only 2-2.5 h.

Thus, in refining under factory conditions an increase in the concentration of alkali and in the time of the process leads to the decomposition of the PLs. Of the complex of PLs in cottonseed [9], the most resistant to the action of alkali are the phosphatidylinositols.

With the phospholipids from soapstocks-1 and -2 were isolated 0.6 and 2.4% of the darkest-colored forms of gossypol pigment, and with the glycolipids 1.7 and 1.8%, respectively. Among the pigments the main components were, as in the crude oil [2], gossypol derivatives having $R_f$ 0.92, 0.89, and 0.59 in system 4.

Earlier, in studying the pigment complex of crude cottonseed oil we detected radical dications of gossypol and $Mn^{2+}$ ions in it by the ESR method [2].

In the pigment fractions of the acid fats from the soapstocks we also recorded under dark conditions the weak ESR signal of stabilized radical ions of gossypol in the form of a singlet with $\Delta H$ = 5 Oe. Illumination of the samples with light from an ordinary incandescent lamp caused a partially reversible increase in the intensities of these signals.

A more complex ESR signal was observed in the pulverulent fraction isolated from the amorphous deposit. The fraction was obtained after the exhaustive washing of the deposit with chloroform–methanol (2:1) and the drying of the residue in the air. The amount of dark-brown powder amounted to 1.8% of the weight of the amorphous deposit from soapstock-2.

In a chloroform–methanolic extract (98.2% on the weight of the deposit), FAs, arylalkanes, and dark-brown pigments were detected by TLC in system 1. The pigments appeared in the form of a continuous band.

In the ESR spectrum of the powder itself, an intensive signal of the radical ion form of gossypol was superposed upon a broad ($\Delta H$ = 500 Oe) signal due to a high concentration of metal ions, including $Mn^{2+}$ [2]. When this sample was illuminated, a partially irreversible increase in the intensity of the signal of the gossypol radical ions was observed.

Part of the powder was treated with 10% alcoholic alkali. A suspension was kept with periodic shaking until a homogeneous reddish-brown solution had been formed (3 days). The solution was acidified with 50% $H_2SO_4$, as a result of which it became decolorized, with the simultaneous evolution of a gas smelling of hydrogen sulfide. An ethereal extract of the acid aqueous solution contained no lipids or pigments. Using the method of analytical chemistry [10], $Fe^{2+}$ and $Fe^{3+}$ were reliably detected in the acid solution, and $Fe^{3+}$ in the powder itself. Perhaps because of their low concentration, $Mn^{2+}$ ions were not detected.

It is known that metals of variable valence in the form of sulfides and hydroxides are capable of existing in the form of colloids and hydrogels [10]. The results of the investigation show that the amorphous deposit formed in the decomposition of the unsaponified soapstock consists of a colloidal solution of the hydroxide of such metals, including $Mn^{2+}$, $Fe^{2+}$, and $Fe^{3+}$ with FAs, arylalkanes, radical ions, and other oxidized forms of gossypol adsorbed on them. The residual amounts of metals may affect the course of the processes involved in the subsequent treatment of the soapstocks, lowering the activity of catalysts in the hydrogenation of the soapstocks themselves or of the soapstock fatty acids.

## EXPERIMENTAL

ESR spectra were recorded as described in [11]. The conditions for taking the mass spectra for GLC analyses and for CC on polyamide were similar to those in [12], CC on silica gel and the identification of the lipid classes have been described in [13], and the pancreatic hydrolysis of the TAGs in [14].

Thin-layer chromatography was performed on Silufol and on L 5/40 silica gel (Chemapol) with the addition of 10% of $CaSO_4$ in the following solvent systems: 1) hexane–ether (7:3), 2) chloroform–methanol–ammonia (65:35:5), 3) chloroform–methanol–acetone–acetic acid–$H_2O$ (10:5:4:2:1), and 4) benzene–methanol (20:5).

The samples of soapstocks were obtained in the processing of cotton seeds of the 1983 harvest. High-grade seeds were processed without storage, and the seeds of the mixed grades after storage for 6 months. The total and neutral fats of the soapstocks were isolated in accordance with [15]. The acid fat was obtained by exhaustive extraction with diethyl ether of the acidified soap residue of the soapstock from which neutral fat had previously been removed. The color, total content of unsaponifiable substances, the tocopherols, and the free gossypol were determined by generally adopted methods [15].

Hydrolysis of the Phospholipids. The phospholipids of the cottonseed oil were mixed with 0.1, 1.0, and 3.0% solutions of NaOH in ratios of PLs to alkali of 2:0.1, 2:1, and 2:3, respectively, and the solutions were kept for a day.

The degree of hydrolysis was monitored by TLC in the systems described. After 2-2.5 h, the 3% NaOH had hydrolyzed the PLs completely; on saponification with the 0.1 and 1% NaOH, of the PLs of the cottonseed oil only the phosphatidylinositols and an unidentified PL remained. After a day, PLs were absent from the samples with the 3% and 1% NaOH, while the PLs mentioned were still detected in trace amounts with the 0.1% alkali.

## CONCLUSIONS

1. The soapstock obtained in the processing of high-grade cotton seeds is enriched with total and neutral fats and contains a small amount of moisture and of non-fat lipophilic substances. The soapstock from low-grade cotton seeds contains a larger amount of acid fat in which saturated fatty acids are concentrated, and also oxygenated fatty acids, nonpolar acylglycerols, glycolipids, sterols, arylalkanes, gossypol pigments, and ions of metals of variable valence.

2. The soapstock obtained under severe refining conditions contains only a small amount of tocopherols and phospholipids mainly consisting of phosphatidylinositols.

3. The combined gossypol pigments of soapstock include stabilized gossypol radical ions.

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