COMPOSITION OF THE CARBONYL COMPOUNDS OF EXTRACTION COTTONSEED MEAL

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After the extraction of cottonseed flake with aqueous acetone or a mixture of solvents containing acetone, the meal acquires a coloration and a permanent unpleasant odor which reduces its fodder value and limits its subsequent use as a raw material for producing food flour.

The unpleasant odor of the meal is due to a complex mixture of carbonyl compounds including residues of acetone, products of its condensation, and substances formed on the interaction of these products with the protein part of the meal [1-3]. Thus, in cottonseed meal treated with pure acetone the presence of mesityl oxide, diacetone alcohol, and a number of unidentified compounds has been shown by gas–liquid chromatography [4].

The present paper gives the results of the qualitative analysis of the volatile carbonyl compounds (VCCs) in samples of meal obtained after the extraction of cottonseed flake with hexane and acetone followed by deodorization.

The samples of meal for analysis were obtained by extracting cottonseed flakes with hexane, successively with hexane–80% aqueous acetone and then with dry acetone, and also with hexane–acetone (85:15), and hexane–dry acetone.

The sum of the VCCs was isolated from the meal by azeotropic distillation with toluene as described by Fore et al. [5] with slight modifications, and the compounds were analyzed in the form of the 2,4-DNPH derivatives by the TLC method and were subjected to the qualitative test with salicylaldehyde.

To separate the derivatives, six solvent systems suitable for these purposes according to the literature were tested: benzene–hexane (1:1) (system 1); petroleum ether (70-100°C)–diethyl ether (7:3) (system 2); diethyl ether (system 3); benzene or toluene with a few drops of diethyl ether (systems 4 and 5, respectively); and ethyl acetate–hexane (1:4) (system 6). The best separation was achieved in system 6, and this was used in subsequent experiments.

As the basic models for the identification of the VCC on the chromatograms we used the 2,4-DNPH derivatives of mesityl oxide, isomesityl oxide, acetone, and diacetone alcohol.

Before the analysis of the samples of meal, all the reagents and solvents taking part in distillation were tested for the presence of VCCs. The blank experiment showed the presence in them of traces of VCCs which, in the form of the 2,4-DNPH derivatives, had R_f values of 0.62, 0.43, 0.41, and 0.05. In the estimation of the VCCs isolated from the samples of meal, the spots of the compounds were corrected for the intensity of coloration and dimensions in the light of the blank experiment.

The meal obtained after the treatment of the cottonseed flake with aqueous or dry acetone was found by TLC to contain 11 substances, giving spots with R_f values from 0.77 to 0.03 (Table 1). In all cases the spots of the mesityl oxide and acetone derivatives (R_f 0.77 and 0.47, respectively) were the main ones in terms both of size and intensity of coloration. We did not detect appreciable amounts of diacetone alcohol (R_f 0.36) in such samples of meal.

The full identification of all the spots of the derivatives is difficult. It must be borne in mind that the VCCs may contain, in addition to the products of the extraction treatment of the cottonseed meal, carbonyl compounds formed by the oxidative degradation of the fatty and protein fractions of the meal, and also various products of their interaction. The presence of such compounds was confirmed by chromatographing the 2,4-DNPH derivatives

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TABLE 1. R_f Values of the 2,4-DNPH Derivatives of the Total Volatile Carbonyl Compounds

R_f	Meal, hexane–acetone (85:15)	Cottonseed flake	Refined oil	Meal after deodorization, 130°C, 2 h
0,85	–	–	+	+
0,82	–	–	+	+
0,79	–	–	+	+
0,77	–	–	–	+
0,75	–	+	+	–
0,74	+	–	+	+
0,71	–	–	–	+
0,57	+	–	–	+
0,53	–	–	–	+
0,47	+	–	–	+
0,44	+	+	–	–
0,40	–	–	+	+
0,36	–	–	+	+
0,29	+	–	+	+
0,26	+	+	+	+
0,22	–	–	+	–
0,17	–	–	+	–
0,12	+	–	–	+
0,08	+	+	–	–
0,07	–	–	+	–
0,05	–	–	–	–
0,04	+	–	–	–
Color of the distillate after the qualitative test	Crimson-red	Orange	Crimson-red	Bright red

of the VCCs obtained in the distillation of commercial refined cottonseed and sunflower seed oils, cottonseed kernels of the variety "Élit" of the 1977 harvest, and cottonseed flake from low-quality seeds not treated with solvents and extraction hexane.

No appreciable amounts of FCCs were detected in a distillate of seeds of the "Élit" variety by the qualitative test (colorless) and the TLC method. On chromatograms of derivatives of the distillates from the low-quality seeds and the corresponding oil, from 6 to 16 spots appeared of derivatives with R_f values similar to those of the eight derivatives from the extraction meal. According to the results of the qualitative test (red color of the distillate), after treatment with hexane of the flake from low-quality seeds the amount of VCCs in the meal increases somewhat. There is information according to which the permanent unpleasant ("cat-like") smell acquired by the meal after acetone treatment is a consequence of the interaction of the products of the condensation of acetone with hydrogen sulfide — the end product of the decomposition of the S-containing protein amino acids cystine, cysteine, and methionine [1, 2]. It has been estimated that mesityl oxide interacts with H_2S under fairly mild conditions to form the evil-smelling mercapto compound 4-mercapto-4-methylpentan-2-one. In order to identify the VCCs and to check the possibility of the formation of mercapto compounds under the conditions of the extraction of the flake with acetone, we treated mesityl oxide with gaseous hydrogen sulfide. The unpleasant smell of the reaction mixture was similar to but not identical with the smell of the acetone-treated meal. When the reaction mixture in the form of the 2,4-DNPH derivatives was chromatographed, five spots, in addition to that of the initial material, were detected with R_f 0.62, 0.51, 0.44, 0.22, and 0.12. The results obtained are insufficient for the reliable identification of the mercapto compounds in the VCCs of the extraction meal.

On comparing chromatograms of the VCCs from distillates from various samples of meal, it was found that the relative amounts of mesityl oxide and acetone increase when the meal is extracted with a binary mixture of hexane and acetone, or after its retreatment with dry acetone. These results were confirmed by a comparison of the intensities of the products of the color reaction of the VCCs of the distillates of the samples of meal analyzed with salicylaldehyde. The distillates from the meal obtained by extraction of the flake with mixtures of hexane and aqueous acetone and of hexane and dry acetone or the binary mixture changed their color after the performance of the qualitative reaction from bright red to crimson-red and crimson-violet.

Since mesityl oxide does not form complexes with salicylaldehyde, the increase in the relative amount of VCCs in the meal can be explained by the assumption that on its retreat-

ment with acetone (or the binary mixture) the protein fraction of the meal binds a large amount of acetone and its condensation products containing the $-CH_2COCH_2-$ group and, because of this, capable of interacting with salicylaldehyde.

The strength of such binding can be judged on the basis of the fact that the complete elimination of VCCs from extraction meal presents certain difficulties. According to the literature, the satisfactory elimination of acetone from the meal is achieved by wetting the sample to a moisture content of 20% followed by thermostating at 70°C for 5 h [6].

In order to select conditions for eliminating VCCs from the meal obtained by extraction of the flake with the binary mixture of solvents, experiments were carried out on its moist heat treatment. The conditions for this treatment were varied in order to take into account the possibilities of the technological equipment of factories. The degree of elimination of the VCCs was checked by the methods described. The moisture content of the samples was increased from 8-9% (initial) to 20-21%, the temperature of drying was from 70 to 130°C, and the time of drying was from 50 min to 2 h at normal atmospheric pressure and with lowering the pressure to 500-400 mm Hg.

In the process of moistening the sample, an intensification of the unpleasant smell and a marked change in the color of the meal from light yellow to dark brown with its simultaneous lumping were observed. It was established that a moisture content of the meal of 13-15% is acceptable. Higher moisture contents of the meal lead to its pronounced caking, which is undesirable for a technological flow. According to chromatography, even at a moisture content of the sample of about 21% and a drying temperature of 110°C for 1.5 h under reduced pressure conditions, traces of mesityl oxide and acetone remain in the meal and are not eliminated completely. When the temperature is raised to 130°C and the pressure is lowered (500 mm Hg), drying of the sample for 1.5-2 h led not only to the retention of traces of acetone and mesityl oxide, but also to a considerable increase in the amount of other VCCs (see Table 1). The intensity of the coloration of the qualitative test intensified. These facts indicate an acceleration of the processes of the degradation of the protein and, especially, the fatty fractions of the meal in the deodorization process.

After deodorization, the meal had a moisture content of from 0.6 to 2.9%.

The moist heat treatment of the meal under severe conditions not only increases the relative amount of VCCs but also leads to various transformations of the protein fraction, the amino acids, the gossypol, the phosphatides, etc. [15]. From samples of meal subjected to moist heat treatment we isolated a protein fraction soluble in 0.2% NaOH solution and we determined its nitrogen content. The cottonseed was also analyzed for its content of free gossypol and of lysine (Table 2).

The total amount of nitrogen in the initial meal was 8.8%. It can be seen from Table 2 that drying the meal even at 110°C leads to a decrease in the total amount of alkali-soluble proteins because of their conversion into the insoluble state. However, with a further rise in the temperature the proteins undergo cleavage and pass into the soluble fraction, which is in complete harmony with information in the literature [15]. The amount of free lysine in the meal after this treatment falls to 45% in comparison with the initial amount, and the free gossypol passes into the bound state.

TABLE 2. Characteristics of the Deodorized Meal

Sample No.	Meal drying temperature, °C	Time of heating, h	Amount of N soluble in 0.2% NaOH, %	Lysine, g/16 g of N	Amount of alkali-soluble proteins, % on the total protein	Free gossypol, %
Initial meal	—	—	7,2	3,8	81,8	0,2
2	110	1,5	6,6	—	76,1	0,08
3	110	2,0	6,5	—	73,9	0,08
4	120	1,5	7,2	—	81,8	0,07
5	120	2,0	7,3	—	82,9	0,07
6	130	1,5	6,9	2,1	78,4	0,05
7	130	2,0	7,1	—	80,7	0,04

EXPERIMENTAL

The preparation of the cottonseed flake and the conditions for its extraction with solvents have been described previously [7, 8]. The samples of meal after extraction were dried in the air at room temperature for 24-36 h and were used for azeotropic distillation.

Cotton seeds of the "Élit" variety were provided by the laboratory for the study of cotton plant seeds of the All-Union Scientific-Research Institute for Cotton-Plant Breeding and Seed Production of the Ministry of Agriculture of the USSR.

Azeotropic Distillation. A sample of meal (10 g) was ground in a mortar to a flour, which was placed in a flat-bottomed conical flask (1 liter) to which 50 ml of 20% Na_2SO_4 solution, 0.7 ml of toluene, and 0.5 g of detergent (sul'fanol NP-1) were added, and the mixture was boiled on a magnetic stirrer with the avoidance of pronounced foaming and overflowing.

The distillate was collected to an amount of 1 ml which consisted of a layer of toluene (∿0.7 ml) and a layer of water (∿0.3 ml), and 0.10-0.15 g of K_2CO_3 was added for the complete transfer of the VCCs into the toluene layer.

After the separation of the layer of toluene, 0.3 ml of the toluene solution of VCCs was taken for the qualitative test, and the remainder of the toluene was treated with a solution of 2,4-dinitrophenylhydrazine.

The qualitative reaction with salicylic aldehyde was performed by a standard method [9].

The 2,4-DNPH derivatives of the total VCCs were obtained by a known method [9]. After the reaction mixture had been diluted with water, the derivatives were re-extracted with toluene (2 × 1 ml), and the combined extracts were washed to neutrality, dried with Na_2SO_4, and concentrated for chromatography.

The analytical TLC of the 2,4-DNPH derivatives was carried out on Silufol plates (12 × 5 and 12 × 10 cm) in the ethyl acetate–hexane (1:4) system [10] in an unsaturated chamber with a twofold ascent of the solvent front.

Of the model compounds, diacetone alcohol was obtained by the method of Levina et al. [11]. Isomesityl oxide, which is present as an impurity in mesityl oxide, was identified by its coloration and the R_f value of its 2,4-DNPH derivative [12].

The mercapto compounds were obtained by the method of Nazarov et al. [13] with treatment by gaseous H_2S for 2 h at room temperature. The samples were moistened with steam on a boiling-water bath, after the meal had first been ground in a mortar.

The moisture content of the meal was determined by the method generally adopted [14].

The alkali-soluble proteins were isolated by a standard method [16] and the nitrogen was determined by an accelerated method [17], the free gossypol by the p-anisidine method, and the free lysine by its conversion into the DNP derivative [18].

SUMMARY

On the basis of a quantitative analysis of the volatile carbonyl compounds present in acetone-treated meal it has been shown that they contain a total of about 11 compounds, the main ones of which are acetone and mesityl oxide. It has been established that the moist heat treatment of extraction meal does not lead to the complete elimination of these compounds, and under severe conditions it actually leads to an increase in the amount of carbonyl compounds through degradation of, mainly, the fatty fraction of the meal.

The amount of assimilable lysine in the meal decreases simultaneously.

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THE GLYCERIDE COMPLEX OF THE SEED OIL OF *Onopordum acanthium*

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The fatty acids (FAs) containing epoxide and hydroxy groups that have been isolated from the seed oils of various plants have *a priori* been considered as components of glycerides [1-3]. However, the direct experimental proof of this hypothesis was published elsewhere [4-11]. It has become known that epoxide- and hydroxyl-containing acyl radicals occupy both the 1,3 and the 2 positions in triglycerides (TGs) [3, 6-10] or exclusively the 1,3 positions [11].

In each of the cited papers, information has been published on those individual glycerides that could be isolated. In the present paper we report the isolation and the investigation of a whole series of individual groups of glycerides found simultaneously in the seed oils of *Onopordum acanthium* L. (Scotch cotton-thistle) family Compositae, growing on the northern slopes of the Chatkal range.

The seed oil (100 g) was subjected to column chromatography. This led to the isolation of nine fractions with solvent systems a-g (wt.% on the oil):

a) fraction I (mixture of hydrocarbons, esters, traces of triglycerides) — 0.18;

b) fraction II (triglycerides) — 88.0;

c) fraction III (epoxyacyl triglycerides) — 4.16;

d) fraction IV (oxoacyl triglycerides, about 0.1; free fatty acids and hydroperoxyacyl triglycerides, about 0.1) — 0.40;

e) fraction V (hydroxyacyl triglycerides, 1.58; pentacyclic alcohols) — 1.62;

fraction V' (hydroxyacyl triglycerides) — 3.42;

f) fraction VI (epoxyacyl-hydroxyacyl triglycerides, 0.36; diglycerides, 0.55; free sterols) — 1.21;

g) fraction VII (oxidized triglycerides) — 0.35;

fraction VIII (oxidized triglycerides, hydroxy acids) — 0.18;

fraction IX (monoglycerides) — 0.17.

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