A METHOD OF EXTRACTING PHOSPHOLIPIDS FROM COTTON SEEDS

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It has been established that a mixture containing water differs from chloroform—methanol by the speed of extraction. In the qualitative respect, the combined phospholipids obtained by the two methods do not differ from one another. In a slowly-extracted fraction the combined material contains no unusual phospholipids or fatty acids.

In order to achieve sufficiently complete extraction of phospholipids (PLs) from plant material use is generally made of highly polar organic solvents — mixtures containing alcohols — which, in complex lipids, break the bonds with other classes of natural compounds in the cell [1]. The extractant usually adopted for PLs is chloroform—methanol (2 : 1) [2].

In order to answer the question of the exhaustive extractability of the total PLs, acetone-defatted ground kernels of cotton plants of varieties 108-F and 5904-I of the 1979 harvest were extracted with various solvents. The results of repeated experiments showed that the bulk of the combined PLs is extracted from cottonseed kernels by 12-13 steepings in the above-mentioned mixture of solvents. At the same time, of course, complete extraction of the PLs is not achieved; Even after 25 steepings the meal continues to show the reaction for PLs. The amount of PLs separated in successive steepings decreases in the following sequence: 1st-5th steepings — PCs → PIs → PEs → N-acyl-PEs → N-acyllyso-PEs → lyso-PEs → unidentified PLs; 6th-13th steepings — PIs → PCs → PEs → N-acyl-PEs → N-acyllyso-PEs → lyso-PCs → unidentified PLs; 14th-18th steepings — PIs → PEs → PCs → N-acyl-PEs; 19-23rd steepings — PIs → PEs; 24th-25th steepings — traces of PIs, traces of PEs.

The qualitative compositions of the fatty acids (FAs) of the main bulk of the PLs (1st-13th steepings) [3, 4] and of the combined material from the 13-25th steepings were the same. In order to achieve complete extraction of the PLs, the meal, after its extraction with $CHCl_3$—CH_3OH (2 : 1) (25 steepings) was treated with water-saturated n-butanol. Water-saturated butanol has been used previously to extract lipids from cereal seeds [5]. In the case of cotton seeds, the butanol extracted the total extractive substances, mainly of nonlipid nature with traces of PLs, from which it was fairly difficult to separate them. In sum, according to TLC, traces of PIs and PEs were present, i.e., butanol extracts the PLs just as slowly as $CHCl_3$—CH_3OH (2 : 1). Thus, for cotton seeds water-saturated butanol is an ineffective agent for the exhaustive extraction of the PLs, as has been observed previously for the cases of the soybean [6] and of wheaten flour [7].

There is information in the literature that the extraction of plant material with water-containing solvents increases the yield of PLs [8, 9, 10]. To compare the extracting power of the mixtures $CHCl_3$—CH_3OH (2 : 1) and $CHCl_3$—CH_3OH—H_2O (3 : 2 : 0.17) as applied to cotton seeds, the defatted kernels of both varieties of cotton plant were extracted in parallel under similar conditions with the two solvent systems. In each case, three extracts were obtained (see the experimental part). It must be mentioned that the qualitative phospholipid compositions of parallel extracts obtained by these solvent systems were basically identical and their fatty acid compositions were also the same (below we give the compositions of the FAs of the combined PLs obtained by the chloroform—methanol and chloroform—methanol—water mixtures). For this reason it is difficult to give preference to one of the solvents used; however, as can be seen from the facts given in the experimental part, the $CHCl_3$—CH_3OH—H_2O mixture extracted a somewhat larger amount of extractive substances altogether and of phospholipids in particular. This means, as was to be expected, that the water-containing mixture possesses a comparatively high extracting capacity, but even after repeated extraction

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of the cottonseed kernels with $CHCl_3-CH_3OH-H_2O$ (3 : 2 : 0.17) mixture a certain amount of PLs remained in it (qualitative test). To determine the nature of the slowly-extracted PLs of cottonseed meal after extraction by the solvent systems mentioned they were extracted with $CHCl_3-CH_3OH$ (2 : 1) containing 0.33% of HNO_3, with the aid of which it is possible to extract the PLs exhaustively [11].

The acid extract obtained consisted of the total extractive substances, mainly of non-lipid nature, and after suitable treatment on TLC it showed the presence of traces of PIs and PEs after extraction with the $CHCl_3-CH_3OH-H_2O$ mixture, and a considerable amount of a mixture of the PLs mentioned after chloroform—methanol extraction. It must be mentioned that, according to our results, the direct concentration of the acid extracts leads to the partial (lyso-PLs are formed) or complete loss of the PLs. This phenomenon is apparently connected with the deacylation of the PLs under the action of the nitric acid the concentration of which increases as the extract is evaporated. Consequently, we first neutralized the acid extract with ammonia and then distilled off the extractant. The qualitative fatty-acid composition of the purified mixture of PLs from the acid extract was identical with that of the total PLs. The fatty-acid composition of the main and the slowly-extracted fractions of the total PLs of the cotton plant obtained by the two systems of solvents [I — total PLs obtained with $CHCl_3-CH_3OH$ (2 : 1); II — with $CHCl_3-CH_3OH-H_2O$ (3 : 2 : 0.17); III — slowly-extracted combined PLs obtained with an acid solvent mixture after extraction of the meal with $CHCl_3-CH_3OH$ (2 : 1); IV — slowly-extracted combined PLs obtained by an acid mixture of solvents after extraction of the meal with $CHCl_3-CH_3OH-H_2O$ (3 : 2 : 0.17)] were as follows:

Total phospholipids	*10:0*	*12:0*	*14:0*	*16:0*	*16:1*	*18:0*	*18:1*	*18:2*	ΣS	ΣU
					Variety 108-F					
I	1.2	2.7	2.2	26.4	1.4	1 7	17.5	46.9	34.2	65,8
II	1 8	2,0	1 9	26.2	2.0	2.2	17.8	46 1	34.1	65.9
III	2.7	3,0	2 7	28.5	2.1	3,5	19.4	38.1	40 4	59,6
IV	—	2,4	2,5	28,9	3,3	3,3	20,0	39,6	37,1	62,9
					Variety 5904-I					
I	—	3 2	4,0	20,7	3 0	4 0	21,1	44,0	31,9	68,1
II	—	2,0	2 0	22.6	1,8	3,8	21 4	46,4	30,4	69.6
III	—	1,2	3 0	25,9	1,2	Tr.	21,4	47,3	30,1	69,9
IV	—	1,0	2,3	24.7	1.0	Tr.	21,7	49,3	28,0	72,0

In both cases, after extraction with the acidified mixture no phospholipids remained in the meal, as was established from the absence of FAs in the products of hydrolysis of the meal with the aid of NaOH. Thus, after extraction of the cottonseed kernels with both $CHCl_3-CH_3OH$ (2 : 1) and with $CHCl_3-CH_3OH-H_2O$ (3 : 2 : 0.17) the meal still contained a certain amount of a mixture of PLs consisting mainly of the more polar components (PIs and PEs). There were no unusual PLs whatever nor FAs in them. The cause of the retention of a certain proportion of the PLs on extraction is possibly a hindrance to the access of solvents, especially those containing no water, to the cell coat.

EXPERIMENTAL

The cotyledons were separated by grinding in a seed mortar that had been treated with liquid nitrogen. The purified kernels were ground in a coffee mill. Solvents were purified by standard methods [12]. The mixtures $CHCl_3-CH_3OH-H_2O$ (3 : 2 : 0.17) and $CHCl_3-CH_3OH$ (2 : 1) with 0.33% of nitric acid each contained 0.01% of the antioxidant Ionol [11]. Analytical TLC was performed on plates coated with KSK silica gel with particle dimensions of about 125 μ containing 5% of gypsum, and column chromatography with silica gel having particle dimensions of 160-250 μ.

The total PLs were deacylated as described by Stahl [13]. Samples of the fatty acid methyl esters were subjected to GLC on a Chrom-41 instrument at 198°C using a column 2.5 m long filled with 17% of PEGS on Celite-545. The following solvent systems were used for TLC: 1) chloroform—methanol—water (65 : 35 : 5) and 2) chloroform—methanol—ammonia (65 : 35 : 5). The chromogenic agents used were: iodine vapor, 50% sulfuric acid solution, Dragendorff's and Vaskovsky's reagents, and ninhydrin solution.

Defatting of the Seed Kernels of the Cotton Plant of Variety 5904-I. The comminuted kernels (100 g) were extracted with freshly-distilled acetone (2 liters) at room temperature by the steeping method. The acetone extract formed a dark-colored oil. TLC in system 2: traces of PCs; amount of P in the oil 0.01%. The meal was dried in the air and divided into two parts.

Extraction of the Meal with $CHCl_3-CH_3OH$ (2 : 1). Part of the defatted meal was extracted first with one liter (extract I) and then with 350 ml (extract II) and 150 ml (extract III) of chloroform—methanol (2 : 1). The solvents were distilled off in a rotary evaporator in an atmosphere of nitrogen to dryness. TLC in systems 1 and 2: extracts I and II — PL, neutral lipids, substances of steroid nature, carbohydrates, and pigments (2.15 g and 0.4 g, respectively); extract III — PLs, traces of neutral lipids (0.1 g).

Elimination of Carbohydrates from the Extracts. Extracts I and II were dissolved in chloroform—methanol—water (90 : 10:1) and the solution was passed through a column containing Mol-Selekt G-25 previously swollen in the same mixture. The completeness of the elimination of carbohydrates was checked by TLC in system 2.

Column Chromatography of the Extracts through Silica Gel. The carbohydrate-free extracts were dissolved in chloroform and passed through a column containing silica gel (1 : 50). The column was eluted with chloroform (neutral lipids), acetone (steroids), chloroform—methanol (1 : 1) and methanol (PLs). The yield of extract I was 0.59 g (68.6% of the total PLs), of II 0.13 g (15.1%), and of III 0.07 g (8.1%). TLC of the extracts freed from impurities (in system 2): I — lyso-PCs (R_f 0.1), PIs (0.2), PCs (0.4), PEs (0.65), N-acyllyso-PEs (0.8), unidentified PLs (0.9), N-acyl-PEs (0.95); II — PIs, PCs, PEs; III — PIs, PEs.

Extraction of the Meal with $CHCl_3-CH_3OH-H_2O$. Another part of the defatted meal was extracted with $CHCl_3-CH_3OH-H_2O$ (3 : 2 : 0.17). Again three extracts were obtained: I — 2.9 g; II — 0.5 g; III — 0.1 g. They were freed from impurities in the form of carbohydrates, neutral lipids, and, particularly, pigments and the substances of steroid nature in a similar manner to the purification of the extracts obtained with $CHCl_3-CH_3OH$ (2 : 1). The purified extracts had the following indices: I — 0.65 g (74.7% of the total PLs), TLC identical with that of the extract I obtained with chloroform—methanol; II — 0.17 g (19.5%), TLC: PIs, traces of PEs, and an insignificant amount of PCs; III — 0.03 g (3.4%) TLC: traces of PIs, traces of PEs.

Extraction of the Meal with an Acidified Mixture. The meal after extraction with $CHCl_3-CH_3OH$ (2 : 1) was extracted with one liter of the same mixture containing 0.33% of nitric acid. The acid extract was neutralized with 25% ammonia, the resulting precipitate was filtered off, and the filtrate was evaporated in a rotary evaporator. The extract obtained was treated several times with chloroform. Yield 0.07 g (8.1% of the total PLs). TLC in system 2: PIs and PEs. For the exhaustive extraction of the difficulty extractable part of the total PLs from the meal that had been extracted with the water-containing mixture we used 500 ml of $CHCl_3-CH_3OH$ (2 : 1) containing 0.33% of nitric acid. After a suitable working up of the extract, the yield of purified total PLs amounted to 0.02 g (2.3% of the weight of the total PLs).

Treatment of the Meal with Alkali. Part of the dried meal was boiled in a 10% solution of NaOH in methanol under reflux for an hour. The meal was filtered off, the alkaline solution was evaporated, and the residue was dissolved in water acidified with 20% HCl and was extracted with ether. No fatty acids were detected (TLC, GLC) in the meal after extraction either with $CHCl_3-CH_3OH$ (2 : 1) or with $CHCl_3-CH_3OH-H_2O$.

SUMMARY

1. It has been established that after the usual extraction of defatted cottonseed kernels with $CHCl_3-CH_3OH$ (2 : 1), about 8% of a mixture of phospholipids (on the total PLs) remains in the meal, and after extraction with a water-containing mixture about 2.3% remains, which is easily extracted with a mixture of solvents acidified with a mineral acid. The slowly-extracted fraction of the total material contains no unusual phospholipids or fatty acids.

2. The working up of an acid extract without preliminary neutralization by ammonia leads to a loss of phospholipids.

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FATTY-ACID COMPOSITION OF THE LIPIDS OF COREGONIDS OF THE OB BASIN

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The fatty acid compositions of the lipids of the muscles of the back and of the abdomen of a pelyad from the lakes of the Ob basin have been studied by gas—liquid chromatography; 29 acids have been detected of which 27 have been identified. It has been established that the lipids of the muscles of the back of the pelyad contain 37.2% of polyunsaturated and 37.3% of monoenoic acids the main components of which are oleic, palmitic, palmitoleic, octadecatetraenoic, and eicosapentaenoic acids, amounting in total to 65%. The lipids of the muscles of the pelyad are unique among lipids of fresh-water fish in their content of tri- and tetraenoic acids. As compared with the usual composition, the neutral lipids are characterized by higher amounts of monoenoic and lower amounts of polyenoic acids.

In spite of the large number of publications on the fatty-acid compositions of fish lipids [1-3]. the compositions of the fatty acids of the lipids of fresh-water fish have been studied inadequately [3, 4], and the composition of the lipids of the coregonids of the Ob basin have not been studied at all. Only the food value of river and lake pelyads [5] and the amount of polyunsaturated compounds in the lipids of the pelyads [6] have been considered.

In the present communication the results are given of a study of the fatty-acid compositions of the total neutral lipids of muscle tissue of the back and abdomen of the pelyad *Coregonus peled* (Umelin) from lakes of the Ob basin by gas—liquid chromatography and IR spectrometry.

In the lipids of the muscles of the back, 29 acids were detected, of which 27 have been identified: 13 acids were present in amounts of more than 1%, the amounts of each of seven other acids did not exceed 1%, and the amounts of the remainder were each less than 0.1%.

In the lipids of the muscles of the back of the pelyad we detected the acids with even numbers of carbon atoms from C_{10} to C_{22}, the acids with odd numbers of carbon atoms from C_{13} to C_{21}, saturated acids with compositions from $C_{10:0}$ to $C_{18:0}$, monounsaturated acids from $C_{12:1}$ to $C_{20:1}$, and polyunsaturated acids from $C_{14:2}$ to $C_{22:6}$. The acids with even numbers of carbon atoms made up 96.36% of the total.

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