HOMOGENEOUS LECITHIN FROM COTTONSEED MEAL

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The possibility of using industrial cottonseed meal for the production of homogeneous lecithin has been shown.

Among the oil crops, cotton seeds occupy one of the leading positions in relation to their phospholipid (PL) content [1, 2]. When the oil is isolated from cotton seed, in addition to phospholipids, pigments of the gossypol group also pass into the oil, which prevents the use of the oil-production wastes for the purposes of obtaining phospholipid concentrates. There are no concrete recommendations for obtaining gossypol-free cotton phosphatide concentrates [3], although the literature contains information on the possibility of obtaining them [4].

Recently, in connection with a study of biological membranes by using membrane models — artificial bilayer membranes and liposomes — and also with the study of membrane enzymes, the demand for homogeneous phospholipids has risen considerably [5]. In an investigation of the metabolism of the PLs and also in order to obtain liposomes it is the phosphatidylcholines (lecithins) which are most frequently used, these being the main components of the total PLs of all higher plants (35-50% of the total PLs) and having the structure of diacyl-sn-glycero-3-phosphocholine. Homogeneous lecithin for scientific investigations is mainly obtained from egg phosphatides [5, 6].

In view of the rising demand for phospholipids with a high degree of purity, we have studied the possibility of using industrial cottonseed meal as a cheap raw material for the isolation of chromatographically homogeneous lecithins. Depending on the conditions of treating the cottonseed, the yield of purified total PLs amounted to 0.45-0.6% of the weight of the meal. On two-dimensional chromatography the total PLs revealed nine phosphorus-containing components. With the aid of qualitative reactions and on the basis of literature information [7] the following PLs were identified: PC, PE, PI, PA, lyso-PC, N-acyl-PE, N-acyl-lyso-PE, two unidentified phospholipids, and also phosphatidic acids (PAs), which were absent from the cottonseeds and were formed in the process of treating them in order to obtain oil.

We isolated chromatographically homogeneous lecithin by subjecting the total phospholipids to column chromatography on alumina. The lecithin of the cottonseed meal consisted of a viscous oil yellowing slightly in the air; it dissolved in chloroform, ethanol, methanol, and ether and was insoluble in anhydrous acetone and in ethyl and methyl acetates. TLC: R_f 0.4 (system 1), 0.5 (system 3); $[\alpha]_D^{20}$ + 3.5° (chloroform). N content 1.6%, P content 3.6%. IR spectrum (cm^{-1}): 3200-3600 (OH); 2860, 2040, 1390 (CH, CH_2, CH_3); 3020 (olefinic bond); 1745 (ester C=O) 1100, 1250 (P=O); 1080 (P–O–C); 980 [$\overset{+}{N}(CH_3)_3$]. The UV spectra lacked the absorption band characteristic for gossypol (355-365 nm) [8]. The lecithin was subjected to alkaline deacylation and the fatty acids split out were analyzed by GLC in the form of their methyl esters. The fatty acid composition was calculated as follows (%):

10:0	0.9	16:1	1.6
12:0	0.9	18:0	3.7
14:0	1.3	18:1	23.8
16:0	21.4	18:2	46.4
	ΣS 28.2		
	ΣU 71.8		

It was established by enzymatic hydrolysis using cobra venom phospholipase A_2 [9] that 97% of the unsaturated fatty acids were esterified in position 2 of the lecithin molecule.

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Thus, a chromatographically homogeneous gossypol-free lecithin with a specific fatty acid distribution has been obtained from industrial cottonseed meal.

EXPERIMENTAL

Thin-layer chromatography (TLC) was performed on type KSK silica gel in the following solvent systems: 1) chloroform–ethanol–25% ammonia (65:35:5); 2) chloroform–methanol–acetone–acetic acid–water (10:5:4:2:1); and 3) chloroform–methanol–water (65:35:5). For two-dimensional TLC we used systems 1 (in direction I) and 2 (in direction II).

The phospholipids were revealed by the Vaskovsky and the Dragendorff reagents and with a solution of ninhydrin.

For column chromatography we used Al_2O_3 (neutral, Brockmann activity grade II). The methyl esters of the fatty acids were analyzed on a Chrom-41 chromatograph in with the solid phase Celite-545 impregnated with 17% of PEGS, the carrier gas being helium.

IR spectra were recorded on a UR-20 spectrophotometer and UV spectra on a Hitachi spectrometer in ethanol. The industrial cottonseed meal was freed from residual oil with acetone, and the phospholipids were extracted from the defatted meal by the method of Folch et al. [10]. The total material was freed from carbohydrate impurities by gel filtration through Molselekt G-25 in chloroform–methanol–water (9:10:1) [11]. Homogeneous lecithin was isolated as described by Singleton et al. [6] from the total PLs that had been freed from impurities.

CONCLUSION

The possibility has been shown of using industrial cottonseed meal for obtaining homogeneous lecithin.

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