PURIFICATION OF TOTAL PHOSPHO- AND PHOSPHONOLIPIDS OF COTTON AND KENAF SEEDS FROM ACCOMPANYING SUBSTANCES

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A method is proposed for freeing total plant phospho- and phosphonolipids from impurities.

The isolation of phospholipids (PLs) with a high degree of purity presents certain difficulties in the methodological respect. Analysis of literature information [1-3] shows PLs are isolated from previously defatted seeds by extraction with chloroform–methanol (2:1). Under these conditions, in addition to the PLs, a certain amount of neutral lipids (NLs), carbohydrates, pigments of phenolic nature, glycolipids (GLs), substances of steroid nature, etc., also pass into the extract.

The freeing of the PLs from impurities is carried out by various methods: stepwise elution of individual fractions of the compounds from a column containing silica gel by solutions of diethyl ether in petroleum ether [4] or successively by chloroform and acetone [5, 6], by washing out with acetone [7] or with isopropanol [8], and by successive liquid–liquid and adsorption extraction [9]. In addition, a method of separating PLs by the precipitation of their petroleum ether solutions with acetone in the cold exists [10].

The application to oilseed lipids of these methods of freeing PLs from contamination from other components does not always give reproducible results and it is difficult to achieve a sufficiently complete purification of the total PLs in these ways.

We have developed a method of purifying the isolated crude total PLs which is based on their preparative separation from accompanying impurities in various systems and is distinguished by a fairly high accuracy. The PLs were obtained by the extraction with chloroform–methanol (2:1) of previously defatted kenaf and cotton seeds. They were freed from carbohydrate impurities by gel filtration through Molselekt G-25. Further purification was achieved by preparative TLC in two solvent systems: in the first stage, plates with the deposited total PLs were subjected to seven ascending runs in hexane–ether (7:3) (system 1) to a height of 12 cm, with the drying of the plate after each run; in the second stage, the chromatograms were subjected to seven ascending treatments in acetone (system 2). The layers of silica gel from the starting and top zones were scraped off, and these were eluted and analyzed by TLC. It was found that the impurities separated from the PLs by system 1 had R_f 0.4-1.0 (the zone was colored on the chromatogram) and, according to the results of TLC in systems 1, 3, and 4, consisted of carbohydrates, sterol esters, TAGs, FFAs, DAGs, and GLs. The total PLs (starting zone) eluted from the adsorbent were enriched with GLs, and in the case of cotton seeds they had a dark color and contained, in addition to GLs (systems 4-6) pigments of the gossypol group. The further purification of the total material from impurities was carried out in system 2. In this system, the PLs remained at the start and the GLs and pigments of the gossypol group ascended at the solvent front.

The purified total PLs removed from the sorbent contained no impurities. It can be seen from what has been said that where it is necessary to isolate a fraction enriched with GLs, the zone of the sorbent with the impurities after the development of the chromatograms in system 1 is removed, and the total PLs remaining at the start are transferred to another plate and are separated in system 2, in which the PLs (starting zone) are separated from the GLs (R_f 0.7-1.0).

We then selected conditions for separating the PLs from their phosphonic analogs.

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It is known that in recent years a new class of lipids – phosphonolipids – has been detected in plant material [11]. Using the CC method proposed by Moschidis [12, 13] we have detected phosphonolipids in the lipids of cotton and kenaf seeds. However, the isolation of the pure combined phosphonolipids by the methods mentioned from the materials that we investigated was complicated, apparently, because of their small amount. We therefore selected the variant of separating the PLs and their phosphono analogs in a thin layer.

For this purpose, the purified total PLs were subjected to further preparative separation in methanol–water (2:1) (system 7) [12]. The experiment showed that in this system the PLs remained at the start and the phosphonolipids were drawn out into a band with R_f 0.4-0.8.

Analysis of the compounds taken from the start and from the zone with R_f 0.4-0.8 showed that to separate the phosphonolipids from the PLs a single ascending run of the chromatogram (TLC, systems 5-7) was sufficient. The highly purified PLs of kenaf seeds consist of seven known components [3], while the combined phosphonolipids are represented by four compounds with R_f values of 0.2, 0.25, 0.35, and 0.5 (system 5), among which the component with R_f 0.5 is present in only trace amounts. The spot with R_f 0.25 was revealed with solution of ninhydrin, which gave grounds for assuming the presence of a phosphono-PE. In cotton seeds, likewise, seven components of the PLs were detected [14], and three spots of phosphonolipids with R_f 0.35, 0.4, and 0.5. Visually, the component with R_f 0.4 was present in trace amounts and those with R_f 0.35 and 0.5 in approximately equal amounts.

Thus, a method is proposed for purifying the total plant PLs which is based on the preparative separation of the crude total PLs by the repeated development of a chromatogram first in the hexane–ether (7:3) system and then in acetone.

Phosphonolipids have been detected in the pure combined PLs of cotton and kenaf seeds.

EXPERIMENTAL

The solvents used were purified by generally adopted methods [15]. Ground kenaf and cotton seeds were defatted by repeated extraction with hexane at room temperature.

The qualitative chromatography of the lipid fractions was performed in a thin layer of Chemapol L silica gel (Czechoslovakia), 5/40 μm. For the preparative separation of the samples by TLC we used plates with dimensions of 13 × 18 cm, with the deposition of 40 mg of the total material on each plate. In system 1, the time for one ascending run of the chromatogram was 20 min, and in acetone (system 2) it was 40-45 min. The spots on the chromatograms were identified on the basis of the R_f values of the substances being analyzed and of markers, and also by color reactions with iodine vapor, by spraying with 50% H_2SO_4 followed by heating to 120°C for 3-5 min, while the phospho- and phosphonolipids were detected with the Vaskovsky reagent and with a solution of ninhydrin, and GLs by successive treatments with α-naphthol and 50% H_2SO_4 with heating. The following solvent systems were used: 1) hexane–ether (7:3); 2) acetone; 3) hexane–ether–acetic acid (70:30:1); 4) acetone–benzene–water (91:30:8); 5) chloroform–methanol–25% ammonia (65:35:5); 6) chloroform–methanol–water (65:35:5); and 7) methanol–water (2:1).

CONCLUSIONS

A method is proposed for freeing the total plant phospho- and phosphonolipids from impurities.

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FEATURES OF SYNTHESIS OF FATTY ACIDS IN ACTIVE LIPID-FORMING YEASTS

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Features of the accumulation of fatty acids and the changes in their composition in seven strains of yeasts that are promising producing agents of lipids have been studied. It has been shown that in the process of accumulation of lipids in the biomass changes take place that lead to a fall in the degree of unsaturation of the pool of fatty acids. This nature of the change in the composition of the fatty acids is, as has been shown by measuring their absolute amounts in the biomass, connected with the intensive synthesis of oleic and palmitic acids as components of the reserve lipids. The results obtained form the basis for an evaluation of the promising nature of individual strains as active producers of fatty acids.

The chemical and physical properties of natural lipids that are responsible for their functional significance and also for the possibility of their use in various sectors of industry are determined primarily by their fatty acid composition.

In recent years, in our country and abroad, active investigations directed discovering substitutes for food oils and fats used for technical purposes have been carried on. Fats of microbial origin, and, especially, yeast lipids, are considered as the most promising from this point of view. The prerequisites for such substitution are a predominance of triacylglycerols in the fractional composition of yeast lipids and the presence among their fatty acids of appreciable amounts of unsaturated acids [1, 2] and, in particular, of linolenic acid [3].

In view of this, in the choice of lipid-producing microorganisms a knowledge of the fatty acid composition of their lipids and the limits of its change with the aid of various technological factors without intervention in the hereditary apparatus of the organism acquires prime importance.

In the present paper we consider the laws of the synthesis of fatty acids by "fatty" strains of yeasts under the conditions of the active synthesis of lipids. Seven strains of yeast were investigated: Candida beechii VKM u-1428, Lipomyces lipofer B-5, Rhodotorula glutinis var. glutinis DKM u-329, 334, 337, 1630, and Rh. gracilis BKM u-335 (all obtained from the All-Union Collection of Nonpathogenic Microorganisms at the Institute of the Biology and Physiology of Microorganisms of the USSR Academy of Sciences) [4], which are capable of accumulating up to 20-50% of lipids [5].

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