COMPOSITION OF PHOSPHOLIPIDS OF DOUBLE LOW RAPESEED.

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The composition of phospholipids /PL/ of the Jantar variety double improved rapeseed from the 1985 crops has been determined. The lipids were extracted from seeds according to the modified method of Folch. Polar lipids were extracted with acidified methanol. Purification and separation of PL into classes with respect to increasing polarity were carried out on a column packed with acid-treated Florisil. Separation of individual phospholipids was carried out by 1-D TLC. Their amount was determined by determination of phosphorus using a modified AOCS method. The quantitative composition of the rapeseed PL looked as follows: phosphatidylcholine /PC/ - 56.5 %, phosphatidylethanolamine /PE/ - 17.9 %, phosphatidylinositol /PI/ - 16.7 %, phosphatidylglycerol /PG/ - 4.3 %, lysophosphatidylcholine /LPC/ - 2.6 % and phosphatidic acid /PA/ - 2.0 %.

INTRODUCTION

Recognition of the qualitative and quantitative composition of lipids of every new rapeseed variety is necessary for proper utilization of this material and rational planning of its processing. The content and composition of phospholipids influence to a high degree the properties of oil, as well as its behaviour during processing

/Goh et al., 1984; List, 1978/. The results of investigations on canola oils /Diosady, 1982, 1984; Mag, 1983/, as well as our preliminary research, proved that the content of PL is higher than in traditional varieties, and hence rafination of oils requires application of more severe conditions. We also established the presence of inorganic phosphorus compounds, which can form nonhydratable phospholipids complexes. The composition of PL of the polish variety of double improved rapeseed constituted the topic of this research.

MATERIALS AND METHODS

The lipids were extracted from ground seeds /after deactivation of phospholipases - 85°C, 30 min./ twice, using a modified method of Folch. The joint extracts were washed with water, dried, and the solvent was evaporated under vacuum. Polar lipids were extracted from oil with acidified methanol /CH_3OH : CH_3COOH, 95:5 v/v/. 10 ml of methanol was added to oil /5 g/, the mixture being heated to 50°C and stirred using a magnetic stirrer in a system comprising a reflux condenser. Extraction time - 30 min. The solvent was evaporated from the extract, and the residue was dissolved in chloroform.

The qualitative composition of PL was determined by a 2-dim. TLC using the following solvent systems: chloroform/methanol/7 N NH_4OH /65:30:4 v/v/v/ in one direction and chloroform/methanol/acetic acid/water /170:25:25:6 v/v/v/v/ in the other direction. The TLC specific spray reagents used were: Zinzades' reagent, Dragendorff's reagent, ninhydrin, $AgNO_3$. Phospholipids standards were de-

livered by Sigma Chemical Company. Separation of lipids was carried out using a column packed with acid-treated Florisil /Carroll, 1963/. The column was first washed with chloroform and acetone, and subsequently the phospholipids were fractionated into PA + PI_{traces}, PG + PI, PE + PI and PC + LPC fractions by an increase of the content of methanol in acetone. The solvent was evaporated and the residue was dissolved in a chloroform - methanol /1:1/ mixture. In order to separate individual phospholipids, each fraction was deposited as a band on TLC plates /20 x 20 cm/ covered with Silica Gel G /purchased from Merck, Darmstadt, Germany/ and developed twice by a 1-D TLC. The following eluents were used in turn: chloroform/acetone/methanol/acetic acid/water /100:100:50:4:10 v/v/v/v/v/ and chloroform/methanol/acetic acid/water /180:150:30:10 v/v/v/v/. The bands were identified in iodine vapours. Each phospholipid was extracted from gel /chloroform/methanol 1:1/, the process being followed by determination of phosphorus by a partly modified AOCS method. A Carl Zeiss Jena VSU 2-P spectrophotometer was used. The composition of the PL fatty acids was determined using a Jeol gas Chromatograph equipped with a Hewlett-Packard integrator.

RESULTS AND DISCUSSION

The content of the phosphorus compounds in lipids /isolated by method of Folch/ of the Jantar variety rapeseed was higher then in case of rapeseed of traditional varietes. Phospholipids constituted ca 80% of the phosphorus compounds /Table I/.

Table I. The content of phosphorus compounds in rapeseed.

Total phosphorus ppm	Phospholipids /phosphorus/ ppm	Inorganic compounds /phosphorus/ ppm	Share of PL phosphorus %
499.6	382.6	117.0	76.6

2-D TLC revealed the presence of 6 phospholipids, viz. phosphatidylcholine /PC/, phosphatidylethanolamine /PE/, phosphatidylinositol /PI/, phosphatidylglycerol /PG/, lysophosphatidylcholine /LPC/ and phosphatidic acid /PA/ /Fig.1/.

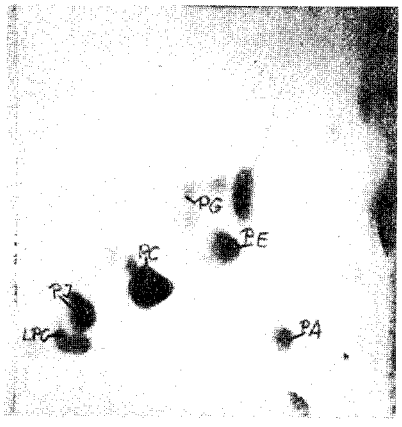

Fig.1. 2D-TLC separation of polar lipids.

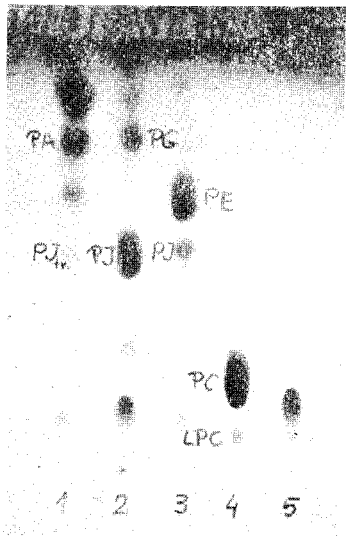

Fig.2. 1D-TLC of polar lipids fractionated on a column.

Developed chromatogram was sprayed with Zinzadés' reagent and heated to 140°C.

Fractionation of PL on a column is illustrated in Fig.2. 700 ug of P was introduced each time onto the co-

lumn. Share of phosphorus in each fraction is listed in Table 2.

Table 2. Separation of PL on a column x/.

Fraction	Composition	Phosphorus ug	Share of P in the fraction %
1	PA + PI_{traces}	38.4	5.7
2	PI + PG	92.5	13.7
3	PI + PE	150.4	22.3
4	PC + LPC	394.5	58.3

x/ The results are means of 6 separations.

The composition of rapeseed PL was the following /Table 3/.

Table 3. Phospholipids of Jantar variety double improved rapeseed.

Phospholipid	PC	PE	PI	PG	LPC	PA
% mol P	56.5	17.9	16.7	4.3	2.6	2.0

A comparison of fatty acids composition of phospholipids and triacylglycerids fractions proved /Table 4/ that PL contained more palmitic and linoleic acids, and less oleic and linolenic acids than TG.

Table 4. Fatty acids composition of phospholipids and triacylglycerids.

acid	16:0	16:1	18:0	18:1	18:2	18:3	20:1
PL	11.0	1.0	0.9	47.8	32.6	6.7	-
TG	4.7	0.4	0.9	60.1	22.8	9.9	1.2

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