

## LIPIDS OF *Arum Korolkowii* TUBERS

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*The compositions of classes and fatty acids in Arum Korolkowii tubers are determined. The principal acids are 16:0, 18:1, and 18:2. Triterpenes, 4-monomethylsterol, and sterols are found among the unsaponified substances.*

**Key words:** Neutral lipids, unsaponified substances, phospholipids, glycolipids.

The plant *Arum Korolkowii* Regel. (Korol'kov arum) belongs to the Araceae family and the *Arum* L. genus. Its tubers are rich in starch [1]. Polysaccharides (10.9%), pectinic substances (10.8%), and fructosans (0.6%) were isolated and characterized structurally during a previous study of the carbohydrates isolated from *Arum korolkowii* tubers [2, 3].

We studied lipids of arum tubers collected near the Chatkal'skii mountains. The moisture content of the raw tubers is 47.9%. The lipid content per absolutely dry substance is 1.6%; protein, 25.0%.

Total lipids were separated by column chromatography on silica gel. Neutral lipids (NL, 50.2%) were eluted by  $\text{CHCl}_3$ ; glycolipids (GL, 35.6%), by  $(\text{CH}_3)_2\text{CO}$ ; phospholipids (PL, 14.2%), by  $\text{CH}_3\text{OH}$ .

The NL were studied by TLC in systems 1-3.

Chromatographically pure substances were assigned to certain lipid groups by comparing the chromatographic mobility of these substances and model preparations, observing qualitative reactions, and using literature data.

The NL contained hydrocarbons, esters, free fatty acids (FFA), triacylglycerols, triterpenes, 4-monomethylsterols, diacylglycerols, and sterols.

The ester fraction obtained from the NL was separated by preparative TLC using system 2 and saponified. Unsaponified substances were extracted by diethyl ether.

The saponified part yielded fatty acids. The ratio of unsaponified substances and fatty acids isolated from the esters was 50.2:49.8.

The TLC of the unsaponified substances showed triterpenes, 4-monomethylsterols, and sterols. These are products of complete hydrolysis of esters.

The Amenta method, which is based on oxidation of lipids by a dichromate reagent [4] and is used by several research groups [5, 6], was used to determine the number of individual NL groups. The quantitative composition of the NL (%) in *Arum korolkowii* tubers is given below:

Lipid classes	Content, %
Hydrocarbons	8.9
Fatty-acid esters, sterols, triterpenes, 4-monomethylsterols and an unidentified compound	9.9
Triacylglycerols	18.1
Free fatty acids	27.1
Triterpenes and 4-monomethylsterols	10.4
Sterols and diacylglycerols	17.3
Unidentified components	8.3

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TABLE 1. Fatty-Acid Composition of Individual Lipid Classes of *Arum Korolkowii* (% , GLC)

Acids	% fatty acids of total lipids	FFA	Esters	DAG	PL	GL
12:0	Tr.	Tr.	1.9	Tr.	Tr.	Tr.
14:0	1.3	1.0	8.7	6.8	1.0	4.7
16:0	42.4	64.4	61.7	35.5	39.7	47.9
18:0	1.5	1.0	2.9	8.4	Tr.	Tr.
18:1	12.3	5.3	7.4	20.5	14.7	23.2
18:2	42.5	28.3	17.4	28.8	44.6	24.2
$\Sigma_{\text{sat}}$	45.2	66.4	75.2	50.7	40.7	52.6
$\Sigma_{\text{unsat}}$	54.8	33.6	24.8	49.3	59.3	47.4

Free fatty acids, triacylglycerols, sterols, and diacylglycerols dominate in the NL.

The presence of a significant quantity of fatty acids was confirmed by determining the acid number of the NL, which was 57.5 mg KOH.

Lipophilic components were studied by saponifying the total lipids and isolating unsaponified substances (US, 8.7%) using diethylether.

A significant amount of the US were hydrocarbons, which appeared in system 2 as three spots with  $R_f$  0.92, 0.83, and 0.77. The spots were brown, rose, and bluish-gray after visualization with 50%  $\text{H}_2\text{SO}_4$  and heating.

Developing the chromatogram using system 4 did not change the positions of the paraffinic and olefinic hydrocarbons. The  $R_f$  values of the other two hydrocarbons were 0.68 and 0.54.

Data from our study and the literature [7, 8] suggested that these hydrocarbons are aromatic.

Unsaponified substances were separated by preparative TLC into the following classes: paraffinic and olefinic (9.3%), aromatic hydrocarbons and an unidentified compound (18.8%), triterpenes and 4-monomethylsterols (24.8%), sterols (21.9%), and unidentified compounds (25.2%). The unidentified compounds contained three substances with characteristic colors: an orange substance ( $R_f$  0.31) in the system with 2%  $\text{CH}_3\text{OH}$  in  $\text{C}_6\text{H}_6$  and two lilac-colored substances ( $R_f$  0.20 and 0.25). Sterols in this system appear as a spot with  $R_f$  0.63. Light brown pigments remained at the origin. These were extracted together with other unidentified components by  $\text{CHCl}_3$ : $\text{CH}_3\text{OH}$  (2:1). It should be noted that the relative content of free triterpenes, 4-monomethylsterols, and sterols that were isolated from the unsaponified fraction of total lipids is greater than in NL. This is explained by the presence of these components in a mixture with components isolated from esters.

Individual lipid classes yielded fatty acids. Their composition was determined by GLC (Table 1). It can be seen that lipids of arum tubers include six fatty acids, the principal ones being 16:0, 18:1, and 18:2. The largest amount of unsaturated acids consists of esters (75.2%) and FFA (66.4%). The most unsaturated is the PL fraction (59.3%).

Qualitative separation of GL using system 5 revealed the presence of sulfolipids, digalactosyldiglycerides, sterylglucosides, monogalactosyldiglycerides, and sterylglucoside esters.

## EXPERIMENTAL

UV spectra were recorded on a Perkin—Elmer Lambda-16 instrument in hexane; IR spectra, on a model 2000 Fourier IR-spectrometer. GLC of methyl esters of fatty acids was performed on a Chrom-4 chromatograph with a flame-ionization detector and a column packed with Chromaton N-AW-DMCS with 15% Reoplex-400. The optical density of solutions of separate NL classes was determined on an SF-4 spectrophotometer at 350 nm.

A calibration curve was constructed using pure palmitic acid.

Total lipids from *Arum* tubers were extracted by  $\text{CHCl}_3$ — $\text{CH}_3\text{OH}$  (2:1, v/v) five times at room temperature. Nonlipid components were removed by washing the  $\text{CHCl}_3$ — $\text{CH}_3\text{OH}$  solution with aqueous  $\text{CaCl}_2$  (0.04%).

The following solvent systems were used:  $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ — $\text{C}_6\text{H}_{14}$  (3:7, 1; 6:4, 2; 1:1, 3),  $\text{C}_7\text{H}_{16}$ — $\text{C}_6\text{H}_6$  (9:1, 4),  $\text{CHCl}_3$ — $(\text{CH}_3)_2\text{CO}$ — $\text{CH}_3\text{OH}$ — $\text{CH}_3\text{CO}_2\text{H}$ — $\text{H}_2\text{O}$  (65:20:10:10:3, 5).

The ester fraction from NL was saponified by heating with alcoholic KOH (10%) for 6 h. Then, the solution was diluted with H<sub>2</sub>O. Unsaponified substances were extracted three times with diethylether.

The saponified part was decomposed with H<sub>2</sub>SO<sub>4</sub> (20%). Isolated fatty acids were extracted with diethylether.

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