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INFLUENCE OF THE METHOD OF ISOLATING LIPIDS ON THEIR COMPOSITION

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The methods of CC, TLC, countercurrent extraction, etc., are usually used to determine the composition of plant lipids [1]. In studying the lipids of the leaves of *Hibiscus* species we noted that the quantitative levels of their classes changed in dependence on the method of their isolation. This induced us to make a comparative investigation of the lipids of hibiscus leaves using CC and PTLC on silica gel.

The total lipids were obtained by extraction with a mixture of chloroform and methanol (2:1), after the fresh leaves had been fixed with hot isopropanol. Nonlipid components were eliminated with a 0.04% aqueous solution of $CaCl_2$. The yield of lipids on the absolutely dry weight was 7.4%. The lipids isolated were dark green. TLC on silica gel in the hexane—methyl ethyl ketone–acetic acid (47.5:7.5:0.5) system revealed the presence of eight pigments with different colors: three were pink, with R_f 0.2, 0.24, 0.28 (anthocyans); two were yellow, with R_f 0.41 and 0.88 (carotenoids); and three were green, with R_f 0.06, 0.09. and 0.13 (chlorophylls). The lipids were separated by countercurrent extraction in the hexane—87% aqueous ethanol system into neutral (NLs) and polar (PLs) fractions. The PLs consisted of phospholipids (PhLs) and glycolipids (GLs), with traces of NLs.

The NLs were separated into individual classes, for which purpose one part of them was passed through a column of alumina prepared by a method excluding the acyl isomerization of the lipids [2]. A second part of the PLs was separated by preparative TLC on silica gel.

The NLs were eluted from the column with chloroform, the GLs with acetone, and the PhLs with methanol. The homogeneity of the individual classes was confirmed by qualitative reactions and chromatographic mobilities.

When PTLC was used, the neutral lipids present in the PLs were separated with a mixture of hexane and diethyl ether (60:40), while, in this system, the PhLs and GLs remained at the start. The PhLs were separated from the GLs in the acetone–toluene–acetic acid–water (60:60:2:1) system [3].

The lipids that had been separated into classes were reextracted from the thin layer, first with a mixture of chloroform and ethanol (1:2) and then with methanol.

Analysis of the individual classes of lipids conducted by PTLC showed that the GL fraction contained 18.3% of NLs consisting of triacylglycerols, free fatty acids, epoxyacyldiacylglycerols, and free sterols. The presence of impurities in this fraction is explained by the fact that part of the NLs was adsorbed together with the PLs and were not separated from them in the neutral system. This phenomenon has also been observed by other authors [4]. The additional separation of these GLs in the hexane–diethyl ether (7:3) system enabled their homogeneity to be achieved.

It must be mentioned that an influence on the yields of the individual classes of lipids was exhibited by the pigments, which behaved differently with different methods of separating the lipids when using the above-mentioned solvents. Thus, in PTLC they were concentrated in the PhLs and in CC they passed into the neutral lipids.

The percentage contents of the lipid classes are given below:

	HPTLC	CC
Neutral lipids	25.6	29.5
Phospholipids	41.0	37.2
Glycolipids	33.4	33.3

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It can be seen that with PTLC a smaller amount of NLs was obtained and a larger amount of PhLs than with CC. The amounts of GLs were practically the same in the two cases. The percentage ratio of GLs and PhLs in PTLC was 44.9:55.1, and in CC 47.3:52.7. The latter ratio is more correct.

Thus, without additional purification, the separation of hibiscus lipids by CC permits purer PLs to obtained than by PTLC.

REFERENCES

1. M. Kates, Techniques of Lipidology, American Elsevier, New York (1972).
2. A. V. Zhukov and A. G. Vereshchagin, Fiziol. Rast., **29**, 212 (1982).
3. T. E. Solov'eva, V. G. Shcherbakov, and V. G. Lobanov, Izv. Vuzov, Pishchev. Tekhnol., **1**, 35 (1986).
4. A. V. Zhukov and A. G. Vereshchagin, Biokhimiya, **40**, 899 (1975).