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LIPIDS OF THE FRUIT OF Diospyros kaki

A. A. Kolesnik, V. N. Golubev,
L. I. Kostinskaya, and M. A. Khalilov

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The composition and amounts of the various groups of liposoluble compounds in the rind and flesh of persimmon fruit of the varieties Hachiya and Hyakume have been established by a combination of chromatographic and chemical methods. The identity of the qualitative composition of the lipids of the flesh and rind of the fruit and quantitative differences in the amounts of individual groups have been found. A total of 24 groups of compounds were identified, the main ones of which were monogalactosyldiglycerides, carotenoids, diacylglycerols, ceramide oligosides, digalactosyldiglycerides, phosphatidylglycerols; phosphatidylcholines, and free and glycosylated sterols. In the fatty acid composition of the lipids unsaturated fatty acids – linolenic, oleic, linoleic, and palmitoleic – predominated (> 70%).

Lipids, which are present in fruit in comparatively low concentrations, largely determine the organoleptic properties and food value of the products and their stability on storage, and also the conditions of technological treatment [1]. In connection with the development of new ways of preserving products based on persimmon fruit possessing high dietetic and medicinal properties [2], we have investigated the lipid spectrum of fresh green fruit of Diospyros kaki L. of the widely grown varieties Hachiya (I), and Hyakume (II), gathered in November, 1985, on the plantations of the training farm of the Azerbaidzhan Agricultural Institute (Kirovobad).

The lipids were isolated from homogenates of the component elements of the fruit (rind, flesh) by a modified Bligh–Dyer method [3]. The purified (washing with 0.5% $CaCl_2$ solution) lipid extracts were separated successively by column and thin-layer chromatography. The preliminary identification of the groups of lipids was carried out by comparing the chromatographic mobilities of the samples under investigation and of markers, and also with the use of specific color reagents. The definitive identification of the chromatographically homogeneous groups of lipids was carried out on the basis of the results of a study of the compositions of the products of severe acid hydrolysis.

The amounts of the total lipids in the flesh of the persimmon fruit were 1195 and 1020 mg/kg, respectively, for varieties (I) and (II). The concentrations of the lipids in the rind were several times higher [4325 mg/kg for variety (I), and 7744 mg/kg for variety (II)]. On the whole, the amounts of lipids in the persimmon fruit were substantially higher than in apples, grapes, and citrus fruits [4].

The predominating groups of lipids in the persimmon are (Table 1) monogalactosyldiglycerols, carotenoids, diacylglycerols, ceramide oligosides, digalactosyldiglycerols, phosphatidylglycerols, phosphatylcholines, and free and glycosylated sterols.

The qualitative compositions of the lipids of the rind and flesh were identical, but the group distributions had certain differences. The largest fraction of the flesh lipids consisted of the neutral lipids (NLs), and of the rind it consisted of the glycolipids (GLs).

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TABLE 1. Group Composition of the Lipids (% of the total) of Fruit of Diospyros kaki, Varieties Hachiya (I) and Hyakume (II)

Group of lipids	Flesh		Rind	
	I	II	I	II
I. Neutral lipids				
Carbohydrates	2,5	1,5	2,5	1,1
Carotenoids	12,5	9,7	10,3	2,6
Sterol ethers	1,6	0,7	0,6	0,5
Fatty acid esters	1,5	0,6	1,2	0,4
Triacylglycerols	4,7	5,6	0,9	0,9
Tocopherols	0,1	0,1	0,1	Ca.
Fatty acids	1,7	2,2	0,9	1,1
Sterols	5,1	3,3	5,8	4,6
Diacylglycerols	10,6	11,3	8,2	5,5
Monoacylglycerols	1,3	1,1	0,6	0,7
Unidentified	0,8	1,1	0,3	0,3
Sum of the neutral lipids	42,4	37,2	31,7	17,7
II. Glycolipds				
Monogalactosyldiglycerols	11,8	14,4	14,2	36,3
Esterified sterol glycosides	1,1	1,3	3,4	3,2
Sterol glycosides	4,3	1,6	7,9	8,8
Cerebrosides	3,3	1,9	6,8	6,5
Ceramide oligosides	7,2	3,4	10,8	12,2
Digalactosyldiglycerols	6,5	3,2	10,2	10,5
Sulfoquinovosyl diglycerols	1,8	0,8	3,5	3,2
Sum of the glycolipids	36,1	26,6	56,8	80,7
III. Phospholipids				
Diphosphatidylglycerols	3,6	1,4	0,4	—
Phosphatidic acids	0,9	0,7	0,2	—
Phosphatidylethanolamines	1,9	1,8	0,8	0,1
Phosphatidylglycerols	6,4	13,4	5,3	0,6
Phosphatidylcholines	4,3	10,2	3,0	0,3
Phosphatidylinositols	2,9	4,0	1,5	0,2
Phosphatidylserines	1,5	4,7	0,2	0,4
Sum of the phospholipids	21,5	36,2	11,4	1,6

TABLE 2. Composition of the Fatty Acids of the Acyl-Containing Lipids of the Fruit of Diospyros kaki, wt%

Fatty acids	Variety I		Variety II	
	flesh	rind	flesh	rind
12:0	0,3	Tr.	0,1	Tr.
14:0	2,9	3,5	3,3	6,9
14:1	0,1	0,2	Tr.	Tr.
14:2	0,5	0,5	0,9	0,9
15:0	0,9	0,3	0,1	0,3
16:0	16,0	18,8	16,7	24,5
16:1	9,7	17,0	15,4	19,5
16:2	5,5	3,9	1,2	3,1
17:0	0,1	0,4	0,2	0,2
18:0	1,0	0,9	2,8	0,6
18:1	33,1	28,3	28,0	17,7
18:2	6,6	4,7	4,9	2,2
18:3	22,7	21,1	25,9	24,0
20:0	0,2	0,3	0,5	0,1
20:1	0,4	0,1	—	—
Sum of the saturated acids	21,4	24,2	23,7	32,6
Sum of the unsaturated acids	78,6	75,8	76,3	67,4

We must also mention the comparatively high relative amount of phosphatidylglycerols and phosphatidylcholines in the flesh lipids, particularly in the case of variety II.

A feature of the composition of the NLs of persimmon fruit was that the amount of diacylglycerols proved to be substantially higher than that of triacylglycerols. Thus, the amount of diacylglycerols in the flesh was double that of the triacylglycerols, while in the case of the rind, depending on the variety, they differed by a factor of 9 (6), which is

normal for cuticular lipids. The diacylglycerols (DAGs) were represented predominantly (60-80%) by the 1,2-isomers.

For identification, the DAGs were hydrolyzed with caustic soda, and the fatty acids were extracted from the hydrolysis products; on chromatography by the TLC method these proved to be identical with the corresponding markers. Glycerol was determined in an hydrolysate of the DAG fraction, the molar ration of this to the fatty acid being 1:2. In the ether-soluble products of alkaline hydrolysis only normal FAs were detected (TLC on Silufol), which indicated a correct identification of the DAGs on chromatography.

The separation of the DAGs (the 1,2- and 1,3-isomers), the FAs, and the glycerol was performed in a thin layer of silica gel impregnated with H_3BO_3 in chloroform–acetone (96:4). For identification we used information on the chromatographic mobilities of the compounds given in the literature [5].

An additional investigation using GLC and IR spectroscopy made it possible, in our opinion, to draw an unambiguous conclusion on the absence of hydroxy acids from the DAG fraction. Thus, in particular, the gas-liquid separation of the diacylglycerols under investigation and control samples in the form on their trimethylsilyl ethers on a nonpolar silicone column under isothermal conditions (300°C) that we performed for checking the purity of the DAGs gave retention parameters in good agreement.

In the IR spectra of the DAGs splitting into two bands was observed in the region corresponding to the vibrations of CH_2 groups (730-710 cm^{-1}), which is characteristic only for diacylglycerols [6].

The IR spectra of the 1,3- and 1,2-DAGs were identical, with the exception of the presence of a weak band at 1060 cm^{-1} in the spectrum of the 1,2-DAGs [7].

The main (>90%) monosaccharide fragments of the GLs were galactose, glucose, and arabinose residues.

In the fatty acids of the lipids of the flesh and rind of the persimmon fruit 15 representatives with C_{12}-C_{20} compositions were detected (Table 2). Palmitic, palmitoleic, oleic, linoleic, and linolenic acids predominated. The lipids studied were characterized by a high unsaturation index (2.4-3.4), which, to all appearance, is one of the factors responsible for the ready spoilage of persimmon fruit during storage and transport [2].

A substantial part of the liposoluble substances identified consists of compounds possessing a high biological value – carotenoids, polyunsaturated fatty acids, phospholipids, and tocopherols – which, in combination with other components (pectin, tanning substances, etc.), are obviously responsible for the medicinal and dietetic properties of persimmon fruit.

EXPERIMENTAL

The column chromatography of the total lipids was carried out as described in [8]. The NLs were separated in a thin layer in the solvent system heptane–methyl ethyl ketone–acetic acid (42.5:7.5:0.5). An aliquot part of the NL extract was used for the identification and isolation of the DAG isomers by chromatography in a thin layer of silica gel impregnated with boric acid [5]. The purity of the DAGs was checked by the GLC of their trimethylsilyl ethers on a nonpolar silicone column under isothermal conditions (300°C) [9]. The IR spectra of the substances in the form of films were taken on a UR-10 instrument.

The phospholipids were separated by two-dimensional TLC in the systems 1) chloroform–methanol–7 N ammonia (65:30:4), and 2) chloroform–methanol–acetic acid–water (170:25:25:6). The glycolipids were also separated by the use of the systems: 1) acetone–toluene–acetic acid–water (60:60:2:1), and 2) chloroform–acetone–methanol–acetic acid–water (6:8:2:2:1). The quantitative determination of the group of NLs was carried out by a spectrophotometric method [10] based on the reaction with a bichromate reagent. The amount of glycolipids was determined with orcinol on the basis of the carbohydrate component [5]. The phospholipids were determined from their phosphorus content [11]. The gas-liquid chromatography of the fatty acid methyl esters and the analysis of the mixture of water-soluble products obtained as the result of severe acid hydrolysis (2 N HCl, 125°C, 48 h) were carried out in accordance with [12]. The compositions of the monosaccharides and glycolipids were determined by PC [13].

CONCLUSION

The composition and amounts of various groups of liposoluble compounds in the rind and flesh of the fruit of the Hachiya and Hyakume varieties of persimmon have been investigated. The identity of the qualitative compositions of the flesh and rind lipids has been established. A total of 24 groups of compounds has been identified, the main representatives of which are monogalactosyldiglycerides, carotenoids, diacylglycerols, ceramide oligosides, digalactosyldiglycerides, phosphatidylglycerols, phosphatidylcholines, and free and glycosylated sterols. A feature of the lipid composition of the rind was the extremely high relative amount of glycolipids. The flesh of the fruit was characterized by a higher relative amount of neutral lipids and phospholipids, which were represented predominately by phosphatidylcholines and phosphatidylglycerols. In the fatty acid composition of the lipids unsaturated fatty acids – linolenic, oleic, linoleic, and palmitoleic – predominated (70%).

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