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LIPIDS OF *Capsicum annuum* FRUIT PULP

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*Lipids from fruit pulp of the sweet red pepper (*Capsicum annuum, *Solanaceae) were characterized. The principal components of the lipids from pepper fruit pulp were neutral lipids with predominance of triacylglycerines (55.8%). The polar lipids contained sulfoquinovosyldiacylglycerines and phosphotidylglycerines. The fatty-acid compositions of the neutral lipids, glycolipids, and phospholipids were determined.*

Key words: *Capsicum annuum*, Solanaceae, sweet pepper fruit, neutral and polar lipids, fatty acids.

Pepper (*Capsicum annuum*, Solanaceae) is a widely cultivated crop. Two varieties, sweet and hot pepper, are differentiated. The pericarp of sweet pepper is valued as a food product for supplying vitamins from groups C, E, B, and P; carotinoids, etc. Sweet red pepper contains more of these physiologically active compounds than other vegetables [1, 2]. Its fruit contains β-carotene (15% of the total pigments); β-cryptoxanthin (12%); and unique ketocarotinoid pigments that explain the red color of the fruit, e.g., capsanthine (33%) and capsarubin (10%); and their mono- and diesters with fatty acids [3, 4]. Furthermore, the total content of acyl-containing lipids in pepper fruit pulp is 0.15% of the dry mass [5]. The principal fatty acids are linoleic, linolenic, and palmitic [6]. The lipids of hot red pepper have been reported [7]. It was found that linoleic acid dominates the lipids of hot pepper fruit and seeds.

Data on the pure lipids of sweet red pepper have not been reported.

We investigated fruit of the sweet red pepper variety "Dar Tashkenta" (medium ripe) cultivated in Uzbekistan. Fruit of 89.9% moisture content freshly collected during ripening was treated with hot $CHCl_3$ to isolate the surface lipids. Then, they were dried. The pulp, pistil, and seeds were separated. The mass of each fraction was determined. Their yields were 84.2, 6.7, and 9.1% of the fruit mass, respectively. Lipids were isolated from each part of the fruit by the Folch method and purified of ballast by the literature method [8]. The calculated content of lipids in pepper fruit pulp considered the surface lipids extracted with $CHCl_3$.

The lipid contents in the various parts of sweet red pepper fruit indicate that seeds contain the most; the fruit pistil, the least (Table 1). The principal fruit mass consists of pulp, making up 1.75 mg/g of fresh mass (1.75% of the dry mass) total lipids. The total lipids accumulated in the fruit is 2.92 mg/g (2.92% of the dry mass). Therefore, lipids of fruit pulp make up >50% of the total lipids synthesized in the various parts.

The presence of tocopherols in extracts of pulp, seeds, and pistil were determined by the literature method [9]; their content, as before [10]. TLC (Silufol, system 3) detected α-, $\beta + \gamma$-, and δ-tocopherols in all extracts. The content was highest (127.0 mg%) in the extract from fruit pulp. The seed extract contained 57.7 mg% tocopherols. They were comparatively low in the pistil extract.

Carotinoid pigments in the extracts were identified and their amounts were estimated using TLC on cellulose and UV spectroscopy. The lipid extract from fruit pulp contained 300.3 mg% of carotinoid pigments calculated as β-carotene. The main components of the pigments were β-carotene (orange, R_f 0.98, system 4), β-cryptoxanthin (yellow, R_f 0.83), capsanthin (red, R_f 0.67), violoxanthin (yellow, R_f 0.35), and capsorubin (red, R_f 0.26). Carotinoid pigments were also found in the lipid extract from fruit pistil, a total of 56.8 mg%.

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TABLE 1. Lipid Content in Various Parts of *C. annuum* Fruit

Fruit part	Lipid content		
	mg/g fresh tissue mass	mg/g fruit mass	% of total fruit lipids
Pulp	1.75	1.47	50.3
Seeds	15.10	1.39	47.6
Pistil	0.89	0.06	2.1

TABLE 2. Content of Lipid Groups in *C. annuum* Fruit Pulp

Lipids	Lipid content		
	mg/g pulp mass	mg/g dry mass	% of lipid mass
Surface	0.07	0.65	3.80
Neutral	1.38	13.46	78.76
Glycolipids	0.25	2.45	14.34
Phospholipids	0.05	0.53	3.10
Total lipids	1.75	17.09	100

The qualitative compositions of lipids from fruit pulp and pistil were identical according to TLC (silica gel, systems 1, 5, and 6). Therefore, the composition of lipids from pepper fruit pulp was investigated further in detail.

Counter-current distribution of lipids by hexane:ethanol (87%) and column chromatography was used to separate the total lipids from fruit pulp into neutral (NL), glycolipids (GL), and phospholipids (PL). Table 2 gives the quantitative amounts of the individual lipid groups of sweet pepper fruit pulp.

It was found that NL are the highest lipid fraction of pepper fruit pulp. The content of GL was much higher than that of PL in the polar lipids.

The GL contained monogalactosyldiacylglycerines, digalactosyldiacylglycerines, sulfoquinovosyldiacylglycerines, sterylglycosides, and esters of sterylglycosides with fatty acids.

Phospholipids were represented by a family of components including phosphatidylcholines, phosphatidylethanolamines, phosphatidylinosites, phosphatidylglycerines (basic), phosphatidic acid, and two N-acylphospholipids. It should be noted that the sulfoquinovosyldiacylglycerines and phosphatidylglycerines observed in the fruit pulp, in addition to galactolipids, are usually membrane structural elements of photosynthetic chloroplasts in higher plant tissues [11].

The composition of the NL of pepper fruit pulp was determined by column chromatography (Table 3). It was found that the NL consisted of compounds typical of reserve lipids. More than half the NL mass was attributed to triacylglycerines. The fraction of free fatty acids in them was also high. Furthermore, fatty alcohols, triterpenols, and sterols, which were present in the free state and as esters, contributed significantly to the NL. Their total was more than one fifth of the pepper fruit pulp NL.

The composition of the fatty acids was determined by isolating them from the total NL, GL, and PL fractions from fruit pulp and pistil; converting them to methyl esters; and analyzing them by GC.

Table 4 shows that the fatty acids of all lipids have the identical set of 12-13 components. There were more saturated acids in the total lipids of fruit pulp than of the pistil. Apparently this is due to the inclusion of surface lipids from the fruit rind. It is also interesting that the content of medium-molecular-weight acids 12:0 and 14:0 in the fruit-pulp lipids is elevated with respect to the pistil lipids.

Saturated acids 16:0 and 18:0 dominated the NL; 16:0, the PL. The composition of the GL fatty acids had the highest content of unsaturated acids. The principal one was linolenic acid. The PL contained equal amounts of 18:1 and 18:2 acids. The fraction of 18:3 acid in them was low.

TABLE 3. Composition of Neutral Lipids of *C. annuum* Fruit Pulp

Component	Lipid content	
	mg/g fruit mass	% of lipid mass
Carotinoids	0.005	0.36
Hydrocarbons	0.112	8.12
Esters of aliphatic and cyclic alcohols with fatty acids	0.150	10.86
Triacylglycerines	0.770	55.80
Tocopherols	0.002	0.14
Free fatty acids	0.197	14.28
Aliphatic alcohols	0.085	6.16
Triterpenols, monomethylsterols, and dimethylsterols	0.053	3.84
Diacylglycerines and monoacylglycerines	0.006	0.44
Total neutral lipids	1.38	100

TABLE 4. Fatty-Acid Composition of Lipids from *C. annuum* Fruit Pulp and Pistil

Acids	Pistil	Fruit pulp			
	Total lipids	Total lipids	NL	GL	PL
12:0	0.3	2.7	0.2	0.4	6.6
14:0	1.0	3.3	2.6	1.9	1.8
15:0	Tr.	Tr.	0.5	0.4	Tr.
16:0	27.4	33.3	26.7	25.2	42.7
16:1	0.4	1.2	1.0	0.7	Tr.
17:0	0.5	0.7	0.3	0.8	Tr.
17:1	Tr.	Tr.	0.2	0.8	-
18:0	1.4	4.1	11.9	0.7	1.7
Unidentified	-	Tr.	0.4	-	-
18:1	7.9	11.3	19.7	7.2	18.6
18:2	51.8	33.2	34.0	19.3	19.3
18:3	8.5	8.4	2.3	42.1	9.3
20:0	0.8	1.8	0.2	0.5	Tr.
$\Sigma_{sat.}$	31.4	45.9	42.4	29.9	52.8
$\Sigma_{unsat.}$	68.6	54.1	57.2	70.1	47.2

NL, neutral lipids; GL, glycolipids; PL, phospholipids

EXPERIMENTAL

UV spectra of pigments were recorded on a Perkin—Elmer Lambda-16 UV/Vis spectrometer.

GC of fatty-acid methyl esters was carried out on a Chrom-4 (Czech Rep.) chromatograph with a flame-ionization detector using a stainless-steel column (2500 mm × 4 mm) packed with Chromaton N-AW-DMCS with 17% ethyleneglycolsuccinate, working temperature 198°C, and He carrier gas.

Column chromatography was performed over L100/160 silica gel (Czech Rep.) washed with $CHCl_3$:CH_3OH (2:1, v/v). NL fractions were eluted with hexane with gradually increasing concentrations of diethylether (4, 6, 10, 15, 20, 50, and 100%); GL, with acetone; PL, with methanol.

TLC was performed on L5/40 silica-gel plates containing $CaSO_4$ (15%) with microcrystalline cellulose for TLC and on prepared Silufol UV-254 (Czech Rep.) plates. The solvent systems (by volume) were: hexane:diethylether:acetic acid (1, 90:10:1) and heptane:methylethylketone:acetic acid (2, 43:7:0.1), for NL; $CHCl_3$ (3), for tocopherols; heptane:propanol (4, 99.9:0.1), for pigments; $CHCl_3$:$(CH_3)_2CO$:CH_3OH:CH_3CO_2H:H_2O (5, 65:20:10:10:3), for GL; and $CHCl_3$:CH_3OH:NH_4OH (25%) (6, 16:6:1, direction I) and $CHCl_3$:CH_3OH:CH_3CO_2H:H_2O (6, 30:10:1:1, direction II), for PL.

Lipids were investigated immediately after collecting pepper fruit. Enzymes were inactivated by hot $CHCl_3$ before isolating lipids. Then, the fruit was dried of solvent and extracted by $CHCl_3$:CH_3OH (2:1, v/v). Nonlipid impurities were removed from the extracts by washing with $CaCl_2$ solution (0.04%) and additionally purified of traces of carbohydrates by gel filtration of the extracts over a column of Dextrantel G-25 molselect (Hungary). Purified lipids were eluted by $CHCl_3$:CH_3OH:H_2O (90:10:1).

Lipid components were identified by specific developers as follows: GL, α-naphthol and H_2SO_4 (50%) with heating; PL, Vaskovsky method and Dragendorff's solution (phosphatidylcholines), ninhydrin (phosphatidylethanolamines); phytosterols and sulfoquinovosyldiacylglycerines, H_2SO_4 (50%) with heating. Tocopherols were identified using phosphomolybdic acid as developer.

Alkaline dehydration of lipids with KOH (10%) in CH_3OH was performed by the literature method [8]. The tocopherol content in unsaponified substances was determined as before [10].

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