LIPIDS OF THE FRUIT OF Citrus unshiu

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The compositions and the amounts of various groups of liposoluble compounds in the component elements of the fruits of Citrus unshiu Marc. [satsuna variety of mandarin orange] – the flavedo, the albedo, the membrane, and the flesh – have been determined by chromatographic and chemical methods. About 30 groups of lipid compounds belonging to the classes of neutral lipids and glyco- and phospholipids have been identified and characterized. The fatty-acid compositions of the main classes of lipids have been studied. About 30 different carotenoids have been determined in the various elements of the satsuma orange.

The not inconsiderable interest in the study of the lipids of citrus fruit is due to their influence on the colloidal stability, oxidizability, time of storage, and organoleptic properties of the juices and the possibility of determining their falsification [1]. However, in the majority of investigations it is the lipid composition of ordinary oranges that has been considered. Information on the chemical composition of the lipids of mandarins bears a fragmentary, incomplete nature and relates to foreign varieties [2]. In view of the prime value of just this species of citrus fruits for the climatic conditions of the Republic of Transcaucasia, the expansion of the volumes of production and variety of products from mandarin raw material, and also the necessity for utilizing the wastes, which in the case of mandarins amount to about 26% of the mass of the fruit [3], we have investigated the liposoluble compounds of individual elements (flavedo, albedo, membrane, flesh) of the fruit of Citrus unshiu Marc.

We used technically ripe (sugar content of the juice not less than 8.5 g/100 g) fruit grown in the experiment plantations of VNIÉKISP (Batumi).

The fresh fruit was separated under laboratory conditions into its four component parts and the total lipids were isolated and separated into neutral lipids (NLs), glycolipids (GLs), and phospholipids (PLs) by column chromatography on silica gel [4].

According to the experimental results (Table 1), the flavedo of the Citrus unshiu fruit was characterized by the largest amount of lipids, and then in order of decreasing amount followed the membrane, the albedo, and the flesh. Such a high level of lipids in the flavedo is due to the presence of a waxy coating on the surface of the fruit and to the specific composition of the chromoplasts of the peel which concentrates various liposoluble substances within itself.

The amounts of lipids in the flesh of the mandarin fruit were closest to the corresponding levels in table varieties of grape [5], figs [6], and persimmons [7]. However, on the whole, taking into account the inedible part of the fruit, mandarins are distinguished by a far higher level of total lipids than the other fruit mentioned. For the majority of the component parts of the mandarin fruit the bulk (more than 50%) of the lipids consisted of the class of neutral lipids. The only exception was the membrane of the fruit, in the lipid composition of which glycolipids predominated. The relative amounts of phospholipids in all the tissues of the mandarin fruit was low, amounting to 8-18%, which is considerably less than in ordinary oranges [1].

The lipids were identified on the basis of a comparison of their chromatographic mobilities with model specimens and also by qualitative reactions and spectral characteristics.

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TABLE 1. Amounts of Lipids in the Elements of Citrus unshiu Fruit

Element of the fruit	Total lipids, mg/100 g	Class of lipids, % on the total mass		
		neutral lipids	glyco-lipids	phospho-lipids
Flavedo	2253,1	73,8	11,3	14,9
Albedo	780,0	52,1	35,8	12,2
Membrane	1241,3	28,6	53,2	18,2
Flesh	443,6	77,2	14,8	8,0

TABLE 2. Group Compositions of the Neutral Lipids of Citrus unshiu Fruit, % on the Total Mass

Group of lipids	Element of the fruit			
	fla-vedo	al-bedo	mem-brane	flesh
Carbohydrates	35 3	27,9	26,3	20,1
Sterol esters	2,7	3,2	5,6	5,9
Wax esters	3,5	2,4	1.5	0,6
Esters of fatty acids and lower alcohols	8,8	9,9	8,0	2,5
Triacylglycerols	5,2	7,5	6,6	15,6
Tocopherols	0,4	0,2	0,1	0,2
Free fatty acids	21,9	30,3	20,8	31,9
Fatty alcohols	8,4	2,4	8,5	8,3
Free sterols	5,8	6,2	4 7	6,6
Diacylglycerols	4,0	3,8	9,9	5,6
Hydroxy acids	2,0	0,6	1,9	1.5
Monoacylglycerols	2,0	5,6	6,1	1,2

In the identification of polar lipids of polar structure we made use of the results of chemical analysis of the water-soluble and liposoluble fragments of the molecules isolated after severe acid hydrolysis [4, 8-11]. The NLs were separated in systems 1 and 2.

Hydrocarbons and free fatty acids predominated in the neutral lipids of all the samples (Table 2). In spite of certain quantitative differences, the ratio of the groups of NLs for the different component parts of the fruit were similar. A feature of the composition of the NLs of mandarin fruit is the low relative amount of glycerides and sterols. However, the NLs of the flesh of the fruit were distinguished by comparatively high levels of triacylglycerols.

In view of the comparatively high level of hydrocarbons in the lipids of Citrus unshiu, this group of compounds was isolated from the unsaponifiable fraction of the NLs by preparative TLC using petroleum ether as eluent and was studied in more detail. The hydrocarbons were separated according to their degree of saturation by chromatography on silica gel plates impregnated with $AgNO_3$, using system 9.

The composition of the hydrocarbons was analyzed by the GLC method. According to the experimental results, saturated long-chain hydrocarbons predominated in the quantitative respect (50-65%). Monounsaturated hydrocarbons amounted to 10-15%, and the remainder of the hydrocarbon fraction consisted of a complex mixture of polyunsaturated hydrocarbons, carotenoids, and unidentified components. In the saturated hydrocarbons the C_{20}-C_{38} components were detected, and of these the C_{23}, C_{25}, and C_{27} species predominated quantitatively. Among the monounsaturated hydrocarbons (C_{20}-C_{35}) the C_{29} and C_{31} components predominated (about 80%).

TABLE 3. Composition of the Carotenoids of the Fruit of the Citrus unshiu, % on the Total Mass

Carotenoids	Flesh	Membrane	Albedo	Flavedo
Phytoene	2,3	13,4	15,8	12,6
Phytofluene	2,9	11,8	14,3	10,0
α-Carotene	0,1	0.4	4,5	3.6
β-Carotene	0,1	0.8	6.0	1,2
γ-Carotene	0,2	0.1	1,0	—
ζ-Carotene	2,8	4,5	3,2	—
Lycopene	0,2	0,1	0,4	1,0
Mutatochrome	0,3	0,2	0,7	—
Cryptoxanthin epoxide	0.4	0,9	0,7	1,0
Cryptoxanthin	26,7	11,2	9,4	16,0
Hydroxy-α-carotene	0,6	0.9	0.2	0,5
Cryptochrome	—	0,1	0,6	0,2
Cryptoflavin	0,4	0,6	0,2	0,1
Rubiflavin	0,6	0,2	0,4	0,3
Rubixanthin	—	—	0,2	0,6
Lutein	5,6	3,0	3,2	3,8
Canthaxanthin	,5	0,6	0,9	0,6
Zeaxanthin	6,0	4,4	3,0	3,8
Antheraxanthin	13,4	10,1	4,7	4,1
Mutatoxanthin	4,6	4,2	3,9	3,1
Violaxanthin	16,0	14,3	9,8	12,1
Luteoxanthin	5.4	6,1	3,7	5,2
Auroxanthin	0 7	0,3	0,2	1,5
β-Citraurin	0,6	1.4	1,9	0,3
Flavoxanthin	1,0	2,3	0,5	2,9
Apocarotene of sintaxanthin	3,0	1,4	5,3	11,6
Unidentified xanthophylls	4,6	6,7	5,3	3,9

The sum of the pigments was studied in the composition of the total lipids. The extract obtained was freed from lipids by saponification [12] and was fractionated into carotenes and xanthophylls by the use of a sucrose-filled column [13]. All the operations were carried out in diffuse illumination with the addition to the extracts of Santoquin, which effectively stabilizes citrus carotenoids [14]. Individual xanthophylls were obtained by the TLC method on celluloses (Nagel, FRG) by means of system 3.

The carotenes were separated on silica gel in system 4. The bands of the separated pigments were removed from the plates and were eluted with acetone.

The pigments were identified by a comparison of their chromatographic mobilities with standard carotenoid preparations (Fluka), from the nature of their absorption spectra in the 200-700 nm region, and from the results of the epoxide test with HCl [15].

Information on the chromatographic mobilities and spectral characteristics of the carotenoids was obtained from the literature [15, 16]. The amount of each pigment was determined spectrophotometrically on the basis of its individual extinction coefficient [16, 17].

The total content of carotenoids (milligrams per 100 g of dry matter) was 2.6 for the flesh, 1.8 for the membrane, 1.3 for the albedo, and 6.2 for the flavedo. Thus, the bulk of the pigments in the mandarin fruit was concentrated in the inedible part – the flavedo. Among the carotenoids about 30 pigments were detected, of which 26 were identified (Table 3). The albedo was characterized by the greatest relative amount of hydrocarbon carotenoids (>40%) and the flesh by the smallest amount (<10%). The flavedo and membrane had relative amounts of carotenoids similar to one another (~30%). In all cases the bulk of the hydrocarbon carotenoids consisted of colorless representatives. In the group of oxygen-containing pigments cryptoxanthin, violaxanthin, antheraxanthin, and the apocarotene of sintaxanthin (flavedo) predominated.

Biologically active carotenoids made up about 30% of the mass of pigments of the flesh and of the order of 20% in the inedible elements of the fruit.

TABLE 4. Group Compositions of the Glycolipids of Citrus unshiu Fruit, % on the Total Mass

Group of lipids	Element of the fruit			
	flavedo	albedo	membrane	flesh
Acylmonogalactosyldiglycerides	11,2	7,5	10,4	3.8
Esterified sterol glycosides	5.4	13,5	13,0	1.9
Monogalactosyldiglycerides	14,4	27,1	23,4	21,5
Sterol glycosides	12,0	10,4	6,2	11,6
Cerebrosides	16,0	15,1	26,0	19,4
Ceramide oligosides	12.8	3,7	3,9	14.5
Digalactosyldiglycerides	15.2	9,8	9.6	12,8
Ceramide phosphate inositol oligosides	2,4	10,5	1,6	8,7
Sulfoquinovosyldiglycerides	10,6	2,4	5,9	5,8

TABLE 5. Group Compositions of the Phospholipids of Citrus unshiu Fruit, % on the Total Mass

Phospholipid	Element of the fruit			
	flavedo	albedo	membrane	flesh
Diphosphatidylglycerols	4,4	6,9	5,7	1,9
Phosphatidic acids	6,9	3,6	1,4	4,9
Phosphatidylethanolamines	37,5	37,0	28,5	32,4
Phosphatidylglycerols	10,6	15,1	19,7	12,4
Phosphatidylcholines	33,9	16,2	26,1	34,9
Phosphatidylserines	1,5	6,7	5,4	2,5
Phosphatidylinositols	4,7	14,5	11,8	7,5
Lysophosphatidylethanolamines	0,2	0,0	0,5	1,2
Lysophosphatidylcholines	0,3	0,0	0,9	2,3

The glycolipids were separated in systems 5 and 6 and were determined quantitatively with respect to their carbohydrate components [10]. In all the component parts of the Citrus unshiu fruit mono- and digalactosyldiglycerides, cerebrosides, and sterol glycosides predominated (Table 4). The amount of other groups of GLs differed substantially for the various structural elements of the mandarin fruit.

Among the carbohydrate components, according to the results of paper chromatography (PC), the main representatives were galactose, glucose, and arabinose.

The phospholipids (PLs) were separated by two-dimensional TLC in systems 7 and 8. In the mandarin fruit the PLs were represented by nine types of compounds (Table 5). The PLs were found to contain a high relative amount (about 80%) of phosphatidylcholines, phosphatidylethanolamines, and phosphatidylglycerols. The quantitative ratios of the groups of PLs were similar for the various component elements of the mandarin fruit.

Components from $C_{9:0}$ to $C_{24:0}$ were detected among the fatty acids of the lipids of the mandarin fruit (Table 6). The lipids of these elements of the mandarin fruit were characterized by high levels of the 18:2, 18:1, 18:3, and 16:0 acids.

TABLE 6. Fatty Acid Compositions of the Main Classes of Lipids from the Component Parts of Citrus unshiu Fruit, Mass %

C_n	Neutral lipids				Glycolipids				Phospholipids			
	Fo*	A	M	Fh	Fo	A	M	Fh	Fo	A	M	Fh
9:0	Tr.	0,1	0,2	0,1	0,1	Tr.	0,2	0,2	0,2	Tr.	0,2	0,2
10:0	0,1	0,3	Tr.	0,2	Tr.	0,3	0,2	0,1	0,1	0,4	0,1	0,4
11:0	0,1	0,2	0,1	0,3	Tr.	0,1	Tr.	0,2	Tr.	Tr.	0,1	0,1
12:0	0,3	0,1	0,1	0,4	0,5	0,2	0,2	0,3	0,2	0,3	0,4	0,5
13:0	0,2	1,3	0,6	0,3	0,1	0,2	0,2	0,1	Tr.	0,9	0,1	0,2
14:0	0,4	2,5	1,3	0,2	1,0	0,2	0,1	0,4	0,5	3,9	1,4	2,0
15:0	0,3	0,8	2,8	0,2	0,1	0,4	0,3	0,1	1,5	0,5	2,0	0,3
15:1	—	1,3	1,9	0,2	—	Tr.	0,5	0,1	—	1,9	0,3	0,2
16:0	13,1	10,3	7,9	10,4	11,3	14,4	12,5	12,7	6,5	17,2	19,8	21,0
16:1	Tr.	3,2	1,5	0,5	Tr.	Tr.	1,3	0,9	Tr.	3,0	0,6	0,3
17:0	—	3,1	6,5	1,3	—	0,8	1,3	1,0	—	—	1,9	1,0
17:1	0,3	—	1,6	1,2	0,1	0,5	1,2	1,6	0,4	—	0,5	0,6
18:0	3,7	7,6	10,9	5,9	2,5	4,8	8,4	4,7	4,6	8,9	11,9	3,8
18:1	21,0	24,2	20,0	27,4	19,4	27,6	28,3	25,8	26,0	17,2	18,4	22,6
18:2	38,8	27,6	22,7	34,4	40,5	23,0	22,9	31,4	37,1	20,4	21,7	30,4
18:3	18,6	13,9	14,6	10,6	20,1	18,2	17,7	11,3	17,7	15,6	15,0	13,3
20:0	0,3	0,4	0,6	0,2	0,2	1,6	0,2	0,2	1,0	0,1	0,1	—
20:2	0,1	—	1,2	0,4	0,5	0,8	0,4	0,6	0,5	1,4	0,2	0,3
20:3	1,6	0,2	3,2	1,6	2,4	0,2	2,4	1,1	0,2	Tr.	3,4	0,1
23:0	0,4	0,1	0,8	0,2	—	2,4	0,6	2,0	0,3	Tr.	0,5	0,4
24:0	0,7	0,9	1,4	3,5	0,9	3,8	0,6	4,7	4,0	5,6	1,4	2,3
Σ_{sat}	19,6	27,7	33,3	23,2	16,7	29,2	24,7	26,7	18,9	37,8	39,9	32,2
Σ_{unsat}	80,4	72,3	66,7	76,8	83,3	70,8	75,3	73,3	81,1	62,2	60,1	67,8

*Fo – flavedo; A – albedo; M – membrane; Fh – flesh.

On the whole, the unsaturated components predominated (60-83%) in the fatty-acid composition. The greatest degree of unsaturation (more than 80%) was characteristic for the lipids of the flavedo. It must be mentioned that in spite of some qualitative differences in the composition of the acids the quantitative ratios were basically similar regardless of the element of the fruit and the class of lipids.

Thus, the inedible part of the fruit (flavedo, albedo) contained considerable amounts of lipids in the fatty acid composition of which there was an average of about 50% of polyunsaturated higher fatty acids possessing vitamin F properties, which are valuable food components.

EXPERIMENTAL

IR spectra were taken on UR-10 instrument using films of the substances.

Hydrocarbons were separated on a 4 × 3050 [mm] column with 2% of SE on the support Gas Chrom Q. Chromatograph with a flame-ionization detector with programming of the temperature (165-270°C).

Column chromatography was conducted on silica gel L 100/160, and thin-layer chromatography on Silufol plates and on silica gel L 5/40 with gypsum in the following solvent systems: 1) heptane–methyl ethyl ketone–acetic acid (47.5:7.5:0.5), two runs; 2) hexane–ether–acetic acid (80:20:1); 3) petroleum ether–acetone–n-propanol (90:10:0.25); 4) hexane–acetone (96:4); 5) acetone–toluene–acetic acid–water (60:60:2:1); 6) chloroform–methanol–water (65:25:4); 7) chloroform–methanol–7 N ammonia (65:30:4) – first direction; 8) chloroform–methanol–acetic acid–water (170:25:25:6) – second direction; 9) petroleum ether–diethyl ether (98:2).

To isolate the lipids, the component parts of the fruit (flavedo, albedo, membrane, flesh) were homogenized with chloroform–methanol in a volume ratio depending on the moisture content of the sample and determined from a ternary diagram [13]. The chloroform extracts obtained were purified by washing with a 0.5% aqueous solution of $CaCl_2$ and were chromatographed on columns of Sephadex G-25 [13].

Methylation and the conditions of performing the GLC of the FA methyl esters have been described previously [18].

The impregnation of the silica gel in the TLC preparation of the hydrocarbons was carried out with the addition of 5% of $AgNO_3$.

The water-soluble products obtained after severe acid hydrolysis of the GLs and PLs (2 N HCl, 125°C, 48 h) were investigated as described in [4].

Phospholipids were determined quantitatively by Bartlett's method [19]. The separation and quantitative determination of the sugars in the GLs was carried out as described in [20].

SUMMARY

The compositions and amounts of various groups of neutral lipids, glycolipids, phospholipids, and carotenoids in the component parts of Citrus unshiu Marc. fruit have been investigated for the first time.

With respect to their lipid content, the component parts of the fruit form the following sequence: flavedo > membrane > albedo > flesh.

The predominant class of lipids in the membrane consists of the glycolipids, while in all the other component parts of the fruit it is the neutral lipids.

Identity of the qualitative compositions and closeness of the quantitative ratios of the groups of lipids have been established for all the component parts of the fruit investigated.

About 30 groups of lipid compounds have been identified, among which hydrocarbons, free fatty acids, mono- and digalactosyldiglycerides, cerebrosides, sterol glycosides, phosphatidylcholines, phosphatidylethanolamines, and phosphatidylglycerols predominate.

Among the fatty acids 22 components from $C_{9:0}$ to $C_{24:0}$ have been detected. The bulk (60-83%) of the acids consisted of unsaturated species – lineolic, oleic, linolenic.

About 30 different carotenoids have been detected, of which quantitatively predominating were cryptoxanthin, violaxanthin, antheraxanthin, phytoene, phytofluene, and the apocarotene of sintaxanthin (flavedo). With respect to the total amount of pigments, the flavedo substantially exceeded (by a factor of 3-5) the other component parts of the fruit.

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COMPOSITION OF LIPIDS, FATTY ACIDS, AND ALDEHYDES OF THE HYDROID "CROSS" MEDUSA Conionemus vertens

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The composition of the lipids, fatty acids, and aldehydes of the hydroid "cross" medusa C. vertens has been investigated. It has been shown that the free sterols are the main components of the phospholipids of the medusa and 78% of them consists of cholesterol. Phosphatidylethanolamine is the main phospholipid of the medusa and 74.4% of it is represented by the plasmalogen form. The amount of phosphatidylcholine is 1.7 times less than that of phosphatidylethanolamine and it contains no plasmalogen form. The 22:4ω6 and 22:5ω3 acids are the main polyenic acids of the medusa. More than 55% of its fatty aldehydes consists of saturated molecular species, the main ones being the 18:0 and 20:1 aldehydes.

The Coelenterata are widely distributed in nature and form an important component of marine ecosystems. The lipids of the Coelenterata have been little studied, however; in the main, information relates to representatives of the class of Anthozoa – actinians and reef-forming corals. There is considerably less information on the composition of the lipids of representatives of other classes of Coelenterata – Hydrozoa, Scyphozoa, and Cubozoa [1]. A paper by É. Ya. Kostetskii [2] has recently appeared which is devoted in part to a comparative analysis of the phospholipids of hydroid and scyphoid Coelenterata. Unfortunately, the author gives quantitative information of the composition of the phospholipids for only five out of the 31 species studied.

In the present paper we describe the results of an investigation of the lipids of the hydroid "cross" medusa Conionemus vertens, which is a characteristic poisonous representative of the hydroid Coelenterata [3].

The micro-TLC of the lipids of C. vertens in a system for neutral lipids showed that the medusa was rich in sterols and contained only a small amount of triacylglycerols. A similar composition of these lipids is known for several representatives of the Coelenterata. Thus, the neutral lipids of the siphonophore Physalia physalis contained 26% of triacylglycerols and 42% of cholesterol [4], while in the medusa Cyanea capillata the triacylglycerols amounted to 14% and the sterols to 47% of all the neutral lipids [1]. Judging from the size and intensities of the spots on a chromatogram, in C. vertens the relative amount of triacylglycerols was even lower. The neutral lipids of C. vertens also contained sterol esters, alkyl glyceryl ether, and free fatty acids. The GLC of the free sterols of the medusa showed that they contained 78% of cholesterol.

Below we give the composition of the phospholipids of C. vertens (% on the lipid phosphorus):

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