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LIPIDS FROM THE PROCESSING WASTES OF *Stevia rebandiana*

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The composition of the lipids in the wastes from the production of a sweetening agent from Stevia rebandiana *Bertoni has been studied. The main components of the resinous wastes are glyco- and phospholipids.*

With the aim of the comprehensive and waste-free use of the plant raw material, we have investigated the wastes from the production of a sweetening agent from the plant *Stevia rebandiana* Bertoni growing in Uzbekistan.

Stevia is a source of calorie-poor sweetening agents. It is a native of Paraguay but at the present time it is cultivated in the Far and Near East, America, the Ukraine, and the Crimea [1].

On the extraction of the sweetening agent with aqueous alcohol the lipids are distributed between the meal and the resinous intermediate formed in the extraction process.

We have mainly studied the lipids of the resinous waste, which, according to our results, contains 1.58% of lipids on the weight of the initial raw material, while the meal contains only 0.3%. It was found by TLC (in systems 1-6) that the qualitative compositions of the lipids of the two samples were identical, but in the meal the phospho- and glycolipids were present in only small amounts.

The lipids of the resinous waste were separated into individual fractions by CC followed by preparative TLC (in systems 1-4) (Table 1).

The predominating component in them consisted of polar lipids. FFAs and chlorophylls were also present in substantial amounts.

The amounts of unsaponifiable substances isolated after the alkaline hydrolysis of the lipids of the meal and of the resinous product were 8.3 and 30.0%, respectively. The compositions of the fatty acids of the lipids are given in Table 2.

The resinous product contained more than 56% of unsaturated fatty acids, among which the 18:3 species predominated, while in the meal lipids the 18:2 species was the main one. In both materials, the 16:0 species was the predominant saturated acid. In the meal lipids the levels of saturated and unsaturated acids were practically the same.

We also determined the compositions of the fatty acids of the acyl-containing classes in the resinous waste (Table 3). In all the classes the main acid was palmitic, except for the class of esters of fatty acids with lower alcohols, in which the 18:1 acid predominated. The largest amount of unsaturated fatty acids was present in the GLs. The second place with respect to this index was occupied by esters of fatty acids with lower alcohols, followed by the PLs and NLs.

The GLs and PLs of the resinous residue were separated into classes with the aid of PTLC (in systems 5 and 6). Identification was based on chromatographic mobilities and specific reactions. Quantitative levels were evaluated gravimetrically (Table 4). In the GLs the DGDGs and SGs predominated, and in the phospholids the PIs, PCs, and PEs.

The mass spectrum of the sterols contained peaks with M^+ 414, 412, and 400, which are characteristic for sitosterol, stigmasterol, and campesterol, respectively [2].

The above facts show that the resinous residue that was studied was enriched with components possessing a high biological activity. Fatty acids and their esters with alcohols exhibit antimicrobial, antifungal, and anticaries activities [4]. In addition, the 18:2 and 18:3 acids are known as group F vitamins [5]. Triterpenols and sterols exhibit hypolipidemic properties [6, 7]. Carotenoids have shown antimutagenic, anticarcinogenic, and immunomodulating effects [8]. Chlorophylls have an antibacterial action [9]. GLs and PLs facilitate the passage of nutrient substances through the skin, activate enzymes, and restore a disturbance of membrane functions [10]. In addition, they possess pronounced surface-active properties [11].

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TABLE 1. Compositions of the Classes of Lipids of the Resinous Waste

Class of lipid	Lipid content, % on the weight of residue
Hydrocarbons	4.0
Carotenoids (calculated as β-carotene)	0.7
Esters of triterpenoids and sterols	4.0
Esters of fatty acids and lower alcohols	2.2
Triacylglycerols	Tr.
Tocopherols	0.1
Free fatty acids (FFAs)	13.2
High-molecular-mass alcohols	1.2
Triterpenols	4.9
Sterols	4.6
Chlorophylls, including	5.7
chlorophyll a	3.4
chlorophyll b	0.4
pheophytin a	1.4
pheophytin b	0.5
Glycolipids (GLs) + pigments	38.0
Phospholipids (PLs) + pigments	21.4
Sweetening agent	Tr.

TABLE 2. Compositions of the Fatty Acids of the Meal and of the Resinous Products

Acid	Resinous product	Meal
12:0	0.7	1.2
14:0	1.1	1.5
15:0	0.2	1.5
16:0	40.5	44.4
16:1	4.1	1.9
18:0	1.2	1.8
18:1	9.2	15.1
18:2	11.6	19.3
18:3	31.4	13.3
$\Sigma_{saturated}$	43.7	50.4
$\Sigma_{unsaturated}$	56.3	49.6

TABLE 3. Fatty Acid Compositions of the Acyl-Containing Classes of Lipids in the Resinous Residue (%GLC)

Acid	Esters of triterpenols and sterols	Esters of FFAs and lower alcohols	FFAS	NLs	PhLs	GLs
12:0	8.2	1.3	9.7	2.2	0.9	0.3
14:0	7.3	0.9	9.6	1.4	0.7	0.8
15:0	1.8	Tr.	Tr.	Tr.	Tr.	0.4
16:0	53.1	44.3	49.0	46.1	44.4	36.1
16:1	2.8	0.7	8.2	2.2	4.4	4.3
18:0	2.6	Tr.	2.8	2.3	2.6	4.2
18:1	10.2	50.6	8.3	36.0	5.4	12.3
18:2	9.7	2.2	6.1	3.4	21.2	8.0
18:3	4.3	Tr.	6.3	6.4	20.4	33.6
$\Sigma_{saturated}$	73.0	46.5	71.1	52.0	48.6	41.8
$\Sigma_{unsaturated}$	27.0	53.5	28.9	48.0	51.4	58.2

Thus, the results obtained indicate the desirability of using the lipids of the resinous waste from the processing of stevia as a biologically active additive in media for skin care.

EXPERIMENTAL

The conditions for recording UV and mass spectra and for CC, and the methods of detecting individual classes of lipids in TLC and for isolating and identifying FAs have been described in [12].

TABLE 4. Compositions of the GLs and PLs of the Resinous Waste

Class of lipid	Amount, % on the weight of GLs and PLs
Glycolipids	
Esters of sterol glycosides	14.5
Monogalactosyldiacylglycerides (MGDGs)	16.2
Steryl glycosides (SGs)	26.2
Digalactosyldiacylglycerides (DGDGs)	29.6
Cerebrosides	6.7
Sulfolipids	6.8
Phospholipids:	
Phosphatidylcholines (PCs)	26.1
Phosphatidylethanolamines (PEs)	24.2
Phosphatidylinositols (PIs)	34.0
Phosphatidylserines (PSs)	8.5
Phosphatidic acids (PAs)	7.2

The samples of stevia wastes were provided by the experimental factory of the Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan.

The lipids were isolated from the meal with a mixture of chloroform and methanol (2:1) and from the resinous waste with chloroform. TLC was conducted on silica gel with the addition of 10% of $CaSO_4$ in the systems: 1) hexane; 2) hexane-diethyl ether (9:1); 3) (7:3); 4) (7:8); 5) $CHCl_3-CH_3OH-NH_4OH$ (65:25:5); and 6) $CHCl_3-CH_3COCH_3-CH_3OH-CH_3COOH-H_2O$ (60:20:10:10:3).

Chlorophylls: UV spectrum (hexane, nm): 663.8 (chlorophyll a); 645.5 (chlorophyll b) [13].

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