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LIPIDS OF THE LEAVES OF Brassica oleracea

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The composition of the various groups of liposoluble compounds in the leaves of wild cabbage of early-, middle-, and late-ripening varieties have been identified and their amounts have been determined by chromatographic and chemical methods. About 30 groups of lipid substances have been identified, among which free and esterified forms of sterols, di- and triacylglycerols, hydrocarbons, lipoquinones, mono- and digalactoside glycerides, cerebrosides, phosphatidylethanolamines, phosphatidylglycerols, and phosphatidylcholines predominate. The presence of 13 components has been established among the fatty acids, linolenic acid being present in the greatest amount (~50%). The influence of the time of ripening of the cabbage variety (early-, middle-, or late-ripening) on the composition of the lipid complex has been elucidated.

The lipids are some of the main nutritional components of food products. The organoleptic evaluation of a foodstuff and its calorie content and also its quality and keeping

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properties during treatment and storage largely depend on their qualitative and quantitative chemical composition.

The information on the lipids of the cabbage found in the foreign literature [1] is of incomplete, nonsystematic nature, while in the domestic literature, only the composition of the liposoluble pigments has been described [2].

At the present time, technological means of processing cabbage raw material with the aim of achieving the possibility of prolonged storage without appreciable loss of the nutritional and biological value of the product are being developed and perfected [3]. Of no little importance here is the correct choice of the variety of raw material and a consideration of its biological characteristics.

We have investigated the qualitative and quantitative compositions of the liposoluble substances of the leaves of white cabbage of different varieties: early-ripening – Dimirskaya (I); middle-ripening – Slava (II); and late-ripening – Amager (III), grown under the conditions of the south of the Ukraine in the 1985-1986 season.

Samples of freshly collected cabbage were used. The outer leaves and the stump were removed from the head, and the remainder was ground and the lipids were extracted by a modified Bligh–Dyer method [4]. The lipids were freed from impurities by washing chloroform extracts with a 0.5% aqueous solution of $CaCl_2$.

According to the experimental results, the total amounts of lipids were 1758 mg/kg for variety I, 977 mg/kg for variety II and 1230 mg/kg for variety III.

The total lipids were separated into neutral lipids (NLs), glycolipids (GLs), and phospholipids (PLs) by column chromatography on silica gel [5]. The individual types of lipid compounds were obtained by TLC.

The assignment of chromatographically individual zones of substances to definite groups of lipids was carried out on the basis of a comparison of the chromatographic mobilities of the substances investigated with the mobilities of model preparations and also be qualitative reactions [6] and on the basis of spectral characteristics.

To identify polar lipids of complex structure we used the results of chemical analysis of the water-soluble and liposoluble fragments of the molecules isolated after the performance of severe acid hydrolysis.

For their quantitative determination, the individual groups of lipids were eluted from the plates and their amounts were estimated. The ratio of the groups of NLs was determined by a spectrophotometric method based on the oxidation of the lipid compounds by the dichromate reagent [7].

The classes of lipids of the leaves of the varieties of Brassica oleracea that we investigated were present in the following ratios (mass %):

	Variety I	Variety II	Variety III
Neutral lipids	70,3	4[illegible],1	43,0
Glycolipids	12,9	33,0	27,2
Phospholipids	16,8	28,9	29,8

Thus, in all the cabbage varieties studied, neutral lipids predominated. The differences between the amounts of glyco- and phospholipids within a given variety were insignificant. It must be mentioned that the relative amount of phospholipids found in the composition of the cabbage lipids was comparatively high, since in the majority of fruits, vegetables, and various plants studied, the amount of phospholipids usually does not exceed 10% of the total lipids [8]. The ratios between the main classes of lipids of the medium- and late-ripening varieties of Brassica oleracea were extremely close. The early-ripening variety was distinguished by an increased amount of neutral lipids.

In the NLs, sterols, di- and triacyl glycerols, hydrocarbons, and lipoquinones predominated. Below we give the group compositions of the neutral lipids (% on the total) of the different varieties of the Brassica oleracea leaves:

Neutral lipids	Variety I	Variety II	Variety III
Hydrocarbons	7,55	9,72	13,45
Carotenoids	0,12	0,20	0,11
Waxes	1,42	1,98	3,13
Sterol esters	1,02	7,93	10,96
Fatty acid esters	7,12	4,96	4,13
Unidentified	0,11	0,89	2,47
Triacylglycerols	13,20	17,46	16,59
Tocopherols	0,08	0,10	0,15
Free fatty acids	1,63	5,16	8,00
Lipoquinones	14,95	10,71	5,36
Fatty alcohols	3,05	2,58	2,07
Diacylglycerols	18,32	14,68	9,96
Sterols	20,36	19,41	20,75
Chlorophylls and their derivatives	0,20	0,15	0,09
Monoacylglycerols	4,89	3,97	3,11

In the sequence of varieties studied I-II-III a rise in the relative amounts of sterol esters, free fatty acids, waxes, tocopherols, and triacylglycerols and a fall in the proportion of lipoquinones, mono- and diacylglycerols, fatty alcohols, and fatty acid esters were observed, which indicated the existence of certain laws in the biosynthesis of lipids depending on the time of ripening of the Brassica oleracea varieties. The amount of sterols proved to be greater than those of the other groups of NLs and was characterized by a constant level in all the cabbage varieties studied.

Analysis of the free and esterified forms of sterol showed that in all cases β-sitosterol predominated (>70%), in addition to which campesterol (~20%) and stigmasterol (~1%) were present in small amounts. The main component of the fatty alcohols was phytol (75-90%). The remainder consisted of saturated and unsaturated alcohols of normal structure and saturated isoprenols.

In the unsaponifiable fraction of the NLs four forms of tocopherol were identified – α-, β-, γ-, and δ-. In all the cabbage varieties, the predominating component (65-85%) was the biologically active α-tocopherol. We had previously identified about 13 compounds in the pigments of the chlorophyll and carotene group [2]. Below we give the composition of the lipoquinones (% on the total) of the different varieties of Brassica oleracea:

Lipoquinone	Variety I	Variety II	Variety III
Plastoquinone A	70,9	54,7	50,8
Plastoquinone B	10,0	14,9	14,1
Plastoquinone C	—	7,5	10,2
Plastoquinone D	—	4,0	4,7
α-Tocopherylquinone	3,8	5,7	2,6
β-Tocopherylquinone	2,5	2,2	1,3
Ubiquinone U_{30}	7,2	—	—
Ubiquinone U_{35}	4,2	2,0	—
Ubiquinone U_{40}	—	4,9	—
Ubiquinone U_{45}	—	3,1	7,4
Ubiquinone U_{50}	—	—	6,7
Phylloquinone	1,4	1,0	2,2

The predominant representative of the lipoquinones was platoquinone A which is characteristic of the chloroplasts of the photosynthetic tissues of various plants [9]. The medium- and late-ripening varieties of Brassica oleracea were distinguished, as compared with the early-ripening variety, by increased amounts of plastoquinones B, C, and D and of long-chain ubiquinones.

The group composition of the glycolipids (% on the total) of the different varieties of Brassica oleracea leaves were as follows:

Glycolipids	Variety I	Variety II	Variety III
Monogalactosyldiglycerides	28,36	25,40	24,00
Sterol glycosides	2,98	4,76	5,34
Cerebrosides	29,85	31,73	26,66
Ceramide oligosides	14,92	19,05	21,34
Digalactosyldiglycerides	17,92	15,88	8,66
Ceramic phosphate inositol-oligosides	5,97	3,18	4,00

Characteristic for the glycolipids of cabbage leaves is a high relative amount of cerebrosides, mono- and digalactosyldiglycerides, and ceramide oligosides.

An increase in the relative amount of sterol glycosides and of ceramide oligosides was observed in the sequence of varieties I-II-III.

The sugar fragments of the GLs determined by PC consisted mainly of galactose (50-65%), arabinose (20-40%), and glucose (10-15%) with trace amounts of uronic acids, amino sugars, and mannose.

A distinguishing feature of the glycolipids that we studied, as compared with A. C. Peng's results [1], is the presence in them of a considerable amount of arabinose localized in the ceramide-containing representatives.

The group compositions of the phospholipids (% on the total) of the different varieties of Brassica oleracea leaves are given below:

Phospholipids	Variety I	Variety II	Variety III
Diphosphatidylglycerols	4,05	1,94	2,08
Phosphatidic acids	16,94	12,36	10,85
Phosphatidylethanolamines	15,07	22,56	30,99
Phosphatidylglycerols	29,87	26,78	21,50
Phosphatidylcholines	11,97	15,88	13,17
Phosphatidylserines	9,14	11,42	5,32
Phosphatidylinositols	7,83	4,57	3,81
Lysophosphatidylethanolamines	3,01	2,45	1,43
Lysophosphatidylcholines	2,13	2,04	0,92

Among the cabbage phospholipids we established the presence of nine compounds. The main representatives in the quantitative respect (>70%) were phosphatidylethanolamines, phosphatidylglycerols, phosphatidylcholines, and phosphatidic acids. In the sequence of varieties I-II-III an increase in the relative amount of phosphatidylethanolamines and phosphatidylcholines together with a decrease in the proportion of diphosphatidylglycerols, phosphatidic acids, phosphatidylglycerols, phosphatidylinositols, and lysophospholipids was observed.

Below we give the fatty acid compositions of the total lipids (mass %) of Brassica oleracea leaves of the different varieties:

Fatty acid	Variety I	Variety II	Variety III
10:1	0,9	—	0,3
12:0	1,7	0,3	0,7
14:0	1,5	0,7	2,6
15:0	1,0	—	3,3
16:0	16,7	18,5	21,5
16:1	6,8	1,6	4,0
17:0	—	—	0,3
18:0	3,2	2,7	1,5
18:1	6,0	17,0	4,1
18:2	8,5	24,8	13,8
18:3	51,1	34,4	46,6
22:3	—	—	0,8
24:0	—	—	0,5
Total saturateds	27,6	22,2	31,5
Total unsaturateds	72,4	77,8	68,5

Among the fatty acids five predominated (~90%): linolenic, palmitic, linoleic, oleic, and palmitoleic. In spite of some differences, the compositions and amounts of the fatty acids of the lipids of the different varieties were extremely close. In comparison with findings given in the literature [1] on the composition of the fatty acids of a cabbage grown in the USA, a considerably higher level of linolenic acid in the Brassica oleracea lipids that we studied must be noted.

On the whole, the unsaturation index (U/S) of the lipids proved to be fairly high, amounting to 2.2-2.6, which may to a substantial degree explain the high lability of this group of substances during the thermal processing and storage of cabbage.

EXPERIMENTAL

Column chromatography was carried out on silica gel L 100/160 and thin-layer chromatography on Silufol and silica gel L 5/40 with gypsum in the following solvent systems: 1) for the NLs – heptane–methyl ethyl ketone–acetic acid (47.5:7.5:0.5), two runs; 2) for the GLs – chloroform–methanol–water (65:25:4); 3) acetone–toluene–acetic acid–water

(60:60:2:1); and 4) chloroform–acetone–methanol–acetic acid–water (6:8:2:2:1); and for PLs – chloroform–methanol–7 N ammonia (65:30:4) in the first direction, and chloroform–methanol–acetic acid–water (170:25:25:6) in the second direction.

The lipoquinones were separated by reversed-phase chromatography on silica gel plates impregnated with a 5% solution of paraffin oil in ether [10]. The mixture for elution was acetone–water (9:1) saturated with paraffin oil. After separation, individual compounds were reduced with $NaBH_4$ and were determined spectrophotometrically with the Emmerie–Engel reagent.

The tocopherols were separated by TLC using the solvent system petroleum ether (60-80°C) → diethyl ether–diisopropyl ether–acetone–acetic acid (254:3:32:12:3). The position isomers β- and γ- tocopherols were differentiated with the aid of the Sonnenschein reagent [11]. Quantitative determinations were made by the iron-pyridine method [12].

Neutral lipids were determined by the method of [7], GLs through their carbohydrate components [13], and PLs on the basis of their phosphorus contents [14]. The water-soluble products obtained after severe acid hydrolysis of the GLs and PLs (2 N HCl, 125°C, 48 h) were separated and identified with the aid of paper chromatography according to Kates [6].

The paper chromatography of the carbohydrate components of the GLs was carried out by the descending method in the benzene–butan-1-ol–pyridine–water (1:5:3:3; upper phase) system. Quantitative determinations were carried out by the colorimetric method using the aniline phthalate reagent [15]. Methylation and the GLC of the methyl esters of fatty acids were carried out as in [5].

The sum of the sterols were separated in the form of acetates on silica gel impregnated with silver nitrate using chloroform–petroleum ether–acetic acid (25:75:0.5) [11]. Quantitative analysis was performed with the GLC method [16].

Fatty alcohols were analyzed in the form of acetates by the TLC/Ag^+ method in the chloroform–carbon tetrachloride (1:1) system.

SUMMARY

The compositions and amounts of the lipid substances in the leaves of early-, middle-, and late-ripening varieties of cabbage have been investigated. About 30 lipid substances have been identified, among which free and esterified forms of sterols, di- and triacyl glycerols, hydrocarbons, lipoquinones, mono- and digalactosyldiglycerides, cerebrosides, phosphatidylethanolamines, phosphatidylglycerols, and phosphatidylquinolenes were predominant.

The compositions of the sterol fragments, the lipoquinones, the fatty alcohols, and the tocopherols have been characterized. The fatty acids of the lipids were represented by 13 components with linolenic acid present in the greatest relative amount. Differences have been found in the lipid complexes of the cabbage in the sequence of early-, middle-, late-ripening varieties.

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CONFIGURATION OF STEREOISOMERS OF NARINGENIN 5-GLUCOSIDE PRESENT IN FLAMIN

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The configurations of the C-2 chiral centers of the stereoisomers of naringenin 5-glucoside isolated from flamin by HPLC have been established by the method of circular dichroism. One of the stereoisomers is (−)-2S-naringenin 5-glucoside and the other has been characterized as a molecular compound containing the 5-glucosides of (+)-2R- and (−)-2S-naringenins in a ratio of ~2:1.

In an investigation of flamin by high-performance liquid chromatography (HPLC) it was established that chalcone-flavanone isomerization [1] took place in its water-containing solutions. In a model example with isosalipurposide it was shown that under suitable conditions the chalcone was converted into two stereoisomers of naringenin 5-glucoside in the course of a month.

The presence of two compounds corresponding to naringenin 5-β-D-glycoside was reported previously for yellow everlasting [2, 3] and in flamin [4]. It was assumed that these were either different hydrate forms [2] or substances having a stereochemical difference [3]. The compounds were separated by column chromatography and paper chromatography [3, 4]. It was established with the aid of chemical methods that one of them (helichrysin A) was a glucoside of (−)-naringenin and it was subsequently ascribed the 2S-configuration [5]. The other compound (helichrysin B) was characterized as a glucoside of racemic naringenin capable of being separated into diastereomers on further chromatographic separation [6]. In a later paper [7] doubt was cast on the existence of the 5-glucoside of racemic naringenin.

Since the pharmacological activity of flamin is due primarily to a chalcone-flavanone pair of compounds, we set ourselves the task of determining the configuration of the C-2 chirality centers of the stereoisomers of naringenin 5-glucoside by using a combination of one of the most informative chiroptic methods — circular dichroism (CD) — and HPLC. An important circumstance is the HPLC provides the possibility not only of effectively separating stereoisomers but also of performing clear qualitative and quantitative monitoring at any stage of the investigation.

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