[10]. The individual groups of PhLs and GLs were identified by comparing their migrational characteristics and also with the aid of specific color reactions. Pure MGDGs and DGDGs isolated from wheat flour were used as markers [11].

For TLC we used the following solvent systems: 1) petroleum ether—diethyl ether (60:40); 2) acetone—toluene—acetic acid—water (60:60:2:1); 3) benzene—methanol (4:1); 4) chloroform—methanol—7 N ammonia (60:30:4) and chloroform—methanol—acetic acid—water (170:25:25:6); 5) chloroform—acetone—methanol—acetic acid—water (65:20:10:3); 6) diethyl ether—hexane (3:7); and 7) pyridine—n-butanol—water (3:2:1). The PC of the carbohydrate components of the GLs was conducted by the descending method in system 7. Quantitative determination was carried out by a colorimetric method using the aniline phthalate reagent [12].

The acid hydrolysis of the GLs was conducted with 2 N H_2SO_4 in the boiling water bath for 4 h. After the solution had cooled the fat-soluble products were extracted with petroleum ether. The insoluble pigments were eliminated by filtering the acidic aqueous solution through a paper filter.

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LIPIDS OF THE LEAF VEGETABLES Spinacea oleracea, Latuca sativa, AND Rumex acetosa

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The class compositions of the lipids of leaf vegetables – Spinacea oleracea (variety Ispolinskii), Latuca sativa (variety Kucheryavets Odesskii), and Rumex acetosa (variety Odesskii-17) have been studied. The compositions of their NLs (17 groups of individual compounds were identified), GLs (nine groups of compounds were found), and PhLs (seven groups of compounds have been found) have been determined by physicochemical methods of analysis. The maximum amount of the total lipids and also of the PhLs was possessed by the spinach. Among the FAs of the leaf vegetables, USFAs predominated.

Leaf vegetables are rich in a number of biologically active substances (folic acid, choline, phylloquinone, ascorbic acid, vitamins of the B group, potassium, iodine, iron, etc.) which are responsible for a complex of curative properties in a number of blood diseases, tuberculosis, diseases of the gastrointestinal tract, nervous breakdown, etc. In

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addition, spinach, lettuce, and sorrel leaves are necessary in the prophylaxis of avitaminoses and tumoral diseases [1]. The leaf vegetables are invaluable raw materials for the development of bioadditives with a curative-prophylactic action and for the creation of new combined products permitting an expansion of the variety and an increase in the consumption of valuable green crops up to the level of the developed countries.

One of the important classes of biologically active substances consists of the lipids, which determine the calorific content and physiological value and also the quality and storage properties of the finished products. We have found no literature information on the lipids of the leaves of Latuca sativa and Rumex acetosa, while investigations of the lipids of Spinacaea oleracea were performed for varieties growing abroad [2, 3].

In view of the fact that in the development of new technologies of food products and curative-prophylactic agents the correct choice of the variety of raw material and consideration of its biological features are not unimportant, we have investigated the qualitative and quantitative compositions of the lipids of the leaves of Spinacea oleracea (variety Ispolinskii), Latuca sativa (variety Kucheryavets Odesskii), and Rumex acetosa (variety Odesskii-17) grown under the conditions of the south of the Ukraine (1987-1991 harvests). Samples of the leaves of freshly gathered vegetables that had reached the technical degree of ripeness were used.

According to the experimental results, the total content of lipids (mg/100 g) amounted to 183.8 for the leaves Spinacaea oleracea, 150.9 for Latuca sativa, and 171.0 for Rumex acetosa, which are extremely close to the results obtained previously for the leaves of different varieties of cabbage [4] and for spinach roots [5].

The classes of lipids of the varieties of spinach, lettuce, and sorrel that we investigated were present in the following proportions (wt.%):

	Spinacea oleracea	*Latuca sativa*	*Rumex acetosa*
Neutral lipids	11,9	38,2	41,8
Glycolipids	30,9	37,4	39,1
Phospholipids	57,2	24,4	19,1

The spinach leaves were distinguished by the largest amount (~90%) of polar lipids, which corresponds to their proportion in the lipids of spinach roots given in [5]. However, in contrast to the roots, where about 90% of the polar lipids were represented by GLs, in the leaf vegetables the PhLs predominated.

The weight concentration of the polar lipids fell in the sequence spinach—lettuce—sorrel. It must be mentioned that the amount of phospholipids in the spinach leaves of some foreign varieties was considerably lower, and made up only 10% of the total polar lipids [6]. The results of the quantitative determination of representatives of the class of lipids for the varieties of leaf vegetables studied are given below.

In the NLs, 17 groups of compounds were identified, among which sterols and their esters, chlorophylls, and free fatty acids predominated in all the species of leaf vegetables studied (% on the total):

Class of compound	*Spinacea oleracea*	*Latuca sativa*	*Rumex acetosa*
Hydrocarbons (HCs)	6,2	12,2	4,1
Carotenes (Cs)	1,6	1,2	0,8
Sterol esters (StEs)	5,3	8,1	7,1
Wax esters (WEs)	1,1	4,2	1,9
Esters of fatty acids and lower alcohols (EFALAs)	2,6	6,2	3,0
Triacylglycerols (TAGs)	3,2	5,3	3,7
Tocopherols (Tph's)	1,3	0,7	1,5
Fatty acids (FAs)	22,1	17,6	12,0
Lipoquinones (LQs)	1,8	0,5	0,7
Fatty alcohols (FAlc's)	1,2	0,7	1,9
1,3-Diacylglycerols (DAGs)	0,9	0,5	0,7
Sterols (St's)	14,3	20,9	37,2
1,2-Diacylglycerols (DAGs)	2,2	0,3	5,9
Hydroxyacids (HAs)	0,9	1,0	0,4
Chlorophylls (Chlp's)	29,2	16,7	16,1
Xanthophylls (Xph's)	5,1	3,3	2,7
Monoacylglycerols (MAGs)	1,0	0,6	0,3

The lipids of the green leaf vegetables were distinguished by fairly low relative amounts of neutral glycerides, in contrast to the lipids of cabbage leaves [4] where these

groups were predominant. On the whole, the composition of the leaf vegetables was similar to that of the mulberry leaves studied previously [5], which may indicate the closeness of their functions in the green leaves. Although the compositions of the NLs of the different species of leaf vegetables were identical and the group ratios were similar, there were, nevertheless, some differences. Thus, a substantial rise in the relative amount of sterols and an increase in the amount of chlorophylls and xanthophylls was observed in the sequence spinach—lettuce—sorrel. Lettuce was distinguished from the other crops by an increased relative amount of weakly polar groups — hydrocarbons, waxes, fatty acid esters, and lower alcohols.

On the whole, all these three species of leaf crops that we have studied were characterized by a complex composition of the NLs, among which a considerable part consisted of compounds possessing a high biological activity, such as carotenoids (provitamin A), tocopherols (vitamin E), to linolenic and linoleic acids (vitamin F activity), and lipoquinones, consisting of ubiquinones (vitamin Q) and phylloquinones (vitamin K), and also phytosterols (with a hypocholesterolemic action) and chlorophylls (with an antitoxic activity) [6, 7].

In the GLs we identified nine groups of compounds, among which digalactosylidiglycerides, cerebrosides, monogalactosyldiglycerides, and esterified sterol glycosides predominated (%):

Class of compounds	*Spinacea oleracea*	*Latuca sativa*	*Rumex acetosa*
Monogalactosyldiglycerides (MGDGs)	24,2	19,5	18,0
Esterified sterol glycosides (ESGs)	15,2	17,8	18,6
Sterol glycosides (SGs)	4,3	3,3	2,7
Cerebrosides (CSs)	13,7	21,3	16,7
Ceramide oligosides (COs)	1,2	1,0	3,5
Digalactosyldiglycerides (DGDGs)	31,0	24,8	25,5
Sulfoquinovosyldiglycerides (SQDGs)	6,5	8,2	9,5
Ceramide phosphate inositol oligosides-I (CPIOs-I)	0,9	1,6	1,3
Ceramide phosphate inositol oligosides-II (CPIOs-II)	3,1	2,5	4,2

As also in the case of the NLs, the qualitative compositions of the group of GLs were identical for all the species of leaf vegetable that were studied, and the group ratios were extremely close. A feature of the composition of the GLs studied was the higher relative amount of ESGs as compared with similar materials studied previously — cabbage and mulberry leaves [4, 5].

Among PhLs we identified seven groups of compounds of which the phosphatidylethanolamines were predominating (% on the total):

Class of compounds	*Spinacea oleracea*	*Latuca sativa*	*Rumex acetosa*
Phosphatidic acids (PAs)	4,8	3,2	1,9
Diphosphatidyldiglycerols (DPDGs)	6,0	4,3	3,6
Phosphatidylethanolamines (PEs)	14 2	7,5	21,3
Phosphatidyldiglycerols (PGs)	39,1	42,0	20,9
Phosphatylcholines (PCs)	28,9	33,2	39,6
Phosphatidylserines (PSs)	3,1	3,4	5 9
Phosphatidylinositols (PIs)	3,9	6,4	6,8

However, the relative amounts of the latter in lettuce were 2-3 times lower than in spinach and sorrel. In spite of this difference, the group proportions of the PLs of the different species of leaf vegetables studied were on the whole closer and close to those of mulberry leaves [5].

Among the fatty acids, 10 components with C_{14}—C_{18} compositions were identified, of which linolenic, linoleic, and palmitic acids made up 80-90% for all the species of leaf crops studied. It must be mentioned that the FAs contained palmitolinolenic (16:3), plamitolinoleic (16:2), and pl;amitoleic acids, which imparts a certain originality to the leaf lipids. Information on the presence of these rare acids in foreign varieties of spinach has been given previously [8].

The fatty acid compositions of the leaf vegetables were as follows (% on the total FAs):

	14:0	15:0	16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
Spinacea oleracea	0.8	0,4	10,2	0,2	0,9	4,1	0,3	3,9	22,5	56,7
Latuca sativa	0,4	0,3	12,1	0,3	0.7	6.0	0,1	3,2	13.0	63,9
Rumex acetosa	0,7	0,5	22,8	0.2	2,1	8,7	0.2	1,8	16 3	46.7

The spinach lipids were distinguished by the highest unsaturation index (U/S) (7.5); then in decreasing order of unsaturation came lettuce (6.7) and sorrel (5.3). It must be mentioned that with respect to this index the lipids of the green crops were somewhat superior to the lipids of cabbage leaves [4].

In addition to the undoubtedly high biological activity that a fatty acid composition with a predominance of polyunsaturated components imparts to the lipids, one must also bear in mind the extremely considerable oxidative lability of the lipids of these vegetables connected with this, which must be taken into account in the development of a technology for their storage, their processing into food products, and their use in the production of curative-prophylactic additives, preparations, etc. On comparing the results that we have obtained for individual groups of GLs with those given in the literature, the following must be observed. The GLs of the Ispolinskii variety of spinach, which has been regionalized for the conditions of south Ukraine, have a higher mass proportion of DGDGs than the varieties described in the literature. Thus, according to German scientists [8], the ratio of the amounts of MGDGs and DGDGs for spinach was 1:1, at 22.6 and 2.2% of the total amount of lipids, respectively, while for spinach regionalized for the Ukraine this ratio was 1:1.28. It follows from the literature that this may be connected with the synthesis of USFAs – in particular, linoleic acid, from the MGDGs [9].

The presence of carotenoids, tocopherols, phylloquinones, sitosterols, and USFAs, in the NL class and of phosphatidylethanolamines and phosphatidylcholines among the polar lipids is responsible for some of the important biological properties as curative-prophylactic components of food – vitamin, antioxidant, immunocorrecting, hypocholesterolemic, carcinogenic-protective, and other properties – and also affects the technological properties of food products using leaf vegetables [10, 11].

EXPERIMENTAL

Extraction and Purification of Lipids. The isolation of the total liposoluble substances was achieved by a modification of the Bligh–Dyer method [12] under conditions preventing the degradation of the labile components of the lipid complex [13]. The lipids were freed from impurities by washing chloroform extracts with a 0.5% aqueous solution of $CaCl_2$.

Separation of the Lipids. The total lipids were separated into neutral lipids (NLs), glycolipids (GLs), and phospholipids (PhLs) by column chromatography on silica gel [14].

The group composition of each class of lipids was determined by TLC on silica gel with gypsum L 5/40 (Czechoslovakia) in various solvent systems: 1) for the NLs – heptane–methyl ethyl ketone–acetic acid (47.5: 7.5: 0.5), two runs; 2) for the GLS – acetone–toluene–acetic acid–water (60:60:2:1); and 3) for the PhLs – 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 chromatograms were revealed by spraying the plates with a 10% solution of molybdophosphoric acid and by treatment with iodine vapor.

The groups of lipid compounds were identified on the basis of chromatographic mobilities in comparison with model preparations of markers, and also from literature information [13, 15] on the R_f values of groups of lipids in the systems used, qualitative color reaction performed on the plates, and the spectral characteristics of the substances eluted from the chromatograms.

In the identification of the lipids with complex structures, they were subjected to severe acid hydrolysis (2 N HCl, 120°C, 48 h), the water- and liposoluble fragments were fractionated and were estimated quantitatively, and the final conclusion concerning the concrete groups to which the compounds under investigation belonged was made on the basis of the molar ratio between the constituent molecules (glycerol, monosaccharides, FAs, sphinogosine bases, phosphoric acid, choline, ethanolamine, etc.).

In the case of spots having R_f values identical with standards of sterol nature, we also carried out a check coloration with the Libermann–Burchard reagent [16].

The presence of carbohydrate-containing fragments in the separation of the GLs was confirmed by specific color reactions when the plates were treated with α-naphthol, the periodate–Schiff reagent, and diphenylamine [13, 15].

All the PhLs, and also the spots with R_f 0.20 and 0.12 (system for GLs) gave the coloration characteristic for phosphorus-containing lipids on being sprayed with the Vas'kovskii-Kostetskii reagent [17]. The mass fraction of glycerol was found by Moore's periodate-chromotropic method [18], which is based on the oxidation of glycerol by sodium periodate followed by the spectrophotometric determination of the colored complex (570 nm) with chromotropic acid.

The compounds that we assigned to the lipoquinones gave a positive reaction when sprayed with a solution of OsO_4 [13], which indicated their unsaturated nature, and had the characteristic absorption bands (1665, 1295 cm^{-1}) for quinones. After separation they were reduced with $NaBH_4$ and were quantitatively determined with the Emmeric—Engel reagent [19]. The tocopherol spots were detected with p-dimethylphenylenediamine.

In the UV spectra of substances from the spots which we assigned to the tocopherols, we observed an absorption maximum in the region of 294 nm, which is characteristic for tocopherols [20]. They were determined quantitatively by spectrophotometry with the iron-pyridine reagent [19].

The zone ascribed to waxes, on being saponified with methanolic NaOH [13], gave a mixture of FAs and higher alcohols the quantitative determination of which enabled their molar ratio to be established (~1:1). The zone with the lowest mobility in the system for NLs, which was characterized by a R_f value corresponding to MAGs, on investigation by IR spectroscopy revealed bands in the region of 3350 and 950-920 cm^{-1}, corresponding to the vibrations of OH groups, and also a band in the 730-710 cm^{-1} region, corresponding to the vibrations of $-CH_2-$ groups [18].

In the IR spectra of the diacylglycerols, in the region corresponding to the vibrations of CH_2 groups, a splitting into two bands, which is characteristic only for DAGs [18], was observed. The IR spectra of the position isomers of the DAGs were identical, with the exception of the presence of a band at 1060 cm^{-1} in the spectra of the 1,2 diacylglycerols, which had a low intensity [21]. In the IR spectra of the substances from the spots that we had identified as TAGs, in contrast to the DAGs there were no bands corresponding to the vibrations of OH groups (3350, 950-920 cm^{-1}), but absorption corresponding to the vibrations of CH_2 groups was observed. The IR spectra of the compounds assigned to the hydroxy acids were characterized by the stretching vibrations of free OH groups in the 3650-3590 cm^{-1} [13] and 930 cm^{-1} [18] regions.

The compounds that had the highest mobility in the system for NLs (R_f 1), coinciding with that for marker hydrocarbons, were distinguished by strong stretching vibrations in the 2926 and 2853 cm^{-1} regions, and also by the deformation (1465 cm^{-1}) and skeletal (750-720 cm^{-1}) vibrations that are characteristic for CH_2 groups [13], which may indicate the presence of saturated hydrocarbons. Furthermore, there were weakened vibrations at frequencies of 960, 1670, and 3020 cm^{-1}, which is typical for a hydrocarbon chain with double bonds [18].

The chromatographic zone identified as free FAs gave the yellow coloration characteristic for fatty acids on treatment with diazotized p-nitroaniline [22].

The liposoluble pigments (Cs, Xph's, and Chlp's) were identified as described in a paper we have published previously [23]. The main groups of the NLs were estimated by Amenta's method from the reaction with a dichromate reagent [24].

The monosaccharides present in the GLs were isolated from the water-soluble fractions of hydrolysates of the glycolipid groups by the TLC method in the solvent system benzene—n-butanol—pyridine—water (1:5:3:3) and were estimated quantitatively by Sevennerholm's method, based on the colorimetric reaction with orcinol [25]. The sphingosine bases from the hydrolysates of the GLs were extracted with diethyl ether according to [18] and were determined quantitatively by Lauter's method [26] in which the colored complex with Methyl Orange is recorded.

The spots with R_f 0.71 and 0.67 (system for GLs) gave a positive reaction with the Leibermann—Burchard reagent, whichshowed the presence of a sterol fragment in the structures of the compounds concerned.

In the IR spectra of the GLs assigned to the COs and CPIOs there were the absorption bands characteristic for α- and β-glycosidic bonds (890 and 776-756 cm^{-1}), which showed the presence in their structures of oligosaccharide chains with different types of glycosidic bonds.

The structures of the CPIO groups (phytoglycolipids) were determined in a similar way to that described previously [27]. The amounts of the main GL groups were estimated from their carbohydrate components, and those of the SQDGs by Kean's method with a toluidine dye [28]. The preliminary identification of the amine- and choline-containing groups of the PhLs was made with the use of specific reagents: ninhydrin and the Dragendorff reagent [15].

Nitrogen in the PEs was determined by Kjeldahl's method [13]. Choline, ethanolamine, inositol, and serine were isolated by PC and were estimated quantitatively as described by Kates [13]. The amounts of PhLs were determined from their phosphorus contents [29].

IR spectra were taken on a UR-20 instrument in chloroform using an NaCl prism in the 2000-700 cm^{-1} range and an LiF prism in the 3600-2000 cm^{-1} range.

Gas-Liquid Chromatography of the Fatty Acids. The esterification of the FAs and the GLC of the FA methyl esters were carried out as described in [30].

Treatment of the Experimental Results. The methods of mathematical statistics were used to treat the results obtained for species of leaf vegetables in different growth years [31, 32].

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SYNERGISM OF PHOSPHOLIPIDS IN EXTRACTS OF MARINE INVERTEBRATES

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The qualitative and quantitative compositions of the phospholipids of prostaglandin extracts of 18 species of marine invertebrates have been determined. A correlation has been shown between the set of phospholipids in the extract and prostaglandin-like activity. For samples with a high prostaglandin-like activity in the set of phospholipids the presence of either diphosphatidylglycerol, phosphatidyl glycerol, phosphatidylethanolamine or a combination of these lipids is necessary.

We have shown previously [1] that the phospholipids (PhLs) in prostaglandin extracts have a pronounced effect on the activity of these extracts and have given a quantitative estimate of the contribution of concrete PhLs to this activity. Investigations on the synergism of PhLs relative to prostaglandins (PGs) have shown the existence of a correlation of the set of PhLs in the extract and its PG-like activity. In the present work we have continued an investigation of this phenomenon and have expanded the range of specimens investigated and the area of their habitats.

The multistage extraction procedure that we use [2] permits the most complete extraction of the PGs from tissue. However, together with the PGs, various substances of lipid nature pass into the extract including PhLs which, obviously, are present in a complex with the PGs [3-7]. We have shown previously for the case of the well-studied coral Plexaura homomalla that the PG activity of the extract from this organism depends substantially on the concrete PhLs present in the extract [1]. Here the greatest synergism is shown by the PhGs.

In the present paper we give the comparative characteristics of the PhLs present in PG extracts from marine invertebrates. The results are shown in Table 1, where figures are presented for the PGs of group B, which is formed as the result of the alkaline treatment of the PGs of groups E and A and also as the result of the storage of the samples and their preparation for analysis, i.e., it is not originally present in the extract [8]. It is known that the PGs of group A do not cause contraction of the smooth musculature of the rat uterus and, accordingly, are not an active form for the biotest that we use.

Two soft corals were distinguished by the lowest amount of PGs in the tissue: Alcyonidae sp. and Plexaura homomalla. The first was inactive, and from the amount in the form of PG inactive in this biotest [(PGB (μg/g of tissue)] it several times exceeded the other specimen. The second was extremely active. This showed that the PGs present in this extract belonged to groups E and F, which are capable of causing the contraction of the smooth musculature of the uterus. The two soft corals differed from one another also by the PhLs that they contained. Of the four representatives of the coelenterate type, the two inactive sam-

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