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# LIPIDS FROM *Halostachys caspica* AND *Halocharis hispida**

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*The composition of lipids from the aerial parts of two species of halophytes from the family Chenopodiaceae,* Halostachys caspica *C. A. Mey. and* Halocharis hispida *Bge. was determined. Neutral lipids (NL, 62.1 and 54.2%, respectively) dominated the total lipids (TL) of these plants. More than a third of the NL were esters of aliphatic alcohols and phytosterols (FAE). Fatty acids 16:0, 18:1, and 18:2 dominated the acids of FAE; 16:0, 18:1, and 18:3, the phospholipids. The principal fatty acids of glycolipids were unsaturated acids (68.3 and 75.1%) with linolenic acid dominating (44.9 and 43.5%).*

**Key words:** *Halostachys caspica*, *Halocharis hispida*, neutral lipids, glycolipids, phospholipids, fatty acids.

The halophytes *Halostachys caspica* C. A. Mey. and *Halocharis hispida* Bge. (Chenopodiaceae) grow in the arid climate of the Mirzachul steppe (Djizak Province). Their habitat is typically hot and dry with elevated amounts of inorganic salts [1]. We studied lipids from the aerial part of these halophytes collected during flowering in August, the driest time of year.

The chemical composition of the aerial organs of Chenopodiaceae plants has been partially reported. *H. caspica* contains 3.47% raw fat, *H. hispida*, 1.38% [1]. Lipids from leaves of *Chenopodium alba* contained 68% neutral fat, 20% unsaponified components, and other compounds [2]. Gentriacontanol and $\beta$-sitosterol and its 3-*O*-glycoside were isolated and identified from the aerial part of *H. hispida* growing in the deserts of Saudia Arabia [3].

The isolation of total lipids (TL) from fresh aerial parts of *H. caspica* and *H. hispida*, fractionation of TL, and analysis of separate lipid groups using counter-current separation, CC, TLC, GC, and GC—MS were performed as before [4-6].

The moisture contents of fresh biomass of these plants were 78.9 and 76.5%, respectively. The yield of TL was 3.5 and 2.5% calculated per dry mass of the compounds. Table 1 lists the fractions of neutral (NL), glyco- (GL), and phospholipids (PL) in addition to the separate components of the NL in TL; Table 2, the fatty-acid composition of the separate lipid groups.

TL of the studied halophytes were dominated by NL (62.1 and 54.2%). The contents of NL calculated per dry biomass of *H. caspica* and *H. hispida* were 2.2 and 1.4%, respectively. Polar lipids (sum of GL and PL) made up 37.9% of the TL mass for *H. caspica* and 45.8% for *H. hispida*. Total PL calculated per dry biomass was 1.33 and 1.15%, respectively. Although the mass fractions of PL in the TL of the two plants are comparable, *H. hispida* has a higher level of GL. The trends in the distributions of lipid groups for the studied salt-tolerant plants and for the previously studied *Atriplex dimorphostegia* follow the order NL > GL > PL [4].

The qualitative compositions of polar lipids from the studied samples were identical according to TLC. The GL contained monogalactosyldiacylglycerines, digalactosyldiacylglycerines, sulfoquinovosylacylglycerines, sterylglycosides, and sterylglycoside esters. These were accompanied by two minor compounds that were detected by a reagent for GL ($\alpha$-naphthol). The PL were identified as phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, phosphatidylglycerols, diphosphatidylglycerols, and phosphatidic acids. Such a composition of PL is typical for photosynthetic tissue of higher plants [7].

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TABLE 1. Composition of Lipids from the Aerial Parts of *Halostachys caspica* and *Halocharis hispida*, % of TL Mass

| Components of total lipids | *Halostachys caspica* | *Halocharis hispida* |
|---|---|---|
| **Neutral lipids**, including: | **62.1** | **54.2** |
| Hydrocarbons and carotinoids | 7.1 | 5.5 |
| Fatty acid esters with alkanols and phytosterols | 21.8 | 21.9 |
| Triacylglycerines | Tr. | Tr. |
| Phytol | 4.3 | 0.1 |
| Free fatty acids | 11.3 | 12.5 |
| Fatty alcohols, triterpenols and sterols | 14.0 | 10.2 |
| Diacylglycerines and monoacylglycerines | Tr. | Tr. |
| Total chlorophylls and unidentified compounds | 3.6 | 4.0 |
| **Glycolipids** with impurity of altered pigments | **22.4** | **28.9** |
| **Phospholipids** | **15.5** | **16.9** |

TABLE 2. Composition of Fatty Acids of Lipids from the Aerial Parts of *Halostachys caspica* and *Halocharis hispida*, % GC

| Acid | *Halostachys caspica* | | | | *Halocharis hispida* | | | |
|---|---|---|---|---|---|---|---|---|
| | NL | | GL | PL | NL | | GL | PL |
| | FFA | FAE | | | FFA | FAE | | |
| 12:0 | 0.3 | 0.2 | Tr. | Tr. | 0.1 | 0.1 | Tr. | 0.5 |
| 14:0 | 0.8 | 5.8 | Tr. | 0.7 | 0.8 | 2.4 | Tr. | 1.9 |
| 16:0 | 45.5 | 32.5 | 29.5 | 46.0 | 37.3 | 35.4 | 21.7 | 38.6 |
| 16:1 | Tr. | 4.1 | Tr. | 6.5 | Tr. | 0.8 | Tr. | 7.7 |
| 18:0 | 5.6 | 4.9 | 2.2 | 3.0 | 2.6 | 7.0 | 3.2 | 1.6 |
| 18:1 | 19.2 | 26.8 | 14.8 | 25.3 | 27.2 | 24.3 | 14.8 | 34.0 |
| 18:2 | 18.7 | 20.7 | 8.6 | 2.4 | 21.2 | 19.6 | 16.8 | 3.7 |
| 18:3 | 7.2 | 5.0 | 44.9 | 16.1 | 10.8 | 10.4 | 43.5 | 12.0 |
| 20:0 | 2.7 | Tr. | – | – | Tr. | Tr. | Tr. | Tr. |
| 22:0 | | Tr. | | | | Tr. | | |
| $\Sigma_{sat.}$ | 54.9 | 43.4 | 31.7 | 49.7 | 40.8 | 44.9 | 24.9 | 40.5 |
| $\Sigma_{unsat.}$ | 45.1 | 56.6 | 68.3 | 50.3 | 59.2 | 55.1 | 75.1 | 54.4 |

The principal NL components of the studied samples were esters of fatty alcohols (waxy esters) and phytosterols with fatty acids (FAE), free fatty acids, free fatty alcohols, phytosterols, and hydrocarbons. The presence of phytol was confirmed by mass spectrometry (*m*/*z* 278 [M - 18], 222, 194, 181, 180, 165, 152, 138, 124, 123, 109, 95, 85, 83, 71, 69, 57, 55, 43). Neutral glycerolipids were present in trace quantities. More than a third of the NL were FAE, which are typically found in surface lipids of photosynthetic tissue of higher plants [7]. Salt-tolerant Chenopodiaceae species growing in saline soils under arid conditions are known to form a waxy cuticle that prevents evaporation of moisture from tissue [1]. Thus, it was confirmed experimentally that the level of cuticle waxes in tobacco and cotton leaves increases with a lack of water [8, 9]. This explains why NL enriched with surface lipids (waxy esters, hydrocarbons, and fatty alcohols) were present in such a large quantity when the samples were collected during the driest time of year.

The fatty-acid compositions of the principal lipid groups of the studied plants (Table 2) showed that they were qualitatively identical but had significant differences in the contents of saturated and unsaturated acids and levels of separate unsaturated acids. The 16:0 acid dominated the saturated acids in all lipid groups.

Unsaturated acids were the main fatty acids of GL, like lipids of photosynthetic tissues of higher plants [7]. As expected, the main one was linolenic acid (18:3, 44%). The amount of total unsaturated acids in PL of *H. hispida* was greater than that of saturated acids. *H. caspica* differed from *H. hispida* by a higher content of 16:0 acid in PL and had an equal ratio of saturated and unsaturated acids. The unsaturated acids of PL of both species were dominated by 18:1 whereas the level of

18:3 was greater than that of 18:2. Furthermore, free fatty acids and acids isolated from FAE had higher amounts of 18:1 and 18:2. The contents of 18:3 acid in these NL classes varied in the range 5.0-10.8% of total acids.

It is well known [10] that plants indigenous to arid climates have certain adaptation mechanisms for various environmental factors. These plants can maintain the required liquid phase state of cell membranes under harsh conditions. Fatty acids of polar lipids of photosynthetic tissue membranes undoubtedly play an important role in this. However, it can be assumed that NL components and their fatty acids also play a certain role in adaptation reactions of cells of resistant plants in order to preserve their vital functions.

## EXPERIMENTAL

GC of fatty acid methyl esters was performed on a Chrom-5 instrument equipped with a steel column packed with Reoplex 400 (15%) on N-AW at 198°C; GC—MS, in an Agilent Technologies GC—MS/HP6890 GC—MS with an AT5973N mass-selective detector using a capillary column with phenylmethylsiloxane (5%) and temperature programming from 150 to 280°C.

Total lipids were extracted from fresh biomass of the studied plants by the well-known Folch method [6]. Enzymes of fresh biomass were inactivated by submersing it for 1-2 min in boiling isopropanol before extracting lipids. TL were extracted using a $CHCl_3$:$CH_3OH$ mixture (2:1, v/v). Combined lipid extracts were condensed, dissolved in $CHCl_3$, and purified of ballast material by multiple rinsings with $CaCl_2$ solution (0.04%).

NL and polar lipids were separated by counter-current methods between hexane and ethanol (87%) followed by CC over silica gel with elution of NL by $CHCl_3$, GL by acetone, and PL by methanol. Separate NL classes were obtained by preparative TLC. Lipid components and lipophilic compounds were identified using the literature methods [4, 5].

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