DOI 10.1007/s10600-019-02818-0
Chemistry of Natural Compounds, Vol. 55, No. 5, September, 2019

LIPIDS FROM CYSTS OF ARAL SEA BRINE SHRIMP *Artemia parthenogenetica*

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The contents and compositions of free (FL) and bound lipids (BL) from cysts of Aral Sea brine shrimp Artemia parthenogenetica *were established. The yields of FL and BL were 12.3 and 2.23 mass% of the cysts. The BL group composition included neutral lipids (0.65%), glycolipids (GL) (0.34%), and phospholipids (PL) (1.24%). The lipophilic components of FL were identified as sterols, hydrocarbons including squalene, triterpenols, and xanthophylls. Fatty acids (FAs) of lipids included 16–17 components. FL were dominated by FAs 18:1, 16:1, and 16:0; GL, 16:0 and total 18:1 and 18:3; PL, total 18:1 and 18:3. Polyunsaturated FAs (20:4, 20:5, and 22:6) were not detected in lipids from the studied sample of* A. parthenogenetica *cysts.*

Keywords: *Artemia parthenogenetica*, cysts, lipids, fatty acids, unsaponifiable substances.

Artemia parthenogenetica Barigozzi is a crustacean of the order Branchiopoda (Artemiidae) [1]. The species is indigenous mainly to Asia in marine and continental aquifers, e.g., chloride, sulfate, and carbonate. Brine shrimp withstand not only high salt concentrations but also acidic and basic media [2]. The systematics of parthenogenetic (fertilized without males) *Artemia* species are poorly developed, in contrast with those of bisexual *Artemia* species (*A. salina*, *A. franciscana*, *A. persimilis*, etc.), so they are all assigned to the species *A. parthenogenetica* [3].

The *A. parthenogenetica* species of brine shrimp appeared in the Aral Sea in 1998 and exhibited signs of successful colonization [4]. This brine shrimp is the only surviving crustacean species in the remaining part of the western Aral Sea.

Biomass of brine shrimp and its cysts (eggs) contains a complex of vitamins, trace elements, biologically active lipids, proteins, and carbohydrates and is widely used as feed for fish, crabs, and shrimp farmed in artificial aquaculture and marine culture [5] and other economic sectors. For example, aqueous and EtOH extracts of *A. salina* cysts possessed antiproliferative activity, as a result of which they were used as components of medicinal preparations and cosmetics for treating and preventing skin diseases [6]. Several biologically active additives (BAAs) for food, medicine [7], bird food [6], and fertilizers [8] were based on *A. salina* cysts.

Lipids are a very important biochemical constituent of *Artemia* cysts. The contents and compositions of *A. salina* cyst lipids varied considerably with respect to temperature, salinity, collection year and month, geographic location of the aquifer, and other factors [9]. The content of total lipids varied from 4.1 to 11.8% in cysts of *Artemia* populations inhabiting 12 saline lakes of Pavlodar Oblast [10]. Cysts that were not decapsulated were reported to contain 3–13% lipids; decapsulated, up to 26% [11].

Triacylglycerides were the main constituents observed in total lipids from cysts of Russian and Italian populations of *A. salina* [9, 11]; phospholipids and sterols, in lipids from brine shrimp cysts from the Great Salt Lake (Utah, USA) [9]. Free fatty acids (FAs), cholesterol and its esters, phospholipids, and squalene were identified qualitatively from other lipid classes in cysts [11]. Total lipids from *A. parthenogenetica* are poorly studied.

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 0009-3130/19/5505-0802

TABLE 1. Characteristics of Cyst Lipids from *A. parthenogenetica*

Parameter	Content
Moisture and volatile substances, mass% of cysts	6.94
Yield of free lipids (FL) with actual moisture, mass% of cysts	11.30
Yield of FL per absolutely dry substance, mass% of cysts	12.13
Acid number of FL, mg KOH/g	53.41
Free fatty acid contents in FL, mass%	24.70
Unsaponifiable substance content, mass% of FL	6.0
Bound lipids (BL), mass% of cysts, including:	2.23
neutral lipids	0.65
glycolipids	0.34
phospholipids	1.24

The FA composition of total lipids from decapsulated cysts of Aral-Sea *A. parthenogenetica* was established for 2003 and 2005 collections. The FA fraction of these cysts was dominated by acids 18:1 (30.3%), 20:5 (12.8), linoleic 18:3 (6.9), 18:2 (6.2), 20:4 (1.5), and docosahexaenoic (22:6, 0.3) [12]. The capability of several *Artemia* species to synthesize polyunsaturated fatty acids (PUFAs, 20:4, 20:5, 22:6) was noted earlier [13]. According to the literature [12], FA compositions of *A. parthenogenetica* decapsulated cysts were practically identical regardless of collection site, year, and month. However, according to the researchers [14], the amount of PUFAs in adult cyst lipids varied depending on collection site and year because of the food composition and quality.

The full group composition of total lipids and compositions of lipophilic compounds and FAs of individual lipid groups from Aral-Sea *A. parthenogenetica* cysts have not been reported in the literature available to us. The present article reports chemical analyses of lipids from *A. parthenogenetica* intact cysts from the Aral Sea shore of the Republic of Karakalpakstan.

Air-dried cyst biomass was milled and extracted first with benzine to remove free lipids (FL). The remaining pulp was extracted by soaking with $CHCl_3$–MeOH (2:1) to extract bound lipids (BLs).

FL contained free FAs and unsaponifiable substances. BLs were separated by column chromatography (CC) over silica gel into neutral lipids (NL), glycolipids (GL), and phospholipids (PL). Table 1 presents the results.

Table 1 shows that the content of total lipids (FL and BL) in *A. parthenogenetica* cyst biomass was significant at 14.36% with FLs containing 24.7% free FAs and BL being mainly PL.

The constituent compositions of FL, GL, and PL were established by TLC on Silufol silica gel plates. FL were separated using solvent systems 1 and 2. Analytical results for *A. parthenogenetica* cyst FL found mainly triacylglycerides and free FAs in association with hydrocarbons, sterols, triterpenols, and xanthophylls.

TLC on Silufol silica gel plates using solvent systems 3 and 5 and comparisons with model compounds (squalene, phytosterols, free FAs) detected sterols as the major constituents of unsaponifiable substances and identified hydrocarbons including squalene, triterpenols, and xanthophylls as minor lipophilic compounds. The carotenoid contents in the unsaponifiable substances were determined using photoelectrocolorimetry (PEC) as 422.76 mg% (or 0.423 mass%).

The GL composition was found using TLC on silica gel and solvent system 6. The predominant constituents of GL were sterylglycosides (R_f 0.49); minor constituents, sterylglycoside esters (R_f 0.86), monogalactosyldiacylglycerides (R_f 0.75), and cerebrosides (R_f 0.38).

PL were analyzed using TLC on silica gel and solvent system 7 and were dominated by phosphatidylcholines (R_f 0.34) and phosphatidylethanolamines (R_f 0.46). Traces of phosphatidic acid (R_f 0.17) and phosphatidylinositol (R_f 0.15) were detected

Parts of FL, GL, and PL were hydrolyzed by alcoholic base. The isolated FAs were methylated by freshly prepared diazomethane [15] and purified. Purified FA methyl esters (FAMEs) were dissolved in hexane and analyzed on an Agilent 6890N chromatograph. Table 2 presents the analytical results.

Table 2 shows that FAs from the three lipid groups were represented by 16–17 constituents. Total unsaturated FAs were greater in FL (73.1%) and considerably lower in GL (55.06%). FL were dominated by FAs 18:1 together with 18:3 (32.31%), 16:1 (22.19), and 16:0 (17.21); GL, by 16:0 (28.16%) together with 18:1 and 18:3 (24.01); PL, by 18:1 and 18:3 (36.9%). Polyunsaturated FAs 20:4, 20:5, and 22:6 were not detected in Aral-Sea *A. parthenogenetica* cyst lipids.

TABLE 2. Fatty Acid Compositions of Free Lipids, Glycolipids, and Phospholipids, mass% of Acids

Fatty acid	FL	GL	PL	Fatty acid	FL	GL	PL
10:0	–	0.25	–	18:2	11.40	9.08	14.97
12:0	0.10	0.6	0.11	X*	6.65	7.5	4.51
14:0	2.84	3.33	1.2	20:0	0.13	0.4	0.27
15:0	1.11	1.13	0.75	20:1	0.55	–	–
16:0	17.21	28.16	15.42	22:0	0.15	1.03	0.7
16:1	22.19	14.47	14.52	24:0	0.09	–	–
17:0	1.24	1.18	1.28	$\Sigma_{sat.}$	26.9	44.94	29.1
18:0	4.03	8.86	9.37	$\Sigma_{unsat.}$	66.45	47.56	66.39
18:1 + 18:3	32.31	24.01	36.9				

*Unidentified acid.

EXPERIMENTAL

FAMEs were analyzed on an Agilent 6890N chromatograph with a flame-ionization detector using a capillary column (30 m × 0.32 mm) packed with HP-5 resin, He carrier gas, and programmed temperature from 150 to 270°C. The carotenoid content was determined by the PEC standard method in hexane in a 10-mm cuvette at 440 nm [16].

Solvent systems: hexane–Et_2O–AcOH (7:3:0.1, 1); hexane–Et_2O (1:1, 2; 8:2, 3; 4:1, 4); heptane–C_6H_6 (9:1, 5); $CHCl_3$–Me_2CO–MeOH–AcOH–H_2O (65:10:20:10:3, 6); and $CHCl_3$–MeOH–NH_3 (65:35:5, 7).

Spots of FL constituents were detected using I_2 vapor and by spraying with aqueous H_2SO_4 solution (50%) followed by heating.

FL were isolated from milled cyst biomass using benzine (bp 72–80°C) in a Soxhlet apparatus for 24 h. The free FA contents in FL were calculated from the acid number [17]. Unsaponifiable substances were isolated from FL via alkaline hydrolysis [17]. Unsaponifiable substances were removed from the FL by alkaline hydrolysis [17]. The $CHCl_3$–MeOH extract of BL was worked up with aqueous $CaCl_2$ solution (0.04%) to remove non-lipid constituents. BL were separated by CC over silica gel into individual groups. NL were eluted by $CHCl_3$; GL, Me_2CO; PL, MeOH. The yields of lipid groups were established gravimetrically.

GL were identified using detection by an aqueous MeOH (1:1) solution (0.5%) of α-naphthol and H_2SO_4 solution (50%); PL, by Vaskovsky's reagent [18]. Constituents of these polar lipids were identified by comparisons with model compounds, qualitative reactions, and chemical conversions [18].

FAMEs were purified twice by preparative TLC on silica gel using solvent system 4. The FAME band on the sorbent was detected by I_2 vapor, scraped from the plates, and desorbed from the silica gel by several elutions with $CHCl_3$. The $CHCl_3$ eluates were combined and evaporated in a rotary evaporator.

Cyst biomass was collected in littoral areas of the Aral Sea in autumn 2017 with 46% humidity. Biomass was dried in a room for several days to 7% moisture and sieved to 0.25 mm size to remove disintegrated cysts, shells, and detritus. Partially purified cysts were further processed to remove as many shells and unconditioned cysts as possible using the literature method [19]. Purified cysts were again dried in air to 5–7% moisture and milled in a ball mill.

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