

Study of the fatty acid composition of *Artemia salina* cysts from Tunisia

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In the present study, decapsulated cysts from eleven Tunisian Artemia salina populations were analysed for their fatty acid profile. Results showed that palmitic (16:0), palmitoleic (16:1n-7), stearic (18:0), cis-vaccenic (18:1n-7), oleic (18:1n-9), linoleic (18:2n-6), linolenic (18:3n-3) and eicosapentaenoic (20:5n-3) were the major fatty acids. The ratio of C16:0/C16:1 fatty acids fluctuated between 0.8 and 3.8. Docosahexaenoic acid (22:6n-3) was absent or found in trace (<0.2%) and arachidonic acids (20:4n-6) was found in higher quantity in all marine-type cysts than in freshwater-type cysts samples. Furthermore, based on the fatty acid profile of the studied Artemia salina populations, we can concluded that Sijoumi, Sahline, Bekalta, Mcheguig and El Adhibet strains could be ascribed to marine-type Artemia, whereas the population from Moknine, Sidi El Hani, Sfax, El Melah, Zarzis and Mhabeul could be categorized as freshwater-type. Principal components analysis showed that palmitoleic acid, linolenic acid, eicosapentaenoic acid, arachidonic acid and C16:0/C16:1 ratio are the most important fatty acids variable between cysts samples, with a total contribution of 68.73% relatively to the first component, whereas, for the second component, palmitic acid, cis-vaccenic acid and oleic acid, are the most important variables, with a total contribution of 56.25%. Moreover, palmitoleic acid, linolenic acid, eicosapentaenoic acid, arachidonic acid and C16:0/C16:1 ratio are the most important fatty acids which contribute to the discrimination between freshwater and marine-type Artemia; while palmitic acid, cis-vaccenic acid and oleic acids represent the major fatty acids permitting differentiation between strains from the same Artemia type, especially for freshwater-type Artemia.

Keywords: *Artemia salina*, fatty acids, marine type *Artemia*, freshwater type *Artemia*, Tunisia

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INTRODUCTION

Species of the brine shrimp, *Artemia*, are found in a variety of harsh environments in many parts of the world (Triantaphyllidis *et al.*, 1998; Van Stappen, 2002) where they encounter severe hypersalinity, high doses of ultraviolet radiation, very low oxygen tensions and extremes of temperature (Abatzopoulos *et al.*, 2002). The temperature range varies widely (5–40°C), the lower limit being consequences of the extreme habitats already discussed such as those in the Tibetan Plateau, Atacama Desert, and Patagonia, in northern and southern Chile, respectively (Gajardo & Beardmore, 2012). *Artemia* environments vary considerably in terms of water anionic composition (Triantaphyllidis *et al.*, 1998), climatological conditions from humid–subhumid (Vanhaecke *et al.*, 1987) to Saharan (Ben Naceur *et al.*, 2009, 2012a) and in altitudes from sea level up to 4500 m (Xin *et al.*, 1994). These stressors on individuals can have an impact on the molecular–cellular and physiological characteristics of *Artemia* (Schuler & Conte, 2009). However, the brine shrimp *Artemia* is one of the very few invertebrates that have the striking ability to adapt, live and reproduce in such extreme

habitats, regulating the osmotic and ionic concentration of their tissues (Vasudevan, 2012).

The brine shrimp *Artemia* is of considerable economic importance in fish and shellfish larviculture (Bengtson *et al.*, 1991). The quality of the *Artemia* product differs from strain to strain and from location to location, in terms of hatching and biometric characteristics (Vanhaecke & Sorgeloos, 1980, 1982), as well as for their nutritional value which is not constant but varies among strains and within batches of each strain, causing unreliable outputs in marine larviculture (Léger *et al.*, 1986).

Modern aquaculture is a young industry that has shown impressive growth in the last three decades. A great deal of interest has been generated in developing an artificial larval diet as an alternative to live feed, but the artificial diet is still generally accepted much less than live food (Tandler & Kolkovski, 1991). *Artemia* is an excellent live food source in larviculture of crustaceans and fish. *Artemia* decapsulated cysts (Stael *et al.*, 1995), juveniles (Lim *et al.*, 2001) and adults (Wouters *et al.*, 1999) have the great advantage of satisfying the nutritional requirements of various aquatic species. However, the nauplii are the most widely used organisms for the fry production of marine as well as freshwater fish and crustaceans, due to their convenience as an off-the-shelf food and requiring only 24 hours of incubation from cysts (Sorgeloos, 1980; Léger *et al.*, 1986).

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Lipids and amino acids are major sources of metabolic energy during the embryonic and pre-feeding larval stages in fish. At hatch, the yolk-sac larvae have high levels of these energy sources, but they are dramatically reduced during the endogenous feeding stage (Evans *et al.*, 2000). Several studies have shown the important role of essential fatty acids (EFA), such as docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6) in larval fish nutrition (McEvoy *et al.*, 1998; Estevez *et al.*, 1999; Sargent *et al.*, 1999). The quantitative and qualitative study of EFA requirements is an active research field in marine larviculture in which important progress has been made (Curé *et al.*, 1996). Levels of this EFA vary tremendously from strain to strain and even from batch to batch, the causal factor being the fluctuations in biochemical composition of the primary producers available to the adult population (Lavens *et al.*, 1989). Previous studies have focused on the factors affecting the fatty acid profile of cysts and nauplii (Léger *et al.*, 1986; Navarro & Amat, 1992; Ruiz *et al.*, 2007a). Specifically, variations in the fatty acid composition of *Artemia* cysts and nauplii are determined by differences in the fatty acid composition of the food ingested by the parental population (Vos *et al.*, 1984; Léger *et al.*, 1986; Lavens *et al.*, 1989), the genotype (Navarro & Amat, 1992) or the selective choice of substrate for feeding (Ruiz *et al.*, 2007a).

There are two limiting factors that constrain the use of *Artemia* nauplii in marine aquaculture. The first is related to the shortage of *Artemia* spp. resources, evidenced by the *Artemia* cyst crisis of the Great Salt Lake of Utah (Van Stappen, 1997), which was exacerbated by a massive increase in cyst demand corresponding with increases in aquaculture world-wide. The second is fatty acid composition, which determines the nutritional value of different stocks as food for marine fish larvae. In recent years, there has been a worldwide effort to find new *Artemia* strains and to characterize them with regard to their potential for application in aquaculture. Watanabe *et al.* (1978) divided *Artemia* cyst samples into two categories according to their fatty acid profiles: freshwater-type *Artemia*, with a high concentration of linolenic acid (ALA, 18:3n-3) and low concentration of eicosapentaenoic acid (EPA, 20:5n-3), which are only suitable for feeding freshwater animals; and marine-type *Artemia*, with a higher EPA content and generally lower ALA, and which are more suitable for culturing marine species.

The aim of this research was to identify the fatty acid profiles of *Artemia* cysts sampled in Tunisia in order to obtain information of interest to aquaculture.

MATERIALS AND METHODS

Cysts location and sampling

Artemia salina cysts samples were collected directly from the banks of salt ponds of eleven Tunisian salt lakes. They were first kept in saturated brine in order to avoid hydration, and then treated following the protocol described by Sorgeloos *et al.* (1986). The biotopes and populations from where cysts were sampled are summarized in Table 1. The biotopes were very diverse: most were located inland, but a few were coastal. Sahline saltworks (SAH) and Sabkhet El Adhibet (ADH) populations, described by Ben Naceur *et al.* (2008, 2010) respectively, are included for the sake of comparison.

Fatty acid analysis

Prior to lipid extraction, cyst samples were hydrated in distilled water under strong aeration until cysts were observed to be completely spherical. They were then decapsulated with sodium hypochlorite (Sorgeloos *et al.*, 1986). Lipid extractions and fatty acid analyses were carried out as in Navarro *et al.* (1992a, b). Total lipids were extracted and stored in chloroform/methanol (ratio 2/1 v/v) with 0.01% butylated hydroxytoluene (BHT) (Sigma Chemical) as an antioxidant. Lipid aliquots were transmethylated overnight after the addition of nonadecanoic fatty acid (19:0) (99% pure; Sigma Chemical) as an internal standard. Fatty acid methyl esters (FAMES) were extracted with hexane/diethyl ether (ratio 1/1 v/v) and purified by thin-layer chromatography (silica gel G60, Merck, Darmstadt, Germany) using hexane/diethyl-ether/acetic acid (ratio 85/15/1.5 v/v) as the solvent system. Analyses of FAMES were performed with a Fisons Instruments GC 8000 (Thermo Electron) gas chromatograph equipped with a fused silica 30 m × 0.25 mm open tubular column (tracer, TR-WAX, film thickness: 0.25 µm; Teknokroma, Barcelona, Spain) and a cold on-column injection system, using helium as carrier and a 50–220°C thermal gradient. Peaks were recorded on a personal computer using the software Chrom-Card for

Table 1. Sources of Tunisian *Artemia* cysts studied for their fatty acids composition.

Sites	Type of habitat	Abbreviation	Geographical coordinates	Area (ha)	Sampling date
Sabkhet Sijoumi	Inland salt lake	SIJ	36°55'38"N 10°15'22"E	2800	2003
Sahline saltworks	Coastal saltwork	SAH	35°45'58"N 10°46'58"E	1200	2006
Sabkhet Sidi El Hani	Inland salt lake	SH	35°37'43"N 10°22'46"E	35,000	2006
Bkalta saltworks	Coastal saltwork	BK	35°34'19"N 11°01'39"E	120	2007
Sabkhet Moknine	Inland salt lake	MOK	35°36'20"N 10°55'37"E	4000	2006
Sfax saltwork	Coastal saltwork	SFX	35°45'N 10°43'E	1500	2005
Sabkhet Mcheguig	Inland salt lake	MCH	34°57'16"N 10°02'28"E	2400	2006
Sabkhet El Adhibet	Inland saltwork	ADH	33°05'42"N 11°24'29"E	12,500	2007
Mhabeul saltwork	Inland saltwork	MHB	33°24'35"N 10°51'20"E	300	2006
Sabkhet El Melah	Inland salt lake	MEL	32°21'34"N 10°55'22"E	15,000	2006
Zarzis saltworks	Inland saltwork	ZAR	33°24'48"N 11°03'43"E	1500	2006

Windows (Fisons CE Instruments, Milan, Italy), and were identified by comparison with known standards. Each fatty acid analysis was done in duplicate except for cysts harvested from Sabkhet Sijoumi (SIJ) and Sabkhet El Adhibet (ADH) where only one sample was subjected to fatty acid analysis.

Statistical analysis

Variables were entered into XLSTAT-Pro 7.5 in order to conduct multivariate principal components analysis (PCA). In this analysis, different variables (relative proportions of selected fatty acids) are displayed on a single plane (principal axis) that accounts for maximum dispersion (variance). Principal component plots are positions of the original variables along the new axes (principal components; PC). Points in the component plot are the variables, and the coordinates of each variable are its factor loadings. Variables at the end of each axis have high loadings on only that factor. Variables near the intersection of the axes are associated with neither factor. The graphical representation (factor score plot) of scores of cases shows the relationships among populations, and is also useful for identifying outliers and unusual cases. The nine most common fatty acids used as variables, for statistical differentiation between population, are the palmitic (16:0), stearic (18:0), palmitoleic (16:1n-7), *cis*-vaccenic (18:1n-7), oleic (18:1n-9), linoleic (18:2n-6), linolenic (18:3n-3), eicosapentaenoic (20:5n-3) and arachidonic (20:4n-6), in addition to the total n-3 fatty acids and C16:0/C16:1 ratio.

RESULTS

Fatty acid profiles of cysts from the Tunisian *Artemia* populations investigated are shown in Tables 2 and 3. In all cases, more than 70% of the peaks were identified, and the total sum of the identified FA vary between 95.6 and 158.1 mg.g⁻¹ of dry weight, for Sfax (SFX) and Sijoumi (SIJ) respectively. Palmitic (16:0), palmitoleic (16:1n-7), stearic (18:0), *cis*-vaccenic (18:1n-7), oleic (18:1n-9), linoleic (18:2n-6), linolenic (18:3n-3) and eicosapentaenoic (20:5n-3) were the major fatty acids. Based on the *Artemia* classification reported by Watanabe *et al.* (1978), *Artemia salina* populations from Sijoumi (SIJ), Sahline (SAH), Bekalta (BK), Mcheuguig (MCH) and El Adhibet (ADH) could be ascribed to marine-type *Artemia* (high EPA, low ALA and C16:0/C16:1 ratio ≤1.1), whereas the population from Moknine (MOK), Sidi El Hani (SH), Sfax (SFX), El Melah (MEL), Zarzis (ZAR) and Mhabeul (MHB) could be categorized as freshwater-type exhibiting a higher quantity of C18:3n-3 than C20:5n-3, and C16:0/C16:1 ratio higher than 1.6. On the other hand, docosahexaenoic acid (22:6n-3) was absent

Table 2. Mean ± SD of fatty acid composition (% of total fatty acids) of total lipids from Tunisian populations of *Artemia* cysts. Data are mean of 2 replicates except for cysts harvested from SIJ and ADH where only one sample was subjected to fatty acid analysis.

	SIJ	SAH*	MOK	BK	SH	MCH	SFX	MEL	ZAR	MHB	ADH**
C14:0	1.5	2.5 ± 0.1	0.5 ± 0.0	1.2 ± 0.2	0.7 ± 0.0	0.4 ± 0.5	0.8 ± 0.2	1.0 ± 0.0	0.9 ± 0.2	0.6 ± 0.1	1.8
C14:1n-5	0.2	0.9 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	Tr	1.0
C15:0	1.0	0.4 ± 0.1	1.7 ± 0.5	1.1 ± 0.0	2.2 ± 0.2	0.8 ± 0.1	0.8 ± 0.0	1.5 ± 0.1	1.2 ± 0.2	1.6 ± 0.3	0.4
C15:1n-5	0.5	0.6 ± 0.1	0.2 ± 0.1	0.6 ± 0.3	0.2 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	Tr	0.6
C16:0	12.5	15.0 ± 1.0	9.3 ± 2.0	12.6 ± 0.8	11.8 ± 0.6	12.3 ± 1.3	11.2 ± 0.3	15.5 ± 0.2	17.8 ± 3.3	14.0 ± 2.4	16.0
C16:1n-7	11.0	18.9 ± 1.0	5.7 ± 1.5	11.6 ± 2.8	7.3 ± 0.5	12.9 ± 1.6	6.0 ± 0.3	5.9 ± 0.2	4.6 ± 0.9	3.8 ± 0.7	14.1
C16:2	0.6	Nd	0.2 ± 0.1	0.4 ± 0.2	0.4 ± 0.0	0.3 ± 0.3	0.7 ± 0.8	0.4 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	Nd
C17:0	0.9	0.7 ± 0.1	0.9 ± 0.3	1.0 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	1.2
C16:3	1.8	Nd	1.2 ± 0.3	1.4 ± 0.6	1.4 ± 0.1	0.9 ± 0.0	0.9 ± 0.0	2.3 ± 0.1	3.1 ± 1.0	1.7 ± 0.3	Nd
C18:0	4.6	4.2 ± 0.1	4.7 ± 0.3	5.1 ± 0.3	5.6 ± 0.2	4.4 ± 0.4	7.1 ± 1.2	4.9 ± 0.4	5.6 ± 0.5	4.8 ± 0.8	4.9
C18:1n-9	12.6	12.6 ± 1.1	12.6 ± 3.4	11.3 ± 1.8	12.6 ± 1.3	12.3 ± 1.8	11.2 ± 0.4	15.7 ± 0.4	17.1 ± 3.1	16.1 ± 2.8	13.1
C18:1n-7	9.5	11.7 ± 2.1	8.2 ± 2.1	14.1 ± 0.1	24.9 ± 1.2	11.6 ± 1.7	12.2 ± 0.7	7.8 ± 0.1	7.0 ± 1.3	7.4 ± 1.3	11.0
C18:2n-6	2.4	3.3 ± 0.1	4.9 ± 0.9	4.1 ± 0.9	5.9 ± 0.5	2.0 ± 0.1	6.7 ± 0.6	7.2 ± 0.1	3.6 ± 0.7	6.2 ± 1.1	4.0
C18:3n-6	0.3	0.7 ± 0.1	0.9 ± 0.9	0.2 ± 0.1	Tr	0.3 ± 0.0	Tr	Tr	0.2 ± 0.0	Tr	0.4
C18:3n-3***	4.5	2.7 ± 0.1	14.8 ± 4.3	3.9 ± 2.2	13.8 ± 1.1	5.4 ± 0.9	13.0 ± 0.8	17.7 ± 0.4	6.1 ± 1.1	15.2 ± 2.7	6.6
C18:4n-3	1.1	1.2 ± 0.1	3.6 ± 0.5	1.0 ± 0.3	2.6 ± 0.2	1.1 ± 0.0	1.7 ± 0.1	3.5 ± 0.1	1.1 ± 0.1	2.2 ± 0.4	1.5
C20:0	0.1	0.2 ± 0.0	0.2 ± 0.2	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
C20:1n-9	0.3	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.0	0.8 ± 0.4	0.5 ± 0.1	0.4
C20:1n-7	0.2	Nd	0.3 ± 0.3	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	Tr	Tr	Tr	Tr	Tr
C20:2n-6	0.3	Nd	0.3 ± 0.4	0.3 ± 0.2	0.3 ± 0.0	0.5 ± 0.4	0.5 ± 0.1	0.3 ± 0.1	0.9 ± 0.7	0.4 ± 0.1	Nd
C20:3n-6	0.1	Tr	0.4 ± 0.4	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.3	Tr	Tr	0.5 ± 0.5	0.2 ± 0.1	Tr
C20:4n-6	1.1	1.2 ± 0.1	0.3 ± 0.3	1.3 ± 0.6	Tr	0.4 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	Tr	0.2 ± 0.0	0.7
C20:3n-3	0.9	Tr	1.0 ± 0.7	1.0 ± 0.3	1.3 ± 0.7	1.3 ± 0.1	2.1 ± 0.4	0.9 ± 0.1	2.2 ± 0.3 ^c	0.7 ± 0.5	0.3
C20:4n-3	0.3	0.3 ± 0.0	0.7 ± 0.3	0.3 ± 0.1	0.4 ± 0.0	0.5 ± 0.2	Tr	0.5 ± 0.1	0.4 ± 0.4	0.2 ± 0.2	0.3
C20:5n-3***	7.6	14.8 ± 0.1	0.6 ± 0.1	9.0 ± 4.9	0.2 ± 0.0	12.3 ± 1.9	2.8 ± 0.3	1.3 ± 0.0	2.2 ± 3.1	2.8 ± 1.0	11.2
C22:5n-3	Nd	Tr	Tr	Nd	Tr	Tr	Nd	Nd	Nd	Tr	0.3
C22:6n-3	0.2	Tr	Tr	0.2 ± 0.0	Tr	0.2 ± 0.3	Nd	Nd	Nd	Tr	0.2
Sum	76.4	92.6	74.4 ± 14.4	83.1 ± 7.8	93.7 ± 4.6	82.2 ± 9.0	80.7 ± 0.9	88.7 ± 1.2	77.2 ± 6.6	79.9 ± 7.6	79.4
Total n-3	14.6	21.5	20.8	15.3	18.3	20.8	19.7	24.0	12.0	21.0	18.6
Total n-6	4.2	4.7	6.4	5.9	6.3	3.2	7.9	7.9	4.8	6.8	5.2
n-3/n-6	3.4	4.5	3.2	2.5	2.9	6.5	2.4	3.0	2.4	3.1	1.6
16:0/16:1	1.1	0.8	1.6	1.1	1.6	0.9	1.8	2.6	3.8	3.6	1.1

Tr, trace amount (<0.2%); Nd, not detected; *, Ben Naceur *et al.* (2008); **, Ben Naceur *et al.* (2010); ***, Ben Naceur *et al.* (2012b).

Table 3. Mean \pm SD of fatty acid composition ($\text{mg}\cdot\text{g}^{-1}$ DW) of total lipids from Tunisian populations of *Artemia* cysts. Data are mean of 2 replicates except for cysts harvested from SIJ and ADH where only one sample was subjected to fatty acid analysis.

Fatty acid	Sij	Sah*	Mok	Bk	Sh	Mch	Sfx	Mel	Zar	Mhb	Adh**
C14:0	3.0	3.5 \pm 0.1	1.1 \pm 0.1	2.3 \pm 0.2	1.0 \pm 0.5	0.4 \pm 0.4	0.9 \pm 0.3	1.2 \pm 0.5	1.3 \pm 0.1	0.7 \pm 0.0	2.5
C14:1n-5	0.4	1.2 \pm 0.1	0.4 \pm 0.3	0.5 \pm 0.2	0.5 \pm 0.4	0.4 \pm 0.4	0.2 \pm 0.1	0.2 \pm 0.1	0.3 \pm 0.2	Tr	1.5
C15:0	2.1	0.6 \pm 0.1	3.5 \pm 0.4	2.2 \pm 0.5	3.2 \pm 1.2	1.1 \pm 0.5	1.0 \pm 0.0	1.7 \pm 0.8	1.8 \pm 0.1	1.9 \pm 0.0	0.6
C15:1	1.0	0.9 \pm 0.1	0.5 \pm 0.0	1.2 \pm 0.4	0.3 \pm 0.2	0.4 \pm 0.2	0.5 \pm 0.1	0.4 \pm 0.2	0.4 \pm 0.0	Tr	0.9
C16:0	25.9	21.4 \pm 0.8	19.1 \pm 0.5	25.9 \pm 4.8	17.6 \pm 7.6	17.7 \pm 8.2	13.3 \pm 1.7	18.2 \pm 7.2	27.0 \pm 1.8	16.3 \pm 0.7	22.7
C16:1n-7	22.7	27.0 \pm 1.1	11.5 \pm 0.9	23.3 \pm 0.3	10.9 \pm 4.6	18.4 \pm 8.2	7.1 \pm 0.4	7.0 \pm 3.0	7.0 \pm 0.5	4.5 \pm 0.2	20.1
C16:2	1.2	Tr	0.4 \pm 0.1	0.8 \pm 0.3	0.6 \pm 0.3	0.3 \pm 0.3	0.9 \pm 1.0	0.4 \pm 0.2	0.6 \pm 0.3	0.2 \pm 0.0	Tr
C17:0	1.9	0.9 \pm 0.1	1.8 \pm 0.2	1.9 \pm 0.1	1.3 \pm 0.5	0.9 \pm 0.4	1.1 \pm 0.0	0.7 \pm 0.4	0.9 \pm 0.0	0.8 \pm 0.0	1.8
C16:3	3.7	Tr	2.5 \pm 0.1	2.7 \pm 0.5	2.1 \pm 0.9	1.3 \pm 0.7	1.0 \pm 0.0	2.2 \pm 1.1	4.7 \pm 0.9	2.0 \pm 0.1	Tr
C18:0	9.4	6.0 \pm 0.2	9.8 \pm 1.2	10.7 \pm 3.2	8.6 \pm 4.5	6.3 \pm 3.0	8.4 \pm 2.3	5.7 \pm 1.9	8.5 \pm 0.2	5.6 \pm 0.2	6.9
C18:1n-9	26.1	17.7 \pm 0.6	25.6 \pm 2.1	23.0 \pm 2.1	19.6 \pm 1.1	17.5 \pm 7.4	13.3 \pm 0.8	18.6 \pm 7.9	25.9 \pm 1.6	18.7 \pm 0.8	18.6
C18:1n-7	19.6	16.7 \pm 0.7	16.8 \pm 1.1	29.3 \pm 7.5	30.8 \pm 25.5	16.6 \pm 7.1	14.4 \pm 0.6	9.2 \pm 3.6	10.6 \pm 0.6	8.5 \pm 0.4	15.6
C18:2n-6	5.1	4.6 \pm 0.1	10.1 \pm 0.0	8.8 \pm 4.0	5.7 \pm 7.9	2.9 \pm 1.5	7.9 \pm 0.0	8.5 \pm 3.5	5.5 \pm 0.4	7.2 \pm 0.2	6.1
C18:3n-6	0.6	1.0 \pm 0.1	1.7 \pm 1.6	0.3 \pm 0.0	Tr	0.4 \pm 0.3	Tr	Tr	0.3 \pm 0.1	Tr	0.6
C18:3n-3	9.2	3.9 \pm 0.1	30.1 \pm 3.1	8.6 \pm 6.6	20.5 \pm 8.6	7.7 \pm 3.2	15.4 \pm 0.5	21.0 \pm 8.9	9.3 \pm 0.6	18.3 \pm 7.1	9.4
C18:4n-3	2.3	1.7 \pm 0.1	7.4 \pm 0.5	2.2 \pm 1.1	2.5 \pm 3.5	1.6 \pm 0.9	2.0 \pm 0.0	4.1 \pm 1.8	1.8 \pm 0.3	2.5 \pm 0.1	2.2
C20:0	Tr	0.3 \pm 0.1	0.5 \pm 0.5	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
C20:1n-9	0.7	0.3 \pm 0.1	1.0 \pm 0.5	1.0 \pm 0.5	0.6 \pm 0.3	0.7 \pm 0.6	0.3 \pm 0.1	0.5 \pm 0.2	1.2 \pm 0.7	0.5 \pm 0.0	0.6
C20:1n-7	0.4	Tr	0.7 \pm 0.7	0.7 \pm 0.3	0.3 \pm 0.2	0.3 \pm 0.2	Tr	Tr	Tr	0.2 \pm 0.0	0.1
C20:2n-6	0.7	Tr	0.8 \pm 0.9	0.7 \pm 0.6	0.5 \pm 0.3	0.8 \pm 0.9	0.6 \pm 0.1	0.4 \pm 0.1	1.4 \pm 1.3	0.5 \pm 0.0	Tr
C20:3n-6	Tr	Tr	0.9 \pm 1.0	0.5 \pm 0.3	0.4 \pm 0.3	0.5 \pm 0.7	Tr	Tr	0.7 \pm 0.8	0.2 \pm 0.0	0.1
C20:4n-6	2.3	1.7 \pm 0.1	0.6 \pm 0.7	2.6 \pm 0.6	Tr	0.6 \pm 0.4	0.9 \pm 0.1	0.3 \pm 0.2	Tr	0.2 \pm 0.0	1.0
C20:3n-3	1.9	Tr	2.3 \pm 1.9	2.1 \pm 1.2	2.2 \pm 2.0	1.8 \pm 0.8	2.5 \pm 0.8	1.0 \pm 0.3	3.4 \pm 0.8	0.7 \pm 0.4	0.1
C20:4n-3	0.6	0.4 \pm 0.1	1.5 \pm 0.8	0.6 \pm 0.3	0.6 \pm 0.3	0.8 \pm 0.7	Tr	0.7 \pm 0.3	0.7 \pm 0.7	0.2 \pm 0.2	0.5
C20:5n-3	15.7	20.8 \pm 0.9	1.2 \pm 0.1	17.4 \pm 5.5	0.3 \pm 0.2	17.5 \pm 7.4	3.3 \pm 0.0	1.6 \pm 0.7	0.6 \pm 0.1	3.4 \pm 1.8	15.9
C22:5n-3	Nd	Tr	Tr	Nd	Tr	0.1 \pm 0.2	Nd	Nd	Nd	Tr	0.4
C22:6n-3	0.3	Tr	Tr	0.4 \pm 0.1	Tr	0.4 \pm 0.6	Nd	Nd	Nd	Tr	0.2
Sum	158.1	131.3	152.4 \pm 0.4	170.0 \pm 26.9	131.5 \pm 69.2	118.4 \pm 54.1	95.7 \pm 8.2	104.6 \pm 43.3	114.6 \pm 1.4	93.7 \pm 11.2	128.5
Total n-3	28.2	26.9	40.5	29.1	23.9	28.2	20.8	27.3	12.2	24.5	28.7
Total n-6	9.0	7.4	14.0	12.9	6.7	5.4	9.4	9.4	8.1	8.0	7.8
n-3/n-6	3.1	3.6	2.8	2.2	3.5	5.2	2.2	2.9	1.5	3.0	3.67
16:0/16:1	1.1	0.8	1.6	1.1	1.6	0.9	1.8	2.6	3.8	3.6	1.1

Tr, trace amount ($<0.2\%$); Nd, not detected; *, Ben Naceur *et al.* (2008); **, Ben Naceur *et al.* (2010).

or found in trace (<0.2%). Proportions of palmitoleic acid (16:1n-7) were higher in marine-type populations. In general, arachidonic acid (20:4n-6) was found in higher quantity in all marine-type cysts (except in those harvested from Mcheguig), than in freshwater-type cysts samples (except in those samples from Sfax).

Principal component analysis revealed two main directions of variation. Variables were most correlated with factor 1 (horizontal axis) rather than with factor 2 (vertical axis), with axis 1 explaining 41.92% and axis 2 explaining an additional 22.95% of the total variance. The component plot (Figure 1A) obtained from a PCA illustrates the variables that were responsible for separation along the two PCs calculated. The two PCs of this analysis accounted for 64.87% of the variation in the data set. Variables defining marine-type (C16:1n-7, C20:5n-3 and C20:4n-6) or freshwater-type (C18:3n-3 and C16:0/C16:1 ratio) are associated with component 1. In fact, relatively to the first component (PCA axis 1), palmitoleic acid (16:1n-7), linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), arachidonic acid (20:4n-6) and C16:0/C16:1 ratio are the most important fatty acids variable between cysts samples differentiation with a total contribution of 68.73%. Whereas, for the second component (PCA axis 2), palmitic acid (16:0), *cis*-vaccenic acid (18:1n-7) and oleic acid (18:1n-9), are the most important variables with a total contribution of 56.25% (Table 4). Negative loadings of linolenic acids (ALA) and C16:0/C16:1 ratio were observed relatively to the principal component 1, whereas palmitoleic acid, eicosapentaenoic acid (EPA) and arachidonic acid (ARA) show positive loadings. When the fatty acids data were projected onto the PCs generated, a factor score plot was obtained (Figure 1B) in which the populations were grouped according to variable (fatty acid) loadings. Marine-type populations are located on the right hand side of component 1, and freshwater-type populations on the left hand side. In fact, the ACP divided studied populations into two groups regarding the principal component 1 (Table 4); the first group with a positive populations component including marine-type populations (SIJ, SAH, BK, MCH and ADH), and the second group with a negative populations component including freshwater-type populations (MOK, SH, SFX, MEL, ZAR and MHB). Note that the freshwater-type populations

were more dispersed than marine-type populations, which can be explained by the difference observed in the quantity of the fatty acids defining freshwater-type such as C18:1n-9 and C18:2n-6, and C16:0/C16:1 ratio.

DISCUSSION

Since Seale (1933) and Rollefson (1939) reported the high nutritional value of freshly hatched nauplii of *Artemia* as food for fish fry, the use of brine shrimp *Artemia* in aquaculture has increased exponentially. Nevertheless, nutritional deficiencies of the freshly hatched *Artemia* nauplii have been reported by some workers such as Dannevig & Hansen (1952), who considered that *Artemia* diet was not adequate for the culture of larval *Clupea*, or Morris (1956), who pointed out that fish larvae did not thrive as well on *Artemia* nauplii which have used up their yolk as they do on freshly hatched nauplii. In fact, in the late 1970s, when many fish and shrimp hatcheries started to be commercial, switching from one source of *Artemia* to another provoked unexpected problems. Japanese, American and European researchers studied these problems and soon confirmed variations in nutritional value when using different geographical sources of *Artemia* for fish and shrimp species (see Merchie, 1996). Studies in Japan and the multidisciplinary 'International Study on *Artemia*' revealed that the concentration of the essential fatty acids (eicosapentaenoic acid, EPA) in *Artemia* nauplii was determining its nutritional value for larvae of various marine fish and crustaceans (Léger *et al.*, 1986). Therefore, *Artemia* nauplii have been offered as food source for more than 85% of the marine animals cultivated so far (Kinne, 1977). Marine fish, particularly at early stages of development, require highly unsaturated fatty acids (HUFA) of the (n-3) family (Léger *et al.*, 1979). In fact, as marine fish have a limited capability to elongate and desaturate linolenic acid (18:3n-3) to (n-3) HUFA, they rely on dietary sources of (n-3) HUFA to satisfy their requirements for eicosapentaenoic and docosahexaenoic acid (Watanabe, 1993). For this fact, the nutritional value of nauplii seems to be determined by their content of (n-3) HUFA.

It is known that the fatty acid composition of *Artemia* nauplii can vary among strains and also from one batch to

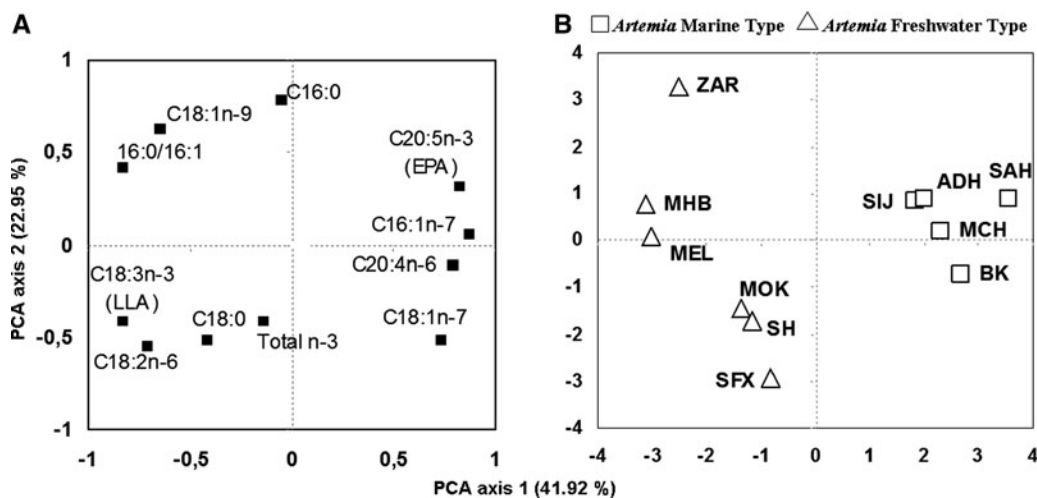


Fig. 1. (A) Component plot and (B) factor score plot, of principal component analysis (PCA) of selected fatty acids (% of total fatty acids) from total lipids of Tunisian *Artemia* cysts.

Table 4. Contribution and portion of each parameter (selected fatty acids in %) used to characterize decapsulated cysts of Tunisian *Artemia* populations.

	Variables contribution (%) of selected fatty acids			Population component	
	F1	F2		F1	F2
C16:0	0.202	26.513	SIJ	1.838	0.839
C16:1n-7	15.633	0.794	SAH	3.550	0.863
C18:0	2.876	9.186	MOK	-1.356	-1.464
C18:1n-9	8.842	19.167	BK	2.676	-0.707
C18:1n-7	9.400	10.571	SH	-1.180	-1.710
C18:2n-6	9.619	9.472	MCH	2.306	0.185
C18:3n-3 (ALA)	12.642	7.962	SFX	-0.827	-2.936
C20:5n-3 (EPA)	14.741	2.941	MEL	-3.033	0.065
C20:4n-6 (ARA)	12.357	0.158	ZAR	-2.521	3.295
Total n-3	0.324	6.387	MHB	-3.116	0.769
16:0/16:1	13.365	6.850	ADH	1.664	0.802
Total variance explained					
% of variance	49.921	22.949			
Cumulative %	49.921	72.869			

another within the same strain (Léger *et al.*, 1986). Several authors reported that the fatty acid profile of *Artemia* reflect the fatty acid profile of their food resulting from the variations in the composition of the microalgae available in their natural habitat (Léger *et al.*, 1986; Lavens *et al.*, 1989; Navarro & Amat, 1992). However, Navarro & Amat (1992) brought attention to a possible genotypic influence on the fatty acid profile of *Artemia*, given the presence and proportion of some fatty acids in cysts, irrespective of dietary levels available to parental populations. Most recently, Ruiz *et al.* (2007b), after examination of the fatty acids profile of two *Artemia* species, *A. persimilis* and *A. franciscana* cultured in coexistence at mesocosm scale, revealed that interspecific differences in fatty acid composition are greater than intraspecific variability, demonstrating that aside from a high phenotypic effect of diet on the fatty acid composition of the animals, a species-specific genotypic effect should not be discarded. In the present study we reported the fatty acid profile of eleven *Artemia salina* populations from Tunisia. The results revealed that, such as for other *Artemia* populations, palmitic (16:0), stearic (18:0), palmitoleic (16:1n-7), *cis*-vaccenic (18:1n-7), oleic (18:1n-9), linoleic (18:2n-6), linolenic (18:3n-3) and/or eicosapentaenoic (20:5n-3) acids were the most important fatty acids on *Artemia* decapsulated cysts (see Navarro *et al.*, 1992a; Abatzopoulos *et al.*, 2006; Ruiz *et al.*, 2007a). In addition, the low rate of the arachidonic acid and/or the lack of the docosahexaenoic acid observed in Tunisian *Artemia* populations were also reported for other *Artemia* strains from different geographical origin (see Navarro *et al.*, 1992a; Kara *et al.*, 2004; Camargo *et al.*, 2005; Abatzopoulos *et al.*, 2006; Ruiz *et al.*, 2007a).

Artemia stocks from different geographical origins were divided into two main categories: 'marine type *Artemia*' with a high content in eicosapentaenoic acid (EPA); and 'freshwater type *Artemia*' with a high concentration of linolenic acid (ALA). Moreover, Navarro *et al.* (1993) stated that the ratio C16:0/C16:1 was lower in marine-type than in freshwater-type cysts. Although, these authors considered in this ratio the sum of C16:1n-9 and C16:1n-7, our results

support this assertion, because the C16:0/C16:1 ratio was lower (0.8–1.1) in all marine-type populations than in freshwater-type (1.6–3.8). It can thus be concluded that the C16:0/C16:1 ratio is a good biomarker that characterizes samples in terms of marine or freshwater-type, especially for *Artemia* cysts which exhibit approximately the same level of ALA and EPA, and, hence, would be classified as mixed type, such as reported by Ruiz *et al.* (2007a). Furthermore, Watanabe (1987) reported that ARA was associated with marine-type *Artemia*. In our case, all marine-type populations described in this work exhibited higher values of ARA than did freshwater types, except for MCH population (marine-type *Artemia*; inland site) and SFX population (freshwater-type *Artemia*; coastal site). This result is in contrast with the results of Navarro *et al.* (1992a), where this fatty acid was present regardless of the inland (freshwater-type) or coastal (marine-type) origin of populations. Otherwise, PCA revealed that palmitoleic acid (16:1n-7), linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), arachidonic acid (20:4n-6) and C16:0/C16:1 ratio are the most important fatty acids that contribute to the discrimination between freshwater and marine-type *Artemia* with a total contribution of 68.73%, according to the first component, explaining 41.92% of the total variance. While, according to the second component (explaining 22.95% of the total variance), palmitic (16:0), *cis*-vaccenic (18:1n-7) and oleic (18:1n-9) represents the major fatty acids permitting the differentiation between strains from the same *Artemia* type, specially for freshwater-type *Artemia*, with a total contribution of 56.25%.

Considering the potential use of the Tunisian *Artemia* population in aquaculture, our results revealed that Sijoumi, Sahline, Bekalta, Mcheguig and El Adhibet populations exhibit higher values of EPA, ranging from 15.7 to 20.8 mg.g⁻¹ dry weight. These values were similar to or even higher than those recorded for other *Artemia* strains: 0.3–2.4 mg.g⁻¹ dry weight for San Francisco Bay and 0.3–8.6 mg.g⁻¹ dry weight for Colombian strains (Camargo *et al.*, 2005), 1.8–7.2 mg.g⁻¹ dry weight for *Artemia urmiana* (Abatzopoulos *et al.*, 2006), 21.6–43.0 mg.g⁻¹ dry weight for *Artemia tibetiana* (Van Stappen *et al.*, 2003) and 2.4–10.96 mg.g⁻¹ dry weight for Spanish strains (Navarro *et al.*, 1992a). Merchie (1996) revealed that commercial provisions of *Artemia* cysts containing high EPA levels are limited and, consequently, these cysts are very expensive. The relationship between fatty acid composition of *Artemia* nauplii used for aquaculture purposes and the successful culture of several fish and crustacean species is well studied, in particular the levels of EPA and DHA, as well as their ratio (DHA/EPA) (Sui *et al.*, 2007). These fatty acids play a crucial role in aquaculture applications for the formation of biological membranes, growth, stress resistance and pigmentation in cultured marine organisms (Mourete *et al.*, 1993). However, as in other natural *Artemia* populations, the ratio DHA/EPA was found in a low level in these Tunisian studied populations. Moreover, apart from these two essential fatty acids, it has been shown that arachidonic acid (ARA) also plays a significant role on the fish larval growth and pigmentation in several marine fish (Koven *et al.*, 2000) since it provides precursors for eicosanoid production (Castell *et al.*, 1994). However, the requirement for ARA in fish seems to depend on the fish species and larval development, and needs to be dosed with extreme care since its effect seems to depend on the DHA concentration (Koven *et al.*, 2000). The

comparison between ARA contents obtained in the studied Tunisian population (from <0.2 to 1.5 mg.g^{-1} dry weight) and those reported for other *Artemia* strain revealed that this fatty acid was present in more or less similar quantity (Navarro *et al.*, 1992a; Camargo *et al.*, 2005; Ruiz *et al.*, 2007a). Therefore, to ensure these quantities in EPA (for freshwater-type *Artemia* cysts), ARA and DHA, special enrichment formulations had to be developed (Sorgeloos *et al.*, 1986).

In conclusion, Tunisian *Artemia* cysts show that, as for the other *Artemia* populations, palmitic (16:0), palmitoleic (16:1n-7), stearic (18:0), *cis*-vaccenic (18:1n-7), oleic (18:1n-9), linoleic (18:2n-6), linolenic (18:3n-3) and eicosapentaenoic (20:5n-3) were the major fatty acids, and that *Artemia salina* populations from Sijoumi, Sahline, Bekalta, Mcheguig and El Adhibet could be ascribed to marine-type *Artemia*, whereas the population from Moknine, Sidi El Hani, Sfax, El Melah, Zarzis and Mhabeul could be categorized as freshwater-type. PCA revealed that palmitoleic acid (16:1n-7), linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), arachidonic acid (20:4n-6) and C16:0/C16:1 ratio are the most important fatty acids which contribute to the discrimination between freshwater and marine-type *Artemia*, and that palmitic (16:0), *cis*-vaccenic (18:1n-7) and oleic (18:1n-9) represents the major fatty acids permitting the differentiation between strains from the same *Artemia* type, specially for freshwater-type *Artemia*. Finally, further research on marine and freshwater-type *Artemia* is needed to clarify the role of environmental factors and genetic characteristics which can affect the fatty acid profile.

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