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Quality characterization of cysts of the brine shrimp *Artemia salina* from Tunisia focusing on their potential use in aquaculture

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The aim of this study was to characterize *Artemia* cysts harvested from Tunisian salt lakes to evaluate their potential use in aquaculture. For this, we examined naupliar size, protein, carbohydrate, total lipids, ash, linolenic acid (LNA), and eicosapentaenoic acid (EPA) content in decapsulated cysts, we determined hatching characteristics, and we made comparisons with commercial cysts of *Artemia franciscana* from Great Salt Lake (GSL), USA. For Tunisian *Artemia* populations, mean naupliar size ranged from 422.6 to 485.4 μm. Protein level ranged from 42.1 to 57.1%, carbohydrate level fluctuated from 8.8 to 21.9%, whereas lipid content varied from 12.1 to 20.2% and ash from 7.2 to 12.1% of the dry weight. Essential fatty acid analysis showed that linolenic and eicosapentaenoic acid percentages varied from 2.7 to 17.7% and from 0.2 to 14.8%, respectively. For untreated cysts the maximum hatching percentage was obtained for Sabkhet Mcheguig with 63.7%. For decapsulated cysts, the maximum hatching percentage was obtained by cysts harvested in Sahline saltwork (81.1%). After H_2O_2 treatment, the highest hatching efficiency was obtained for Sfax with 143111 nauplii g^{-1} of dry cysts. The comparison between Tunisian and commercial GSL cysts using principal component analysis showed that linolenic acid, eicosapentaenoic acid, and hatching characteristics (hatching efficiency, hatching percentage, and synchronization time) are the most important variables for cyst quality differentiation, with a total contribution of 93.36%.

Key words: *Artemia*, nutritional quality, hatching performance, aquaculture, Tunisia.

INTRODUCTION

The primary problem in rearing fish larvae is that of food supply (Léger *et al*., 1986). The brine shrimp *Artemia* is widely used in aquaculture as a live feed, with the nauplius stages being the most utilized food for crustacean and fish larvae (Sorgeloos *et al*., 2001). In fact, since the introduction of *Artemia* nauplii as a food source for fish larvae by Seale (1933), development of hatchery activities of fish and shrimp has greatly accelerated (Sorgeloos, 1980).

Biotopes of the brine shrimp *Artemia* vary considerably with regard to physical and chemical parameters (Bowen *et al*., 1985; Lenz & Browne, 1991; Xin *et al*., 1994; Abatzopoulos *et al*., 1998). This variability results in numerous different strains with various phenotypes and different biochemical and physiological characteristics which are related to many ecological changes (Triantaphyllidis *et al*., 1998). The characterization of an *Artemia* population, from an aquaculture perspective, can be done using cyst and naupliar biometry, nutritional profile (e.g. fatty acids, total lipids, ash content etc.), buoyancy, and hatching characteristics. Aquaculture activities, especially rearing of economically important marine fish and shellfish, have shown a constantly increasing trend over the last few decades. The decline of *Artemia* cyst harvests from the Great Salt Lake in Utah, USA since 1977 (Lavens & Sorgeloos, 2000) has intensified the search for alternative cyst sources, especially in inland lakes that are sufficiently large and productive

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to justify commercial exploitation. Moreover, Dhont & Sorgeloos (2002) reported that numerous managed ponds and saltworks worldwide can yield small quantities of cysts (1-20 metric tons each), thus providing interesting opportunities for the development of local economies. In Tunisia, the brine shrimp *Artemia* has been reported from several inland and costal salt lakes (sebkha) and saltworks (see Ben Naceur *et al*., 2009a). However, limited information is available about *Artemia* density, biomass, composition etc., in these sites. For example, concerning *Artemia* density, only four Tunisian *Artemia* populations have been studied so far (i.e. Sahline Saltwork: 357.25 individuals l^{-1}, Mahdhi *et al*., 2010; Sabkhet Moknine: 504 individuals l^{-1}, Mahdhi *et al*., 2010; Sfax Saltwork: 1414 individuals l^{-1}, Guermazi, 2008; Sabkhet El Adibet: 60.27 individuals l^{-1}, Ben Naceur *et al*., 2009b). Moreover, little data exist on the biometry, biochemical composition, hatching characteristics, and biomass of these populations.

In the current paper, 11 *Artemia* populations from Tunisia are characterized, based on protein, carbohydrate, total lipids, ash, linolenic acid (LNA), and eicosapentaenoic acid (EPA) content in decapsulated cysts as well as on hatching characteristics. The Tunisian *Artemia* populations were also compared with commercial cysts of *Artemia franciscana* from Great Salt Lake (Maniatsi *et al*., 2009) in order to determine their potential use in aquaculture. The data presented here might prove useful for the aquaculture industry in the context of providing new *Artemia* sources.

MATERIALS AND METHODS

Collection of samples

The biotopes, characteristics, and location from where cysts were sampled as well as taxonomical status of *Artemia* populations are summarized in Table 1. Sahline saltwork and Sabkhet El Adhibet, described by Ben Naceur *et al*. (2008, 2010) are included for the sake of comparison. Once harvested from the shore of the sabkha or saltworks, cysts were mixed with salt and transported to the laboratory where they were cleaned by differential flotation in saturated brine and freshwater as described by Sorgeloos *et al*. (1986). Commercial cysts from Great Salt Lake (GSL) were used as reference for comparisons with the Tunisian *Artemia* populations.

Nauplii characterization

To analyze the biometrical characteristics of nauplii, cysts were hatched by incubating them in natural seawater (32 g l^{-1}) at 28°C with continuous illumination of 2000 lux. The length of instar-I nauplii ($n = 100$) was measured under a microscope equipped with a calibrated micrometer eye piece. Data obtained were treated by analysis of variance (one-way ANOVA), and averages compared with Duncan's test using Sta-

TABLE 1. Sources of Tunisian *Artemia* populations studied for their potential use in aquaculture. Cyst material from Great Salt Lake, USA was used for comparison

Sites	Type of habitat	Abbreviation	Geographical coordinates	Approximate surface (km^2)	Sampling date	Taxonomical status
Sabkhet Sijoumi	Inland salt lake	SIJ	36°55′38″N-10°15′22″E	28-30	2003	*A. salina* [1,3]
Sahline Saltwork	Coastal saltwork	SAH	35°45′58″N-10°46′58″E	12	2006	*A. salina* [1,2,3]
Sabkhet Moknine	Inland salt lake	MOK	35°36′20″N-10°55′37″E	40	2006	*A. salina* [3]
Bkalta Saltwork	Coastal saltwork	BK	35°34′19″N-11°01′39″E	1.2	2007	*A. salina* [3]
Sabkhet Sidi El Hani	Inland salt lake	SH	35°37′43″N-10°22′46″E	350	2006	*A. salina* [3]
Sabkhet Mcheguig	Inland salt lake	MCH	34°57′16″N-10°02′28″E	24	2006	*A. salina* [3]
Sfax Saltwork	Coastal saltwork	SFX	35°45′N-10°43′E	15	2005	*A. salina* [3]
Sabkhet El Melah	Inland salt lake	MEL	32°21′34″N-10°55′22″E	150	2006	*A. salina* [3]
Zarzis Saltwork	Coastal saltwork	ZAR	33°24′48″N-11°03′43″E	1.5	2006	*A. salina* [3]
Mhabeul Saltwork	Inland salt lake	MHB	33°24′35″N-10°51′20″E	3	2006	*A. salina* [3]
Sabkhet El Adhibet	Inland saltwork	ADH	33°05′42″N-11°24′29″E	125	2007	*A. salina* [1,2,3]
Great Salt Lake	Commercial cysts	GSL	–	–	–	–

[1] Romdhane *et al*. (2004); [2] Muñoz *et al*. (2008); [3] Ben Naceur (2010)

tistica (version 5.0). Significance was accepted at $p < 0.05$.

Biochemical analysis

Prior to the biochemical study, cyst samples were hydrated in freshwater for 2 hrs. They were then decapsulated according to procedures described in Sorgeloos *et al*. (1986).

Protein, carbohydrate, lipid, and ash content of decapsulated cysts were estimated following Lowry *et al*. (1951), Dubois *et al*. (1956), Folch *et al*. (1957), and AOAC (1995), respectively. Each test was done in triplicate and results were expressed as percentages of dry weight. Data obtained were treated by analysis of variance (one-way ANOVA), and averages compared with Duncan's test using Statistica (version 5.0). Significance was accepted at $p < 0.05$. Fatty acid analyses were carried out as reported 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) as an antioxidant. Each fatty acid analysis was done in duplicate except for cysts harvested from Sabkhet Sijoumi where only one sample was subjected to fatty acid analysis.

Hatching characteristics

Cyst hatching quality was evaluated for each population: untreated cysts, decapsulated cysts, and cysts whose embryonic diapause had been deactivated with hydrogen peroxide treatment (H_2O_2, 3%) for 5 min (Van Stappen *et al*., 1998). Percentage and hatching efficiency were determined for each treatment. The hatching test was repeated three times in conical plastic tubes, according to standard conditions defined by Sorgeloos *et al*. (1986). Data obtained were treated by analysis of variance (ANOVA) with *post hoc* Duncan's test using Statistica (version 5.0). Significance was accepted at $p < 0.05$. The hatching rate allowed the determination of T_{10} and T_{90} (incubation time until appearance of 10% and 90% of total hatchable nauplii). The synchronization time *Ts* was also calculated.

Potential use of Tunisian Artemia in aquaculture

In order to determine the potential use in aquaculture of the different Tunisian populations studied, nauplii length, protein, carbohydrate, lipid, ash, linolenic acid (LNA), and eicosapentaenoic acid (EPA) content as well as hatching characteristics for decapsulated cysts (hatching efficiency, hatching rate, and synchronization time) were analyzed statistically and compared with commercial cysts from Great Salt Lake (GSL). Variables were entered into the XLSTAT-Pro 7.5 computer program in order to conduct multivariate principal component analysis (PCA). The graphical representation (factor score plot) of scores of cases shows the relationship between populations, and is also useful for identifying outliers and unusual cases.

RESULTS

Nutritional quality characterization

Instar-I nauplii length measurements are presented in Table 2. The lowest mean length of instar-I nauplii was scored for MEL with 422.6 μm, whereas the highest value was obtained for MCH (480.1 μm) and MHB (485.4 μm). The ANOVA for naupliar length indicated significant differences among populations ($p < 0.05$, $F = 40.369$). Duncan's test revealed that nauplii from BK, ZAR, and ADH are the most similar in length to nauplii from GSL.

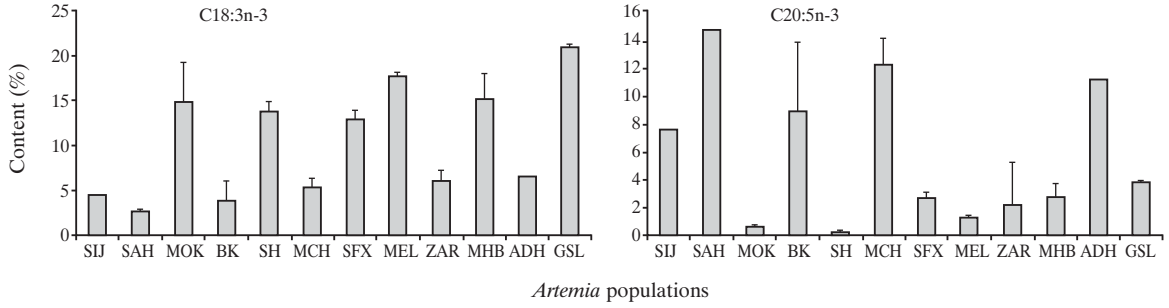

FIG. 1. Percentage of the main fatty acids (% from total fatty acid methyl ester) from the total lipids of decapsulated *Artemia* cysts from Tunisia. Abbreviations of populations in Table 1.

TABLE 2. Mean values (± standard deviation) of instar-I nauplii length, protein, carbohydrate, lipid, and ash content estimation (% on DW basis). Same letters show non-significant differences between mean in each row of main column (Duncan test, $p < 0.05$). Abbreviations of populations in Table 1

	Instar-I nauplii length	Protein	Carbohydrate	Lipid	Ash
SIJ	453.5 ± 34.3^{c}	49.6 ± 0.4^{abc}	15.5 ± 1.6^{d}	18.0 ± 1.3^{bc}	8.0 ± 0.9^{abc}
SAH*	432.8 ± 29.8^{b}	56.1 ± 4.2^{bc}	10.9 ± 1.7^{abc}	17.5 ± 0.8^{bc}	8.7 ± 0.1^{abc}
MOK	435.8 ± 31.5^{b}	49.6 ± 3.2^{abc}	10.6 ± 0.6^{abc}	18.5 ± 1.1^{bc}	8.0 ± 1.8^{abc}
SH	455.2 ± 22.7^{c}	57.1 ± 15.1^{c}	10.6 ± 2.3^{abc}	16.9 ± 0.3^{b}	7.9 ± 0.1^{ab}
BK	465.2 ± 26.3^{de}	45.7 ± 2.9^{ab}	12.2 ± 0.7^{bcd}	20.2 ± 0.9^{d}	8.8 ± 1.5^{abc}
MCH	480.1 ± 24.3^{f}	49.6 ± 0.2^{abc}	11.2 ± 1.4^{bc}	18.3 ± 0.3^{bc}	11.5 ± 1.5^{bc}
SFX	441.4 ± 30.1^{b}	50.3 ± 3.2^{abc}	9.4 ± 3.8^{abc}	16.8 ± 0.3^{b}	12.1 ± 4.1^{c}
MEL	422.6 ± 22.0^{a}	42.1 ± 1.6^{a}	21.9 ± 0.9^{e}	17.2 ± 1.8^{b}	7.5 ± 0.6^{ab}
ZAR	470.4 ± 32.0^{e}	45.8 ± 4.4^{ab}	8.8 ± 1.6^{ab}	12.1 ± 0.4^{a}	11.0 ± 2.7^{abc}
MHB	485.4 ± 34.8^{f}	47.4 ± 2.2^{abc}	13.3 ± 3.9^{cd}	19.1 ± 0.4^{cd}	7.2 ± 1.0^{a}
ADH**	458.8 ± 37.4^{cd}	51.2 ± 7.3^{abc}	12.5 ± 2.1^{bcd}	17.4 ± 0.6^{b}	8.6 ± 0.1^{abc}
GSL	464.5 ± 34.1^{de}	48.9 ± 1.0^{abc}	7.0 ± 0.3^{a}	18.0 ± 0.9^{bc}	7.1 ± 0.2^{a}
F-value	40.369	1.753	9.839	13.419	2.035
p-value	0.000	0.121	0.000	0.000	0.119

* Ben Naceur *et al*. (2008); ** Ben Naceur *et al*. (2010)

The biochemical contents of protein, carbohydrate, lipids, and ash are given in Table 2. Protein levels ranged from 42.1 to 57.1%, carbohydrate fluctuated from 8.8 to 21.9%, whereas lipid content varied from 12.1 to 20.2% and ash content from 7.2 to 12.1% of the dry weight. Statistical analysis (one-way ANOVA, $p < 0.05$) revealed no significant differences among the *Artemia* populations studied (Tunisian and commercial cysts) for protein and ash content ($F = 1.753$, $p = 0.121$ and $F = 2.035$, $p = 0.119$, respectively). This was not the case for lipid and carbohydrate levels, where significant differences were found among the studied *Artemia* populations (one-way ANOVA, $p < 0.05$). The Duncan's test ($p < 0.05$) confirmed these results and grouped all the studied populations into four and five distinctive groups for carbohydrates and lipids, respectively.

Figure 1 shows linolenic acid (LNA) and eicosapentaenoic acid (EPA) percentages found in Tunisian and commercial *Artemia* decapsulated cysts. Eicosapentaenoic acid [20:5n-3] was more abundant than linolenic acid [18:3n-3] in SIJ, SAH, BK, MCH, and ADH. In contrast, cysts harvested from MOK, SH, SFX, MEL, ZAR, MHB, and GSL showed a higher content of linolenic than eicosapentaenoic acid. Linolenic acid fluctuated from 2.7 to 21.1% for SAH and GSL, respectively, while eicosapentaenoic acid content varied from 0.2 to 14.8% for SH and SAH, respectively.

Hatching characteristics

Artemia cyst samples collected from Tunisian salt lakes appeared to have a moderate hatching quality (Table 3). The hatching percentage (H%) and efficiency (HE) obtained 48 hrs after incubation differed considerably from one strain to another. The hatching efficiency of untreated cysts fluctuated from a minimum of 31111 nauplii g^{-1} of cysts for Sabkhet Sijoumi, up to a maximum of 154666 nauplii g^{-1} of cysts for Sabkhet El Mcheguig. The maximum hatching percentage of untreated cysts was obtained for Sabkhet Mcheguig with 63.7%, whereas the minimum was observed for Sabkhet Sijoumi with 14.3%. The hatching efficiency and percentage were improved after decapsulation. The maximum hatching percentage and efficiency were obtained by decapsulated cysts harvested in Sahline saltwork (81.1%) and Sabkhet Sidi El Hani (198667 nauplii g^{-1} of cysts), respectively. After H_2O_2 treatment, the lowest hatching efficiency was obtained for SIJ with 54222 nauplii g^{-1} of cysts, while the highest was for SFX with 143111 nauplii g^{-1} of cysts. However, we could see different degrees of diapause deactivation between the studied strains. In fact, the impact of H_2O_2 on hatching characteristics varied from one population to another; hatching efficiency and percentage (after H_2O_2 treatment) increased compared with the untreated cysts for SIJ, SAH, MOK, ZAR, MHB, and ADH, while

TABLE 3. Mean values (± standard deviation) of hatching quality evaluation results for *Artemia* cysts harvested from Tunisian salt lakes compared with GSL commercial cysts. HE: hatching efficiency (nauplii g^{-1} of cysts); H%: hatching percentage (%); Ts: synchronization time (hrs). Same letters show non-significant differences between mean in each row of main column (Duncan test, $p < 0.05$). Abbreviations of populations in Table 1

	Untreated cysts		Decapsulated cysts		Cysts treated with H_2O_2 (3%)	
	HE	Ts	HE	Ts	HE	Ts
SIJ	31111 ± 5048^{a}	20.8	57778 ± 1540^{a}	19.7	54222 ± 3079^{a}	23.5
SAH*	81750 ± 13242^{b}	17.8	162500 ± 39363^{de}	18.6	88500 ± 3152^{b}	17.2
MOK	88000 ± 11718^{b}	21.0	117111 ± 5092^{db}	14.9	128667 ± 8083^{de}	12.6
BK	142666 ± 6666^{cd}	19.8	159111 ± 9076^{de}	21.1	140000 ± 19732^{de}	24.6
SH	142666 ± 17022^{cd}	18.0	198667 ± 6928^{f}	18.8	131111 ± 13488^{de}	26.3
MCH	154666 ± 3527^{d}	23.8	166667 ± 4619^{de}	20.7	138667 ± 8110^{de}	24.9
SFX	144977 ± 23636^{cd}	18.0	172444 ± 10357^{de}	17.0	143111 ± 8878^{e}	21.1
MEL	118667 ± 13856^{bc}	21.8	182222 ± 6012^{ef}	20.0	114667 ± 17487^{cd}	15.4
ZAR	92444 ± 19428^{b}	20.0	127556 ± 5048^{bc}	16.6	93333 ± 6110^{bc}	19.5
MHB	105777 ± 11495^{b}	18.7	174222 ± 12673^{d}	17.4	122222 ± 6842^{de}	26.8
ADH**	52500 ± 6907^{a}	23.9	139500 ± 9200^{c}	25.0	108000 ± 18961^{c}	24.6
GSL	228888 ± 53171^{e}	10.5	246667 ± 16707^{g}	10.0	236000 ± 8327^{f}	10.6
F-value	30.104		56.791		33.589	
p-value	0.000		0.000		0.000	
	H%		H%		H%	
SIJ	14.3 ± 1.9^{a}		35.3 ± 1.5^{a}		24.3 ± 1.3^{a}	
SAH*	38.2 ± 1.3^{c}		81.1 ± 10.9^{f}		44.5 ± 14^{b}	
MOK	31.2 ± 3.3^{c}		57.5 ± 1.3^{b}		52.5 ± 0.6^{cd}	
BK	62.3 ± 2.7^{f}		73.4 ± 2.4^{e}		57.2 ± 6.0^{d}	
SH	59.3 ± 3.4^{f}		75.5 ± 3.9^{e}		57.5 ± 2.3^{d}	
MCH	63.7 ± 5.3^{f}		68.1 ± 2.9^{d}		66.9 ± 3.4^{e}	
SFX	51.0 ± 1.8^{e}		78.6 ± 0.7^{ef}		51.1 ± 0.7^{bcd}	
MEL	49.0 ± 4.8^{e}		66.0 ± 2.1^{cd}		47.3 ± 4.6^{bc}	
ZAR	41.6 ± 4.9^{d}		60.4 ± 2.5^{bc}		41.8 ± 0.3^{b}	
MHB	52.1 ± 3.8^{e}		63.5 ± 1.2^{cd}		57.0 ± 2.7^{cd}	
ADH**	22.0 ± 2.4^{b}		55.6 ± 3.6^{b}		42.9 ± 4.5^{b}	
GSL	90.4 ± 1.1^{g}		92.3 ± 3.1^{g}		91.2 ± 1.2^{f}	
F-value	147.986		65.722		29.677	
p-value	0.000		0.000		0.000	

* Ben Naceur *et al*. (2008); ** Ben Naceur *et al*. (2010)

there was no evident improvement for SH, BK, SFX MCH, and MEL hatching parameters.

Not only hatching efficiency and percentage but also hatching synchronies varied across strains and treatments. For untreated cysts, *Ts* varied from 17.8 to 23.9 hrs for SAH and ADH, respectively. After decapsulation, synchronization time showed a minimum value for MOK with 14.9 hrs and a maximum value for ADH ($Ts = 25$ hrs). Nevertheless, after H_2O_2 treatment, *Ts* ranged between 12.6 hrs (MOK) and 26.8 hrs (MHB). Statistical analysis showed differences (ANOVA, $p < 0.05$) between different populations and with different treatment: $F = 30.104$, $p < 0.05$ for untreated cysts, $F = 56.791$, $p < 0.05$ for decapsulated cysts, and $F = 33.589$, $p < 0.05$ for cysts treated with H_2O_2. The Duncan's test showed that there are five groups for untreated cysts, while for decapsulated and diapause deactivated cysts, Duncan's test divided *Artemia* populations into seven and six groups, respectively. The comparison between the hatching characteristics for the Tunisian populations and commercial cysts from GSL showed that, in all

cases, commercial cysts presented better hatching quality. Duncan's test confirmed these results by placing the commercial cysts in a separate group for all treatments.

Potential use of Tunisian Artemia in aquaculture

Table 4 shows the loadings of the nutritional and hatching parameters used to determine the potential use of Tunisian *Artemia* in aquaculture. Principal component analysis (PCA) revealed two main directions of variation, with axis 1 explaining 31.25% and axis 2 explaining an additional 21.92% of the total variance. Relative to the first component (PCA axis 1), linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), and hatching characteristics (hatching efficiency, percentage, and Ts) are the most important variables for cyst quality differentiation with a total contribution of 93.36%. For the second component (PCA axis 2), carbohydrate, protein, and ash content are the most important variables with a total contri-

TABLE 4. Results of principal component analysis of the contribution and portion of each parameter used to characterize decapsulated cysts for aquaculture in the two first components

	Variables contribution (%)		Component	
	F1	F2	F1	F2
Nauplii length	0.025	7.076	–0.028	–0.394
Total lipids	0.008	1.569	0.016	0.185
Carbohydrate	4.223	30.242	–0.363	0.814
Total protein	0.001	14.877	0.004	–0.571
Ash	2.381	15.775	–0.273	–0.588
C18:3n-3	23.029	8.890	0.848	0.441
C20:5n-3	10.776	10.758	–0.580	–0.486
Hatching efficiency	21.322	1.780	0.816	–0.198
Hatching percentage	17.503	8.890	0.740	–0.441
Synchronisation time (Ts)	20.733	0.142	–0.805	0.056
Total variance explained				
% of variance	31.245	21.919		
Cumulative %	31.245	53.165		

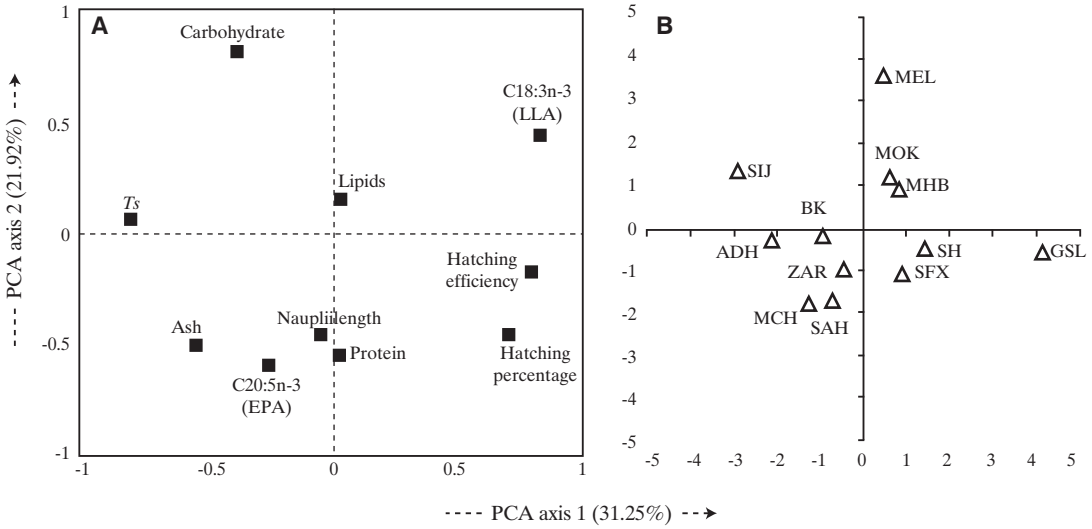

FIG. 2. Principal component analysis (PCA) of (A): different variables used for cyst characterization, and (B): *Artemia* strains (sources) studied. Abbreviations of populations in Table 1.

TABLE 5. Results of principal component analysis of the contribution and portion of each population from the constituent parameters used to characterize decapsulated cysts for aquaculture in the two first components. Abbreviations of populations in Table 1

	Variables contribution (%)		Component	
	F1	F2	F1	F2
SIJ	22.402	6.902	–2.898	1.347
SAH	1.371	11.460	–0.717	–1.736
MOK	0.979	5.386	0.606	1.190
SH	5.338	0.920	1.415	–0.492
BK	2.290	0.140	–0.927	–0.192
SFX	2.182	4.383	0.904	–1.074
MCH	4.331	12.203	–1.274	–1.792
ZAR	0.590	3.579	–0.470	–0.970
MEL	0.604	50.119	0.476	3.631
MHB	1.780	3.359	0.817	0.940
ADH	11.774	0.296	–2.101	–0.279
GSL	46.360	1.253	4.169	–0.574

bution of 60.89%. Figure 2A illustrates the factor loading plot for the variables used to determine the potential use of Tunisian *Artemia* cysts in aquaculture. Variables correlated mostly with factor 1 (horizontal axis) than with factor 2 (vertical axis). Positive loadings of linolenic acid, hatching efficiency, and hatching percentage were observed relative to the principal component 1, whereas eicosapentaenoic acid and synchronization time (*Ts*) showed negative loadings. Figure 2B, shows the factor scores plot, based on the population scores from the constituent variables. The PCA divided the studied populations into four groups (Table 5). According to the PCA axis 1, two groups were evident: group 1, formed by the commercial cysts from GSL (with variable contribution of 46.36%), characterized by high hatching quality (hatching efficiency, hatching percentage, and synchronization time), and group 2, formed by *Artemia* from Sabkhet Sijoumi (with variable contribution of 22.40%), characterized by low hatching quality. According to PCA axis 2, one group was evident (group 3), formed by *Artemia* from Sabkhet El Melah (with variable contribution of 50.12%), characterized by high levels of carbohydrate. The 4th group was composed of all the other populations and where the variable contribution varied from one population to another according to the combined effect of PCA axis 1 and 2.

DISCUSSION

Larviculture appears to be the major bottleneck for the industrial up-scaling of the culture of fish and shellfish. Among live food routinely used in fish hatcheries, *Artemia* nauplii are the most frequent. *Artemia* nauplii can be easily hatched from cysts and they are easy to use mainly because of the knowledge accumulated on the factors that influence the quality of the harvested cysts (Sorgeloos *et al*., 1986). They can be also enriched with nutrients (e.g. essential fatty acids and vitamins to improve their nutritional value to cultured fish larvae or juveniles; Dhont & Sorgeloos, 2002) and they have been used as carriers of spawning hormones to treat fish diseases or induce spawning in adult fish (Burton *et al*., 1998).

Considering the results obtained in this work, the SIJ, SAH, MOK, SH, SFX, and MEL nauplii are smaller than those of GSL. However, based on GSL instar-I nauplii length obtained from literature (486 μm; Van Stappen, 1996), all Tunisian *Artemia* populations show a smaller nauplii length. Comparisons with other *Artemia* strains revealed that the average size of instar-I nauplii from Tunisia is larger than that of *A. franciscana* from San Francisco Bay (428 μm) reported by Van Stappen (1996), except for *Artemia* from Sabkhet El Melah. However, nauplii from Tunisian populations are significantly smaller than those of *A. tibetiana* (667 μm; Abatzopoulos *et al*., 1998) and *A. urmiana* (483.5, 502.1, and 592.7 μm; Abatzopoulos *et al*., 2006) but in the same range as nauplii of *A. salina* from Mégrine (Tunisia) with 467.7 μm (Van Ballaer *et al*., 1987) and from Sabkhet Abu Kammash (Libya) with 468.2 μm (El-Magsodi *et al*., 2005).

Considerable differences in nutritional value have been found among different *Artemia* strains (De Quei-

roz, 1989). The biochemical composition in proteins, carbohydrates, and lipids of *Artemia* nauplii generally varies considerably from 37 to 71%, 11 to 23%, and 12 to 30%, respectively (Bengtson *et al*., 1991; Evjemo & Olsen, 1997). In our study, decapsulated cysts, harvested from Tunisian sites, showed that protein level varies from 42.1% (MEL) to 57.1% (SH), carbohydrate from 8.8% (ZAR) to 21.9% (MEL), and lipids from 12.1% (ZAR) to 20.2% (BK). Based on analysis of variance (one-way ANOVA) with *post hoc* Duncan's test, protein levels between Tunisian *Artemia* cysts and those from GSL did not differ (ANOVA, $p = 0.121$). Nevertheless, for carbohydrate and lipids, statistical differences can be seen among SAH, MOK, SH, SFX, and ZAR, a similarity of carbohydrate content with GSL, and BK and MCH, and a difference for lipid content with GSL. Léger *et al*. (1986) reported that ash content in *Artemia* nauplii varies from 4 to 21%. In our study, commercial cysts showed lowest ash content compared with the Tunisian populations. For protein levels, the analysis of variance did not show a significant difference between the studied *Artemia* strains ($p = 0.119$).

Fatty acid content is an important trait of *Artemia* strains used 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 (C18:3n-3, LLA) and low concentration of eicosapentaenoic acid (C20:5n-3, EPA), which are suitable for feeding freshwater fish and crustacean larvae; and marine-type *Artemia*, with a higher EPA content and lower LLA, which are more suitable for culturing marine species. Based on this division, *Artemia* cysts harvested from SIJ, SAH, BK, MCH, and ADH belong to marine-type *Artemia*, whereas *Artemia* from MOK, SH, SFX, MEL, ZAR, and MHB belong to freshwater-type *Artemia*.

The most practical criterion for evaluation of the economic value of cysts is the hatching quality. For untreated cysts from Tunisia, the results showed that hatching quality (efficiency, percentage, and *Ts*) varied considerably across strains. Concerning hatching percentage (H%), we can see that cysts harvested from SH, BK, SFX, MCH, MEL, and MHB presented a better H% compared with those of SIJ, SAH, MOK, ZAR, and ADH with values varying from 49 to 67.6% and 14.3 to 41.6%, respectively. However, except for cysts from SIJ, the hatching characteristics were within the average of the limits as reported by Vanhaecke & Sorgeloos (1983), which are between 20% and 90%. Nevertheless, it should be emphasized that all Tunisian cysts were harvested between 2005 and 2007 apart from those of SIJ which were collected in 2003. In fact, Van Stappen (1996) reported that hatching behaviour is strain specific as they are influenced by a wide array of factors like harvesting, processing, storage, and hatching techniques, as well as production conditions affecting the parental generation. On the other hand, the comparison between the Tunisian *Artemia* populations and other *Artemia* strains showed that the hatching quality of Tunisian cysts is better than that of cysts harvested from Urmia Lake (35%, Abatzopoulos *et al*., 2006), Sabkhet Abu Kammash 'Libya' (60300 nauplii g^{-1}, El-Magsodi *et al*., 2005), from Colombian Caribbean sites (46.7-53.1%, Camargo *et al*., 2005), and from GSL 'non-commercial cysts' (43.9%, Sorgeloos *et al*., 1986).

Although cysts from the Tunisian strains showed rather moderate hatching quality, improvement was observed after decapsulation and treatment with H_2O_2. The best efficiency was obtained with decapsulated cysts harvested from SH (198667 ± 6928 nauplii g^{-1}). However, we can see that H_2O_2 treatment does not improve the hatching quality of cysts from SH, BK, SFX, and MCH. The same result was obtained by Van Stappen *et al*. (1998). These authors reported that differences in tolerance and responsiveness to H_2O_2 treatment may be genetic and thus strain-specific, or due to environmental factors, including processing and storage (of a few months).

The synchronization time (*Ts*) for Tunisian *Artemia* cysts is relatively long (18-23.9 hrs, untreated cysts). Under conditions of weakly synchronized hatches, the first nauplii to hatch move from instar-I to instar-II, thus becoming less accessible to predators and losing, approximately, 27% of their energetic value (Benijts *et al*., 1976). The comparison between Tunisian and commercial cysts shows that, whatever the type of treatment, commercial cysts present better hatching quality. Several factors could influence the hatching performance of the cysts, e.g. exposing the cysts to suboptimal conditions before harvesting, the harvest period, the presence of other components (empty shells, salt, sand), hydration/dehydration cycles, and storage conditions (Sorgeloos *et al*., 1986). Moreover, *Artemia* cysts should be stored dry (water content lower than 9%, preferably between 2 and 5%) and in oxygen-free conditions (Van Stappen, 1996). When *Artemia* cysts are not stored in vacuum sealed cans or under nitrogen atmosphere (as is the case for us), hatching rates and efficiencies start to drop after a few months of storage at room temperature (Van Stappen, 1996).

The comparison between Tunisian and commercial cysts using PCA for studying their potential use for aquaculture showed that hatching characteristics (hatching efficiency, percentage, and *Ts*) are the most important variables for cyst quality differentiation. In fact, this variable showed a total contribution of 59.55% according to the first component, explaining 31.25% of the total variance. Otherwise, the high LLA and EPA contribution observed, according to the first component (23.02 and 10.77%, respectively), can be explained by the differences found in these two parameters between the studied populations. In fact, Watanabe *et al*. (1978) reported that higher EPA content is generally accompanied with a lower LLA content. Based on the populations' scores, we can see that GSL and SIJ were the most divergent populations because of their high and low hatching qualities, respectively. Moreover, except for *Artemia* from Sabkhet El Melah, with its separation from the other populations based on its high carbohydrate content, for all the other populations (which present a moderate hatching quality) the separation was based on the EPA and LLA content (freshwater and marine-type) and all the other parameters (lipids, protein, carbohydrate).

CONCLUSIONS

Considering nutritional quality (nauplii size, lipids, protein, carbohydrate, ash, LLA, and EPA), Tunisian *Artemia* populations exhibit a good potential for commercial exploitation, especially for *Artemia* strains with high levels of EPA. However, hatching quality can be considered as a handicap for their use in aquaculture. Nevertheless, it is very important to underline that our study was based on the comparison between Tunisian cysts, harvested on the shores of salt lakes, and commercial cysts. Indeed, the comparison between commercial cysts and cysts harvested at the shore of GSL revealed that commercial cysts show much larger hatching qualities than cysts harvested along the shore, as reported in the literature (Sorgeloos *et al*., 1986; Van Stappen, 1996). Moreover, the hatching quality of cysts from Tunisian sites can be improved by concentrating efforts on cyst processing techniques such as harvesting condition, storage condition, and different cysts treatments.

REFERENCES

Abatzopoulos TJ, Zhang B, Sorgeloos P, 1998. International Study on *Artemia.* LIX. *Artemia tibetiana*: preliminary characterization of a new *Artemia* species found in Tibet (People's Republic of China). *International Journal of Salt Lake Research*, 7: 41-44.

Abatzopoulos TJ, Baxevanis AD, Triantaphyllidis GV, Criel G, Pador EL, Van Stappen G, Sorgeloos P, 2006. International Study on *Artemia.* LXIX. Quality evaluation of *Artemia urmiana* Günther (Urmia Lake, Iran) with special emphasis on its particular cyst characteristics. *Aquaculture*, 254: 442-454.

AOAC (Association of Official Analytical Chemists), 1995. *Official methods of analysis of AOAC International, 16th edn*. AOAC International, Arlington, Virginia, USA.

Ben Naceur H, 2010. Caractérisation taxonomique, biochimique et éco-biologique pour la valorisation aquacole de l'*Artemia* en Tunisie: cas de Sabkhet El Adhibet (sud-est Tunisien). Ph. D. Thesis, National Institute of Agricultural Sciences of Tunisia.

Ben Naceur H, Ben Rejeb Jenhani A, Romdhane MS, 2008. Valorisation de l'*Artemia* (Crustacea; Branchiopoda) de la saline de Sahline (Sahel Tunisien). *Bulletin de la Société Zoologique de France*, 133: 181-188.

Ben Naceur H, Ben Rejeb Jenhani A, Romdhane MS, 2009a. New distribution record of the brine shrimp *Artemia* (Crustacea, Branchiopoda, Anostraca) in Tunisia. *Check List*, 5: 281-288.

Ben Naceur H, Ben Rejeb Jenhani A, Romdhane MS, 2009b. Ecobiological survey of the brine shrimp *Artemia salina* from Sabkhet El Adhibet (south-east Tunisia). *Journal of the Marine Biological Association of the United Kingdom*, 89: 1109-1116.

Ben Naceur H, Ben Rejeb Jenhani A, Romdhane MS, 2010. Variability of *Artemia salina* cysts from Sabkhet El Adhibet (southeast Tunisia) with special regard to their use in aquaculture. *Inland Water Biology*, 3: 70-78.

Bengtson DA, Léger P, Sorgeloos P, 1991. Use of *Artemia* as a food source for aquaculture. In: Browne RA, Sorgeloos P, Trotman CAN, eds. *Artemia biology*. CRC Press, Boca Raton, Florida: 255-285.

Benijts F, Vanvoorden E, Sorgeloos P, 1976. Changes in the biochemical composition of the early larval stages of the brine shrimp *Artemia salina* L. In: Persoone G, Jaspers E, eds. *Proceedings of the 10th European synposium on marine biology. Vol. 1. Research in Mariculture at laboratory and pilot scale*. Universa Press, Wetteren, Belgium: 1-9.

Bowen ST, Fogarino EA, Hitchner KN, Dana GL, Chow VHS, Buoncristiani MR, Carl JR, 1985. Ecological isolation in *Artemia*: population differences in tolerance of anion concentration. *Journal of Crustacean Biology*, 5: 106-129.

Burton S, Kaiser H, Hecht T, 1998. The potential of *Artemia*-mediated delivery of gonadotropin hormone analogue to induce ovulation in the cardinal tetra (*Paracheirodon axelrodi*). *Aquarium Sciences and Conservation*, 2: 89-92.

Camargo WN, Duran GC, Rada OC, Hernandez LC, Linero J-CG, Muelle IM, Sorgeloos P, 2005. Determination of biological and physicochemical parameters of *Artemia franciscana* strain in hypersaline environments for aquaculture in the Colombian Caribbean. *Saline Systems*, 1: 9.

De Queiroz JF, 1989. The effect of *Artemia* diets on the re-

productive activity and offspring quality in the marine crustacean *Mysidopsis bahia* (M.), in function of shrimp farming. Ph. D. Thesis, Faculteit van de Landbouwwetenschappen, Centrum voor *Artemia* – Onderzoek, Belgium.

Dhont J, Sorgeloos P, 2002. Applications of *Artemia*. In: Abatzopoulos TJ, Beardmore JA, Clegg JS, Sorgeloos P, eds. *Artemia: basic and applied biology*. Kluwer Academic Publishers, Dordrecht, The Netherlands: 251-277.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F, 1956. Colorimetric method for determination of sugar and related substances. *Analytical Chemistry*, 28: 350-356.

El-Magsodi MO, El-Ghebli HM, Hamza M, Van Stappen G, Sorgeloos P, 2005. Characterization of Libyan *Artemia* from Abu Cammash Sabkha. *Libyan Journal of Marine Science*, 10: 19-30.

Evjemo JO, Olsen Y, 1997. Lipid and fatty acid content in cultivated live feed organisms compared to marine copepods. *Hydrobiologia*, 358: 159-162.

Folch J, Lees N, Sloane-Stanley GH, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 266: 497-509.

Guermazi W, 2008. Etude de la distribution et de la composition biochimique d'*Artemia salina* (Anostracé) dans la saline de Sfax. Essais de détermination des relations trophiques existantes entre *Artemia* et ses proies potentielles. Ph. D. Thesis, Sciences University of Sfax, Tunisia.

Lavens P, Sorgeloos P, 2000. The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. *Aquaculture*, 181: 397-403.

Léger P, Bengtson DA, Simpson KL, Sorgeloos P, 1986. The use and nutritional value of *Artemia* as food source. *Oceanography and Marine Biology*, 24: 521-623.

Lenz PH, Browne RA, 1991. Ecology of *Artemia*. In: Browne RA, Sorgeloos P, Trotman CNA, eds. *Artemia biology*. CRC Press, Boca Raton, Florida: 237-253.

Lowry OH, Rosebrough WJ, Fair HL, Randall RJ, 1951. Protein measurement with Folin-phenol reagent. *Journal of Biological Chemistry*, 195: 265-275.

Mahdhi A, Chaieb K, Charfeddine R, Laachkar H, Kammoun F, Bakhrouf A, 2010. Preliminary characterization of two Tunisian *Artemia salina* populations. *Marine Life*, 17: 3-11.

Maniatsi S, Baxevanis AD, Abatzopoulos TJ, 2009. The intron 2 of p26 gene: a novel genetic marker for discriminating the two most commercially important *Artemia franciscana* subspecies. *Journal of Biological Research-Thessaloniki*, 11: 73-82.

Muñoz J, Gómez A, Green AJ, Figuerola J, Amat F, Rico C, 2008. Phylogeography and local endemism of the native Mediterranean brine shrimp *Artemia salina* (Branchiopoda: Anostraca). *Molecular Ecology*, 17: 3160-3177.

Navarro JC, Amat F, Sargent JR, 1992a. Fatty acid composition of coastal and inland *Artemia* sp. populations from Spain. *Aquaculture*, 102: 219-230.

Navarro JC, Amat F, Sargent JR, 1992b. Lipid composition of cysts of the brine shrimp *Artemia* sp. from Spanish populations. *Journal of Experimental Marine Biology and Ecology*, 155: 123-131.

Romdhane MS, Ben Naceur H, Hamrouni S, Ben Rejeb Jenhani A, El Cafsi M, 2004. Biological and biochemical characterization of *Artemia* from Tunisian wetlands. In: Agh N, Sorgeloos P, eds. *International workshop on Artemia. INCO-DEV Project on Artemia biodiversity.* Urmia, Iran: 89-91.

Seale A, 1933. Brine shrimp *Artemia* as satisfactory live food for fish. *Transactions of the American Fisheries Society*, 63: 129-130.

Sorgeloos P, 1980. The use of the brine shrimp *Artemia* in aquaculture. In: Persoone G, Sorgeloos P, Roels O, Jaspers E, eds. *The brine shrimp Artemia. Vol. 3. Ecology, culturing, use in aquaculture*. Universa Press, Wetteren, Belgium: 25-46.

Sorgeloos P, Lavens P, Léger P, Tackaert W, Versichele D, 1986. *Manual for the culture and use of brine shrimp Artemia in aquaculture*. The Belgian Administration for Development Cooperation. The Food and Agriculture Organization of the United Nations. State University of Ghent, Belgium.

Sorgeloos P, Dhert P, Candreva P, 2001. Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture*, 200: 147-159.

Triantaphyllidis GV, Abatzopoulos TJ, Sorgeloos P, 1998. Review of the biogeography of the genus *Artemia* (Crustacea, Anostraca). *Journal of Biogeography*, 25: 213-226.

Van Ballaer E, Versichele D, Vanhaecke P, Léger P, Ben Abdelkader N, Turki S, Sorgeloos P, 1987. Characterization of *Artemia* from different localities in Tunisia with regard to their use in local aquaculture. In: Sorgeloos P, Bengtson DA, Decleir W, Jaspers E, eds. *Artemia research and its applications. Vol. 1. Morphology, genetics, strain characterization, toxicology*. Universa Press, Wetteren, Belgium: 199-209.

Van Stappen G, 1996. *Artemia*. In: Lavens P, Sorgeloos P, eds. *Manual on the production and use of life food for the aquaculture*. FAO Fishery Technical Paper 361: 79-163.

Van Stappen G, Lavens P, Sorgeloos P, 1998. Effects of hydrogen peroxide treatment in *Artemia* cysts of different geographical origin. *Archives of Hydrobiology Special Issues: Advances in Limnology*, 52: 281-296.

Vanhaecke P, Sorgeloos P, 1983. International Study on *Artemia*. XIX. Hatching data for ten commercial sources of brine shrimp cysts and re-evaluation of the "hatching efficiency" concept. *Aquaculture*, 30: 43-52.

Watanabe T, Arakawa T, Kitajima C, Fukusho K, Fujita S, 1978. Nutritional quality of living feeds from the viewpoint of essential fatty acids for fish. *Bulletin of the Japanese Society of Scientific Fisheries*, 44: 1223-1227.

Xin N, Sun J, Zhang B, Triantaphyllidis GV, Van Stappen G, Sorgeloos P, 1994. International study on *Artemia*. LI. New survey of *Artemia* resources in the People's Republic of China. *International Journal of Salt Lake Research*, 3: 105-112.