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# Sulfuric acid treatment for Artemia cyst decapsulation

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**Abstract:** In the present study, sulfuric acid was used for Artemia cysts decapsulation. Cysts of *Artemia franciscana* were hatched out in regular manner or following hypochlorite or acid decapsulation. Two acid concentrations (1 and 5%), three acid immersion times (10, 30 and 50 min) were used and hatching rates were recorded after 15, 18 and 24 h incubation. Hatching rates increased but hatching time decreased in line with acid concentration and acid immersion time increment. Hypochlorite-treated cysts had significantly higher hatching rate (97%) compared to other groups. However, among the acid- treated cysts, the best hatching rate (92.4%) was achieved in cysts treated with 1% acid over 50 min. Acid treatment could be used as a decapsulation method which saves cost and labor because of increasing the hatching rate and speed.

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## Introduction

Live food is necessary for fish larvae, especially in marine species. Undeveloped digestive system of larvae, makes them completely dependent on live food. Live foods have other advantages over the formulated feed including appropriate size, palatability due to high water content, immersion in water column and stimulation of larval predatory behavior (Bengston, 2003).

Nauplius of *Artemia* sp. is one of the important live foods with the widespread use in aquaculture. It contains 41-62% protein and 12-23% lipid which is a good source of linolenic acid and eicosapentaenoic acid (Dhot and Van Stappen, 2003). Small size, acceptable nutritional values, easy to produce and suitability for use in bio-encapsulation process are the advantages of Artemia nauplii. The common technique to produce Artemia nauplii is to collect and hatch the cysts under artificial condition. The cysts have a chorion shell constituted of lipoproteins, chitin and haematin (García-Ortega et al., 1998). This layer is completely indigestible by all known cultured species and may cause gut obstruction (Dhot and Van Stappen, 2003). During nauplii production, some cysts might not hatch. Consumption of these cysts may cause gut obstruction in fish larvae, which should be considered.

Decapsulation is a process in which the chorion layer is dissolved and removed. Decapsulated cysts could be used as a food source for fish larvae, although its application is limited compared to the nauplii. Decapsulated cysts have been used to rear larvae of freshwater catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*), and marine shrimp (*Penaeus indicus* and *Penaeus monodon*) and milkfish (*Chanos chanos*) (Verreth et al., 1987; Vanhaecke et al., 1990; Stael et al., 1995; Ribeiro and Jones, 1998; Sui, 2000). The decapsulated cysts offer a number of advantages over nauplii and non-decapsulated cysts, in larval production (Dhot and Van Stappen, 2003):

1. Use of decapsulated cysts instead of nauplii eliminates the need for labor and additional hatching-related facilities.
2. Cyst shells are not introduced into the culture tanks. Non-decapsulated cysts introduce shell to the

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rearing tanks. It is not easy to separate all shells. In addition, hatching rate is not always 100% and the remaining unhatched cysts may cause gut obstruction in larvae.

3. Decapsulated nauplii have higher energy content and individual weight (30–55% depending on strain) compared to nauplii hatched out of non-decapsulated cysts. Where cysts have relatively a low energy content, the hatchability could be improved by decapsulation as the larvae need a lower energy to break out of a decapsulated cyst.

4. Cysts would be completely disinfected by decapsulation.

5. Cysts with poor hatching quality or even non-hatching cysts can still be used as a food source.

Decapsulation is normally performed by hypochlorite solution. The detailed protocol was described by Dhot and Van Stappen (2003). It would be of interest to investigate other solvent for decapsulation. Although cysts are washed following hypochlorite exposure, the risk of hypochlorite residual is not eliminated. Hypochlorite releases chlorine, which is highly toxic to fish (Brungs, 1973). The present study aimed to investigate the efficiency of sulfuric acid on decapsulation of *Artemia* cysts. The residual of sulfuric acid is sulfate which is a natural water anion.

## Materials and methods

Hatching technique was based on Dhont and Van Stappen (2003). Briefly, cysts of *A. franciscana* were artificially hatched by adding 1 g cyst to 1 liter water (33 ppt). Cysts were incubated for 24 h. Temperature and light intensity was maintained at 28 ºC and 2000 lux, respectively. Continuous aeration was provided to ensure dissolved oxygen up to 5 ppm and suitable turbulence for cysts. Hatching rate was determined by counting and averaging the nauplii in three samples (0.1 ml) under stereoscopic loupe (Olympus, SZX7, Japan).

Control cysts were hatched following the aforementioned method. The cysts were decapsulated using the method by Dhont and Van Stappen, 2003), cysts were allowed to hydrate for 1 h in clean aerating water. Then, cysts were added to hypochlorite (liquid bleach, NaOCl, 12%) or acid solution (1 and 5% sulfuric acid, Mihanjaz Co., Iran). Cysts were removed from hypochlorite solution after 5 min. The cysts were washed with clean water 5 times prior to incubation. In acid treatments, cysts remained 10, 30 and 50 min in both 1 and 5% acid solution. Thereafter, cysts were washed 5 times prior to incubation. Hatching rates were recorded at 15, 18 and 24 h after incubation.

To compare hatching rates of acid treated cysts, data were analyzed using a split-plot design, which incubation time considered as the whole plot and acid immersion time and concentration as split plots

Table 1. Analyses of variance of effect of acid concentration (%), acid immersion time (min) and incubation time (h) on hatching rate of *Artemia* cysts.

| Source | Type III Sum of Squares | df | Mean Square | F | $P_{value}$ |
|---|---|---|---|---|---|
| IT[1] | 24298.7 | 2 | 12149.3 | 830 | <0.0001 |
| Error | 87.7 | 6 | 14.6 | 354.8 | |
| AT[2] | 4250.5 | 2 | 2125.2 | 35.2 | <0.0001 |
| IT × AT | 844.35 | 4 | 211.1 | 10.3 | <0.0001 |
| CON[3] | 61.65 | 1 | 61.65 | 0.25 | 0.003 |
| IT × CON | 2.96 | 2 | 1.48 | 59.5 | 0.486 |
| AT × CON | 712.65 | 2 | 356.32 | 2.24 | <0.0001 |
| IT × CON × AT | 53.61 | 4 | 13.4 | | 0.09 |
| Error | 179.69 | 30 | 5.99 | | |
| Corrected total | 30491.84 | 53 | | | |

1. Incubation time, 2. Acid immersion time, 3. Acid concentration.

Table 2. Hatching rate (mean and SE) in different acid concentration × acid immersion time × incubation time combinations.

| CON (%) | AT (min) | IT (h) | Mean | SE | 95% Confidence Interval Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| 1 | 10 | 15 | 12.16 a | 1.499218 | 9.126112 | 15.20722 |
| | | 18 | 25.90 bc | 1.499218 | 22.85945 | 28.94055 |
| | | 24 | 51.23 f | 1.499218 | 48.19278 | 54.27389 |
| | 30 | 15 | 29.46 c | 1.499218 | 26.42611 | 32.50722 |
| | | 18 | 43.43 e | 1.499218 | 40.39278 | 46.47389 |
| | | 24 | 80.10 h | 1.499218 | 77.05945 | 83.14055 |
| | 50 | 15 | 34.16 d | 1.499218 | 31.12611 | 37.20722 |
| | | 18 | 50.93 f | 1.499218 | 47.89278 | 53.97389 |
| | | 24 | 92.40 j | 1.499218 | 89.35945 | 95.44055 |
| 5 | 10 | 15 | 23.36 b | 1.499218 | 20.32611 | 26.40722 |
| | | 18 | 29.90 cd | 1.499218 | 26.85945 | 32.94055 |
| | | 24 | 59.36 g | 1.499218 | 56.32611 | 62.40722 |
| | 30 | 15 | 23.60 b | 1.499218 | 20.55945 | 26.64055 |
| | | 18 | 40.16 e | 1.499218 | 37.12611 | 43.20722 |
| | | 24 | 77.53 h | 1.499218 | 74.49278 | 80.57389 |
| | 50 | 15 | 22.56 b | 1.499218 | 19.52611 | 25.60722 |
| | | 18 | 41.96 e | 1.499218 | 38.92611 | 45.00722 |
| | | 24 | 84.76 i | 1.499218 | 81.72611 | 87.80722 |

on the incubation time. To compare hatching rate of acid-treated groups, control group and hypochlorite-treated group, data of 24 h incubation were analyzed using a one-way ANOVA. Duncan test was used as Post Hoc to determine significant difference among the treatments. $P<0.05$ was considered as significant difference. Data are presented as mean ± SE.

## Results

Results showed that acid concentration, acid immersion time, incubation time, interaction between acid concentration × acid immersion time as well as acid immersion time × incubation time had significant effect on hatching rate (Table 1). Hatching rates of acid-treated cysts are shown in table 2.

There was significant difference in hatching rate among 15, 18 and 24 h incubated cysts as hatching rate increased with increase of the incubation time (Fig. 1). Hatching rates increased with increase of the acid immersion time from 10 to 50 min (Fig. 2). Hatching rate of the cysts exposed to 5% acid was significantly higher than those exposed to 1% acid (Fig. 3). Comparison of hatching rates among acid-treated, hypochlorite-treated and control cysts are shown in Fig. 4. The hatching rates were in following order:

1% acid over 10 min < 5% acid over 10 min < control < 1% acid over 30 min and 5% acid over 30 min < 5% acid over 50 min < 1% acid over 50 min < hypochlorite.

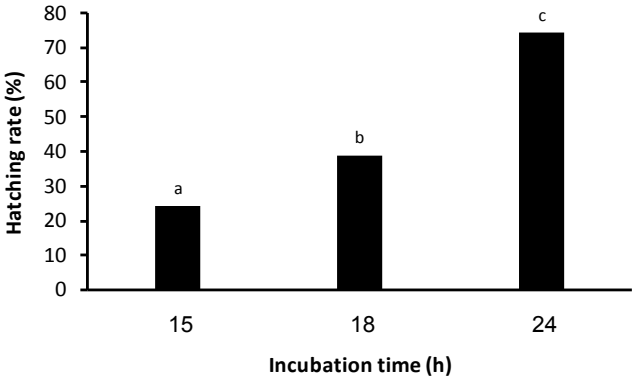


Figure 1. Effect of incubation time on hatching rate of acid-treated cysts. Different letters above the bars show significant difference. $P<0.05$. SE=3.63.combinations.

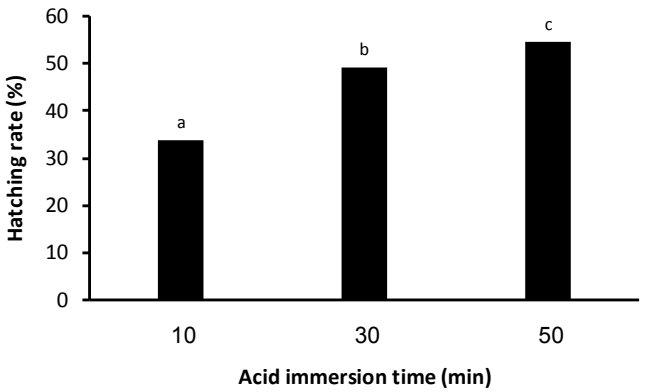


Figure 2. Effect of acid immersion time on hatching rate of acid-treated cysts. Different letters above the bars show significant difference. $P<0.05$. SE = 7.48.

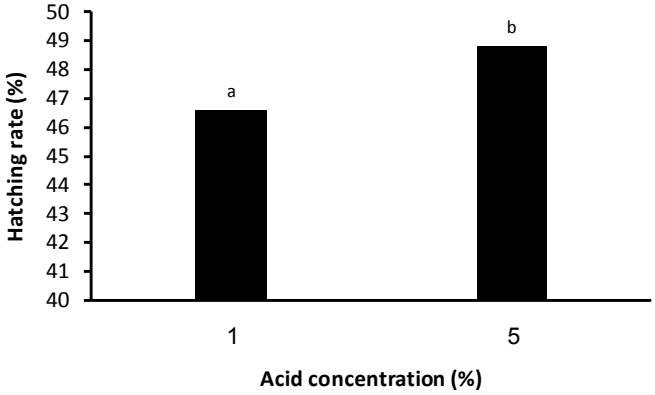


Figure 3. Effects of acid concentration on hatching rate of Artemia cysts. Different letters above the bars show significant difference. $P<0.05$. SE = 0.50

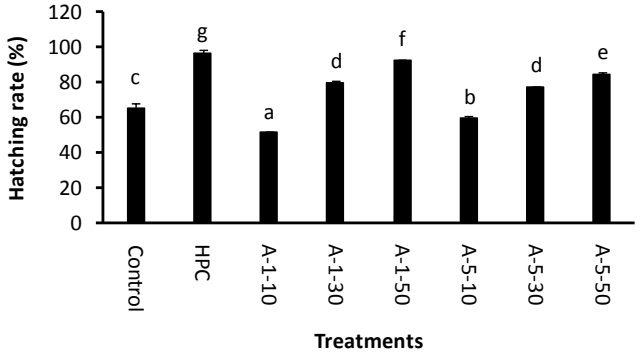


Figure 4. Twenty four hours hatching rate of control, hypochlorite-treated (HPC) and acid-treated (A-1-10 = 1% acid over 10 min, A-1-30 = 1% acid over 30 min, A-1-50 = 1% acid over 50 min, A-5-10 = 5% acid over 10 min, A-5-30 = 5% acid over 30 min, A-5-50 = 5% acid over 50 min) cysts. Different letters above the bars show significant difference. $P< 0.05$.

## Discussion

Hypochlorite oxidation is the common method for *Artemia* cysts decapsulation. However, if hypochlorite is not neutralized appropriately, it may damage the fish larvae. The present study showed that acid digestion could be used instead of hypochlorite oxidation for *Artemia* cyst decapsulation. This method would be safe, since residual of acid, if any, may only change the pH slightly, depending on the water alkalinity. However, 3-5 times washing would completely eliminate the risk of pH change.

Previous studies showed that hatching rate of *A. franciscana* cysts were about 70 % (Bruggeman et al., 1980; Triantaphyllidis et al., 1994). In the present study, similar hatching rate was also obtained in non-decapsulated cysts. Data of this study showed that decapsulation has a significant impact on hatching rate of *Artemia* cysts. On the other hand, the present results showed that increase in acid immersion could speed up hatching process. This feature is important because this could be cost and labor saving in practice.

Increase in acid concentration and acid immersion time cause increase in hatching rate. This could be due to increased shell digestion allowing nauplii to hatch out spending lees energy and over a shorter time. Similar results were reported in *Artemia parthenogenetica* decapsulated with hypochlorite solution over 2-5 min (Hosseini Najd Gerami and Agh, 2008).

The best hatching rate (97%) was observed in hypochlorite-treated cysts. Although, hatching rate of the cysts treated with 1% acid over 50 min was significantly lower than hypochlorite-treated ones, it was still high (92.4%). However, all acid-treated cysts showed significantly higher hatching rate compared with control, except those exposed to acid over 10 min. This suggests that 10 min acid exposure is not suitable for decapsulation. More studies are needed to illustrate effects of acid-treatment on cysts in term of pH, shell thickness and embryo condition. It is concluded that acid-treatment could be a useful method for cysts decapsulation. There is no risk of hypochlorite residuals in this method. Decapsulation with acid cause higher hatching rate and shorter hatching time compared to control. Acid concentration and acid immersion time are important

factors affecting hatching rate and time. The best hatching rate (92.4%) in acid-treated cysts was achieved when cysts were exposed to 1% acid over 50 min.

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