



## Extending the shelf life of *Artemia urmiana* during frozen storage using Vitamin E treatment

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Received: July 2023

Accepted: October 2023

### Abstract

*Artemia* is one of the most important live foods used for feeding larvae and also broodstocks of shrimp, marine fish, sturgeon and ornamental fish species, which can improve the growth performance and survival of larvae and also improve reproductive performance of these species. However, small scale centers need to purchase *Artemia* products from the market due to the time-consuming *Artemia* hatching and the need for science and equipment for *Artemia* hatching and cultivation. Considering the seasonal fluctuation of *Artemia* production and consumption, as well as the high sensitivity and perishability of *Artemia* even during frozen storage, this product underwent quality loss. In the present study, the effect of immersion in vitamin E solution at concentrations of 0.02, 0.2 and 0.4% on shelf life and quality of *Artemia urmiana* biomass during frozen storage (-18°C) was investigated. Total volatile basic nitrogen (TVB-N), peroxide value (PV), thiobarbituric acid (TBA), free fatty acid (FFA) values, pH, and proximate composition of *Artemia* tissue (moisture, protein, fat, ash) and the profile of fatty acids were determined for all studied treatments until the end of 8 months of storage. The results showed that vitamin E in the studied concentrations could effectively prevent the deterioration of *Artemia* quality during frozen storage compared to the control samples. The results indicated that vitamin E treatments in terms of pH, TVB-N, PV, TBA, FFA and nutritional value had a better condition compared to the control and with an increase in the concentration of vitamin E, better preservative effects were observed. In conclusion, the use of vitamin E is suggested to maintain the quality and extend the shelf life of *Artemia* during frozen storage, which is expected to show positive effects after feeding the aquatic animals with this product due to the transfer of vitamin E to the host.

**Keywords:** *Artemia* biomass, frozen storage, quality, Vitamin E

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## Introduction

*Artemia* or brine shrimp, an aquatic crustacean genus, is fed as an important live food source to the larvae of many valuable aquatic species such as shrimp, sturgeon, marine fish and ornamental fish to increase survival and growth during the larval stages. It can also have a positive effect on the sexual maturation process and the productivity of broodstocks of these species. The necessity and popularity of using *Artemia* in aquaculture is due to ease of access and transportation, long-term storage of dry dormant *Artemia* cyst which easily can be hatched to fresh nauplii after 24 hours in sea water, various sizes depending on its stage of life that is suitable for different species of fish, high nutritional value, high digestibility for larvae that have not yet developed the gastrointestinal tract, the possibility to be enriched due to non-selective filter feeding habit which can play an important role as a carrier of essential nutrients, vitamins, pigments and vaccines (Bengtson *et al.*, 2018; Madkour *et al.*, 2022).

*Artemia* trading is a growing business and due to its increasing demand in the aquaculture industry along with the limitations of harvesting from natural environments, *Artemia* cultivation in earth ponds has been being started in several parts of the world (Toi *et al.*, 2021). Like most fisheries products, *Artemia* biomass is highly perishable and deteriorates quickly if not managed (handled, stored and prepared) appropriately. Freezing is an effective method for long storage preservation of

the products (Valencia-Perez *et al.*, 2015), however, unfavorable changes may be observed in the product even during frozen storage especially if the suitable freezing (quick freezing) and cold storage facilities are inaccessible, like in small units of culturing *Artemia* in earth ponds in developing countries. Therefore, the treatment with antioxidant compounds either synthetic or natural ones can be a helpful solution before freezing process (Songsaeng *et al.*, 2010; Badii and Howell, 2002; Gunathilake *et al.*, 2022). However, due to the negative side effects of synthetic preservatives on human health, attention has been shifted towards natural preservatives (Bahram *et al.*, 2016; Rhamani- Manglano *et al.*, 2024). Vitamin E or  $\alpha$ -tocopherol is one of the major natural compounds with antioxidant property that is safe and provides multiple health benefits when used in the food and feed industry (Sadeghi *et al.*, 2018; Habeebullah *et al.*, 2023). Therefore, the present study aimed at investigation the effect of vitamin E as a natural antioxidant on the changes in quality of *Artemia* biomass during frozen storage.

## Material and methods

### *Preparation of vitamin E*

(+)- $\alpha$ -tocopherol acetate or Vitamin E acetate (1360 IU/g) was purchased from Sigma-Aldrich.

### *Preparation of Artemia and treatment groups*

In this study, *Artemia* biomass was caught/harvested from the geo-

membrane earth ponds of *Artemia* culture at National Artemia Research Center, Urmia, Iran. For frozen storage, the harvested *Artemia* biomass was washed in cleaning systems to remove any accompanying materials and fauna and then de-watered, packaged and freeze. However, in our study, *Artemia* were immersed in vitamin E solutions (1:1 w/w) at different doses of 0.02, 0.2 and 0.4% to prepare study treatments and then dewatered and freeze as mentioned above. A treatment prepared the same without vitamin E addition was used as control. After freezing, the samples were stored at -18°C up to 8 months. *Artemia* quality related tests including measurement of pH, total volatile basic nitrogen (TVB-N), peroxide value (PV), thiobarbituric acid (TBA), free fatty acids (FFA) were performed for all treatments at monthly intervals during 8 months of frozen storage. Also, proximate composition analysis of *Artemia* (moisture, protein, fat, ash) and fatty acid profiles were determined at the beginning, in the middle and at the end of the storage period.

#### *Determining of proximate composition*

Proximate analysis of *Artemia* biomass

$$PV = \frac{\text{Volume of thiosulfate consumption} \times \text{Normality}}{\text{oil sample weight}} \times 100$$

#### *Measurement of thiobarbituric acid (TBA)*

Thiobarbituric acid (TBA) was determined according the method described by Egan *et al.* (1997) (Egan). This method is based on the

which includes determination of moisture, protein, fat and ash contents was performed according to the standard method. The moisture content, crude protein and ash were determined according to AOAC, 2005 (AOAC. Official Method of Analysis (17th ed). Washington). The lipid content was measured according to Bligh and Dyer 1959 (Bligh and Dyer, 1959).

#### *Chemical analysis*

##### *Peroxide value (PV)*

The peroxide value (PV) was determined according to the method described by Egan *et al.*(1997). To determine the PV, 20 cc of the lower phase of the decanter used to extract the fat samples was carefully transferred to a 250 mL Erlenmeyer, to which about 25 mL of the chloroformic acetic acid solution (ratio of chloroform to acetic acid 2:3) was added. Then 0.5 mL of saturated potassium iodine solution, 30 mL of distilled water and 0.5 mL of 1% starch solution were added to the complex and the amount of iodine released was titrated with 0.01 normal sodium thiosulfate solution. The amount of peroxide was calculated as meq oxygen/Kg lipid as follow:

spectrophotometric quantification of the pink complex formed after the reaction of one molecule of malondialdehyde, a product of distillation, with two molecules of TBA

added to the distillate (heated in a water bath, 90C for 35 min).

#### *Measuring the number of free fatty acids (FFA)*

For measurement of free fatty acids, 25 cc of neutralized ethyl alcohol (by 1N NaOH) was added to the oil sample. The mixture was heated and after the first

sign of boiling, 2 to 3 drops of phenolphthalein reagent was added and then it was tittered with 0.1N NaOH. The amount of acidity was determined as the percentage of oleic acid as follow(Egan):

$$\text{FFA} = \text{Volume of N NaOH} \times 28.2 \times (\text{N}/10) / \text{oil sample weight}$$

#### *pH measurement*

After homogenizing 5 g of the sample with 45 mL of distilled water, the above mixture was filtered, then the pH of the samples was measured at room temperature using a pH meter (model 3510, Jenway, UK) (Suvanich *et al.*, 2000; Bahram *et al.*, 2016).

#### *Measuring of total volatile basic nitrogen (TVB-N)*

Measurement of TVB-N was carried out using Kejeldal microdiffusion technique by placing 10 g of sample plus 2 g of

magnesium oxide and adding 500 cc of distilled water in a balloon and finally collecting volatile basic nitrogen in solution containing 2% boric acid and methylred as a reagent indicator. The resulting yellow color was tited with sulfuric acid until a purple color was obtained and expressed as milligrams of nitrogen per 100 grams of *Artemia* sample. The amount of volatile nitrogen bases was calculated as follows (Goulas and Kontominas, 2005; Bahram *et al.*, 2016):

$$\text{TVB-N} = \text{volume of sulfuric acid consumed} \times 14$$

#### *Determination of fatty acid composition*

To extract fish fat, 1 g of the tissue sample was transferred to a container, to which 5 mL of methanol was added. The container was shaken vigorously for 1 minute. Then 10 mL of chloroform was added and shaken vigorously again for 1 minute. The samples were then placed in a quiet, dark place for 24 hours. Then, 5 mL of distilled water was added and then the contents of the container were transferred to a decanter, left in a quiet

place for 2 to 3 hours to form 3 separate phases. The fat solubilized in solvent phase, which was located at the bottom of the decanter, was collected and transferred to a clean glass tubes. To separate the fat from the solvent, glass tubes containing the fat and solvent were placed in a water bath and nitrogen gas was introduced into the tubes. In this way, after a few minutes, the solvent evaporated, and finally the fat collected. In order to esterify the fat, 5 mL of 2%

methanolic NaOH (2 g of NaOH in 100 mL of methanol) was added to the collected oils. The tube lid was then closed and shaken vigorously and then placed in a boiling water bath for 10 minutes. 2.2 mL of BF<sub>3</sub> solution (triborofluoride) was added to the above mixture and then it was placed in a boiling water bath for another 2-3 minutes. To the resulting mixture was added 1 mL of n-hexane and after shaking, 1 mL of saturated NaCl solution (300 g NaCl in 1 liter of distilled water) was added. The resulting solution was shaken vigorously and settled in a stationary place. After the appearance of two separate phases, the upper phase was carefully separated.

VARIAN gas chromatograph (GC) (CP3800 Walnut Creek model) was used to investigate and identify the fatty acids in the sample. The temperature of detector and injection site was set at 280 and 240 °C, respectively. 0.2 µL of the esterified sample was injected into the gas chromatography apparatus using a 1 µL syringe. The initial temperature of the column was set at 160 °C and after 5 minutes, the temperature of the column reached to 180 °C (at a rate of 20 degrees per minute). The temperature remained at this temperature for 10 minutes, and then it reached to 200 degrees at a rate of 1°C per minute. After one minute the temperature at a rate of 30°C per minute increased to 230 degrees. At the end, the column was left at 230 for 5 minutes to remove all the compounds. In this method, helium gas (with a purity of 99.999%) was used as a carrier gas and hydrogen gas was used as a fuel,

nitrogen (with a purity of 99.999%) was used as an auxiliary gas and dry air was used. By comparing the retention time of chromatograms of the injected sample with the chromatograms obtained in the standard solution of methyl ester fatty acids, the fatty acids of oil samples were identified and the results were expressed as a percentage of the sub-peak area of the total (AOAC. 2005. Official Method of Analysis (17th ed). Washington).

#### *Data analysis*

The experiment was designed and implemented in a completely randomized design. Each treatment was tested in triplicate. SPSS version 22 software was used for statistical analysis of the data. The normality distribution of the obtained data was checked using the Kolmogorov-Smirnov test, and the homogeneity of the variances was determined using the Leven test. One-way ANOVA followed by Duncan test was used for normal data to check the significant difference between different treatments. *P* value < 0.05 was considered statistically significant.

#### **Results**

In the present study, the effect of vitamin E on maintaining the quality and shelf life of *Artemia urmiana* biomass during frozen storage (-18°C) was investigated.

#### *Changes in total volatile basic nitrogen (TVB-N)*

The results of total volatile basic nitrogen (TVB-N) of different treatments of *Artemia* biomass during frozen storage are shown in Table 1.

TVB-N showed an increasing trend in all treatments with increasing storage time, although this trend was slower and had a slower slope in samples treated with vitamin E. The follow-up of the experiment until the end of 8 months of storage under freezing condition showed that the control treatment always had the

highest total TVB-N compared to the other treatments. Vitamin E was able to effectively delay the deterioration process and it was observed that better effects were observed by increasing the concentration of vitamin E.

**Table 1: The values of total volatile basic nitrogen (TVB-N) of different treatments of *Artemia* biomass during storage under freezing conditions**

Treatments	Time (month)						
	0	1	2	3	4	6	8
Vitamin E 0.4 %	7 ± 0.70 <sup>a</sup>	8.63 ± 0.40 <sup>a</sup>	10.03 ± 0.40 <sup>b</sup>	12.13 ± 0.40 <sup>a</sup>	12.36 ± 0.80 <sup>b</sup>	13.06 ± 0.80 <sup>b</sup>	14.46 ± 0.80 <sup>b</sup>
Vitamin E 0.2 %	7 ± 0.70 <sup>a</sup>	8.63 ± 1.06 <sup>a</sup>	10.73 ± 0.40 <sup>b</sup>	12.13 ± 0.40 <sup>a</sup>	12.36 ± 0.80 <sup>b</sup>	13.06 ± 0.80 <sup>b</sup>	15.63 ± 1.06 <sup>b</sup>
Vitamin E 0.02 %	7 ± 0.70 <sup>a</sup>	10.03 ± 0.80 <sup>a</sup>	10.73 ± 0.40 <sup>b</sup>	12.83 ± 0.80 <sup>a</sup>	13.06 ± 0.40 <sup>b</sup>	13.76 ± 0.40 <sup>b</sup>	16.10 ± 1.21 <sup>b</sup>
Control	7 ± 0.70 <sup>a</sup>	9.80 ± 0.70 <sup>a</sup>	16.33 ± 0.80 <sup>a</sup>	13.76 ± 1.45 <sup>a</sup>	18.43 ± 1.43 <sup>a</sup>	21.70 ± 1.40 <sup>a</sup>	25.66 ± 1.06 <sup>a</sup>

a, b, c, ... indicating a significant difference at the 0.05 level in each column

#### Changes in pH values

The pH values of different *Artemia* biomass treatments during frozen storage are shown in Table 2. During the storage period, the pH level for all treatments showed first a decreasing

trend and then an increasing trend. The increase in pH at the end of the storage period was higher in the control treatment compared to the vitamin E treated samples.

**Table 2: The pH of different treatments of *Artemia* biomass during storage under freezing conditions**

Treatments	Time (month)						
	0	1	2	3	4	6	8
Vitamin E 0.4 %	7.06 ± 0.04 <sup>a</sup>	6.62 ± 0.05 <sup>b</sup>	6.62 ± 0.04 <sup>b</sup>	6.68 ± 0.01 <sup>b</sup>	6.68 ± 0.00 <sup>a</sup>	6.66 ± 0.03 <sup>b</sup>	6.72 ± 0.04 <sup>b</sup>
Vitamin E 0.2 %	7.06 ± 0.04 <sup>a</sup>	6.66 ± 0.04 <sup>b</sup>	6.64 ± 0.04 <sup>b</sup>	6.67 ± 0.01 <sup>b</sup>	6.67 ± 0.04 <sup>a</sup>	6.69 ± 0.02 <sup>b</sup>	6.75 ± 0.03 <sup>b</sup>
Vitamin E 0.02 %	7.06 ± 0.04 <sup>a</sup>	6.65 ± 0.02 <sup>b</sup>	6.68 ± 0.03 <sup>b</sup>	6.73 ± 0.02 <sup>b</sup>	6.76 ± 0.03 <sup>a</sup>	6.75 ± 0.02 <sup>b</sup>	6.84 ± 0.04 <sup>b</sup>
Control	7.06 ± 0.04 <sup>a</sup>	6.91 ± 0.05 <sup>a</sup>	6.84 ± 0.03 <sup>a</sup>	6.83 ± 0.05 <sup>a</sup>	6.76 ± 0.04 <sup>a</sup>	6.93 ± 0.04 <sup>a</sup>	7.16 ± 0.06 <sup>a</sup>

a, b, c, indicating a significant difference at the 0.05 level in each column

#### Changes in peroxide value (PV)

The results of peroxide value (PV) of different treatments of *Artemia* biomass during storage under freezing conditions are shown in Table 3. The amount of

peroxide showed an increasing trend for all studied treatments with the increase in the storage period, but this trend was not linear and with the same slope.

**Table 3: The values of peroxide value (PV) of different treatments of *Artemia* biomass during storage under freezing conditions**

Treatments	Time (month)						
	0	1	2	3	4	6	8
Vitamin E 0.4 %	2.40 ± 0.32 <sup>a</sup>	3.88 ± 0.55 <sup>a</sup>	3.88 ± 0.55 <sup>a</sup>	3.33 ± 0.55 <sup>a</sup>	3.88 ± 0.55 <sup>a</sup>	3.51 ± 0.84 <sup>a</sup>	3.70 ± 0.32 <sup>ab</sup>
Vitamin E 0.2 %	2.40 ± 0.32 <sup>a</sup>	2.22 ± 0.55 <sup>b</sup>	4.44 ± 0.78 <sup>a</sup>	3.61 ± 0.39 <sup>a</sup>	3.88 ± 0.55 <sup>a</sup>	3.88 ± 0.55 <sup>a</sup>	3.05 ± 0.39 <sup>ab</sup>
Vitamin E 0.02 %	2.40 ± 0.32 <sup>a</sup>	3.88 ± 0.55 <sup>a</sup>	4.44 ± 0.55 <sup>a</sup>	2.59 ± 0.64 <sup>a</sup>	4.62 ± 0.32 <sup>a</sup>	4.16 ± 0.39 <sup>a</sup>	3.88 ± 0.55 <sup>a</sup>
Control	2.40 ± 0.32 <sup>a</sup>	4.07 ± 0.32 <sup>a</sup>	5.27 ± 1.17 <sup>a</sup>	3.51 ± 0.64 <sup>a</sup>	3.88 ± 0.55 <sup>a</sup>	3.61 ± 0.39 <sup>a</sup>	2.96 ± 0.32 <sup>b</sup>

a, b, c, indicating a significant difference at the 0.05 level in each column

Considering that the amount of peroxide value is an indicator of primary oxidation and the formation of hydroperoxides, then the increase of peroxide values was also expected. On the other hand, given that the primary oxidation products include hydroperoxides, are very unstable and thus quickly decompose or break down, ultimately accelerating secondary oxidation. In the current research, vitamin E showed a positive effect in delaying the process of quality loss by preventing and delaying the increase of peroxide as well as delaying the breakdown and decomposition of hydroperoxides.

#### *Changes in thiobarbituric acid (TBA)*

The amounts of thiobarbituric acid (TBA) of different treatments of *Artemia*

biomass during storage under freezing conditions are shown in Table 4. The amount of secondary oxidation products measured by thiobarbituric acid (TBA) index showed an increasing trend for all treatments with the increasing in storage time, but the amount of this increase in the control treatment was significantly higher compared to vitamin E treatments. Therefore, the treatment of *Artemia* with vitamin E at different concentrations was able to show a positive effect on preventing oxidation and reducing the quality of frozen *Artemia* during storage in freezing conditions, and in general, these effects were better with increasing the concentration of vitamin E.

**Table 4: The values of thiobarbituric acid (TBA) of different treatments of *Artemia* biomass during storage under freezing conditions.**

Treatments	Time (month)						
	0	1	2	3	4	6	8
Vitamin E 0.4 %	0.029 ± 0.001 <sup>a</sup>	0.033 ± 0.003 <sup>b</sup>	0.030 ± 0.004 <sup>b</sup>	0.035 ± 0.005 <sup>c</sup>	0.037 ± 0.005 <sup>b</sup>	0.054 ± 0.006 <sup>b</sup>	0.070 ± 0.006 <sup>b</sup>
Vitamin E 0.2 %	0.029 ± 0.001 <sup>a</sup>	0.035 ± 0.004 <sup>b</sup>	0.036 ± 0.001 <sup>b</sup>	0.045 ± 0.002 <sup>bc</sup>	0.043 ± 0.002 <sup>b</sup>	0.049 ± 0.006 <sup>b</sup>	0.073 ± 0.012 <sup>b</sup>
Vitamin E 0.02 %	0.029 ± 0.001 <sup>a</sup>	0.040 ± 0.002 <sup>b</sup>	0.039 ± 0.003 <sup>b</sup>	0.049 ± 0.001 <sup>b</sup>	0.048 ± 0.003 <sup>b</sup>	0.055 ± 0.010 <sup>b</sup>	0.083 ± 0.004 <sup>b</sup>
Control	0.029 ± 0.001 <sup>a</sup>	0.063 ± 0.005 <sup>a</sup>	0.086 ± 0.006 <sup>a</sup>	0.110 ± 0.010 <sup>a</sup>	0.134 ± 0.014 <sup>a</sup>	0.176 ± 0.012 <sup>a</sup>	0.226 ± 0.014 <sup>a</sup>

a, b, c, indicating a significant difference at the 0.05 level in each column

### Changes in free fatty acids (FFA)

The results of free fatty acids (FFA) of different treatments of *Artemia* biomass during storage under freezing condition are shown in Table 5. The amounts of free fatty acids showed an increasing trend with the increase in storage time. The highest increase of free fatty acids was observed in the control treatment, so that at the end of 8 months of storage

under freezing condition, the control treatment had a significantly higher amount of free fatty acids compared to the other treatments. The use of vitamin E showed a positive effect in preventing the rapid increase of free fatty acids compared to the control treatment, and in the meantime, better effects were observed with increasing the concentration of vitamin E.

**Table 5: The values of free fatty acids (FFA) of different treatments of *Artemia* biomass during storage under freezing conditions.**

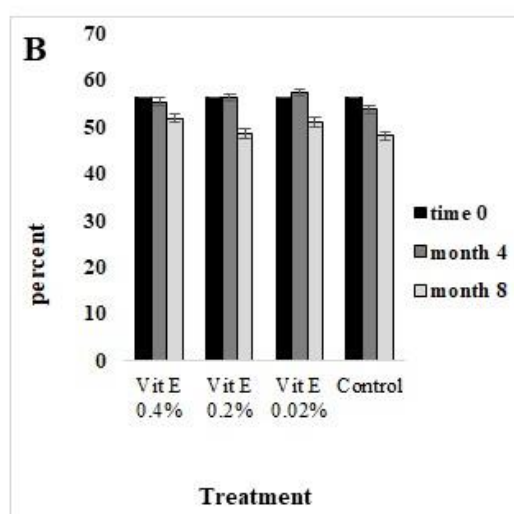
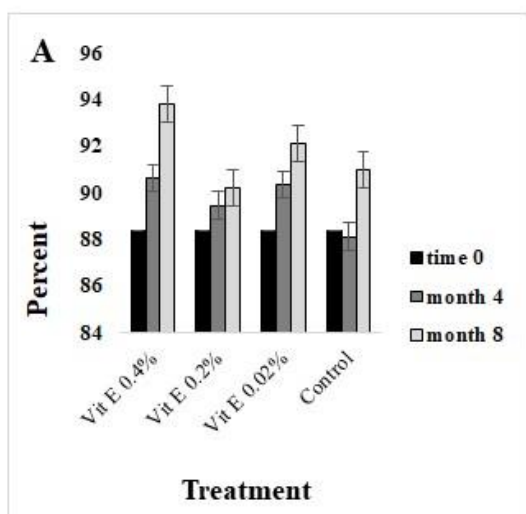
Treatments	Time (month)						
	0	1	2	3	4	6	8
Vitamin E 0.4 %	0.51 ± 0.06 <sup>a</sup>	0.90 ± 0.15 <sup>a</sup>	0.93 ± 0.13 <sup>a</sup>	0.76 ± 0.12 <sup>a</sup>	0.83 ± 0.09 <sup>b</sup>	0.90 ± 0.19 <sup>b</sup>	0.94 ± 0.18 <sup>b</sup>
Vitamin E 0.2 %	0.51 ± 0.06 <sup>a</sup>	0.70 ± 0.21 <sup>a</sup>	0.78 ± 0.16 <sup>a</sup>	0.90 ± 0.08 <sup>a</sup>	0.90 ± 0.09 <sup>b</sup>	0.63 ± 0.18 <sup>b</sup>	1.04 ± 0.22 <sup>b</sup>
Vitamin E 0.02 %	0.51 ± 0.06 <sup>a</sup>	0.98 ± 0.06 <sup>a</sup>	0.89 ± 0.13 <sup>a</sup>	0.98 ± 0.18 <sup>a</sup>	1.00 ± 0.08 <sup>b</sup>	0.86 ± 0.18 <sup>b</sup>	1.09 ± 0.04 <sup>b</sup>
Control	0.51 ± 0.06 <sup>a</sup>	1.12 ± 0.38 <sup>a</sup>	0.73 ± 0.11 <sup>a</sup>	0.88 ± 0.10 <sup>a</sup>	1.25 ± 0.07 <sup>a</sup>	1.67 ± 0.17 <sup>a</sup>	2.31 ± 0.36 <sup>a</sup>

a, b, c, indicating a significant difference at the 0.05 level in each column

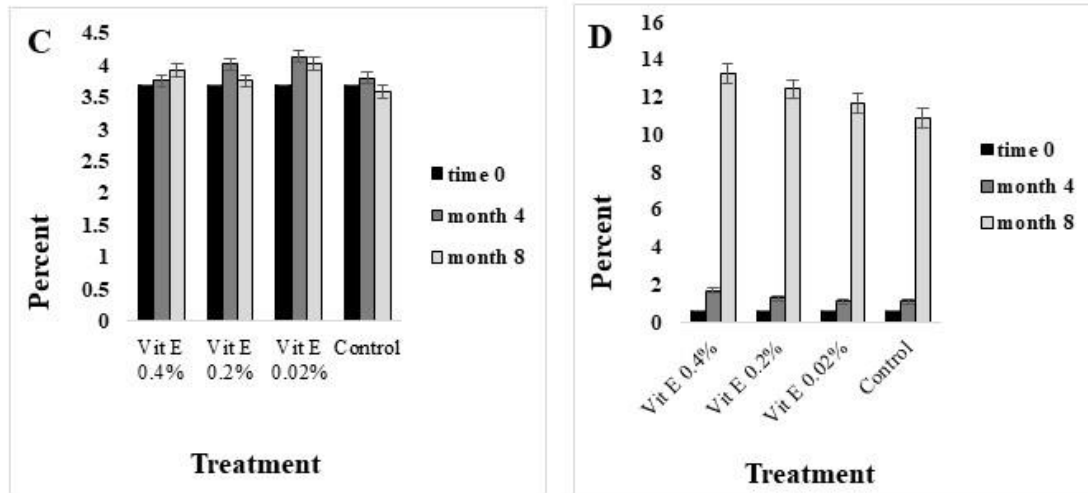
### Changes in the approximate analysis

The results of approximate analysis (including moisture, protein, fat and ash)

of different treatments of *Artemia* biomass during storage under freezing condition are shown in Figure 1.





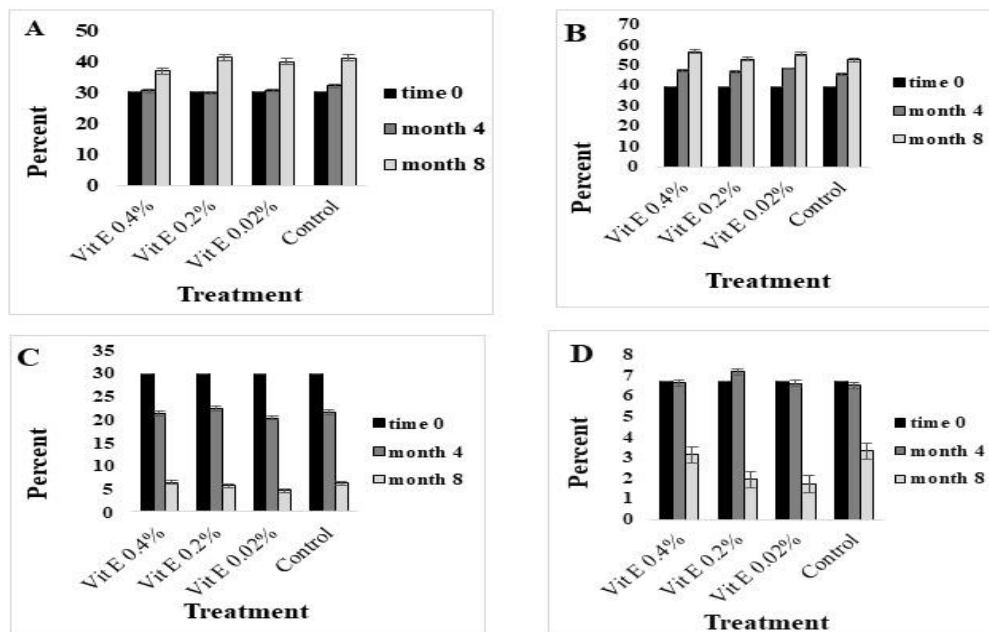


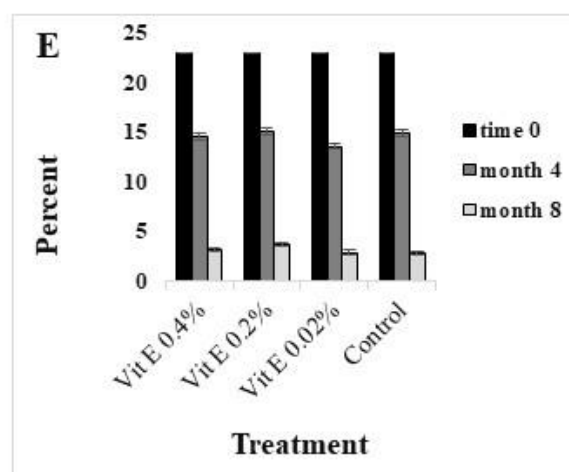
**Figure 1:** Changes in proximate composition of different treatments of *Artemia* biomass during storage under freezing condition. A; moisture, B; protein, C; fat and D; ash.

As shown, during the storage period, the amount of moisture, protein and fat showed a similar pattern in all research treatments. In all treatments, with the increase of storage time, the amount of moisture increased, the amount of protein and fat was almost constant, although they showed a slight decrease and the amount of ash increased.

The results of the changes in fatty acid profiles of different *Artemia* biomass treatments during storage under freezing condition are shown in Figure 2. During storage period, the amounts of SFA and MUFA showed an increasing trend in all treatments, but the amounts of PUFA, omega-6 and omega-3 fatty acids showed a decreasing trend.

#### Changes in fatty acid profile





**Figure 2: Changes in fatty acid profiles of different treatments of *Artemia* biomass during storage under freezing condition. A; saturated fatty acids, B; mono unsaturated fatty acids, C; poly unsaturated fatty acids, D; n-6 fatty acids and E; n-3 fatty acids.**

## Discussion

The results of this study showed that vitamin E in the studied concentrations could effectively prevent the deterioration of *Artemia* quality during frozen storage compared to the control samples. The results indicated that vitamin E treatments in terms of pH, volatile basic nitrogen (TVB-N), peroxide value (PV), thiobarbituric acid (TBA), free fatty acids (FFA) and nutritional value had a better condition compared to the control and with an increase in the concentration of vitamin E, better preservative effects were observed. In line with our results, the preservation and maintaining nauplii and adult *Artemia* at ambient or refrigerator temperature using preservative liquid based on garlic extract has been developed commercially (named nauplii guard and *Artemia* guard, respectively). However, it should be noted that there is no study on the use of vitamin E or other natural preservatives to maintain *Artemia* quality during frozen storage.

The results of studies related to the use of natural preservatives, including antioxidant vitamins and plant extracts, for the preservation and extending the shelf life of other fishery products are in line with the results of the present study. In a study by Paktermani *et al.* (2017),  $\alpha$ -tocopherol was used in combination with sodium alginate to maintain the quality characteristics of rainbow trout fillets, and the results showed that the use of this antioxidant reduced the rate of peroxide and thiobarbituric acid production compared to the control (Hermund *et al.*, 2019). Zuta *et al.* (2007) also reported that the primary oxidation products in mackerel fish oil were reduced in the presence of a low concentration of  $\alpha$ -tocopherol. Similarly, the use of  $\alpha$ -tocopherol in combination with chitosan coating greatly controlled the process of fatty acid production in rainbow trout fillets during storage at refrigerator temperature compared to the control (Tolouie *et al.*, 2012). The above results

are consistent with the results of the present study regarding the effectiveness of vitamin E in preventing the increase of peroxide and ultimately maintaining the quality of *Artemia* during frozen storage.

In another study, muscle  $\alpha$ -tocopherol of trout fish fed a diet containing  $\alpha$ -tocopheryl acetate at 5000 mg/kg diet increased through 9 wks. of feeding. Reduced muscle  $\alpha$ -tocopherol and moisture, and increased muscle redness and fat were observed in frozen-refrigerated fillets compared with fresh fillets. Feeding with higher Vitamin E or increased feeding duration resulted respectively in lower TBARS and increased proportion of unsaturated fatty acids and omega-3 fatty acids (Nartea *et al.*, 2023; Abdul Rahim *et al.*, 2024). Similarly, in another study it was reported that diet containing fish oil for enrichment of meat of broiler chickens enhanced meat lipids peroxidation and using vitamin E in diet showed lower levels of MDA during the storage and increased meat stability in fish oil enriched meat (Armin *et al.*, 2015; Rahmani-Manglano *et al.*, 2020).

The set of studies mentioned above confirms the results of the present study on the positive effect of vitamin E on maintaining the quality and extending the shelf-life of *Artemia* biomass during frozen storage. Moreover, considering the positive effects of vitamin E in diet on the growth, survival and resistance to diseases of different fish species, it can be expected that fed fish with vitamin E treated *Artemia* can show the positive

effects due to the transfer of vitamin E to the host.

### Conclusion

In conclusion, vitamin E in the studied concentrations could effectively prevent the deterioration of *Artemia* quality during frozen storage compared to the control samples. The results indicated that vitamin E treatments in terms of pH, TVB-N, PV, TBA, FFA and nutritional value had a better condition compared to the control and with an increase in the concentration of vitamin E, better preservative effects were observed. In conclusion, the use of vitamin E is suggested to maintain the quality and extend the shelf life of *Artemia* during frozen storage, which is expected to show positive effects after feeding the aquatic animals with this product due to the transfer of vitamin E to the host.

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