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The study of frozen *Astacus leptodactylus* tail fillet quality changes

[1]Ali Nekuie Fard, [1]Masoud Seidgar, [2]Dariush Azadikhah

[1] National Artemia Research Center, Iranian Fisheries Science Research Institute, Agricultural Research Education and Extension Organization (AREEO), Urmia, Iran; [2] Department of veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran. Corresponding author: A. Nekuie Fard, dr.nekuiefard@gmail.com

Abstract. Chemical and microbial quality changes of freshwater crayfish (*Astacus leptodactylus*) tail fillet were studded during storage at freezing temperature. Samples were packaged and stored in -18 ° C in the freezer after fishing, preparation and peeling in the presence of air. Quality and microbial tests were performed on frozen fillets in time zero and once every month for 6 months with one treatment including three replicates for each test period. The results showed that at the end of six-months storage in freezer, the values (Mean±SE) of thiobarbituric acid (TBARS), peroxide value (PV) and total volatile nitrogenous bases (TVB-N) had reached from 0.05±0.005 to 1.45±0.25 mg, malondialdehyde / kg; 0 to 2.2±0.30 meqO2 kg /fish fat and 11.25±.05 to 26.6±1.40 mg per 100 g of meat, respectively. These values were within the acceptable range for use in spite of exesting significant difference with each other ($p<0.05$). pH value had no significant changes ($p>0.05$) until the end of fifth months (6.2±0.25), but this value had a significant increase (6.57±0.55) after the sixth month ($p<0.05$). At the same time, Total viable of bacteria (TVC) and psychrotropic bacteria (PTC) were reached from 3.49±0.01 to 6.86±0.08 log CFU/g and from 1±0.0 to 6.75±0.25 log CFU/g., respectively. Comparison of within group parameters during test period showed significant difference with each other ($p<0.05$) but from the sixth month psychrotropic bacteria were on the verge of taking the risk for human consumption.
Key Words: freshwater crayfish, meat, spoilage, chemical, microbial.

Introduction. Crayfish are classified in different 1200 genera and 10,000 species. Among them, there are about 500 species in the world that are taken place in 29 genera (Skurdal & Taugbøl 2002). Crayfish is considered as one of the special fish foods in America in the last decade (Martin et al 2000). Annually, 110 thousand tons of fresh water crayfish are fishing commercially. Meanwhile, processed ones are provided for human consumption by America 55%, China 36% and also Europe and Australia (Martin et al 2000). *Astacus leptodactylus* is widely consumed in many countries as one of the commercial species due to high levels of omega 3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid. This species has reached to water resources in several countries, such as Poland, Italy, Germany, England, Spain and France unexpectedly (escape from shopping centers) and has created certain populations (Harlioğlu 2004). Today, it can be found in 27 countries around the world that its presence is possible as introducing to water resources in 14 countries (Skurdal & Taugbøl 2002). *A. leptodactylus* is the only species of *Astacus* in Iran that fishes from inland fresh water resources. Among water resources, Reservoir Lake behind the Aras dam located in the Poldasht city, West Azerbaijan province, which is the original habitat and its only location for commercial fishing in Iran that annually more than 200 tons are exported to European countries (Nekuie Fard et al 2011).

Commercial value of fresh water crayfish in Europe caused to consider it as an important economic product. Russia, Turkey, France and Spain are among the main producers of *A. leptodactylus* that have almost the ability to supply all the needs of Western

Europe and Scandinavia (Nekuie Fard et al 2011). European consumers are preferred to consume *A. astacus* that is a more valuable European species than *A. leptodactylus*, but *A. leptodactylus* always has had an special and important place among consumers of freshwater crayfish in Europe (Harlioğlu 2004). Statistics show that commercial interest of *A. leptodactylus* has increased dramatically with *A. astacus* population decline due to disease and contaminated water (Harlioğlu 2004). So, European consumers has begun to import *A. leptodactylus* to supply their own internal meat consumption (SamCookiyaei et al 2012).

Increased demand for the product has challenged distributors to optimize and provide various products in accordance with the tastes of their customers. So, in addition to product, they should be guarantee the health of consumer (Flick 2008). Since the spoilage in aquatic animals is depending on temperature and time and there is a long distance between producing, processing and demanding countries, using freezing system, packaging crayfish in a vacuum is considered as an assistant technology to increase the life shelf and durability and prevent chemical, oxidation and microbial spoilage (Gates 2012).

The most important part of the crayfish meat in processing factories is of tail area (McClain 2000). In addition to tail, arm meat is also used (Martin et al 2000). The amount of tail flesh in freshwater crayfish is included about 9-13% of wet body weight (Harlioğlu 2004). Mostly in the processing centers after cooking (dropping in boiling water) for about 4 minutes, exterior skeleton of tail area is removed and the staff in addition to separating the intestine from meat of the tail, adhesions of hepatopancreas are also isolated and then packed and frozen (Martin et al 2000; McClain 2000). As the arm meat is very difficult to separate, the action is performed by mechanical devices. The amount of arm meat varies depending on the crayfish species (Martin et al 2000). Freezing causes the crayfish tail meat to tighten as other products of aquatic animals. The mechanism of this process is not yet well known, but it seems denaturation and protein aggregations are the initial causes of hardening. This process may be due to mechanical damage associated with freezing rate or related to the formation of ice crystals and the effect of ion concentration or interaction of chemicals such as formaldehyde or products derived from fat oxidation (Godber et al 1989).

TBA represents as an index of lipid oxidation is one of the most important tests in the detection of meat spoilage (Tokur et al 2006). A very low initial level of TBA represents freshness and good quality of crayfish (Ojagh et al 2010).

Generally 1–2 mg MDA.kg^{-1} is considered as acceptable TBA limit so that higher than the amount cause bad odor in the product (Olafsdottir et al 1997).

This study was performed to determine the shelf-life of raw tail meat of *A. leptodactylus* during freezing temperatures -18°C in vacuum packaging conditions to help distribution and processing factories will be performed necessary exploitation toward optimizing processing methods in terms of features of species.

Material and Method

Preparation, packaging and storage of freshwater crayfish tail fillet. 130 freshwater crayfish with an average weight of 50±10 g were captured from Reservoir Lake of Aras dam. Captured crayfish are transferred to the National Artemia Research Center immediately adjacent to ice and after washing with water and peeling the tail, the tail fillets were removed. Average weight of each fillet tail was 4.5±1.5 g. Fillets placed in packs of 20 g of polyamide material randomly and after labeling were packed exposed to air. Samples were stored in the freezer for 6 months at -18 °C. For each test period one treatment including three replicates was considered. Diagnostic tests were performed for chemical and microbial spoilage on zero days (initiate research) and once every month for 6 consecutive months. Tests are done in zero time approximately 4 hours after the procedure.

Measurement of pH. 2 g of tail fillets from each treatment were added to 10 mL of distilled water and were homogenized by homogenizer device for 30 seconds, then PH value of samples were measured by a pH meter model Metrohm 713 (Sallam et al 2007).

Measurement of total volatile nitrogen bases (TVB-N). 10 g tail fillet are placed into 1000 mL distilled balloon and 2 g of magnesium oxide, 300 mL distilled water with small boil pebbles and a little defoamer were added. Balloon was heated for 15 minutes to reach boiling point. Vapors emitted from distilled balloon had been directly collected into the Erlenmeyer containing 25 mL of 2% boric acid solution and a few drops of red methyl, so that the volume of boric acid and steam condenses inside reached to 150 mL. Boric acid color containing red methyl indicator that in the beginning was red due to acidic, slowly became Alkaline and was green by accumulation of steams caused by distillation. In the end, the solution obtained by accumulation of steams distillation was titrated by 0.1 normal acids sulfuric until reaching onion skin color. Volatile nitrogen content was obtained in milligram of one hundred grams of sample (Etemadian et al 2011).

Thiobarbituric acid index measurement (TBARS). 97.5 mL distilled water was added to 10 g crayfish tail fillet sample in one liter distilled balloon and was stirred for 2 minutes. Then 2.5 mL of 4 M chloridric acid was added to it. After adding a few drops of anti-foam, balloon heated to obtain 50 mL of distilled liquid in 10 minutes of boiling time. Then 5 mL of distilled liquid with 4 mL TBARS indicator (this indicator is obtained by dissolving 0.288 g powder TBARS indicator in 100 mL of 90% glacial acetic acid) was mixed in a test tube. Test tubes that contain distilled liquid and indicator associated with control tube were placed for 30 minutes into benmary at a temperature of 100°C. After cooling the samples for 10 minutes, their absorption was read by spectrophotometer device against control solution at wavelength 538 nm. The amount of thiobarbituric acid were obtained in terms of milligrams malondialdehyde per kilogram samples (Elkashef & Wyatt 1999).

Peroxide measurements (PV). Extracted crayfish oil samples from acid acetic chloroform solution are used for measuring peroxide (acid acetic to chloroform rate 3 to 2) and the liberated iodine was tittered with normal sodium thiosulfate solution 0.01 (Elkashef & Wyatt 1999).

Microbial tests. 10 g of tail fillet were placed into a Stomacher Bag Mixed and then 90 mL of sterile salt solution (0.85 g in 100 mL) were added. After homogenization for 1 min using Stomacher device, serial dilutions were prepared to 10^{-10} dilution. One mL of each dilution was placed in plate count agar medium for bacterial culture by pour pellet method. Cultured plate counts related to total viable counts (TVC) were counted after 48 h incubation at 37°C, plates related to the Psychrothrophic counts (PTC) were counted after 10 days incubation at 6°C (Arashisar et al 2004).

Statistical analysis. Statistical analysis of data was performed using the Medcalc software version 13. Initially normality of data was surveyed using the Kolmogorov - Smirnov test and homogeneity of variance data was analyzed by Leuven test. For analysis of the data ANOVA test and to investigate the difference between means of a treatment in different times and between different treatments at one time Duncan test was used. In all stages of analysis, the allowable error to reject the null hypothesis was considered (Schoonjans 2008).

Results and Discussion

In a research conducted on Australian lobster or red arm crayfish kept in freezing conditions, increasing the amount of Thiobarbituric acid was observed in storage period of 6 months (Tseng et al 2005). This increase can be related to the interaction between the protein and

lipid oxidation products and reduced the protein solubility (Siddaiah et al 2001). In another study on the effects of freezing at -21°C for 6 months on crayfish fillet, the increase in TBARS was observed in each month and in the seventh month it reached to 1.64 mg/kg (Tseng et al 2005).

In the present research TBARS Thiobarbituric acid increased with time in frozen samples during storage period (Figure 1). This amount indicated significant difference in zero time with other test periods ($p<0.05$).

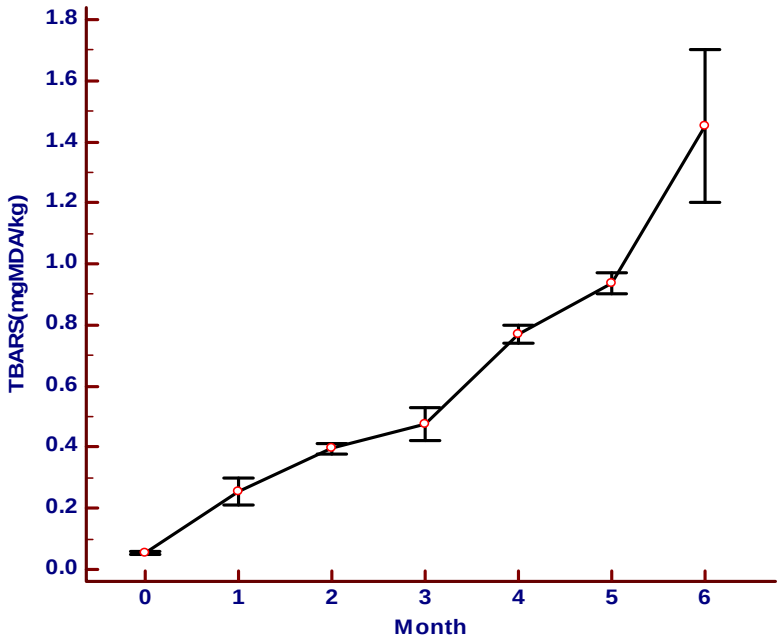

Figure 1. Thiobarbituric acid index changes (Mean±SE) of *Astacus leptodactylus* fillet during test period.

Comparing TBARS results of the tests showed that 1 to 3 months storage time had no significant differences with each other ($p>0.05$) but from the fourth month onwards showed significant differences with each other ($p<0.05$). So, at the end of the storage period it reached to 1.4±0.25 mg malondialdehyde/kg (Table 1). These results are consistent with other researchers in studies on other species of crayfish and crustaceans (Amr & Rutledge 1980; Baboli et al 2012; Gandotra et al 2012; Tseng et al 2005).

The amount of peroxide is the indicator of oxidation of lipids and is used to measure the oxidation of primary products, hydro-peroxides (Ehsani & Jasour 2014). Hydro-peroxides are formed at the first phase of oxidation by binding of oxygen to the double bond of unsaturated fatty acids (Lin & Lin 2005). Peroxides are compounds with no taste and odor and are not recognized by consumers, but by making secondary compounds such as aldehydes and ketones can cause bad taste and odor of the product. Another study conducted in 2005 by Tseng showed the impact of plunging crayfish red meat (*Cherax quadricarinatus*) with tocopherol and propil galat antioxidants that was stored for 6 months at -20°C that mentioned antioxidants had an effect in preventing lipid oxidation compared with the group without antioxidants, but these changes were not significant. Comparing peroxide value (PV) at time zero, with other periods showed a significant difference ($p<0.05$) (Table 1).

Table 1

The comparison of quality indexes changes including chemical and acidity of *A. leptodactylus* fillet during test period

Month	Test	PV meq O_2/kg	TBA mg MDA/kg	TVB-N mg/100 gr	pH
0	Mean	0.000 [a]	0.055 [a]	11.250 [a]	6.350 [a]
	SE	0.00	0.005	0.050	0.0500
1	Mean	0.815 [b]	0.255 [b]	14.215 [b]	6.250 [a]
	SE	0.2150	0.045	0.215	0.150
2	Mean	1.105 [c]	0.395 [b]	15.115 [b]	6.315 [a]
	SE	0.005	0.015	0.01500	0.0150
3	Mean	1.140 [c]	0.475 [Ab]	15.295 [b]	6.285 [a]
	SE	0.02	0.055	0.0150	0.0250
4	Mean	1.220 [c]	0.770 [ABc]	16.770 [c]	6.225 [a]
	SE	0.060	0.030	1.430	0.0050
5	Mean	1.340 [c]	0.935 [Bc]	19.775 [d]	6.225 [a]
	SE	0.040	0.035	0.175	0.0250
6	Mean	2.200 [d]	1.450 [d]	26.600 [e]	6.575 [b]
	SE	0.300	0.250	1.400	0.0550

Similar letters show no significant differences and dissimilar characters show a significant difference at 95%.

The second to fifth months together showed no significant difference ($p>0.05$). Peroxide value in the sixth month reached the highest amount 2.2±0.3 mEq kg of oxygen in kg fish fat, which had significant difference ($p<0.05$) with all test periods (Figure 2). This increase was consistent with other studies conducted by Huss (1995) and Jeyasekaran et al (2002).

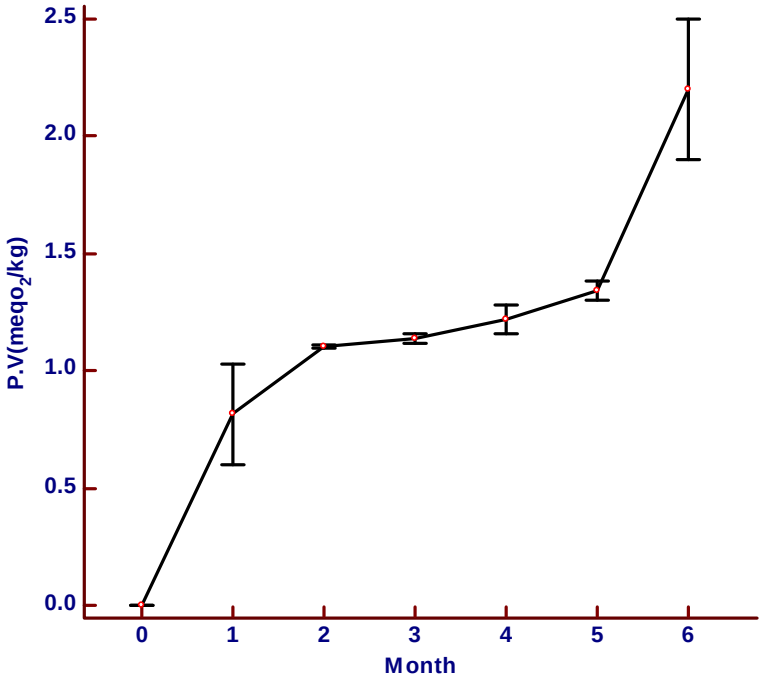

Figure 2. Peroxide (Mean±SE) changes of *Astacus leptodactylus* fillet during test period.

TVB-N includes trimethyl amine (produced by bacterial spoilage), dimethyl amine (produced by the autolytic enzymes during storage period), and ammonia (produced by the

deamination of amino acids and nucleotides and other nitrogenous volatile bases) related to corruption of marine products (Huss 1995). The maximum acceptable level of TVB-N is declared 25 mg of nitrogen per 100 g of meat (Ojagh et al 2010).

In a survey by Moeeni & Pazira (2004), the effect of cold storage on the quality of cultured and wild shrimp of Persian Gulf were examined for 120 days that on day 60 reached to 31 milligrams per hundred grams that showed the increase of this index during freezing period.

The result of total volatile base nitrogen (TVB-N) represents an increase in this criterion during storage in freezing temperature (Figure 3). So, zero time had significant differences with all of the times ($p<0.05$). The results obtained from the second to fourth months had no significant differences with each other ($p>0.05$). From the fifth month a significant difference ($p<0.05$) was observed with the other test periods with an ascending trend as in the sixth month it reached to the highest value 26.6±1.4 mg per 100 g crayfish fillet (Table 1). Obtained results were consistent with the findings of Martínez-Álvarez et al in 2008 on Norwegian lobster and with Tsironi et al (2009).

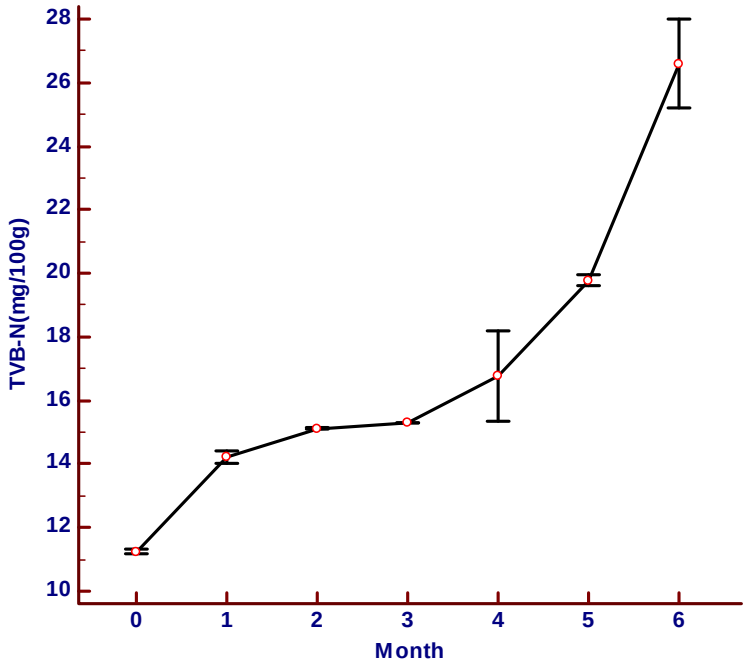

Figure 3. TVB-N (Mean±SE) Changes of *Astacus leptodactylus* fillet during test period.

Initially low pH was due to lactic acid production, at the end of the storage period the pH was high while increasing the buffering compounds produced during the decay process was due to enzymatic degradation of the meat content (Simeonidou et al 1997). Comparing the results obtained from pH changes of freshwater crayfish fillets during storage in freezing conditions (Figure 4) showed no significant difference between zero times to the fifth month ($p>0.05$). But, from the sixth month the amount reached to 6.57±0.5 that showed a significant increase ($p<0.05$) with other test periods (Table 1). The results obtained are consistent with Martínez-Álvarez et al (2008) on Norwegian lobster.

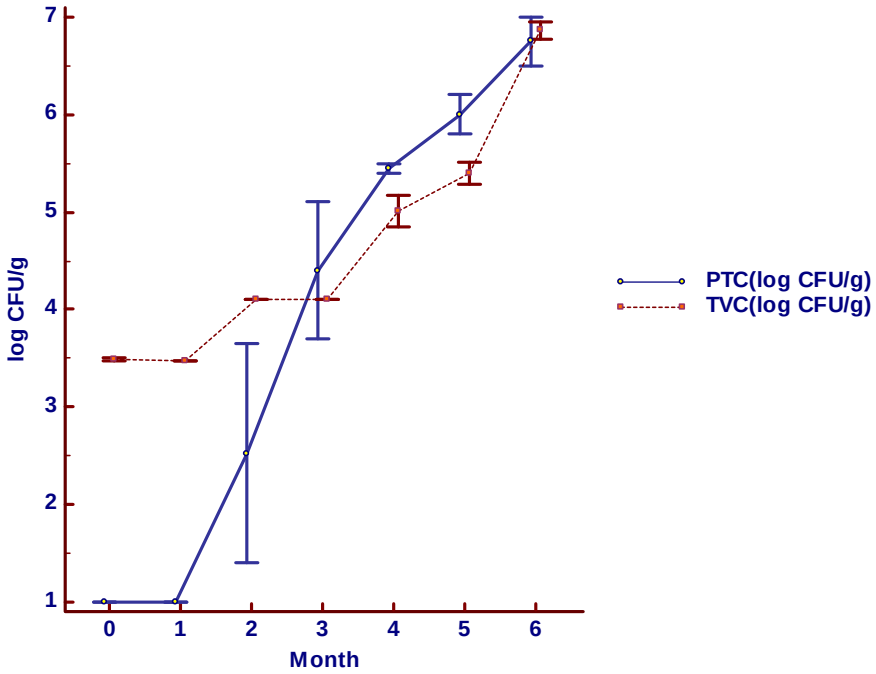

Figure 4. PTC and TVC changes (log CFU/g) of *Astacus leptodactylus* fillet during test period.

Microbial tests. Bacteria are considered as the first group of tissue destruction and corruption in seafood, thus in all meats the sanitary criteria from fishing to packaging should be conducted under controlled conditions to minimize the amount of bacterial contamination (González-Escalona et al 2008). The acceptable maximum level of mesophilic aerobic bacteria counts in freshwater fish meat by the International Committee of foods and biological features is proposed 7 log CFU/g by the International Committee of foods and biological features (Reij & Den Aantrekker 2004). Mean primary total count of mesophilic aerobic bacteria before freezing was 3.49±0.01 log CFU/g, which represents the good quality of crayfish studied (Huss 1995).

Total count of mesophilic aerobic bacteria (total viable count) TVC showed an increase during storage period between different treatments (Figure 4).

Comparison of TVC means at different storage times showed no significant difference ($p>0.05$) at time zero and the first month and also showed a significant difference from the third month to the end of the period with all treatments ($p<0.05$) so that in the sixth month this value reached 6.86±0.85 (log CFU/g) (Table 2). This increase was consistent with Treece et al (1985) and also with studies of other researchers in other aquatic animals (Huss 1995; Jeyasekaran & Ayyappan 2002).

Gram-negative psychotrophic bacteria are of other main microorganisms responsible for spoilage of fresh aquatic animals stored in cold condition (Mexis et al 2009). Especially, pseudomonas species producing lipase and phospholipase enzymes increasing free fatty acids (Kykkidou et al 2009). Psychrophilic bacteria have global distribution and are present in all freshwater resources (Onishchenko & Kiprianova 2004). The presence of these bacteria in frozen aquatic products can be due to transition through contaminated water supplies (Makarios-Laham & Lee 1993).

The results of Psychrothrophic count (PTC) in this study showed an increasing trend during storage period (Figure 4).

Table 2

Compression of microbial indexes changes (Mean±SE) of *Astacus leptodactylus* fillet during test period

Month	*Test*	*TVC (log CFU/gr)*	*PTC (log CFU/gr)*
0	Mean	3.490 [a]	1.000 [a]
	SE	0.010	0.00
1	Mean	3.480 [a]	1.000 [a]
	SE	0.00	0.00
2	Mean	4.110 [b]	2.525 [b]
	SE	0.00	1.1250
3	Mean	4.110 [b]	4.400 [c]
	SE	0.00	0.700
4	Mean	5.015 [c]	5.450 [d]
	SE	0.1650	0.050
5	Mean	5.395 [d]	6.000 [e]
	SE	0.1150	0.200
6	Mean	6.865 [e]	6.750 [f]
	SE	0.085	`0.250

* The same letters show no significant differences and dissimilar characters show a significant difference at 95%.

Comparison of PTC means in different storage periods showed no significant difference ($p>0.05$) at zero time and the first month and also, the significant difference from the third month until the end of the period with all treatments ($p<0.05$). As, in the sixth month this value reached to 6.75±0.25 log CFU/g (Table 2). The results were consistent with the results of (Huss 1995; Shamshad et al 1990; Tseng et al 2002) regarding the shrimp and other aquatic products. This study revealed that the maximum long-term sustainability of freshwater crayfish tail fillet in package exposed to air was 5 months after freezing at -18°C.

Conclusions. The results showed that microbial (TVC, PTC) and chemical (TVB-N, TBA, PV, pH) spoilage indicators of *A. leptodactylus* frozen fillets were in the acceptable range, although the index showed upward trend until the end of the fifth month. But from the sixth month these changes turned into the scope of corruption. Therefore it can be concluded that the maximum long- term sustainability of freshwater crayfish tail fillets packaged in air is 5 months at -18°C.

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Authors:
Ali Nekuie Fard, National Artemia Research Center, Iranian Fisheries Science Research Institute, Agricultural Research Education and Extension Organization (AREEO), Iran, Urmia, e-mail: dr.nekuiefard@gmail.com
Masoud Seidgar, National Artemia Research Center, Iranian Fisheries Science Research Institute, Agricultural Research Education and Extension Organization (AREEO), Iran, Urmia, e-mail: seidgar21007@yahoo.com
Dariush Azadikhah, Department of Veterinary Medicine, Urmia Branch, Islamic Azad University, Iran, Urmia, A82, e-mail: d_azadikhah@yahoo.com

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