# Comparison of nutritional value base on alpha linolenic acid and some heavy metal contamination in farmed rainbow trout and tuna fish in markets

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## Abstract

Considering the importance of the role of nutrition for the health and the prevention of diseases, food should first be examined for the chemical and microbial safety and then for the amount of bioactive components according to the factors affecting its stability.In other words, the presence of nutrients in the food substance cannot be a reason to supply all of these resources to the bodyby reducing of bioactive content from the farm to the table. For this purpose, nutritional characteristic base on alpha linolenic acid (ALA) and human health risk base on some heavy metal as lead, mercury, cadmium in rainbow trout and tuna fish with respect to storing time in refrigerator and heat treatment method were analyzed by gas chromatography and atomic absorption spectrometry, respectively. Data were analyzed by T test in SPSS software. The results showed that the amount of heavy metals in these two fish species is less than the permissible FAO/WHO levels. But the accumulation of these metals in the rainbow trout skin was higher than the muscle. ($P \geq 0.05$).In tuna fish, sterilization heating could not obviously affect on the amount of heavy metals contents ($P \geq 0.05$). The mean percentage value of the ALA reduction in farmed rainbow trout was 11.9% in 5$^{th}$ day in refrigerator away from light that wasn't usually used in markets. Sterilization heating in tuna fish processing has significant effect on ALA reduction ($P \leq 0.05$). Eventually, ALA content was decreased by storage time and processing method in farmed rainbow trout and tuna fish and ALA amount in farmed rainbow trout was higher than tuna fish ($P \leq 0.05$) which could be due to difference in species, nutrition addition to environmental factors such as fishing time, transport and storage conditions.

**Key words**: *heavy metals, omega3, rainbow trout, tuna fish*

## Introduction





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One of the important hygiene indicators is development of the human health in the community. Human health can be influenced by two factors, the genetic and environmental factors that the most important environmental factors can be nutrition (Das *et al.*, 2016). Today, the role of food is proven in health protection and diseases prevention in consumers. Among the type of foods, some of them due to bioactive substances are very important than others. Bioactive substances as food components are effective in diseases prevention, especially chronic diseases such as cancer, heart disease, etc., and sometimes in the treatment of certain diseases (Jitendra and Amit, 2015). Fish is a natural animal functional food by the presence of bioactive substances such as essential fatty acids, vitamins, minerals and proteins. These food naturally and without fortification protect the health of the consumer, in addition to nutritional value (Heidmannsoccol and Oetterer, 2003). One of bioactive component of fish is omega3 that including α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid. These essential fatty acids help to stability and fluidity of the cell membrane for the exchanging nutrients and waste of the cells. Cells without healthy membrane lose the ability to water and nutrients storage and also lose connectivity to other cells. Therefore essential fatty acids play a vital role in the function of the nervous system, reproductive system, immunity system, cardiovascular system and many of the biochemical processes of the body (Murillo *et al.*, 2014). In spite of these good properties, the long-chain PUFA are especially susceptible to oxidation and this chemical spoilage occurs due to different factors as fatty acid profile, processing and storage conditions. Lipid oxidation can change the color and taste of fish products and decrease their nutritional value by degrading these fatty acids. Therefore, the stability of omega-3 is important until consumption (Hsieh and Kinsella, 1986: Schneedorferová *et al.*, 2015). Existence of omega-3 in a foodstuff cannot provide the needs of human body, because it could be reduced for inappropriate storage and supply method.

Today, in addition to nutritional value, the safety of food products is also important. On the other hand, one of the functional food criterions is that there are no harmful components in it. Therefore, the nutritional value of the fish should be evaluated with the chemical contamination. Measurement of heavy metals as the most important chemical contamination in fish is worth for public health (Sadeghi *et al.*, 2011; Sanjar *et al.*, 2009).



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Aquaculture is one of the important sources of human nutrition, which all people like to increase its consumption due to beneficial properties. Heavy metals contamination in fish occurs through the leakage of petroleum products, atomic waste, and industrial wastewater into the aquatic environment. Heavy metals persist in environment and they are rapidly released to all fish meat and human body (Askari *et al.,* 2012; Fallah *et al.,* 2011). These pollutants are accumulated in the human tissues and cause many diseases and complications in human because of they are not metabolized and excreted from body. Heavy metals can be transmitted from the cell membranes by protein carriers and bind to essential components like proteins and enzymes and thereby the activity of enzymes and the synthesis of essential compounds are disordered in the human body. Among the heavy metals, lead, arsenic and mercury are more dangerous than others (Vieira *et al.,* 2011). Hence, many studies have been done on these metals in fish because many factors such as age, physiological factors, nutrition and geographical location and etc. are effect on heavy metal contamination in fish (Miklavc *et al.*, 2011; Najm *et al.,* 2014).

In this study, rainbow trout and tuna fish were analyzed because rainbow trout is one of the most popular kinds of farmed fish in Iran and tuna fish are considered as convenience of consumption. In addition they have different aquaculture environment that can be compared together. The aim of this study was to evaluate and compare the omega-3 levels base on the linolenic acid and some heavy metals in farmed rainbow trout and tuna fish according to post-harvest preservation and heat processing methods. These data can determine nutritional value and safety in farmed and pelagic fish.

## Materials and Methods

### Sample collection

For this research, 10 tuna fish and 25 farmed rainbow trout were randomly collected from fish markets in Isfahan city. All samples were kept in polyethylene bags and placed on ice until transferred to laboratory and immediately analyzed. For ALA test, rainbow trout samples were analyzed on $1^{st}$, $3^{th}$ and $5^{th}$ days after fishing. They were stored in a refrigerator away from light ($4^{℃}$) and Tuna fish, were tested before and after sterilization treatment in autoclave (121 $^{℃}$, 20 min,) for ALA content. Heavy metals were measure in meat and skins of farmed rainbow trout and tuna fish before and after sterilization heating.

**Preparation of fish for the measurement of heavy metals**

In the laboratory, the fish samples were washed and cleaned and then, 20 g of meat or skins of rainbow trout were weighed and stored in the oven at 105$^{℃}$ for 44 hours. Subsequently the samples were transferred to the desiccator until constant weight and samples were completely powdered. 10 ml of nitric acid 65% was injected into 1 gram of fish powder and heated to140$^{\circ C}$on a stove for heavy metals solution. The supernatants were transferred from filter paper and diluted with deionized water so that the volume became 25 ml. The uniformity solution was injected into the atomic absorption system and the amount of absorbance was read. Finally, heavy metal concentration was determined according to the calibration curve for each element (Mol, 2011a; Shiber, 2011; Karim *et al.*, 2012).

**Sample Preparation for Alpha-Linolenic Acid Assay**

The fish oil samples were extracted according to the Soxhlet protocol (using n-hexane for 6 hours). The solvent separated from fish oil was distillated by rotary evaporator and residual oil was used as the methylated fatty acid for the GC analysis. For this purpose, hydrolysis reaction must be completed in alcoholic potash as a solvent for half an hour in a reflux system. After hydrolysis, the solvent was again evaporated with rotary evaporator. The remaining was dissolved in 10ml ether and extracted with 15ml of distilled water for 3 minutes (three times) in a separator funnel. Aqueous phase containing the hydrolyzed fatty acids were collected in a 50ml beaker. Hydrolyzed fatty acids were methylated by the addition of 15ml of methanol, 2mL of $H_2SO_4$ and 5ml of benzene for 6 hours under reflux condition. After methylation, the residue of methanol was evaporated and the solution was dissolved in 10ml of diethyl ether, and then rinsed with water to wash out any remaining acid and salts. After evaporation of ether, the sample was ready to be injected into the GC instrument (Ramos *et al.*, 2008, Saberiet *al.*, 2011). Fatty acid composition based on linolenic acid was determined by gas chromatography using the method of Morrison and Smith (1964) with slight modifications (Morrison and Smith, 1964). The GC

Varian CP- 3800 (Varian, USA) fitted with a flame ionization detector and a fused silica capillary column (CP-Sil 5 CB dimethyl siloxane) of 60 m length, 0.32 mm i.d. and 0.25 µm film thicknesses from Varian (Varian, USA) were used for the separation and identification of fatty acids. The operating conditions were injector temperature of $230^{\circ}C$ and detector temperature of $250^{\circ}C$. A temperature gradient program was followed with initial oven temperature set at $70^{\circ}C$ (hold for 2 min) at $5^{\circ}C\,min^{-1}$ to $170^{\circ}C$ without holding time, then with a rate 3/min to $220^{\circ}C$ (hold for 15 min). Therefore, the total run time was 53.6 min. The carrier gas was helium at 25 psi pressure. Peaks were identified by comparing their retention times with those of authentic fatty acid standard. Omega-3 contents of fish were determined based on the standard solution of linolenic acid using the described method. Calibration curves were plotted using five levels of the methyl linolenate in the concentrations ranging from 100 to 3000 mg $L^{-1}$ in CCl4 (Fig. 1).

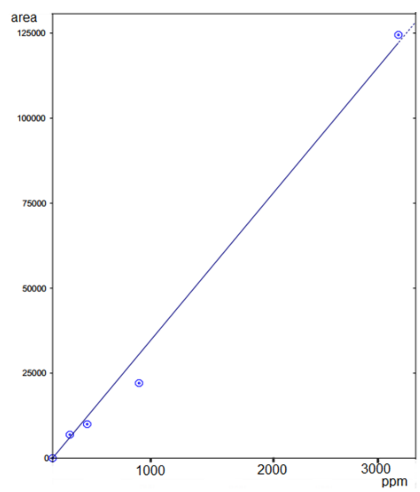


Diagram 1: Calibration curves using five levels of the methyl linolenate in the concentrations ranging from 100 to 3000 mg $L^{-1}$ in CCl4.

## Statistical Analyses

All experiments were performed in triplicate. Data were analyzed by t-test, and any significant difference was determined at 0.05 levels by Tukey's test. The results were expressed as mean ± standard deviation (SD).

## Results

### 1. Alpha linolenic acid in farmed rainbow trout and tuna fish

According to Fig. 1 there are different kinds of omega-3 fatty acids in Iranian rainbow trout, including three major acids of alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid.

Table 1 show that there was an early increase in ALA content in third day and then the reduction started in fifth day but despite this decrease, there was no significant difference between day 5 and 1.

ALA levels in fresh and heat-treated tuna fish were 2225.32 ± 65.59 and 298.96± 60.54 (mg/kg) respectively, this data indicate that the thermal process has a significant reduction in the amount of omega-3 in tuna fish (P<0.05)

Also, comparison of ALA values in these two fish species shows that despite the fact that both fish are rich in essential fatty acids, there is a statistically significant difference between farmed rainbow trout and tuna fish (P<0.05)

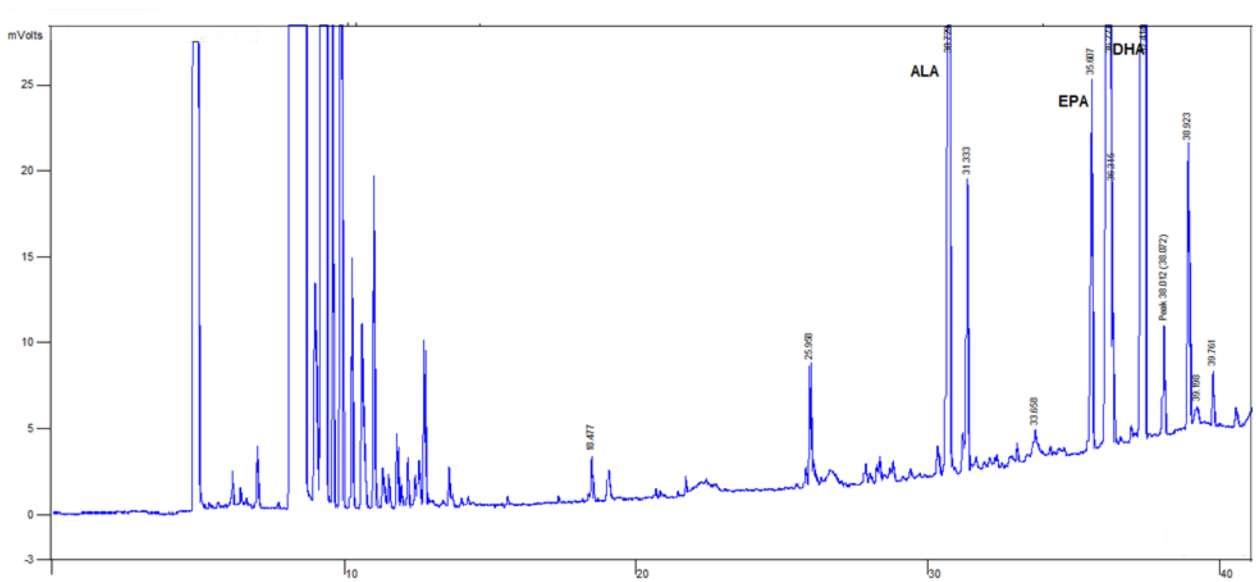


**Diagram 2:** The GC_FID chromatogram for extracted oil of Iranian farmed rainbow trout in which three types of acid that identified are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)

**Table1:** Content of ALA (mg/kg) in farmed rainbow trout maintained in refrigerated

| Time of storage (day) | Means ±SD | Percentage of ALA reduction compared to the first day |
|---|---|---|
| **1** | 2864.09±18.3[a] | - |
| **3** | 2974.99±20.6[a] | 3.8% increased |
| **5** | 2521.60±22.1[a] | 11.9% decrease |

**2. Heavy metal content in farmed rainbow trout and tuna fish**

The measurement of heavy metals in meat and skins of farmed rainbow trout was carried out regarding the fact that skins of fried farmed rainbow trout are sometimes consumed. The results of Table 2 indicate that these metals are higher in the skin than meat of fish, although this difference was not significant statistically.

The sterilization treatment used in the production of tuna does not have a significant effect on the reduction of heavy metal contamination according to Table 3 and there is no significant difference in the amount of heavy metals before and after heating.

**Table 2:** Assessment of heavy metal concentrations (mg/Kg wet weight) in skin and meat of rainbow trout *(*P<0.05)

| **Heavy metals** | **skin** | **meat** |
|---|---|---|
| Pb | 0.438±0.072 | 0.248±0.077 |
| Cd | | 0.0664±0.01 |
| Hg | 0.0798±0.01 | 0.0536±0.007 |

**Table 3:** Assessment of heavy metal concentrations (mg/Kg wet weight) in tuna fish *(*P>0.05)

| **Heavy metals** | **Tuna fish** | **Processed tuna** |
|---|---|---|
| Pb | 0.244±0.043 | 0.248±0.077 |
| Cd | 0.0732±0.015 | 0.0664±0.01 |
| Hg | 0.0446±0.016 | 0.0536±0.007 |







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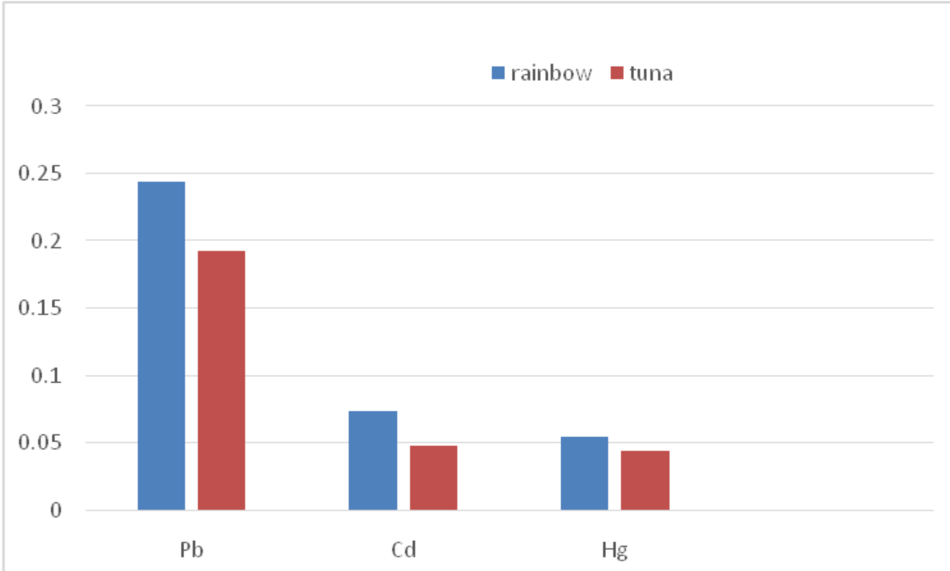


**Diagram 3:** Comparison of heavy metal concentrations of rainbow trout and tuna fish



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## Discussion

Plants and aquatic animals are one of the most important sources of fatty acids. Linoleic acid is one of the essential fatty acids for the cells. This fatty acid is able to convert to other fatty acids in omega-3 fatty acid group, but it enters the human body only through food (Danabas, 2011; Timberg, *et al.*, 2011).

Among fish, depending on the species, nutritional conditions, environmental factors and culture conditions, the amount of omega 3 may be varied. But unfortunately, after fishing, environmental factors are very effective in reducing the amount of omega-3 fatty acids group. Therefore, factors that affect nutritional values of fish base on omega3 from the farm to the table should be considered because they can be effective in omega-3 levels that could be reached to the consumer's body (Chen, *et al.*, 2008; Khoramgah *et al.*, 2007).

One of the effective factors in the amount of omega3 and ALA content was fish species so that Saberi *et al*. (2011) evaluated the fatty acids in meat from three types of fish as carp, phytophagus and rainbow trout. Their results showed that rainbow trout had the highest amount of unsaturated fatty acids and carp meat had the lowest amount. Also, according to Fig. 2 in this study there are appropriate amount of different kinds of omega-3 fatty acids in Iranian farmed rainbow trout, including three major acids of alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid. In this group ALA is an essential fatty acid, which means human body is not able to make it by itself. ALA can be partially converted into EPA and then to DHA. Therefore, the investigation of speed reduction of ALA is useful for the nutritional index (Schneedorferová *et al.,* 2015; Chen *et al.,* 2008). Based on this results, maximum ratio of ALA to total lipid was (0.3%) in Iranian rainbow trout which was lower than the findings of Turkish and Estonian researches in 2011 because the composition of fatty acids in rainbow trout depends on food and its additives, age, growth conditions and environmental conditions. In addition, in those studies, rainbow trout samples were acquired from aquaculture centers but the fish in this study were prepared from markets, therefore the effect of time was less on chemically reaction on their studies (Danabas, 2011; Timberg *et al.,* 2011).

Fish growth conditions are another factor that influences the amount of ALA. According to this, Khoramgah *et al*. (2007) compared the profiles of different fatty acids between wild and farmed carp. The results indicated that there was no difference between the two different culture environments, and each species of fish contained relatively good amounts of unsaturated fatty acids (Khoramgah *et al.,* 2007). Therefore, it could be said that the higher amount of ALA in farmed rainbow trout in compartment to tuna fish depends on other factors. On the other hand, according to the Caponio *et al*. (2011) research, the final level of unsaturated fatty acids in fish is more dependent on environmental conditions than their initial level in fish. They said that unsaturated fatty acids in plants and fish can be oxidized and hydrolyzed under environmental conditions and the amount of these changes in fatty acids is more dependent on environmental parameters, so that proper environmental conditions in storage time of fish can prevent the reduction of these useful fatty acids (Caponio *et al.,* 2011; Chen *et al.,* 2007).

It seems that in this study, one of the important reasons of low ALA content in tuna fish is fishing and post fishing conditions. The tuna fish usually reached the coast after a few days and can easily be affected by inappropriate environmental conditions in the ship. Tuna fish come later from the south of the country to markets, especially in the center of the Iran. So ALA content was decrease in tuna fish when reach to markets under these improper conditions.

Also, the increase in ALA content in third day in rainbow trout can be attributed to the reduction of moisture and increase of dry matters, and the reduction of 11.9% in fifth day was related to chemical reactions by using the t-test ($P \leq 0.05$). This data showed that storage time is important factor in ALA protection.

Chen *et al.* also had a study on the effect of rainbow trout nutrition and fish packaging on the physic-chemical variations of omega3 on 12-daysof shelf life in refrigerator. The results showed that antioxidant-rich nutrition and vacuum packing could help to maintain the amount of omega-3 in the fish fillet. However, the effect of alpha-tocopherol antioxidant was more effective than the type of packaging (Chen *et al.*, 2007). Miklavc *et al*. (2011) concluded that omega-3 differ in fish species by examining essential fatty acids in a number of fish species, and reported that this amount was lower in canned fish than fresh fish. So that in some canned Tuna, the amount of essential fatty acid was lower than the detection limit of gas chromatography method (Miklavc *et al.*, 2011). These results are in agreement with the data of the present study, which showed that the amount of omega after heating of tuna was significantly lower than fresh ones (86.6% reductionn) Therefore, canned tuna, although has long-term storage without using preservatives, but due to the use of heat in the production process and increasing the contact surface of the fish with oxygen during skin and meat separation, the oxidation of fatty acids in canned tuna is high and finally cause to reduce the omega-3 content. Neff *et al*. (2014) and Schneedorferova *et al*. (2015) also examined the effects of various cooking methods such as frying, grilling, and boiling on the amount of essential fatty acids in fish. The results showed that cooking methods, especially frying, reduced the amount of omega but the rate of reduction was different in different methods. According to their study the percentage of omega-3 in fresh rainbow trout was 2.7%, grilled fish 2.6%, dry cooking 2.3%, frying 1.2%, therefore they reported that reducing omega3 with frying cooking method is more than others (Neff *et al.*, 2014; Schneedorferova *et al.*, 2015).

In this study, the levels of lead, mercury and cadmium were investigated in rainbow trout and tuna fish with regard to their toxic effects on humans. Fortunately, all of these elements were in the global standards level. Sadeghiet *et al*. (2011) described the accumulation of lead, cadmium, nickel in liver and muscle of Halvasiah (*Parastromateus niger*) and Ghobad



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(*Scomberomorus guttatus)* fish as the most important commercial fish in the Persian Gulf were less than the standard level (Sadeghi *et al.*, 2011). The amount of heavy metals of mercury, cadmium and lead in the muscle of Shoorideh fish (*Otolithes ruber*) in Abadan and Bandarabbas was investigated. The results showed that, cadmium content was higher than the global standard (Askari *et al.,* 2012). Miklavc *et al.* (2011) evaluate the amount of some heavy metals in fish such as rainbow trout and tuna fish. Accordingly, the amount of mercury and selenium was obtained 0.225 mg/kg 0.164mg/kg respectively in rainbow trout and were reported as 0.5795 mg/kg and 0.747mg/kg respectively in tuna fish. In the study of Miklavc *et al.* (2011) the amount of mercury in tuna fish was obtained higher than rainbow trout, while in this study, cadmium contamination was higher in tuna fish and also Pb and Hg was higher in rainbow trout. Source of contamination of cadmium is usually in sewage, this metal is easily accessed into the seas, which is the tuna's cultureenvironment, but the entry of lead and mercury into the water is dependent on air pollution, which is higher in urban areas. This seems to these differencein heavy metals contamination is due to the location of the two species of fish (Miklavc *et al.*, 2011).

Due to the type of nutrition and environmental factors, the amount of heavy metals and essential elements in farmed rainbow trout and wild trout was different, and the levels of lead, mercury and cadmium were higher in the wild type (Fallah *et al.,* 2011).

In addition to the difference in the accumulation of heavy metals in different fish, the accumulation of heavy metals can also vary in different parts of fish. A series of studies have also been conducted to investigate the contamination of muscle, gills, skin, liver and kidney of fish. So that Velayatzadeh *et al*. (2011) examined the accumulation of heavy metals of mercury, cadmium and lead in the meat and liver of the Lutak fish (*Cyprinion macrostomum*) in the Karoon River. The results showed that these metals were higher in the liver than muscle. Of course, these values were reported below the international standard. They were obtained the amount of mercury 20.06, cadmium 124.6 and lead 232.86 µg/kg dry weight in the liver. In another study, the pattern of accumulation of some metals in muscle, liver, kidney, gills and scales in the silver carp showed that the highest accumulation of lead was in the liver (0.19 µg/g) and nickel was in the kidney. The accumulation of most of the essential metals (copper and zinc) was in the liver and gills. The accumulation of unnecessary metals in liver and kidney tissues was similar to scales. So scales could be an appropriate index for determining the amount of contamination in aquatic animals (Pakzad Tukhayeshi *et al.,* 2013).

On the other hand the amount of fat and omega-3 in the skin (22.8 g/kg) was higher than muscle (21.4 g/kg) of fish (Rebole *et al.,* 2015). Rainbow trout skin was sometimes consumed by people. For these reasons, in the present study, the amount of heavy metal in the skin and muscle of rainbow trout was examined. According to the results, it was found that the amount of heavy metals in the skin is more than the muscle. However, there was no significant difference in the results. These results were different from the results of the same research on lead and cadmium contamination in the Domnavary fish (*Platycephalus indicus*) by Sanjar *et al*. (2009). They stated that the amount of these metals in the muscle is greater than the skin, which can be related to the type of fish and the amount of pollution in the region. However, Caspian Sea Kilka and Sekhareh (*Gasterosteus aculeatus*) contaminated with lead, chromium and cadmium elements. Based on this research, the effect of different sexes and organs such as skin, liver, muscle and intestine was not significant (Najm *et al.,* 2014).

Some food processes can affect the amount of heavy metals. Ganjavi *et al*. (2010) examined this issue in canned tuna. The results showed that cooking, defrost and sterilization with autoclave can reduce the levels of lead and cadmium. In this study, the comparison of contamination of fresh tuna and canned showed that the sterilization process can reduce the amount of contamination; however, this difference was not statistically significant. Determination of fish contamination of mercury, nickel, cadmium, tin, zinc and iron in canned fish produced in Shooshtar, Isfahan and Hamedan by Velayatzadeh *et al*. (2010) determined that canned tuna were safe to for people consumption. Tuzen *et al*. (2007) reported the amount of lead in canned tuna between 0.09-0.4 μg/g and cadmium levels in the range of 0.06-0.25 μg/g in Turkey. The amount of some elements was above the permitted limit, which determined the need for periodic studies and the level of Pb contamination was similar to the current study, but cadmium contamination was higher in their report). In one study the mercury contamination of Tuna fish was in the range of 0.06-0.3 mg/kg, 0.48-0.58 for lead and 0.01-0.02 mg/kg for cadmium (Mole, 2011a). Compared with this study, mercury and lead contamination in Turkey was higher and cadmium was lower. In another study, the average amount of cadmium, mercury, lead in canned rainbow trout was obtained 0.001, 0.026, 0.54 mg/kg, respectively. This contamination for mercury and cadmium was lower than the amount of these elements in the present study, which could be partly related to environmental and cultural condition (Mole, 2011b).

## Conclusion

According to the results of this study, the amount of heavy metals as lead, cadmium and mercury in farmed rainbow trout and tuna fish was less than the international standard. All of these metals in the skin were more than meat of fish. But considering the harmful effects of these metals on human health especially in the long term, continuous monitoring should be one of the priorities programs of food safety. The amount of omega in farmed rainbow trout was more than tuna fish. This statistically significant difference can be attributed to environmental factors. Storage time even in dark refrigerator could reduce ALA in farmed rainbow trout and in tuna fish heating treatment could have significant effect in ALA content. However these factors did not change the heavy metals contamination.

Finally, it can be said that today, one of the reasons for reducing the health of consumers, despite the use of more and more foodstuffs, is the presence of environmental pollution and subsequent chemical pollution of food, as well as the lack of attention to the duration and environmental conditions in food storage method for bioactive compound stability.

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