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Nutritional value and heavy metal content of fishmeal from the Southwest Caspian Sea

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ABSTRACT

This study aimed to evaluate some nutritional values and heavy metal contents of fishmeal produced by the commercial factories in Guilan Province, the Southwest Caspian Sea. The fishmeal samples were randomly collected from five factories producing fishmeal by Kilka (*Clupeonella* spp.) (units 1-4) and tuna canning offal (unit 5) as the raw materials. Proximate fish composition, amino acid contents and four heavy metal concentrations including chromium (Cr), cadmium (Cd), lead (Pb), and mercury (Hg) were measured in the fishmeal specimens. Crude protein contents were found to be in the range of 53.61-68.82% and the lowest value belonged to the unit 5. Also, the highest level of fat and ash contents were 22.49% and 18.05%, respectively (p < 0.05). The lowest essential: nonessential amino acid ratio was 0.71 in unit 5. Fishmeal specimens from unit 5 showed the highest metal concentrations with the following descending order: Cr > Cd > Pb > Hg. Results indicated that the heavy metal concentrations in the fishmeal are dependent on the source of raw materials. The heavy metal concentrations in the examined fishmeal did not exceed the permissible limits proposed by National Research Council (NRC) for animal feedstuff requirements.

Key words: Fishmeal, Kilka, Heavy metal, Amino acid composition, Caspian Sea.

INTRODUCTION

The Caspian Sea is the largest landlocked endorheic body of water and known as a largest lake in the world (Dumont 1998). Recently, water quality of the Caspian Sea has been changed mainly due to human activities, such as offshore exploration and exploitation of oil and gas resources as well as chlor-alkali plants and altered ecosystem (Bespalova & Mousavi 2013). There is also a relation between water pollution and contamination of the living aquatic animals. Although, substitution of fishmeal by herbal protein is core of attention (Gatlin et al. 2007), but fishmeal is still a major source of protein used in the commercial diets of fish, livestock and poultry due to balance amounts of amino acids, fatty acids, phospholipids and minerals trace

(Moghaddam et al. 2007; Usydus et al. 2011; Khan et al. 2012; Mahdabi & Hosseini Shekarabi 2018). Kilka, Clupeonella spp., are the most abundant and widespread zooplanktivorous fish in the Caspian Sea, and supported major raw material for the fishmeal factories (Mahdabi & Hosseini Shekarabi However, Kilka stocks has virtually collapsed due to environmental condition, overfishing and adverse effect of comb jellyfish bloom (Roohi et al. 2010). Globally, fishmeal can be made from almost any type of seafood and divided into three groups: inedible feed grade fish, small fish and surplus resource of fish and shellfish (IFFO 2008). There are two types of fishmeal produced in the north of Iran: fishmeal prepared from whole kilka and its solid waste or by-products discharged from

tuna canning factories. Hence, probable contamination of kilka are tied together through the food web and can potentially harmful for wildlife and human health.

Heavy metals are usually occurred naturally and artificially in water bodies. Aquatic animals have been found to be a good bioindicator for monitoring water pollution. Some heavy metals including cadmium, lead, chromium and mercury are potentially bioaccumulative substances that may cause severe health problems even at low concentrations for human consumption (Ali *et al.* 2013; Wang *et al.* 2013). Some heavy metals including, Co, Ni, Cr, Pb, Cd and Hg have been reported from kilka in the Caspian Sea (Bespalova & Mousavi 2013; Taghavi Jelodar *et al.* 2016; Dadar *et al.* 2017). As the fishmeal is a part of the food chain, assessing for any contamination is crucial.

Several studies reported the nutritional values of different fishmeal samples (Moghaddam *et al.* 2007; Usydus *et al.* 2011; Khan *et al.* 2012; Mika *et al.* 2016). However, there is still lack of information concerning probable heavy metal contamination of commercially produced fishmeal. Chemical compositions of fishmeal are highly depend on fish species, seasonal variations, processing technique and fishery

location (Samuelsen & Oterhals 2014). The objective of this study was to determine the proximate composition, amino acid profile and heavy metals contents (Cr, Cd, Pb and Hg) of Kilka fishmeal compared to tuna canning offal meals which are produced by some commercial fishmeal factories in the southwest shores of the Caspian Sea (Guilan Province, Iran).

MATERIALS AND METHODS Sampling

Five fishmeal factories were randomly selected for sampling in Guilan Province, from the southwest basin of the Caspian Sea in December 2016. The source of fishmeal of four factories (units 1-4) was whole fish species (Clupeonella spp.) and one factory used canned tuna waste (unit 5) (Table 1). All fishmeal samples were produced by six steps including heating, pressing, separation, evaporation, drying and grinding. In some sampled fishmeal factories this process was run continuously, while some steps can be manipulated in batchtype of production. Four samples were randomly taken from each processing unit, packed in labeled polythene bags (100 g) to avoid humidity absorption, and stored at 4 \pm 1°C until chemical analyses.

Table 1. Information of sampled fishmeal factories in Guilan Province, Iran.

Samples	Species used	Processing techniques	Start time
Unit 1	Kilka	continuous	1991
Unit 2	Kilka	continuous	2000
Unit 3	Kilka	batch-type	1999
Unit 4	Kilka	continuous	1992
Unit 5	Tuna (mixed species) canning offal	batch-type	1990

Proximate Composition and Amino Acid Contents

Proximate composition of the fishmeal samples was analyzed according to AOAC (1990). In summary, moisture (%) was determined by oven drying for 6 hours at $105 \pm 1^{\circ}$ C and ash content (%) by burning 1.0 g sample in electrical furnace at 550°C overnight. Also, total fat content (%) was measured by Soxhlet apparatus and the solvent used for the lipid extraction was n-hexane. Crude protein (%)

was determined by Kjeldhal methods (N×6.25) using copper sulfate and magnesium sulfate as a digestion mixture.

Near-infrared reflectance spectroscopy (NIR, Model 5000, Foss, USA) was used for estimating amino acid compositions of the fishmeal samples according to Fontaine *et al.* (2001). Briefly, the samples (15 g) were kept at room temperature (25°C) for 6 h prior to NIR analysis to balance the temperature and moisture, because these factors can affect the

reflectance and absorbance of the NIR waves. Reflectance spectra were recorded every 2 min from 400 to 2500 nm for scanning of the samples.

Heavy metal content analysis

Three grams of dried powdered sample was weighed in to Teflon tubes (100 mL) and digested overnight with 6 mL nitric acid and 3 mL hydrogen peroxide (30% H₂O₂), then the samples were digested using a laboratory microwave digester (Milestone ETHOS 1, Leutkirch, Germany).

The microwave parameters were 545 \pm 10 W power with minimum of 3 rpm and 760 \pm 70 kPa pressure. The digested contents were transferred to acid-washed polyethylene tubes and reached to 50 ml by double distilled water, then subjected to various metal analyses in an atomic absorption spectrophotometer (Varion AA 100, Melbourne, Australia) following the Jorhem & Engman (2000) method. The atomic absorption spectrometer equipped with Cr, Cd and Pb hollow cathode lamps. The operating parameters for the spectrometer elements were set as recommended instruction by the manufacturer. Total Hg contents were measured using an advanced mercury analyzer (Leco 254 AMA, USA) according to Houserova et al. (2007). In summary, the dried samples (5-10 mg) were decomposed at 550°C for 5 min under a pure oxygen carrier flow to an Auamalgamator.

Trapped mercury was released from the amalgamator by heating at 850°C (measuring cycle, 45 s) and the cold vapor was measured at 253.7 nm wave length. Heavy metal concentrations were expressed as milligrams per kilogram (mg kg⁻¹) of fishmeal on dry basis. All containers and equipment were carefully soaked overnight in 2% nitric acid and later rinsed with deionized water before use.

Statistical analyses

The data were subjected to statistical analysis using the SPSS software (SPSS version 20, Chicago, USA). One-Way Analysis of variance (ANOVA) along with Duncan's post hoc method was carried out to examine mean

differences among the fishmeal samples at 95% confidence level (p value <0.05).

RESULTS AND DISCUSSION

Proximate Composition

Protein is considered as an important component of aquatic feed (NRC 1993). Fishmeal generally contains 60-72% protein, 5-12% fat and 10-20% ash in dry weight (Shepherd & Jacksona 2013).

As shown in Fig. 2, the crude protein content of kilka fishmeal samples (unit 1-4) ranged from $65.11 \pm 0.64\%$ to $68.82 \pm 0.21\%$ while, in tuna canning waste fishmeal processing unit (unit 5) the lowest protein content was $53.61 \pm 0.658\%$ (p < 0.05). This range of protein content is higher than some fishmeal products such as Pakistani fishmeal as reported by Khan et al. (2012) and Rahim et al. (2017). Moghaddam & Mesgaran (2007) reported that the Kilka fishmeal crude protein in Iran was 59.1% which is different from our results. The variation in protein content of different fish meals may be due to different raw material types and the methods of fishmeal preparation (Rahim et al. 2017).

Lipids are the best energy source as well as developing essential fatty acid compositions in animal feed (Rodriguez-Barreto *et al.* 2012; Rahim *et al.* 2017). In the present study, fat content of kilka fishmeal samples were found to be from 11.30 ± 0.283 to $14.70 \pm 0.141\%$, while unit 5 showed the highest fat content (22.49 \pm 0.219%) (p < 0.05). These results are in agreement with Rahim *et al.* (2017) who found that fat content of Pakistani fishmeal was 15.29-26.23% from 23 different mixed fish species. Similarly, Moghaddam & Mesgaran (2007) reported that fat content of kilka fishmeal in Iran was 22.9% in dry weight.

However, Abbas and Siddiqui (2001) showed lower fat content (2-10% dry weight) in Pakistani fishmeal using different under-sized fish. The variation in fat content is highly depend on chemical composition of raw material and manufacturing process of fishmeal in separating oil from the raw material.

The level of ash content in fishmeal is associated with the mineral and inorganic compound levels. In the present study, the highest ash content was obtained in unit 5 (p <

0.05) due to high level of inorganic substances in tuna canning processing wastes i.e. head bones, backbone and viscera used for producing fishmeal.

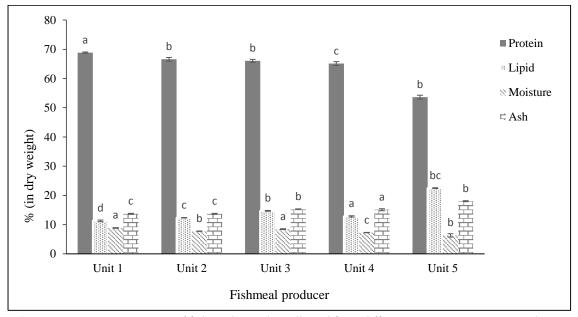


Fig. 1. Proximate composition of fishmeal samples collected from different processing units in Guilan Province, Iran. Means (\pm SD) with different superscripts are significantly different (n = 4, p < 0.05).

Amino acid profile

The nutritive value of protein-based products are directly depend on the amino acids composition especially essential ones. The amino acids composition of different fishmeal samples are presented in Table 2. The analysis of amino acids content of Kilka fishmeal samples showed that they contain all the essential amino acids required by farm-raised fish according to FAO standard (FAO 1985; Tacon 1994). Also, the lowest essential/nonessential amino acids ratio was obtained in unit 5. The lower amino acids content may be attributed to the additional heating of cooked tuna canning wastes in fishmeal processing, therefore proportionally diminishing amino acids composition in the final product (Vidotti et al. 2003).

Lysine, cysteine and methionine are limiting amino acid in most of the animal feed (NRC 1993). From the results, all Kilka fishmeal specimens showed higher values of these amino acids content compared to tuna canning waste meal.

These values were within the standard range reported for different fishmeal brands (BOANR, 1993). Results showed that the highest amount of amino acids was glutamic acid in all fishmeal samples. It seems that this non-essential amino acid may be responsible for fishmeal palatability (Miles & Chapman 2006). Also, tryptophan level was above FAO and NRC standards in all samples (Tacon 1994). Similar results were also observed by Vidotti et al. (2003) in freshwater fish silage. Amino acids profile of fish tissues can be affected by fish species, spawning period and feeding behavior (Shirai et al. 2002). Also, fishmeal amino acid levels from same fish species depend on the raw material freshness, catching, handling and processing methods (Anderson et al. 1993).

Heavy metal contamination

Bioaccumulation and bio-magnification of heavy metals in aquatic food webs are core of attention for human health (FAO/WHO 1983).

The highest metal concentration was observed in unit 5 (Fig. 2, p < 0.05). Heavy metal concentrations are known to be vary in fish tissues depending on species, habitat, feeding behavior and life span. The higher heavy metal contents in fishmeal, compared to fish muscle can be explained by existing a concentrated protein and heavy metals in former product which can be easily bound to protein as metal-binding proteins (Dallinger *et al.* 1987).

Although, Cr is an essential micronutrient in some animals and human nutrition, but can be toxic at high levels (Akoto et al. 2014). Cr can also be accumulated in fish tissues by industrial activities and polluted area such as steel production, wood preservation, and leather tanning sectors (Viti et al. 2003; Rahman et al. 2012). The highest $(4.67 \pm 0.010 \text{ mg kg}^{-1})$ and lowest $(0.67 \pm 0.010 \text{ mg kg}^{-1})$ Cr concentrations were calculated in fishmeal samples produced in units 5 and 3, respectively (Fig. 2, p < 0.05). Cr concentration at 0.461-0.486 mg g-1 was reported in kilka muscle from the Southern Caspian Sea (Taghavi Jelodar et al. 2016). Level of Cr (3.32 mg kg-1) was determined in fishmeal samples in Texas (Dai et al. 2016) which is higher than in the present study. The Western authorities Australian proposed concentration of 5.5 mg kg⁻¹ for Cr (Plaskett & Potter 1979). However, the estimated daily intake of Cr is recommended 1.5 mg kg⁻¹ day⁻¹ for human according to WHO (1992). Although, Cr concentration in all examined fishmeal samples were exceeded the permissible limits for human consumption, Cr concentration was much lower than the permissible limits for poultry proposed by NRC (1993) (500 mg kg-1 of feed). It was also reported that some birds are unable to tolerate Cr content at higher than 10 mg kg-1 in their diets (Eisler 1994).

Sullivan *et al.* (1994) reported that mineral premixes used for animal feedstuffs should be contained Cr level in range of 60-500 mg kg⁻¹. Cr concentration was found to be 0.28-7.71 mg kg⁻¹ among 21 samples of poultry feed in Pakistan (Imran *et al.* 2014). Most of the processing fishmeal units in north of Iran are

old and with outdated machineries. Stainless steel is used in the fishmeal factories equipment due to their thermal conductivity, easy cleaning and disinfecting. Nickel and chromium are the major metal compositions of stainless steel products and they can probably leach into the fishmeal during processing procedures as shown by Kamerud et al. (2013). The highest amount of Cr level was found in fishmeal samples from tuna canning offal due to high content of bones in the raw materials. The high mineral contents lead to increased concentration in final product (Dai et al. 2016) which is similar to our results in unit 5. Lead is known as a neurotoxin element and can lead to decreased survival, growth, and metabolism of animals as well as increased cardiovascular disease in adults (Eisler 1994; Garcia-Leston et al. 2010).

These results showed that Pb levels ranged from 0.195 ± 0.020 to 0.42 ± 0.014 mg kg⁻¹ in all fishmeal samples. Anan et al. (2001) reported a similar results about Pb concentrations at the range of <0.001-0.575 $\mu g g^{-1}$ (dry weight) in various fish collected from coastal waters of the Caspian Sea. However, the higher Pb level was reported in fishmeal (4.6-18.2 mg kg-1) and squid meal (0.46-1.77 mg kg-1) which are produced in India (Murthy et al. 2013). The lower range of Pb concentrations (0.0162-0.072 μg g-1 of wet weight) was reported from canned Kilka in Iran (Bespalova & Mousavi 2013). According to Cronin et al. (1998) Pb concentration in fish muscle should not be exceed 2 mg kg-1 in UK for human consumption with the acceptable maximum limit of 3 mg kg week-1 for adults (FAO/WHO 1983). Averaged amount of Pb concentration in all samples examined in this study was not exceeded the standard limits for human and poultry nutrition.

Cadmium is regarded as a chronic toxic element when it is present at approximately 1 mg kg⁻¹ (Friberg *et al.* 1971). Birds may be less sensitive to Cd up to 1.0 mg kg⁻¹ in their diet than mammals (Eisler 1994). Our results showed that Cd content in all fishmeal samples was lower than recommended levels for human

and poultry nutrition. However, higher content of Cd was reported in squid meal (2.45-14.1 mg kg⁻¹) and fishmeal (0.22-4.4 mg kg⁻¹) in India (Murthy *et al.* 2013). Dadar *et al.* (2017) determined Cd concentrations in common kilka muscle to be 0.74 mg kg⁻¹ (wet weight) which is higher than both Kilka fishmeal and tuna waste fishmeal in the present study.

Cd like other metals is absorbed into the fish body from water and feed, but is slowly excreted, while more accumulates in the internal organs. Therefore, tuna canning offal fishmeal from unit 5 as a raw material for producing fishmeal had more viscera as well as high amount of Cd and other metal concentrations. Mercury is a non-essential element and has an undesirable fatal effects. Harmful effect of Hg can be occurred at dietary 0.05-0.5 mg kg⁻¹ (wet weight) in fish muscle (Eisler

1987). The regulatory limits of Hg in fish tissue for non-predatory fish, such as Kilka is 0.5 mg kg⁻¹ (FAO/WHO 1983). The present study showed that Hg level in Kilka fish meals were < 0.001-0.04 mg kg⁻¹ which is similar to that of previous reports in canned kilka samples (0.0065 mg kg⁻¹) (Bespalova & Mousavi 2013) and common Kilka muscle (0.010 mg kg⁻¹ wet weight) in Iran (Dadar *et al.* 2017). However, tuna canning wastes meal samples showed the higher Hg level (0.15 \pm 0.075 mg kg⁻¹) (p < 0.05) than the other (Kilka) samples.

The higher Hg concentrations in tuna canning offal fishmeal can be explained by the larger fish size, longer life span and feeding behavior (piscivore) of tuna than Kilka species. Hg bioaccumulation in fish tissue is reported to be highly dependent on fish species, size, age and feeding behavior (Ikem & Egilla 2008; Burger & Gochfeld 2011).

Table 2. Amino acid content of fishmeal specimens collected from different examined processing units in Guilan Province, Iran (g 100g-1 of protein).

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Amino acid	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5	
Methionine	2.04 ± 0.002^a	1.98 ± 0.019^{ab}	1.92 ± 0.006 ^b	1.92 ± 0.019 ^b	1.47 ± 0.029^{c}	
Cysteine	$0.62\pm0.004^{\rm a}$	0.60 ± 0.006^a	0.51 ± 0.001^{b}	0.56 ± 0.008^{ab}	0.41 ± 0.012^{c}	
Lysine	5.69 ± 0.007^{a}	5.59 ± 0.025^a	5.25 ± 0.008^{b}	5.46 ± 0.025^{c}	2.76 ± 0.035 ^d	
Threonine	5.27 ± 0.016^a	5.05 ± 0.037^{b}	5.06 ± 0.016 ^b	4.90 ± 0.049^{c}	$3.83 \pm 0.076^{\rm d}$	
Tryptophan	2.91 ± 0.009^a	2.80 ± 0.027^{b}	2.65 ± 0.008^{c}	2.67 ± 0.027^{c}	$2.21\pm0.044^{\rm d}$	
Arginine	0.78 ± 0.001^a	0.75 ± 0.007^a	$0.68\pm0.005^{\mathrm{b}}$	0.71 ± 0.007^{ab}	0.56 ± 0.011^{c}	
Isoleucine	4.06 ± 0.005^a	3.95 ± 0.038^{b}	3.81 ± 0.013^{c}	3.74 ± 0.037^{c}	3.11 ± 0.067^{d}	
Leucine	2.97 ± 0.004^a	$2.87\pm0.028^{\mathrm{b}}$	2.61 ± 0.008 c	2.74 ± 0.028^{bc}	$2.14\pm0.043^{\rm d}$	
Valine	5.15 ± 0.008^a	5.00 ± 0.021 ^b	4.55 ± 0.005^{c}	4.73 ± 0.047^{c}	$3.69 \pm 0.074^{\rm d}$	
Histidine	3.45 ± 0.002^a	3.35 ± 0.033 b	3.03 ± 0.009^{c}	$3.19\pm0.033^{\rm d}$	$2.55 \pm 0.050^{\rm e}$	
Phenylalanine	1.58 ± 0.012^a	$1.49\pm0.014^{\mathrm{b}}$	$1.59\pm0.005^{\mathrm{a}}$	$1.46\pm0.015^{\mathrm{b}}$	1.51 ± 0.030^{ab}	
Glycine	2.84 ± 0.002^a	2.71 ± 0.026^{b}	$2.45\pm0.008\text{bc}$	2.59 ± 0.026^{b}	$2.05\pm0.041^{\rm d}$	
Serine	3.74 ± 0.004^a	3.84 ± 0.037 b	$4.37\pm0.014^{\rm d}$	3.68 ± 0.037^{c}	$4.04 \pm 0.074^{\rm e}$	
Proline	2.66 ± 0.004^a	2.63 ± 0.025^a	2.46 ± 0.008^{b}	2.43 ± 0.024^{b}	1.99 ± 0.040^{c}	
Alanine	2.75 ± 0.003^a	2.75 ± 0.027^a	2.68 ± 0.008^{b}	2.53 ± 0.025^{c}	2.61 ± 0.052^{b}	
Aspartic acid	4.05 ± 0.012^a	3.89 ± 0.037^{b}	$4.09\pm0.013 a$	3.89 ± 0.011^{b}	3.58 ± 0.071^{c}	
Glutamic acid	6.38 ± 0.013^a	6.13 ± 0.060 b	5.86 ± 0.019^{d}	$5.91 \pm 0.038^{\circ}$	$4.67\pm0.093^{\rm e}$	
E/NE	0.82	0.80	0.79	0.80	0.71	

The data were expressed as mean \pm standard deviation. Data with different superscripts within a row are significantly different (n = 4, p < 0.05.

E/NE: essential/nonessential amino acids.

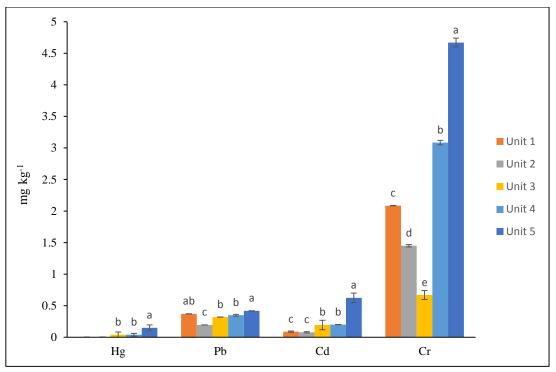


Fig. 2. Heavy metal concentrations in fishmeal specimens collected from different processing fishmeal units in Guilan Province, Iran. Means (\pm SD) with different superscripts are significantly different (n=4, p < 0.05).

CONCLUSION

Determination of proximate amino acid composition and content along with heavy metals in fishmeal products are crucial for both animal and human health.

Fishmeal is widely used in aquaculture industries and low nutritional value leads to a decrease in aquatic animal production. The proximate composition of fishmeal samples in Kilka (units 1-4) and tuna waste (unit 5) were acceptable for animal feedstuffs. Also, amino acid profile of kilka fishmeal was in accordance with aquatic and poultry feed standards.

The results showed that the heavy metal contents were dependent on the raw material types and the age of the producing fishmeal machines.

So that, tuna canning offal meal exhibited the highest Cr, Cd, Pb and Hg concentrations compared to kilka fishmeal. Also, the possible heavy metal contaminations seem to be found in the higher trophic levels. Therefore, it is necessary to monitor fishmeal products continuously for the nutritional quality and heavy metal concentrations.

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ارزش غذایی و آلودگی به فلزات سنگین پودر ماهی تولید شده از بخش جنوب غربی دریای خزر

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چکیده

ترکیبات تقریبی، پروفایل اسیدهای آمینه و غلظت ۴ فلز سنگین کروم (Cr)، کادمیوم (Cd)، سرب (Pb) و جیوه (Pb) در ناحیب توریبی، پروفایل اسیدهای آمینه و غلظت ۴ فلز سنگین کروم (Cr)، کادمیوم (Cr)، سرب (Cd) و جیوه (Cr)، سد. نمونه از کارخانه تولید کننده پودر ماهی کیلکا (واحدهای (-4)) و یک نمونه از کارخانه تولید کننده پودر ماهی تون (واحد ۵) به صورت کاملاً تصادفی نمونهگیری شد. مقدار پروتئین نمونههای پودر ماهی در محدوده (-4)7/۲۱ کنسروسازی ماهی تون (واحد ۵) به صورت کاملاً تصادفی نمونهگیری شد. مقدار چربی (-4)7/۲۲/۴۹) و خاکستر ماهی در محدوده (-4)7/۲۲/۴۹) بود، درحالی که واحد ۵ کمترین مقدار پروتئین و بیشترین مقدار چربی (-4)7/۲۱) اندازهگیری شده در نمونههای پودر ماهی غلظت (-4)1 و همه بیشتر، درحالی که (-4)2 از همه کمتر بود شد. در میان فلزات اندازهگیری شده در نمونههای پودر ماهی غلظت (-4)3 و همه بیشتر، درحالی که (-4)4 و مشاهده (-4)5 و خاکستر شد. در نتیجه، کیفیت پودر ماهی تولید شده از ماهی کیلکا در مقایسه با ضایعات کنسروسازی تون بهتر بود و با توجه به غلظت پایین فلزات سنگین برای استفاده در فرمولاسیون جیره غذایی حیوانات مناسب تر است. در تحقیقات آینده باید به نوع ماده پایین فلزات تولید پودر ماهی توجه بیشتری شود.

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