The Reduction of Lead in Fish Meat from Floating Net Cages in Cirata Reservoir by Citric Acid

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Abstract
The objective of the research is to reduce the lead content in the meat of carp, tilapia and catfish cultured in floating net cages (FNCA) in Cirata Reservoir by citric acid solution. The experimental method uses two treatments. The first treatment is by citric acid solution at four level concentration of 15, 20, 25 and 30%. The second treatment is the arrangement of the duration period of the soaking process at five condition of 30, 60, 90, 120 and 150 minutes. Each combination of the treatments is repeated twice. The observation has been done to determine the lead content of fish meat using AAS (Atomic Absorption Spectrometry). The lead content data is analyzed descriptively and compared to the standard.

The results indicate that the lead content in carp meat is under the allowed standard when treated with 15% citric acid and 150 minutes soaking time. The initial concentration of lead in the carp meat is 3.152 ppm. The lead content in the tilapia meat is under the allowed standard when treated with 25% citric acid and 150 minutes of soaking time. The initial concentration of lead in the tilapia meat is 3.878 ppm. The lead content in the catfish meat is below the permissible quality standard at the treatment of 25% concentration of citric acid with the duration of soaking time of 120 minutes. The initial content of lead in the catfish meat is 3.911 ppm.

Keywords: Heavy metal, FNCA, Cirata Reservoir, Citric Acid.

Introduction

Fish freshwater species commodities that are generally traded in Bandung and West Java Province are the Carp (*Cyprinus carpio*), Tilapia (*Oreochromis niloticus*) and Pangasius (*Pangasius pangasius*). These species are the post-harvested products from the intensive aquaculture in the floating cage system (FCS) in Cirata reservoir. Nevertheless, several studies reveal that the water quality in this reservoir is contaminated by the heavy metals i.e. cadmium, zinc, lead and mercury.

The concentrations of those heavy metals are extremely above the allowed national standard water quality according to the Indonesian government regulations. The heavy metals in the water of Cirata reservoir can be bio-accumulated in the aquatic organisms including the cultured fish species. The bio-accumulation of heavy metals may occur in the meat part[1]. Furthermore, it is estimated that the heavy metals uptake from the waters into the body tissue can be through the skin, digestive system and respiration[2]. Moreover, the bioaccumulation levels in fish meat were affected species, metals-specific and also by the physical parameters of water such as pH, temperature and salinity[3].

The accumulation of heavy metals in the fish meat can have a fatal impact to the human who consumed it[4]. The heavy metals contained in fish meat cannot be metabolized or accumulated in the human body[5]. Moreover, they can significantly affect the health condition. Several health disorders can be observed such as dizziness, allergies and impaired growth of abnormal cells[6].

Therefore, efforts should be made to overcome this health problem. One possible solution may be conducted such as the heavy metals binding from the heavy metals contaminated fish meat to decrease their concentration. This alternative solution can be implemented before the contaminated fish meat is processed and consumed by human.

A study from Junianto et al[7] estimated that intensively cultured Tilapia, Carp and Pangasius in Cirata reservoir are heavily contaminated by the lead metal. The concentration of this contaminant has extremely surpassed the allowed national standard quality (2 ppm).

Heavy metals binding in fish meat can be performed using acid compounds such as citric acid[8]. The effectiveness of heavy metals reduction in fish meat can be influenced by the type of fish, acid concentration and submersion time as well as the preparation technique either in a whole fish form or in fillet form[9].

However, this research on heavy metals binding to reduce the metal concentration in fish meat is still less documented. Most importantly, this technique is expected to support the food security in the future. Therefore, this study is aimed to determine the concentration levels, submersion time and preparation technique in order to decrease the lead concentration in the Carp, Tilapia and Pangasius fish meat by using citric acid as the binding agent.

Material and Methods

The present study consists of two stages. First is by evaluating the heavy metals contamination on the three most consumed freshwater fish, namely the Carp, Tilapia and

Pangasius in the West Java Province. Secondly, is by evaluation on citric acid as lead binder by acidification technique.

Lead binding in the fish meat using acidification technique: This stage is aimed to determine the concentration levels and submersion time as well as preparation technique of three fish species in order to reduce the lead concentration until the allowed standard (2 ppm) by using citric acid as the binder.

Samples preparation

Fish preparation: Fish samples are obtained from the two sampling sites that correspond to the FCS where the three fish species are cultured and also the higher lead concentration compared to other sampling points in Cirata reservoir, namely Calincing and Maleber Site for the Carp, Pangasius and Tilapia. The obtained fish samples are then transported to the laboratory of the Faculty of Fisheries and Marine Sciences of Universitas Padjadjaran. Afterwards, the whole fishes from the samples are prepared by exterminating the fish. Then, the fish gills, scales, guts and fins are removed. The fish samples are then washed.

Citric Acid Preparation: Different concentrations of citric acid are prepared by weighing the citric acid in powder form with respect to the expected concentration and then mixed it with the t. Briefly a 1.5, 2, 2.5 and 3 kg of citric acid is weighed and mixed with 10 L of distilled water to prepare 15, 20, 25 and 30% concentration of citric acid solution.

Experimental set up: Briefly, the fish meat samples are prepared and then inserted in each plastic container corresponding to different concentration of citric acid (15, 20, 25 and 30%), submersion time (30, 60, 90, 120 and 150 minutes). Afterwards, the prepared fish meat samples are then inserted into the plastic container according to its treatment. The treatment is repeated in two times. After the submersion, the analysis of the lead content in the fish meat is then carried out.

Fish meat samples preparation: The snippet of fish meat from the sample is washed, dried and ground using the grinder and then sieved in 100 μm mesh size. The powder is then homogenized and weighed 0.5 g in a teflon bomb digester. A drop of distilled water and 1 mL of concentrated citric acid are added into the sample. Afterwards, the teflon bomb digester is closed tightly and then inserted into the furnace prior to combustion in 150°C for 4 hours. When the sample is cold, it is poured into the beaker glass and then heated with the addition of distilled water repeatedly. The sample is then inserted into 10 mL of flask and is ready for lead analysis.

Analysis of lead concentration: Analysis of the lead is carried out in the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. The lead concentration is measured by using the Atomic Absorption Spectrometry (AAS) method[10].

Results and Discussion

Binding of lead in Carp fish meat: The carp fish is highly demanded by some people in Bandung. Half of the carp supplies is obtained from the FCS in Cirata. The preliminary study in 2015 estimated that the lead concentration in the Carp fish meat for the consumption size (250 – 300 g $ind.^{-1}$) ranged between 2.5041–3.0014 ppm. Moreover, the highest lead concentration in the Carp fish meat was obtained from the FCS Calincing Site of Cirata reservoir.

In the present study, the initial lead concentration in the Carp fish meat prior to treatment is 3.152 ppm. The observation of lead concentration in fish meat at different levels of citric acid concentration, submersion time and preparation technique are presented in table 1.

In this study, the lead concentration in the carp fish meat is decreased proportionally to the increased concentration of citric acid at similar submersion time. Additionally, at similar citric acid concentration, submersion time is inversely proportional to the lead concentration in fish meat. The result implies that the binding of lead in the fish meat is changed by the citric acid during the submersion. According to Saputri et al[11], the lead bound by citric acid is caused by the reaction between the citrate ions with lead ion resulting in the formation of citrate salts. In addition, the binding of lead highly depends on the concentration of ion as metal chelator, in this case, the citrate ion from the citric acid[12]. These two previous results support our study where higher concentration of citric acid is negatively correlated to the lead concentration in the Carp fish meat.

In table 1, at the concentration of citric acid 30%, the reduction of lead until the allowed levels (< 2 ppm) for the fish meat achieved at 90 minutes of submersion time. Thus, citric acid at this concentration is able to decrease the lead concentration up to 40 % in a fish meat.

Binding of the lead heavy metal in the Tilapia meat: Tilapia fish is the most preferred freshwater fish by the inhabitants of Bandung. This fish has a thick meat texture and less thorny compared to the Carp fish. Moreover, the color of meat is not too red and almost resembles to meat of Snapper fish. Based on its characteristics, Tilapia meat is considerably suitable to be made as fillet. Fillet of Tilapia is enormously served in many restaurants and hotels in Bandung. So far, the Tilapia produced from FCS in Cirata reservoir is supplied to Bandung in order to meet the demand of this fish.

Previously, Junianto et al[7] informed that Tilapia from Cirata reservoir contained lead which surpassed the allowed national standard. In general, the consumed size of Tilapia (250 – 300 g $ind.^{-1}$) contains lead ranging from 3.683to 3.971 ppm. The harvested Tilapia from the FCS of Maleber Site has the highest lead (3.971 ppm) as compared to other sites in Cirata reservoir. In addition, same authors suggest that lead in the fish is significantly correlated to the-lead in the water body and on the Tilapia's source of foods. This fact is confirmed byYirgu[13] who explains that heavy metals uptake into the fish body can be from the gills as respiratory tools, body surface and foods.

The lead metal bound from the fish meat harvested from FCS in Cirata reservoir needs to be conducted, until at least the allowed standard level (2 ppm at maximum) for consumption purpose. The metal binder used in this study is citric acid. Our observation on lead content in Tilapia meat at different concentration, submersion time and preparation technique is described in table 2. The initial content from the harvested Tilapia in FCS Maeleber Site prior to the treatment is 3.878 ppm.

The lead content in Tilapia meat at whole fish form attains the allowed level at two treatments (table 2). The first combination is observed at concentration of citric acid 30% and 120 minutes of submersion time whereas the second combination is at concentration 25% and 150 minutes of submersion time. In contrast, lead in the fillet form of Tilapia achieved the allowed level in three combinations of treatments. First combination was achieved in 90 minutes of submersion time at 30% of citric acid. The second combination is at 25% with 120 minutes of submersion time while the third combination was achieved at concentration 20% with 120 minutes of submersion.

Reduction percentage of lead in Tilapia meat for 120 minutes of submersion at 25% of citric acid is 51%. This percentage is considerably higher than the similar treatment combination conducted at oyster meat, which is only 18%[14]. This happens probably due to the lead that binds to the protein in oyster meat, is more persistent than to that of Tilapia. The oyster meat has indeed more stromal protein than the one compared to Tilapia meat. Thus, it produces stronger and dense texture. This stromal protein is more difficult to denature than the miofibrillar protein and sarcoplasm. Additionally, protein in oyster meat has more cysteine. The amino acid has sulfhydryl group with strong affinity against the lead ion[11].

Binding of lead in Pangasius meat: Pangasius fish is considered as one of the most economically important fish in Indonesia. This fish has various product diversifications from a simple fried fish to the meatball main ingredient. The production of Pangasius fish is mainly dependant on the aquaculture site on the three main reservoirs in West Java Province such as Saguling, Cirata, Jatiluhur and Darma reservoirs. Particularly in Cirata reservoir, the harvested Pangasius fish is supplied to meet the demand of this fish in Bandung.

Several studies revealed that Cirata reservoir has been contaminated by excessive amount of lead. Therefore, it is assumed that the cultured fish in this reservoir has also been contaminated by this metal. Junianto et al[7] have studied the availability of four heavy metals (zinc, lead, cadmium and mercury) in the three commercially important fishes from Cirata reservoir. These authors reveal that the presence of lead in Pangasius meat surpasses the allowed level of standard from the Inspectorate of Foods and Drugs.

The binding of lead in Pangasius meat until the allowed level is strongly recommended. In fact, Pangasius meat is contaminated by high amount of lead which may endanger the consumer. According to Sari et al[15], lead can disrupt the kidney working system and its nerve. Moreover, Saputri[11] also explained the adverse effect of lead on human health. Lead can affect the kidney working system by formation of excesses amino acids and leads to kidney channel enclosure. Lead is also very sensitive to the brain neurons and may cause accelerating hallucination and epilepsy. Additionally, Pb can also inhibit the formation of hemoglobin hence it increases the risk of anemia.

The binding strategy by using citric acid can be an option to reduce lead concentration in fish meat. According to Abadiana et al[14], citric acid is considered as a weak organic compound and safe to be used in food materials either as preservative or as chelating agent. The excess amount of citric acid in human body can be metabolized instead of being accumulated. Furthermore, Sari et al[15] explained that the ability of citric acid to bind heavy metals is due to its OH^- and COOH functional groups. These groups then react to the metal ions to form citric salts. However, during the washing process, these citric salts disappeared from the surface of food materials.

Table 3 presents the effect of treatments on the lead content in the Pangasius fish meat. The initial of lead concentration from the sample fish in FCS of Calincing site prior to treatment is 3.911 ppm. In the present study, the fastest submersion time needed to reduce lead in Pangasius meat until the allowed level (< 2 ppm) is 90 minutes at 30% of citric acid (table 3). Additionally, the lowest concentration of citric acid that can be used to reduce lead in Pangasius meat is at 25%, at the submersion time of 120 minutes.

The present findings indicate that the submersion time is correlated to the concentration of citric acid used. Indeed, a higher citric acid concentration proportionally decreases the submersion time. This result is confirmed by the previous research by Saputri et al[11] who demonstrated that the tangerine extract at 100% can reduce the lead concentration faster than at 75 and 50% in the Tilapia meat.

Conclusion

The present study shows that lead concentration in Carp meat can be decreased from 3.152 ppm to the allowed level (< 2 ppm) at 15% of citric acid with 150 minutes of submersion time.

Table 1
The lead content (in ppm) in the Carp fish meat sample at the various concentrations of citric acid (15, 20, 25 and 30%) and submersion times (30, 60, 90, 120 and 150 minutes).

Submersion time (minutes)	Citric acid concentration 15%	20%	25%	30%
30	2.842	2.704	2.545	2.311
60	2.615	2.485	2.225	2.098
90	2.379	2.239	2.075	1.888
120	2.165	2.035	1.866	1.754
150	1.943	1.843	1.685	1.576

Note: The lead concentration with light blue color indicates the concentration is below the allowed national standard quality according to the Inspectorate of Foods and Drugs[16]

Table 2
The lead content (in ppm) in the Tilapia meat sample at the various concentration of citric acid (15, 20, 25 and 30%) and submersion times (30, 60, 90, 120 and 150 minutes).

Submersion time (minutes)	Citric acid concentration			
	15%	20%	25%	30%
30	3.511	3.366	3.300	3.183
60	3.255	3.109	2.913	2.688
90	3.032	2.889	2.638	2.205
120	2.877	2.633	2.297	1.905
150	2.708	2.460	1.989	1.822

Note : The lead concentration with yellow color indicates that the concentration is below the allowed national standard quality according to the Inspectorate of Foods and Drugs[16]

Table 3
The lead content (in ppm) in the Pangasius meat sample at the various concentrations of citric acid (15, 20, 25 and 30%) and submersion times (30, 60, 90, 120 and 150 minutes).

Submersion time (minutes)	Citric acid concentration			
	15%	20%	25%	30%
30	3.483	3.318	3.099	2.875
60	3.111	2.951	2.575	2.275
90	3.111	2.725	2.025	1.903
120	2.500	2.433	1.933	1.799
150	2.311	2.145	1.772	1.655

Note : The lead concentration with red color indicates that the concentration is below the allowed national standard quality according to the inspectorate of Foods and Drugs[16]

As for Tilapia, lead concentration in this fish is reduced from 3.878 ppm to the allowed level at 25% of citric acid with 150 minutes of submersion time. On the other hand, lead concentration in the Pangasius meat is successfully reduced from 3.911 ppm to the allowed level at 25% of citric acid concentration with 120 minutes of submersion time.

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