

THE EFFECTIVENESS OF LIME (*Citrus aurantifolia*) SOLUTION ON QUALITY OF *Pterygoplichthys pardalis* FLESH FROM CILIWUNG RIVER, JAKARTA, INDONESIA

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ABSTRACT

Rivers play an important role in life, but they are highly polluted by increased activities in the surrounding. For example, Ciliwung River is experiencing heavy metal pollution which has affected aquatic organisms including the pleco fish widely used by local communities as food ingredients such as dumplings, *dimsum*, and *otak-otak*. This element's content rises in pleco fish flesh yearly and the accumulation after entering the human body interferes with health. Even though the usage of citric acid-containing lime reduces heavy metals, currently, no information explains the fruit's effectiveness on metal content in pleco fish. Therefore, this research aims to prove the effectiveness of lime solution on pleco fish flesh quality. It was conducted by soaking the fish flesh with control and the lime solution at different concentrations, namely 0, 50, 75, and 100% for 10 and 30 minutes. Proximate analysis was performed followed by metal and mineral content testing through the XRF method. Based on the results, soaking the sample object with a higher concentration of lime and at a longer time caused a decrease in ash content, while water, protein, and fat increased. After soaking, Pb and Hg content had chelation in each treatment but still exceeded the SNI threshold value, while Sn was in accordance with the SNI. The content of macrominerals (K, Cl, Mg, and P) and microminerals (Cu, Zn, and Se) became different.

Keywords: Ciliwung River, lime, metal and mineral content, *Pterygoplichthys pardalis*, proximate.

INTRODUCTION

Ciliwung River flows from Bogor District to Jakarta bay, and the rapid development of community activities in its surrounding leads to increased pollution. This has been identified as an environmental hazard to humans and aquatic organisms such as fish. The pollution sources include heavy metals

which move from the environment to other organisms through food chains (Elfidasari, Shabira, et al., 2019). This river is one of the waters that has been dominated by pleco fish (*Pterygoplichthys pardalis*). This freshwater fish has a very wide distribution in several countries in the world and originates from the Amazon River in South America (Elfidasari et al., 2020). Furthermore, it is included in the type of

invasive species that are predators or competitors to native species. The usual experience of population explosion makes pleco fish become a separate threat to the native species (Hasrianti *et al.*, 2020).

P. pardalis flesh is used as one of the basic ingredients of processed food products, such as *dimsum*, *otak-otak*, and dumplings by most people around Ciliwung River (Ismi *et al.*, 2019; Putri *et al.*, 2020; Tunjung Sari, 2007). It has an abundant population, affordable prices, and high nutritional content (Elfidasari *et al.*, 2019a). However, the heavy metal content of As, Cd, Hg, and Pb in this fish exceeds the maximum limit for fishery flesh products set by the government (Ismi *et al.*, 2019).

Efforts are needed to reduce the reported high heavy metals in this fish while still maintaining the body's needed nutrients, including protein, fat, and minerals. Currently, natural compounds are widely used for food processing such as acid solutions which bind metals, known as chelating agents (Sipa *et al.*, 2016).

Furthermore, lime (*Citrus aurantifolia*) reduces heavy metals due to the citric acid content and is commonly employed by Indonesians to remove fishy odors (Nurvita *et al.*, 2015). No information has explained the fruit's exact effect, therefore, this research aims to prove the effectiveness of lime solution on pleco fish flesh quality.

MATERIALS AND METHODS

Research Objects and Locations

The object analyzed was pleco fish flesh obtained from fishermen around the Depok area in Sadar street, West Java with longitude coordinates of 6°21'40.96" S and latitude of 106°50'21.56" E (Figure 1). This research was carried out at the Center for the Application of Isotopes and Radiation - National Nuclear Energy Agency (PAIR BATAN) laboratory. Meanwhile, metal contents were identified using XRF equipment at PTBGN BATAN, known as the Center for Nuclear Minerals Technology - National Nuclear Energy Agency.

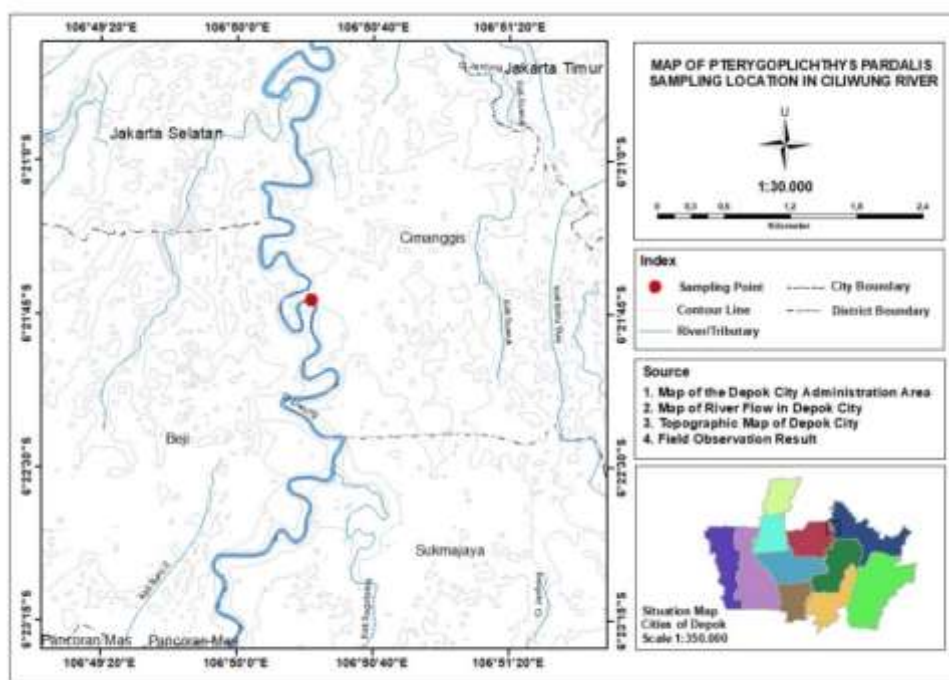


Figure 1. The sampling location of *Pterygoplichthys pardalis* in Ciliwung River

Tools and Materials

The tools used for the laboratory analysis were analytical balance [Sartorius] with an accuracy of 0.0001 g, volumetric pipette, Erlenmeyer, beaker glass, dropper pipette, spatula, oven, desiccator [Duran Normax], XRF device, the crucible, crucible cup and tongs, pens, and a plastic spoon. Other tools included a label, plastic ziplock with size 6x8 cm, ashing furnace, filter paper, Soxhlet apparatus, destruction apparatus, Kjeldahl flask, distillation flask, titration apparatus, petri dish, measuring cup, basin, surgical board, and juicer. The materials used were fish flesh samples from the Ciliwung River, lime, petroleum ether, selenium mixture, H₂SO₄, distilled water, 0.1N NaOH, 0.1N HCl, and methyl red.

P. pardalis Samples Flesh Soaking

P. pardalis flesh soaking was carried out at the PAIR BATAN Laboratory by preparing a fish fillet of 2500 g and cutting it into two parts. The treatments used were control, i.e. fresh fish flesh and distilled water, and soaking with lime. A 100% lime stock solution was prepared by weighing 10000 g of lime, which was peeled and put into a juicer to obtain the fruit juice. This was followed by double filtration to separate the juice from the pulp. During soaking, the first factor considered was lime juice concentrations including 0, 50, 75, and 100%. The second factor was the difference in soaking time lengths of 10 and 30 minutes. Every 100 g of the flesh was soaked in 100 ml of lime solution at various treatment concentration levels and then repeated 3 times.

Analysis of Nutritional Content and Heavy Metals in *P. pardalis* Flesh

Proximate analysis of the nutritional content of *P. pardalis* flesh included tests for ash, water, protein, and fat (AOAC, 2005). Heavy metals and minerals were

analyzed using the X-Ray Fluorescence (XRF) method.

Data Analysis

Proximate and mineral analysis of *P. pardalis* flesh after soaking produced data in percent and ppm units, which were processed manually using Microsoft Excel 2013.

RESULTS AND DISCUSSION

Ash, Water, Protein, and Fat Content in *P. pardalis* Flesh

Based on the testing results, the highest ash content was 1.05% in fresh flesh and the lowest was 0.78% after soaking for 30 minutes with a concentration of 100% as presented in Figure 1. Tarigan *et al.*, 2016 stated that higher ash content in food demonstrates the presence of higher minerals. The decrease in ash content was caused by soaking lime with a certain concentration and time. Lime solution contains an organic acid, namely citric acid (Pomanto *et al.*, 2016), which reduces heavy metal content due to the carboxyl group (COO⁻) forming complex bonds with metals or minerals.

The highest water content was detected in fish flesh soaked in 100% lime for 30 minutes and the lowest was obtained from fresh flesh as shown in Figure 1. This was probably due to the low pH value causing an increase in the water content during lime treatment (Hutapea *et al.*, 2019). According to Al Chusein & Ibrahim, (2012), the longer soaking time elevates the water content as water keeps entering the fish flesh and replacing metal ions.

The highest protein content was found in the control and fresh flesh. Control with distilled water for 10 and 30 minutes generated protein content of 79.44% and 77.07%, while the fresh flesh had 77.51%. The lowest value was obtained at a

concentration of 50% with a soaking time of 30 minutes as presented in Figure 1. Because lime juice has a high acid concentration and low pH, the addition to *P. pardalis* flesh causes protein denaturation and degradation into simpler water-soluble forms (Petalia et al., 2017). Several protein breakdown factors are heat, pH, pressure, electricity, and chemicals (Kunsah, 2017). The content of this nutrient in *P. pardalis* flesh depends on its physiological ability to synthesize protein (Elfidasari, Ismi, et al., 2019).

The highest value of *P. pardalis* fat content was 13.75% in fresh flesh and the lowest was 10.82%, obtained from a 50% concentration with a soaking time of 30 minutes (Figure 1). During soaking, citric acid compounds are absorbed into the flesh fiber and a breakdown process occurs which decreases the fat content. The addition of lime containing organic acids in citric acid form causes fat hydrolysis and subsequent decrease (Supirman *et al.*, 2013). The nutrient turns into a smooth emulsion and dissolves in the acid solution due to soaking (Setiawan *et al.*, 2012). This result is different with their research conducted by Ferbrian et.al (2016), that give us the information soaking lime solution did not have a different effect on fat content in Naniura goldfish carp.

***P. pardalis* Flesh Heavy Metal Content**

According to Table 1, the lead (Pb) content of *P. pardalis* flesh after soaking with lime did not decrease but all soaking treatments experienced Pb content elevation. The analysis results showed that *P. pardalis* flesh has a Pb concentration exceeding the safe consumption threshold value of 0.30 ppm set by the government (SNI, 2009). The highest Hg loss occurred

at a soaking time of 10 minutes with 100% lime, while Hg content increased within 10 and 30 minutes at 75%. Also, the metal concentration value of Hg exceeded the 0.5 ppm safe threshold value (SNI, 2009). The highest decrease in Sn content to 30% occurred after soaking with 50% lime for 10 minutes while using 75% concentration for 10 minutes did not decrease the metal. Sn concentration did not exceed the safe threshold value of 40 ppm once viewed in other processed foods that are not packaged in cans (SNI, 2009).

The effectiveness of lime solution with different concentrations and times is considered to reduce some heavy metal content in *P. pardalis* flesh. The results showed that a decrease in Pb did not occur after soaking but the increased content was because lime contains Pb naturally. Heavy metal accumulation also tends to occur in plants (Eludoyin & Odimegwu, 2018).

Furthermore, the increase in *P. pardalis* flesh metals was due to the increased protein content. The Pb elevation discovered was due to the acid solution being unable to damage protein metal complex bonds. In organisms' bodies, almost all metal ions become attached to proteins (Ilyasa *et al.*, 2016). Pb leftover in the fish flesh after soaking in lime juice was caused by a strong interaction of Pb with the sulfhydryl group. Metallothionein is a protein that binds metals in living things (Suratno *et al.*, 2017).

Heavy metals Hg and Sn decreased after lime juice addition, but the remaining Hg was still above the threshold. The metals decreased due to soaking with citric acid-containing lime (Saputri *et al.*, 2015). Based on their structure, the three carboxyl acids (COOH) combine with metals to produce

complexes. The carboxyl group releases a proton (H^+) or is deprotonated in the solution to generate citrate ions ($-COO^-$). The total released protons are determined by the number of carboxyl groups. Citric acid often releases three protons, hence, because of the highly acidic solution used in this research, protons were released.

Hydrolysis presence separates Hg^{2+} ions from the complex bonds and they subsequently react with the initial citrate ion to form the citrate salt as presented in Figure 2. The Hg metal bound to the carboxyl group dissolves in citric acid solution and becomes wasted after soaking (Masduqi & Ngabekti, 2015).

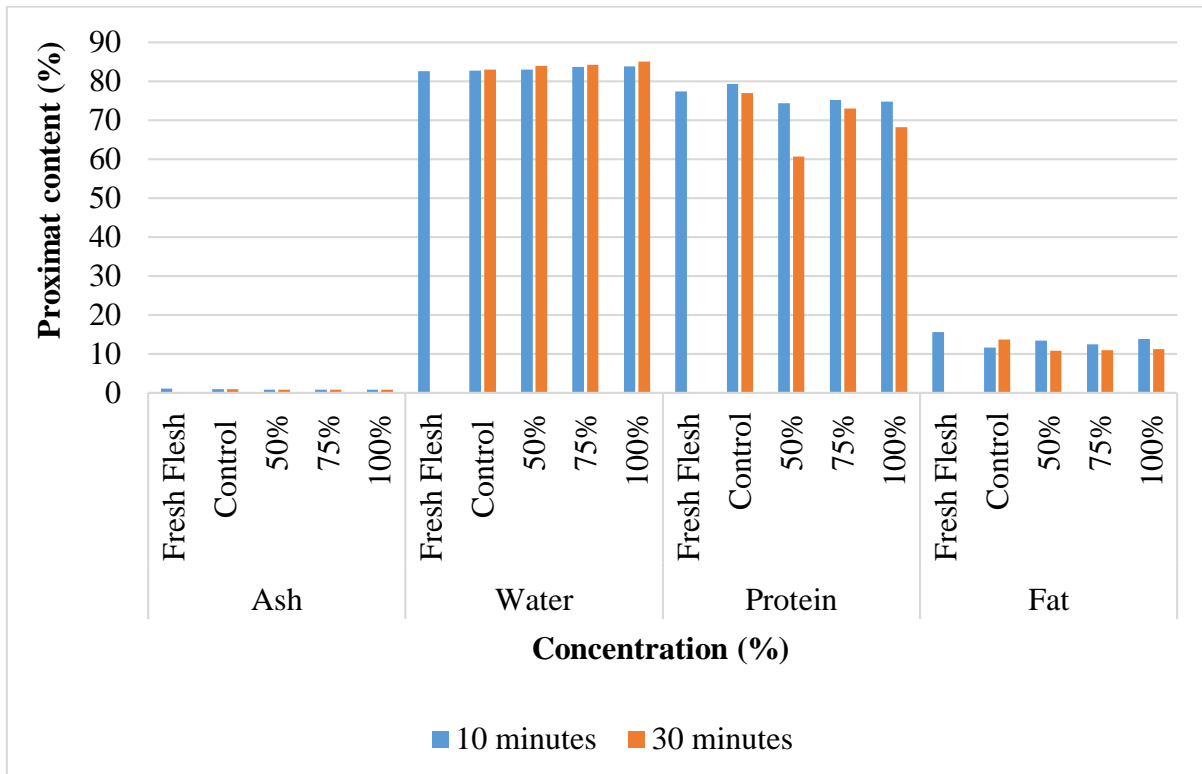


Figure 1. Ash, Water, Protein, and Fat Content in *P. pardalis* Flesh

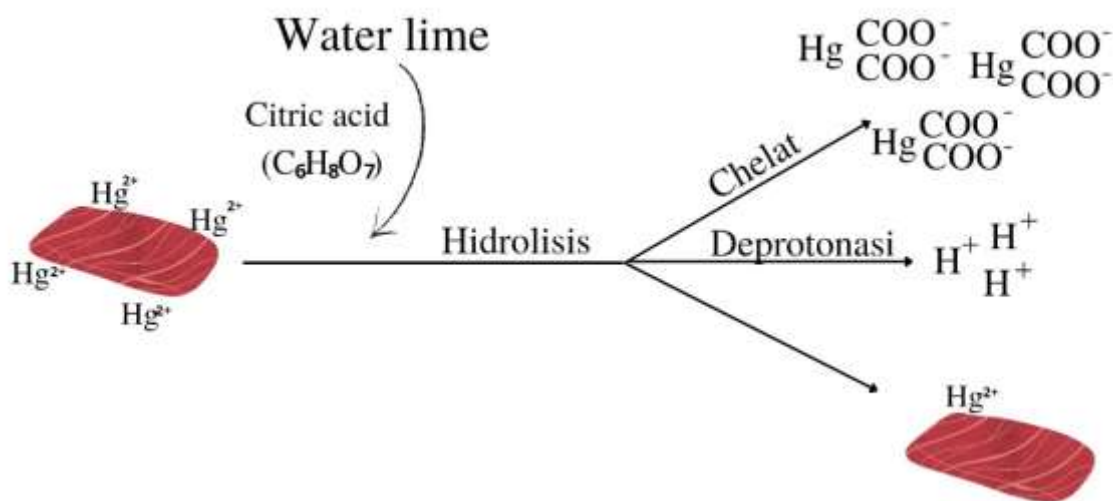


Figure 2. The reaction of citric acid with Hg

***P. pardalis* Mineral Content**

Table 1 shows that K, Cl, Mg, and P with various treatments increased and decreased. The increase discovered after soaking was due to lime containing nutrients such as K, Mg, and P. According

to Kurnia (2014), the macromineral content in 50 g of lime was K (75 g), Mg (5 g), and P (9 g). Priyadi *et al.*, (2013) also stated that citric acid has a relatively low affinity for the alkali metals Ca, K, and Mg.

Table 1. Pb, Hg, and Sn content of *P. pardalis* flesh after soaking in lime solution for 10 and 30 minutes

Lime Concentration (%)	Heavy metal content (ppm)			(SNI, 2009)	Chelation (%)	
	0 minute	10 minutes	30 minutes		10 minutes	30 minutes
Pb	0	2.1	1.9	0.30	-31.25	-18.75
	50	2	1.7		-25	-6.25
	75	2.4	2.6		-50	-62.5
	100	2	1.9		-25	-18.75
Hg	0	4	3.5	0.5	18.4	28.6
	50	5.7	3.2		-16.3	34.7
	75	15.2	11.1		-210.2	-126.5
	100	2.6	3.9		47	20.4
Sn	0	3.3	3.7	40	17.5	7.5
	50	2.8	3.5		30	12.5
	75	4	3.7		0	7.5
	100	3.5	3.8		12.5	5

Macro and microminerals are essential, hence the body needs large amounts of macrominerals including potassium (K), chloride (Cl), magnesium (Mg), and phosphorus (P) which were chelated by the used lime solution. Meanwhile, microminerals such as copper (Cu), zinc (Zn), and selenium (Se) are needed in little amounts. According to Table 2, the fish flesh analysis results showed that the highest and lowest K decrease occurred after soaking with 0% and 75% lime solution for 10 minutes. Furthermore, the increase and highest decrease in Cl was at 0% and 75% in 10

minutes. The elevation and highest reduction in Mg content occurred at 100% soaking lime within 10 and 30 minutes. P decreased at a concentration of 0% in 10 and 30 minutes but increased at 75% within that same time.

Microminerals in the pleco fish flesh included copper (Cu), zinc (Zn), and selenium (Se). Based on the analysis results in Table 3, Cu content decreased after soaking with 0% and 100% lime concentrations for 30 minutes. Meanwhile, it increased at 75% lime usage in 30 minutes and 50% in 10 minutes. The highest increase in Zn content occurred at 75%

while the decrease was at 100% in 10 minutes and 50% in 30 minutes. Se content decreased at 0% and 50% within 10 and 30 minutes, respectively, but increased at 75% and 100% within 30 minutes.

Table 3 shows different results for Cu, Zn, and Se which their chelation was caused by citric acid forming a complex with metal ions. The carboxyl group – COOH is released as H⁺ ions and often

generates complex compounds after combining with metal ions (Anggraini & Fitriana, 2021). The ions are released due to a decrease in metal bonds strength in the protein (Sabila & Kusuma, 2019). The increased content of Cu, Zn, and Se was initiated by lime containing these nutrients (Kurnia, 2014). Priyadi *et al* (2013) stated that citric acid has the most effective ability to remove Cd, Cu, and Zn.

Table 2. Contents of K, Cl, Mg, and P of *P. pardalis* flesh after soaking in lime solution for 10 and 30 minutes

Lime concentration (%)	Macromineral content (ppm)			Chelation (%)		
	0 minutes	10 minutes	30 minutes	10 minutes	30 minutes	
K	0	2.82	3.25	29.07	18.30	
	50	3.98	4.50	3.14	-13.09	21
	75		4.76	4.51	-19.60	-13.19
	100		3.24	3.83	18.61	3.73
Cl	0		1136	1243	43.15	37.78
	50	1998	1842	1651	7.8	17.36
	75		2307	2193	-15.47	-9.75
	100		1495	1862	25.18	6.80
Mg	0		0.887	0.971	3.90	-5.20
	50	0.92	0.917	0.94	0.65	-1.84
	75		1.092	0.904	-18.31	2.05
	100		1.206	0.822	-30.66	10.94
P	0		1.924	2.218	25.55	14.16
	50	2.58	2.906	2.315	-12.46	10.41
	75		3.236	3.239	-25.23	-25.34
	100		2.338	2.716	9.52	-5.10

Another method that can be used to reduce the heavy metal content in pineapple (*Ananas comosus*) is one of the fruits that have high concentrations of citric acid. Interaction of citric acid with heavy metals (Pb & Hg) can be enhanced by the soaking and boiling process at high temperatures. Lead (Pb) and mercury (Hg) in juaro fish were successfully removed above 80% after

soaked and boiled with 100% pineapple extract for 45 min at 100°C. The increasing of boiled temperature and a longer period of treatment at any concentration of citric acid from pineapple increase of removal heavy metals from juaro fish (Mariadi & Sebayang, 2017). Another method was proposed for reducing mercury in fish fillets using mixed solutions containing mercury

absorbent agents. It can remove mercury from fishes fillet up to 91%. Effect of pH, cysteine concentration, EDTA

concentration, salt concentration, and time on mercury removal were found to be significant (Hajeb & Jinap, 2012).

Table 3. Cu, Zn, and Se content of *P. pardalis* flesh after soaking in lime solution for 10 and 30 minutes

Lime concentration (%)	Micromineral content (ppm)			Chelation (%)	
	0 minutes	10 minutes	30 minutes	10 minutes	30 minutes
Cu	0	3.7	0.6	11.90	85.71
	50	4.6	3	-9.52	28.57
	75	4.1	4.8	2.38	-14.28
	100	2.2	1.7	47.61	59.52
Zn	0	75.1	69.5	-16.43	-7.75
	50	66.9	63.6	-3.72	1.39
	75	79.4	74.3	-23.10	-15.19
	100	62.7	79.2	2.79	-22.79
Se	0	4.7	4.9	11.32	7.54
	50	4.8	5.2	9.43	1.88
	75	5.7	5.6	-7.54	-5.66
	100	5	5.8	5.66	-9.43

CONCLUSION

Soaking lime solution with a certain concentration and time provides effectiveness on the pleco fish flesh quality. The proximate content of *P. pardalis* flesh after soaking was best at a concentration of 100% within 10 minutes, namely 0.82% ash, 83.91% water, 74.81% protein, and 13.84% fat. The lime solution failed to reduce Pb and Hg below SNI threshold 7387: 2009, but kept Sn content within the threshold. Lime addition with 0, 50, 75, and 100% concentrations at 10 and 30 minutes did not cause high chelation of

macrominerals, i.e K, Cl, Mg, and P as well as microminerals, i.e. Cu, Zn, and Se.

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