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Penyerapan Pb dalam Daging Kupang Putih (*Potamocorbula faba*) dengan Pemberian Kitosan dari Cangkang Kupang Putih

Absorption of Pb in the Flesh of White Kupang (Potamocorbula faba) By Applying Chitosan Extracted from White Kupang's Shells

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ABSTRAK

Kupang putih (*Potamocorbula faba*) dikenal sebagai produk perikanan yang potensial, tetapi juga berbahaya karena terkontaminasi oleh Pb. Penelitian ini bertujuan untuk menguji pengaruh perlakuan konsentrasi dan waktu perendaman kitosan untuk menurunkan kadar Pb dalam daging kupang putih serta untuk menentukan kombinasi perlakuan terbaik dalam menurunkan kandungan Pb dalam daging kupang putih. Kitosan yang digunakan dalam penelitian ini adalah optimalisasi kitin hasil transformasi cangkang kupang dengan derajat deasetilasi 70,21%. Sampel kupang diambil dari muara sungai Kepetingan, Sidoarjo. Penelitian ini menggunakan rancangan acak lengkap dengan dua faktor, yaitu konsentrasi (0%, 1,5%, 2,0%, dan 2,5%) dan waktu perendaman (30, 60, dan 90 menit). Kadar Pb dianalisis dengan menggunakan metode AAS (*Atomic Absorption Spectrometry*). Pengaruh konsentrasi dan waktu perendaman terhadap kandungan Pb dalam daging kupang putih dianalisis dengan ANOVA dua arah dan dilanjutkan dengan uji LSD untuk menentukan pengobatan kombinasi terbaik. Hasil penelitian menunjukkan bahwa konsentrasi dan waktu perendaman berpengaruh signifikan terhadap kandungan Pb di kupang putih. Kombinasi perlakuan kitosan yang dioptimalkan pada konsentrasi 2,0% dengan waktu perendaman 60 menit memberikan pengurangan Pb yang optimal, sebesar 94,53%.

Kata kunci: kupang putih; Pb; kitosan; konsentrasi

ABSTRACT

White Kupang (Potamocorbula faba) is known as potential fishery products, but on the other hand It is also dangerous because It's contaminated by Pb. This research aimed to describe the treatment effect of concentration and immersion time of chitosan to decrease the content of Pb in the white kupang's flesh as well as to define the best treatment combination in decreasing the content of Pb in the white kupang's flesh. The chitosan used in this research was optimized chitin resulted from the transformation of kupang shell with the degree of deacetylation 70.21%. The samples of kupang were taken from the estuary of the river Kepetingan, Sidoarjo. This research used completely randomized design with two factors treatment items, namely concentration (0%, 1.5%, 2.0%, and 2.5%) and immersion time (30, 60, and 90 minutes). The content of Pb was analyzed by using AAS (Atomic Absorption Spectrometry) method. The influence of concentration and immersion time to the content of Pb in the white kupang's flesh was Analyzed by two-way ANOVA and Followed by LSD test to determine the best combination treatment. The results showed that the concentration and immersion time significantly affected on the content of Pb in white kupang. The combined treatment of optimized-chitosan at concentration of 2.0% with immersion time of 60 minutes gave the optimal reduction of Pb, amounting to 94.53%.

Key words: white kupang; content of Pb; chitosan; concentration; immersion time

PENDAHULUAN

White Kupang (*Potamocorbula faba*) is one the species of mussel that is abundant in the waters of Sidoarjo. White kupang has long been used as a raw material for making "kupang lontong", typical food from Sidoarjo and Surabaya. However, the estusry, as a habitat of white kupang is often contaminated by inorganic components of the waste from surrounding industries, including Pb.

White kupang that live in polluted estuary will lead these metals accumulate in the tissues of kupang's flesh. If those kupang consumed by humans continuously, it will lead to the accumulation of heavy metals in the body and cause various diseases such as anemia, hemoglobin

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deficiency and brain damage as proposed by Darmono (2001). Sikana et al. (2016) reported that the shell of kupang putih (*Potamocorbula faba*) was potential as the sources of chitin and chitosan.

The results of preliminary research indicated that the boiled flesh of white kupang containing Pb at 1.094 ppm. The value has exceeded the maximum threshold issued by FAO / WHO regarding content of Pb in marine animals that can be consumed by humans in the amount of 1 ppm. Therefore, contaminants Pb on flesh of white kupang needs to be reduced in order to safely consume. One alternative method of decreased levels of Pb is using metal-binding compounds (chelating agent), such as chitosan.

Chitosan is a biopolymer of chitin derivative that is polikation so it can be used as a binder of heavy metals. Much research has been done to extract and analyze the ability of chitosan as absorbing heavy metals. Riswanda et al. (2014) have analyzed the ability of chitosan extracted from the white shrimp (*Lithopannaeus vannamei*) in binding heavy metals Pb in flesh of "kerang tahu". Apsari and Fitriasti (2010) had conducted a study the kinetics of absorption of chromium and copper ions using a product chitosan from crab shells. The results showed that the decreased levels of chromium and copper amounted to 99.61% and 99.95%. The study was conducted to describe the effect of treatment concentration and the immersion time of chitosan to decreased levels of Pb in flesh of white kupang and determine the best treatment combination in lowering levels of Pb in flesh of white kupang.

METHODS

The research was conducted on the analysis of Pb in the flesh of white kupang before treatment, the flesh of white kupang soaking treatment using chitosan, and analysis of Pb in the the flesh of white kupang after treatment that was carried out in the Laboratory of Ecology, Department Biology, Surabaya State University and Nutrition Laboratory, FKM Airlangga University.

Samples of white kupang were taken from the estuary Kepetingan, Sidoarjo. Flesh of white kupang was separated from the shell by boiling for ± 3 minutes. The content of Pb in the flesh of white kupang prior to treatment was measured by AAS method (Atomic Absorption Spectrometry). The chitosan used were extracted from the shell waste of white kupang with levels of 22% and the degree of deacetylation of 70.21% (Sikana et al., 2014).

Application of chitosan to reduce the content of Pb in the flesh of white kupang was done by soaking method. Flesh of white kupang that had been boiled then soaked in various concentrations of chitosan (1.5%; 2.0% and 2.5%) and immersion times (30 minutes, 60 minutes, 90 minutes). After the treatments, the flesh of white kupang destructed with HNO_3 and then analyzed by AAS method (Atomic Absorption Spectrophotometry). The percentage value of decreased levels of Pb between treatments was calculated using the formula:

$$\text{\% of decrease} = \frac{\text{Pb content before treatment} - \text{Pb content after treatment}}{\text{Pb content before treatment}} x\ 100\%$$

Data obtained was in the percentage of decreased levels of Pb. Analysis of the influence of concentration and the immersion time of chitosan to decreased levels of Pb in flesh of white kupang was done with 2-way ANOVA test and continued with Least Significant Difference (LSD) to determine the best combination treatment.

RESULTS AND DISCUSSION

The use of chitosan resulted from white kupang's shell (*P. faba*) with a degree of deacetylation of 70.21% was proven to reduce the levels of Pb in the flesh of white kupang with various concentrations (1.5%, 2.0%, and 2, 5%) as well as the immersion times (30, 60, and 90 minutes) given. AAS analysis results showed a mean decrease in Pb (%) after soaking with chitosan of each treatment was 49.84 to 95.79% (Table 1).

Results of analysis of variance showed that the concentration and the immersion time of chitosan were proven to influence the decreasing levels of Pb in flesh of white kupang. F-count value for chitosan concentration was 122,498.2 with a significance value of 0.000 (less than 0.05), so that it could be concluded that Ho refused and the various concentration of chitosan had a significantly different effect on decreasing the levels of Pb in flesh of white kupang. The value of the F-count for immersion time of chitosan was 815.974 with a significance value of 0.000 (less than 0.05), so that Ho refused and it could be concluded that the variation of immersion time of chitosan had a significantly different effect on decreasing the levels of Pb in flesh of white kupang.

Combination of concentration and the immersion time of chitosan gave a significantly different effect on reducing the levels of Pb in flesh of white kupang. The LSD value to combination of concentration and the immersion time of chitosan showed the most optimal treatment in lowering levels of Pb in flesh of white kupang was in concentration of 2.0% and the immersion time of 60 minutes (Table 2).

Pb is a toxic and non-biodegradable material that can be a threat to the lives of all living beings through the process of bioaccumulation and biomagnification in the food chain. Results showed that flesh of white kupang taken from estuary of Kepetingan, Sidoarjo containing average levels of 1.271 ppm Pb (Table 1). While the maximum threshold value of Pb contamination in the marine animals that can be used for human consumption according to FAO/ WHO is only 1 ppm. This means that the levels of lead in the flesh of white kupang has exceeded the threshold and is not safe for consumption. One of the most effective ways to reduce or eliminate Pb in flesh of white kupang is treated with chitosan that can function as chelating agent, as well as to maintain the physical condition, protein and micronutrients, as well as an antimicrobial in flesh of white kupang (Alfie, 2003; Ariyani and Yusma, 2008).

Table 1. Decreased levels of Pb (%) in the flesh of white kupang after soaking in a solution of chitosan from white kupang's shells at various concentrations and immersion times

Chitosan concentrations	Times (min)	Average Levels Initial Pb (ppm)	Average Levels End Pb (ppm)	Decreased levels of Pb (ppm)	Decreased levels of Pb (%)
0%	30	1.271	1.257	0.014	28.20
	60	1.271	1.259	0.012	28.04
	90	1.271	1.260	0.011	27.96
1,50%	30	1.271	0.982	0.289	49.84
	60	1.271	0.657	0.614	75.41
	90	1.271	0.654	0.617	75.64
2,00%	30	1.271	0.510	0.761	86.97
	60	1.271	0.414	0.857	94.53
	90	1.271	0.408	0.863	95.00
2,50%	30	1.271	0.490	0.781	88.55
	60	1.271	0.402	0.869	95.47
	90	1.271	0.398	0.873	95.79

Table 2 Effect of interaction of the concentration and immersion time of chitosan to decreased levels of Pb (%) in flesh of white kupang

Chitosan concentrations	Immersion times 30 min	60 min	90 min
0%	28.18±0,28 [a]	28,01±0,32 [a]	27,96±0,21 [a]
1,5%	49.86±0,16 [b]	75,38±0,16 [c]	75,67±0,28 [c]
2,0%	86.97±0,24 [d]	94,53±0,08 [f]	94,97±0,12 [fg]
2,5%	88.52±0,48 [e]	95,50±0,35 [gh]	95,81±0,20 [h]

Description: The number followed by the same notation showed that the average was not significantly different ($p \leq 0.05$)

The ability of chitosan to absorb heavy metals (in this case Pb) related to its active side (Mekawati et al., 2000). There are two active sides on chitosan, the N atom of the amine group (-NH2) and the O atom of the hydroxyl group (OH), both of which have free electrons that can bind protons in the form of metal ions (Pb) and form complexes $Pb(NH_2)_2$ and $Pb(OH)_2$ which is stable (Volesky and Holan, 1995; Li and Bai, 2002). Reaction or chelate complex formation between chitosan-metal ions (Pb) in detail is as follows (Li and Bai, 2002):

$$R\text{-}NH_2 + M^{2+} \longrightarrow R\text{-}NH_2M^{2+}$$
$$R\text{-}NH_3^{+} + M^{2+} \longrightarrow R\text{-}NH_2M^{2+} + H^{+}$$
$$R\text{-}O^{-} + M^{+} \longrightarrow R\text{-}OM$$

Description: R = components in addition to the group - NH_2 in chitosan; M = Pb

The ability of chitosan from white kupang's shells (*Potamocorbula faba*) with a degree of deacetylation of 70.21% to absorb heavy metals is known yet. Application of various concentrations (1.5%, 2.0% and 2.5%) and immersion times (30 minutes, 60 minutes and 90 minutes) showed that the results of the chitosan of white kupang's shell can absorb Pb in flesh of white kupang.

The combination of concentration (1.5%, 2.0% and 2.5%) and the immersion times (30, 60, and 90 minutes) of chitosan given proved significant effect on decreasing levels of Pb in flesh of white kupang. The chitosan treatment with a concentration of 2.0% and immersion time of 60 minutes resulted the most optimal in decreasing levels of Pb in the flesh of white kupang, in the amount of 94.53% (Table 3).

Statistical analysis showed that variations in the concentration of chitosan (covering 1.5%, 2.0% and 2.5%) effected on decreasing levels of Pb in flesh of white kupang. The decreased levels of Pb in flesh of white kupang were shown the highest (optimal) value at a concentration of 2.5%, while the reduction in the lowest levels of Pb (minimum) at a concentration of 1.5%. Decreasing levels of Pb in flesh of white kupang is directly proportional to the concentration of chitosan given. That was, the greater concentration of chitosan given made the higher reduction in the levels of Pb. It is related to the number of active groups of chitosan, ie the greater concentration of chitosan led to the greater number of amine groups and hydroxyl chitosan roling in process of metal ion chelating (Schmuhl et al., 2001). Therefore, the metal would be efficiency absorbed and the decreasing levels of Pb in the flesh of white kupang also increased.

The decreasing levels of Pb in the flesh of white kupang were not significantly different in application of chitosan concentration of 2.0% and 2.5%. It was alleged to have occurred due to the saturation of the absorbent chitosan at concentrations above 2.0%, because of the viscosity of solution increased along with the concentration of optimation chitosan. The increasing viscosity resulted in the ability of chitosan to get into the flesh of white kupang was hampered. High viscosity caused the water potential of chitosan solution is low, so that the internal fluid of white kupang's flesh out and not vice versa. This resulted NH_2 or OH molecules of chitosan could not bind perfectly with the Pb, so the ability of chitosan to bind metal was actually reduced (Apsari and Fitriasti, 2010) and the provision of chitosan treatment above 2.0% became ineffective and ineconomic to do.

Statistical analysis showed that the immersion times of chitosan (covering 30, 60 and 90 minutes) effected on decreasing levels of Pb in flesh of white kupang. The highest (optimal) percentage of the decreasing levels of Pb in flesh of white kupang shown in 90 minutes of immersion times, while the lowest (minimum) percentage of decreasing levels of Pb was in 30 minutes of immersion times. The immersion time of chitosan given is proportional to the percentage of the decreasing levels of Pb in flesh of white kupang. That was, the longer time of immersion time given makes the higher percentage of the decreasing levels of Pb. This was related to the contact time between chitosan and metal ions, which was the time it took the active side of chitosan to bind to metal ions. The contact times to achieve equilibrium in the process of metal ion uptaken by different absorbent were various, could range from a few minutes up to several hours. Longer contact time allowed the diffusion process and attachment of molecules of chitosan-metal ion took place better (Schmuhl et al., 2001) so that the metal was absorbed higher and the decreasing percentage of Pb in flesh of white kupang arose.

The decreasing percentage of Pb in flesh of white kupang on longer application of immersion times of 60 minutes and 90 minutes were not significantly different. This was allegedly due to the absorbent chitosan have reached the saturation when in 60 minutes of immersion times. This was due to the diffusion process and attachment of molecules of chitosan-metal ions has been perfect, so that no ion exchange that occured in the flesh of white kupang and the network in the Pb binding ability of chitosan would be reduced (Apsari and Fitriasti, 2010). Therefore, chitosan treatment of immersion times with 60 minutes over was not effective and noneconomic to do.

Statistical analysis showed that there was an interaction between the concentration and immersion times of chitosan to decrease the levels of Pb in flesh of white kupang. The most optimal combination treatment is using chitosan concentration of 2.0% with 60 minutes of immersion time. Apsari and Fitriasti (2010) stated that the solubility of the polymer in a solvent is limited, so that at certain concentrations will experience saturation (absorbency decreases). Saturation of absorbent chitosan showed that the process of diffusion and pasting between chitosan molecules with metal ions has been perfect, so that no ion exchange that occurred in the flesh of white kupang and resulted the network in Pb binding ability of optimation chitosan would be reduced on a immersion time of 60 minutes over.

CONCLUSION

Chitosan extracted from white kupang's shell (*Potamocorbula faba*) with a degree of deacetylation of 70.21%, at various concentrations (1.5%, 2.0% and 2.5%) and immersion times (30, 60, and 90 minutes) was proven to effect on the decreasing levels of Pb in flesh of white kupang. Concentration of 2.0% chitosan with 60 minutes of immersion time resulted the most optimal in decreasing the Pb levels, amounting to 94.53%.

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