October – December, 2024
Original Article
DOI: http://dx.doi.org/10.30867/action.v9i4.1948
Pages: 739 – 746
AcTion: Aceh Nutrition Journal
p-issn 2527-3310; e-issn 2548-5741

The effetiveness of various sequestrants on the reduction of cadmium and lead levels in Remis (*Donax sp.*)

Efektivitas sekuestran spesifik dalam menurunan kadar logam kadmium dan timbal pada Remis (Donax sp.)

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Article History:
Received: July 11, 2024; Revised: September 14, 2024; Accepted: October 13, 2024; Published: December 05, 2024.

Publisher:
Politeknik Kesehatan Aceh Kementerian Kesehatan RI

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Abstract

Mussels (*Donax sp.*) are marine shellfish that tend to be contaminated with heavy metals as they are filter feeders. Heavy metal levels can be reduced by immersion in sequestrants. This study aimed to measure the effectiveness of the optimal sequestrant to reduce heavy metal levels in mussels. This experimental study was conducted at the Food Technology Laboratory of Padjadjaran University in January-April 2024 using samples of mussels (*Donax* sp.) from Cirebon, Indonesia. The data processing method used was a Randomized Group Design with five treatments consisting of soaking mussels in distilled water, 25% citric acid, 25% tamarind, 25% tomato, and 25% Pidada; each repeated 3x. The results showed that the most optimal organic sequestrant in reducing heavy metals was Pidada fruit with the effectiveness of reducing cadmium metal 21,195%, lead metal 6,543%, total citric acid 0.552%, water content 76.953%, and antioxidant activity based on IC50 value of 19,537 ppm with a very strong category. In conclusion, the Pidada fruit is the most optimal organic sequestrant for reducing heavy metal levels in mussels (*Donax sp.*) and has very strong antioxidant activity, making it effective for reducing heavy metal contamination.

Keywords: Cadmium, clams, mangrove, lead, sequestrants

Abstrak

Remis (*Donax sp.*) adalah kerang laut yang cenderung terkontaminasi logam berat karena bersifat filter feeder. Kadar logam berat dapat direduksi melalui perendaman pada sekuestran. Penelitian bertujuan untuk mengukur efektivitas sekuestran paling optimal terhadap penurunan kadar logam berat remis. Penelitian eksperimen ini dilaksanakan di Laboratorium Teknologi Pangan Universitas Padjadjaran bulan Januari-April 2024 dengan menggunakan sampel Remis (*Donax* sp.) dari Cirebon – Indonesia. Metode Pengolahan data yang digunakan yaitu Rancangan Acak Kelompok dengan 5 perlakuan terdiri dari perendaman remis dengan akuades, asam sitrat 25%, asam jawa 25%, tomat 25%, dan Pidada 25%, masing-masing diulang sebanyak 3x. Hasil penelitian menunjukkan sekuestran organik yang paling optimal dalam menurunkan logam berat yaitu buah Pidada dengan efektivitas penurunan logam kadmium 21,195%, logam timbal 6,543%, total asam sitrat 0,552%, kadar air 76,953%, dan aktivitas antioksidan berdasarkan nilai IC50 sebesar 19,537 ppm dengan kategori sangat kuat. Kesimpulan, menunjukkan bahwa buah Pidada merupakan sekuestran organik paling optimal dalam menurunkan kadar logam berat pada remis (*Donax sp.*) serta memiliki aktivitas antioksidan yang sangat kuat, menjadikannya efektif untuk mengurangi kontaminasi logam berat.

Kata Kunci: bioaktif, logam berat, kerang, mangrove, organik

Introduction

Mussels (*Donax sp.*) are a type of sea shell that is often used by coastal communities in Cirebon and Karawang as processed food. Shellfish are a food source in the complete protein category because they contain proteins in the form of essential amino acids reaching 85-95%

consisting of glutamate, aspartate, lysine, arginine, and leucine (Tari et al., 2018). Sea shells are categorized as filter feeder animals that filter aquatic substrates or phytoplankton found at the bottom of the water to obtain food (Suarman et al., 2019). Mussels can also be used as aquatic bioindicators because they can bioaccumulate heavy metals (Sari et al., 2021).

The presence of high concentrations of heavy metals in the bodies of marine animals can be dangerous to the health of humans who consume them over long periods of time (Hasanah, 2022). Heavy metals that accumulate in marine animals can be reduced by soaking them in sequestrants (chelating agents) such as citric acid. Consuming shellfish exposed to heavy metals can accumulate in the body, causing higher toxicity, and in the long term can cause cancer (Elvira et al., 2021).

Citric acid is an organic acid that can bind metals by having three carboxyl (-COOH) and hydroxyl (OH-) groups (Ernawati et al., 2022). Citric acid is present in natural fruit juices such as tamarind, tomatoes, and Pidada fruit. Carboxyl and hydroxyl ions in fruit juice can bond with metal ions to form citrate complex compounds which are easily soluble in water. Organic compounds contained in fruit juice can reduce the levels of heavy metals such as ascorbic acid, oxalic acid, and malic acid (Putri, 2014). Fruits that have been used as natural sequestrant alternatives include starfruit, tamarind, and tomato (Susanti & Priamsari, 2016).

Pidada fruit has high nutritional content such as 221,97 IU of vitamin A, 5,04 mg of vitamin B, 7,65 mg of vitamin B2, and 56,74 mg of vitamin C and contains phytochemical compounds such as flavonoids, steroids and terpenoids. (Wulandari et al., 2020). Pidada fruit tends to have a very sour taste because it is influenced by its high ascorbic acid content (Susanto et al., 2020). The total acid content of Pidada fruit (Sonneratia caseolaris) is 0,2697% (Basyuni et al., 2019). Ascorbic acid has a hydroxyl group (-OH), such as a hydroxyl group like citric acid, so it is hoped that Pidada fruit can be used as an alternative natural sequestrant to reduce heavy metal content in mussels (Ondu et al., 2019).

This study aimed to determine the effects of immersion in various sequestrants on cadmium and lead metal levels in mussels (*Donax sp.*) and determine their effects on water content, total titrated acid, antioxidant activity, and organoleptic assessment.

Methods

The ingredients used in this study were mussels (*Donax sp.*) from Cirebon-Indonesia, tamarind, tomatoes, Pidada fruit, and distilled water. The equipment used was an Atomic Absorption Spectrophotometer), 50 ml burette (Pyrex), freeze dryer (Christ Gamma), oven, rotary evaporator (BUCHI R-300, Switzerland), UV-Vis spectrophotometer (UV-9200 Beijing Ray Leigh Analytical Instrument Corporation, China), and ultrasound-assisted extraction (UAE) (QSonica Model CL-334, USA).

Sample Preparation

Mussel meat samples were boiled to separate the shell from the meat. The mussel meat was first washed and then soaked in various 25% sequestrant solutions of synthetic sequestrant (citric acid) and natural sequestrant (tomato, Pidada fruit, and tamarind) for 60 min.

Analysis of Heavy Metal Levels (SNI 2354.5:2011)

The sample was weighed to as much as 5 g in a porcelain cup and then placed in an ashing furnace at 450 °C for 18 h. The sample was dissolved in 1 ml of 65% HNO3 and evaporated at 100 °C until dry. The sample was then placed back in an ashing furnace for 3 h. After the ash was formed, 5 ml of 6 M HCl was added to the sample and evaporated until it was dry.

The sample was added to 10 ml of 0,1 M HNO3, and the solution was transferred to a 50 ml polyphenylene measuring flask and a matrix modifier was added, adjusted to the limit mark using 0,1 M HNO3. The standard solution, blank, and sample were analyzed using an Atomic Absorption Spectrophotometer (AAS).

Total Acid Analysis (AOAC, 2000)

The fruit sample (10 g) was weighed and dissolved in distilled water to the limit. The sample solution was filtered using filter paper, and 20 ml of the filtrate was taken. The sample was added to the PP indicator (phenolphthalein) and titrated with 0,1 N NaOH solution until the color changed to pink.

Antioxidant Analysis (Gümüşay et al., 2015; Nurhadi et al., 2020)

The samples were dried using the freeze-drying method, subjected to size reduction, and sieved using a 60 mesh size. The samples were macerated using ultrasound-assisted extraction (UAE) for 30 min and then filtered. The macerated solution was evaporated at a temperature of 45-50oC using a rotary evaporator to obtain a thick extract. Antioxidant analysis using 1,1-diphenyl picrylhydrazyl (DPPH). The sample extract was dissolved in methanol in a 25 ml volumetric flask. The samples, DPPH, and methanol were added to a total volume of 2,5 ml. The solution was poured into a cuvette, and the absorbance was measured using a UV-9200 spectrophotometer at a wavelength of 517 nm.

Water Content Analysis (SNI 2354.2:2015)

The aluminum cup was dried for 2 h at a temperature of 105 °C, transferred to a desiccator for 30 min, and weighed until constant. The sample was weighed ± 2 g into a cup and then dried in an oven at 105 °C for 16 – 24 h. The cup was moved into a desiccator for ± 30 min and then weighed until constant.

Organoleptic Analysis (SNI 2346:2011)

Organoleptic analysis used a hedonic quality test, using an assessment sheet with 45 panelists. Organoleptic parameters such as color, aroma, texture, and overall appearance were observed.

Statistical Analysis

The research used a Randomized Block Design (RBD) with three repetitions. The data were analyzed statistically using one-way ANOVA to determine significantly different results, followed by Dunnett's test at a 95% confidence level, and organoleptic data were analyzed using Duncan's multiple area test at a 95% confidence level using SPSS 29.

Result and Discussion

Cadmium Metal Levels (Cd)

The natural sequestrant 25% Pidada solution showed the highest reduction in Cd metal levels of 21,195% (Table 1). Pidada contains phytochemical compounds such as flavonoids, steroids, and terpenoids (Wulandari et al., 2020). The research results showed that pisada fruit contains a total acid content of 0,552% with very strong antioxidant activity at an IC50 of 19,537 μg/mL. Bioactive compounds in fruit juice, such as phenolic compounds and antioxidants in mussels, can act as chelating agents because they contain carboxyl, phenolic, or hydroxyl groups that can react with heavy metal ions (Parwata, 2016).

Table 1. Average results of chemical analysis tests on various sequestrants

Treatment	Effectiveness of Reducing Metal Levels (%)		Total Citric Acid (%)	Water content (%)	Antioxidant Activity IC50 (ppm)
	Kadnium (Cd)	Timbal (Pb)			
P1 (Control)	-	-	0,000 ± 0,000	78,493 ± 0,372*	-
P2 (Citric Acid)	60,018 ± 1,034*	17,258 ± 0,373*	22,314 ± 0,188*	70,288 ± 0,780*	-
P3 (Tamarind)	6,468 ± 0,720*	10,175 ± 0,328*	1,224 ± 0,003*	72,556 ± 1,285*	2522,005 ± 16,268 (Very weak)
P4 (Tomato)	11,099 ± 0,593*	4,411 ± 0,478*	0,063 ± 0,001*	76,840 ± 1,833*	450,977 ± 0,450 (Very weak)
P5 (Pidada)	21,195 ± 2,737*	6,543 ± 0,028*	0,552 ± 0,028*	76,953 ± 1,275*	19,537 ± 0,064 (Very gravy)

*SD (standard deviation)

In addition, a decrease in Cd levels in mussels, such as citric acid and ascorbic acid, can influence the decrease in Cd levels in mussels. Citric acid has three carboxyl functional groups (–COOH) that can undergo deprotonation with the release of protons (H+) when dissolved in water. The proton (H+) can be released and form citrate ions that react with metal ions in the mussels, and a chelation process occurs so that Cd can be reduced (S. A. Putri et al., 2023).

The 25% citric acid solution, a sequestrant, showed the highest effectiveness in reducing Cd levels, reaching 60,018% (Table 1). The permitted use of citric acid BTP in food is 3000 ppm/kg or 0,33%/kg (BPOM, 2024). Consuming foods containing synthetic citric acid over a long period can cause inflammation, which affects the respiratory, digestive, nervous, bone, joint, muscle, and connective tissue systems (Sweis & Cressey, 2018).

Lead Metal Content (Pb)

Soaking with various sequestrants resulted in a decrease in Pb levels in mussels, but the natural sequestrant that was able to reduce the highest Pb levels was tamarind (Table 1). The reduction in Pb in mussels is due to the presence of organic acid compounds and bioactive compounds in various sequestrants with properties such as chelators or metal binders. When mussels are soaked in a sequestrant solution, Pb metal ions are released and bound to the OH- and COOH contained in citric acid (Indasah et al., 2011). Pb metal forms a Pb citrate complex compound, which is easily soluble in water, so that the Pb in the mussels will be released and reduced.

Javanese acid has a total acid content of 1,224%, possibly consisting of organic acid compounds found in tamarind, namely succinic, tartaric, malic, citric, formic, and acetic acids (Devi & Boruah, 2020). The high organic acid content in tamarind can make it an alternative sequestrant or chelating agent (Edina et al., 2017). The difference in the efficiency of fruit juice solutions in reducing the heavy metal content with synthetic citric acid can be influenced by the cellulose and lignin contents of various fruit juices. (Mopoung & Kengkhetkit, 2016).

The Cd metal content in mussels tends to be easier to reduce using acidic sequestrants than the Pb metal. This is influenced by the natural nature of the Cd ion, which has a lower ionic radius than the Pb ion, so it can affect the ability of the adsorbent functional groups to bind (Swastawati et al., 2021).

The factor that causes the reduction of Pb metal to be less effective is the sequestrant solution, which has reached the saturation point before reaching the soaking time of 60 min. A saturated sequestrant solution has a low ability to interact with metal ions, so that the bonds between the functional groups of the sequestrant solution and heavy metals can be easily separated (Swastawati et al., 2021).

Total Titrated Acid

Treatment of mussels soaked in various sequestrant solutions caused a decrease in citric acid levels in the soaking solution. The highest acid absorption occurred in the mussel soaking treatment using a synthetic citric acid solution, with a total acid reduction of 10,31% (Table 1). There was less citric acid in natural sequestrant solutions than in synthetic sequestrant solutions. The difference in citric acid levels between fruit juice solutions and synthetic citric acid solutions can be caused by the presence of other compounds contained in fruit juice, such as water, carbohydrates, fiber, vitamins, and other bioactive compounds.

Each natural sequestrant has a different citric acid content, including tamarind (2,20 %) (Hakim et al., 2023), fruit Pidada (0,279 %) (Basyuni et al., 2019b), and tomato (0,230 %) (Mahardhika et al., 2016). Soaking mussels with various sequestrant solutions caused a decrease in citric acid levels in the soaking solution. This shows that the acid content in the soaking solution was absorbed by the mussel meat (Table 1).

Antioxidant Activity

The antioxidant activity can be determined by the IC50 value; the lower the IC50 value, the higher the antioxidant activity. The very strong antioxidant activity of the fruit can be used as a natural sequestrant to reduce heavy metal levels in mussels.

Pidada is a natural sequestrant with strong antioxidant activity (Table 1) and is rich in bioactive compounds such as steroids, triterpenoids, saponins, tannins, alkaloids (Halifah et al., 2019), and flavonoids, which can function as chelating agents for metals (Parwata, 2016). This causes Pidada fruit to have a higher ability to reduce Cd metal compared to other natural sequestrants (Požgajová et al., 2022).

Tomatoes and tamarind have very weak antioxidant activity. This can be caused by various factors such as the extraction method used, which cannot extract the maximum bioactive components (antioxidants) (Tukiran et al., 2021). The decrease in antioxidant activity can be influenced by an inappropriate drying process, so that the extraction process can be hampered and the bioactive compounds obtained are less than they should be (Limesri et al., 2023).

Water Content

Mussels soaked in the 25% Pidada solution had the highest water content compared to those soaked in other natural seuentrants (Table 1). The difference in water content is influenced by the citric acid solution and tamarind, which have a stronger acid effectiveness compared to the tomato juice solution and Pidada. The difference in the concentrations of the sequestrant solution and mussels causes water movement and osmotic pressure.

Soaking with various acidic sequestrants can break the hydrogen bonds in collagen and weaken the bonds between amino acids. Therefore, soaking mussels with various acidic sequestrants reduces the strength of the bonds that bind water molecules and causes the water content to decrease (Pomanto et al., 2016).

Organoleptic

The color of mussel flesh after soaking was preferred by panelists in treatment P2 (Citric Acid), with a score of 3,711 ≈ 4 (yellowish white). Soaking treatment with citric acid solution resulted in a brighter color of mussel meat, which could be due to protein denaturation and dissolution of myoglobin during the soaking process (Jayanti et al., 2018). Mussels are categorized as white meat because they contain low myoglobin levels. When marinated with acid, the low myoglobin content brightens the color of the meat to become whiter (Patriani & Apsari, 2022).

The smell of mussel meat after soaking was most liked by panelists in treatment P4 (tomato), with a score of 4,000 (not fishy). Tomatoes can disguise or eliminate the fishy aroma of the mussel meat. This can be influenced by aroma-producing volatile compounds in tomatoes. The volatile compounds found in tomatoes are acetals, esters, carbonyls, ketals, lactones, and sulfur (Cahyo et al., 2022).

Table 2. Effect of sequestrant soaking on the average organoleptic test

Treatment	Color	Aroma	Texture	Overall Appearance
P1 (Control)	3,400 ± 1,116^{bc}	3,467 ± 0,991^{c}	3,133 ± 0,757^{b}	3,111 ± 1,005^{ab}
P2 (Citric Acid)	3,711 ± 1,014^{c}	1,533 ± 0,661^{a}	3,733 ± 0,986^{c}	3,511 ± 0,757^{c}
P3 (Tamarind)	3,111 ± 0,832^{b}	2,489 ± 0,968^{b}	2,378 ± 0,747^{a}	2,844 ± 0,903^{a}
P4 (Tomato)	3,200 ± 0,919^{b}	4,000 ± 1,000^{d}	2,978 ± 0,839^{b}	3,267 ± 0,809^{bc}
P5 (Pidada)	2,444 ± 1,078^{a}	2,222 ± 1,106^{b}	2,444 ± 1,013^{a}	3,089 ± 0,925^{ab}

Note: Different superscript letters indicate significant differences at the Duncan multiple area test level with a confidence level of 95%. The organoleptic values are the average of 45 panelists with standard deviations.

The texture of mussel meat after soaking was most preferred by panelists in treatment P2 (Citric Acid), with a score of 3,733 ≈ 4 (chewy). The texture of a product can be influenced by its water content contained in the material (Manganti et al., 2021). This is in accordance with the water content obtained that soaking with citric acid (P2) can cause a diffusion process of water transfer from the mussel meat, so that the water content decreases and produces a chewier texture in the mussel meat. The overall appearance of mussel meat after soaking was preferred by panelists in treatment P2 (Citric Acid), with a score of 3,511 ≈ 4 (Uniform). The overall appearance of the mussel meat after soaking was the least liked by panelists in treatment P3 (Tamsamic Tamarind), with a score of 2,844 ≈ 3 (somewhat uniform). The significant difference in the P2 (Citric Acid) treatment can be influenced by the color of the mussel meat, which is brighter, and the P3 (Tamon Tamarind) treatment can be influenced by the color of the tamarind solution, which is quite dark, thus making the mussel meat darker after soaking. The test Hedonic quality has the weakness of varying panelist perceptions with the same sample; therefore, it is important to select panelists to obtain an objective sensory assessment (Gupta et al., 2021).

Conclusion

Soaking treatment with various 25% sequestrant solutions (citric acid, tamarind, tomato, and Pidada) can reduce the levels of the heavy metals Cd and Pb in mussels (Donax sp.). The most optimal sequestrant for reducing heavy metal levels in mussels is citric acid, with an effectiveness of reducing Cd metal levels of 60,018% and Pb metal levels of 17,258%. The most optimal organic sequestrant to reduce heavy metal levels in mussels is Pidada fruit, which is effective in reducing Cd metal (21,195 %), Pb (6,543 %), total citric acid (0,552 %), water content (76,953 %), and antioxidant activity (IC50 = 19,537 ppm).

Acknowledgements

We thank Pertamina Hulu Energi Offshore North West Java for research funding support and the Chancellor of Padjadjaran University for providing the laboratory facilities.

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