SPIN
JURNAL KIMIA & PENDIDIKAN KIMIA
https://journal.uinmataram.ac.id/index.php/spin

DETERMINATION OF MERCURY (Hg) IN GREEN MUSSELS (*Perna Viridis*) USING REFLUX DIGESTION BY COLD VAPOUR ATOMIC ABSORPTION SPECTROPHOTOMETRY (CV-AAS)

Atina K. Nisa[1*], Diana C. Dewi[2], Umaiyatus Syarifah[2]
[1*]Produksi Tekstil, Akademi Komunitas Darussalam, Banyuwangi, Indonesia, 68485,
[2]Kimia, UIN Maulana Malik Ibrahim Malang, Malang, Indonesia, 65114,
DOI: 10.20414/spin.v5i2.8369

History Article
Accepted:
September 26, 2023
reviewed:
November 14, 2023
Published:
December 23, 2023

Keywords:
Green mussels, mercury, refluks digestion.

ABSTRACT

Green mussels, or Perna viridis, are a well-liked fishery product in the community. It can grow happily in a loch, seabord, or mangrove downstream. Through its eggshell, it takes in metals, including mercury (Hg). This study measures the effect of temperature and digestion composition on the amount of mercury (Hg) in green mussels using reflux digestion by cold vapour absorption spectrophotometry (CV-AAS). Utilizing Cold Vapour Atomic Absorption Spectrophotometry (CV-AAS) at a wavelength of 253,7 nm, the study's methodology entails figuring out the ideal digestion composition and temperature for various HNO_3 p.a.: H_2O_2 p.a. destructor compositions (1:0; 2:1; 4:1; 10:3). The results of the investigation indicated that the optimal temperature was 80^0C. With a p-value (0,023) > a (0,05), the t-test result demonstrated a significant effect utilizing the variation in digestion composition. The general public can still safely consume green mussels from Muncar Beach because the sample, which was taken at the ideal temperature and with different digestion variations, contained 0.359 parts per million of mercury. The Indonesian National Standard's maximum limit is not met by these results.

ABSTRAK

Kerang hijau (Perna Viridis) merupakan salah satu komoditi perikanan yang digemari Masyarakat. Kerang hijau dapat tumbuh subur pada daerah pesisir Pantai, teluk, mangrove, ataupun muara. Kerang hijau memiliki kemampuan mengadsorpsi logam dengan menggunakan cangkangnya dengan sifat yang menetap, salah satu logam yang diadsorpsi adalah merkuri (Hg). Penelitian ini bertujuan untuk mengetahui pengaruh variasi suhu dan komposisi pendestruksi terhadap kandungan logam merkuri (Hg) pada kerang hijau menggunakan destruksi refluks secara Spektrofotometri Serapan Atom Uap-Dingin (SSA-UD). Metode penelitian meliputi: penentuan suhu terbaik proses destruksi, penentuan komposisi pendestruksi terbaik meliputi HNO_3 p.a:H_2O_2 p.a (1:0; 2:1; 4:1; 10:3) secara Spektrofotometri Serapan Atom Uap-Dingin (SSA-UD) dengan panjang gelombang 253,7 nm. Hasil penelitian menunjukkan bahwa variasi suhu terbaik menggunakan suhu 80^0C dengan rerata konsentrasi Hg yang terukur sebesar 0,961 ppm. Hasil uji t adalah p-value (0,759)> α (0,05) yang menunjukkan tidak adanya pengaruh yang signifikan antara ketiga variasi suhu. Variasi komposisi pendestruksi terbaik menggunakan HNO_3 p.a:H_2O_2 p.a (1:0) dengan rerata kadar logam Hg sebesar 0,961 ppm. Hasil uji t adalah p-value (0,023)> α (0,05) yang menunjukkan adanya pengaruh yang signifikan antara variasi komposisi pendestruksi. Penentuan kandungan logam Hg dalam sampel dengan menggunakan suhu dan komposisi pendestruksi terbaik diperoleh 0,359 ppm. Hasil tersebut berada di bawah batas maksimum yang ditetapkan Standar Nasional Indonesia, sehingga kerang hijau yang berada di Pantai Muncar masih aman untuk dikonsumsi masyarakat.

How to Cite
Nisa, A. K., Dewi, D. C., Syarifah, U. (2023). Determination of Mercury (Hg) in Green Mussels (Perna Viridis) Using Reflux Digestion by Cold Vapour Atomic Absorption Spectrophotometry (CV-AAS). *SPIN-Jurnal Kimia & Pendidikan Kimia*. 5(2). 267-274.

*Coresspondence Author:
Email: atinaniesa@gmail.com

p-ISSN: 2580-2623
e-ISSN: 2745-6854

INTRODUCTION

One type of shellfish that is well-liked in Indonesian society is the green mussel (P. viridis). It has a high nutritional value for human consumption, containing 40% water, 21.9% protein, 14.5% fat, 18.5% carbohydrates, and 4.3% ash, making it an excellent source of protein (Ervany et al., 2014). One of the districts in Banyuwangi Regency, which is situated next to the Bali Strait and at the extremity of East Java Province's east coast, is Muncar District. The largest fish auction site in Indonesia is located at the coastal fishing port of Muncar. The Technical Implementation Unit Coastal Fishing Harbor (UPT PPP) Muncar's working area includes Banyuwangi. Purse seine nets or purse seines are active fishing tools that are used in most of the catch methods used by Muncar fishermen. Fish behavior in the work area indicates that fresh quality is not being paid attention to, so handling fish on flotsam is still not good. The total amount of fish caught by fishermen in February 2020 was 235,092 tons of flying fish, 207,280 tons of tuna, and 74,474 tons of lemuru fish (Hadi, et al., 2020).

The water pollution near Muncar Beach might be impacted by these production activities, at the very least. When a substance enters water, pollution occurs because it alters the water's structure, flavor, and aroma and prevents it from being used to its full potential. Heavy metal pollution from production activities near Muncar Beach has led to negative effects as well, such as an increase in the amount of heavy metal pollution entering the port. Activities related to production and marine transportation in the vicinity of ports may be exposed to heavy metals. Sediments will be formed when heavy metals present in water bodies go through a deposition process. Mercury (Hg) was one of them.

Hazardous compounds like hydroquinone and mercury can lead to cancer, allergies, rashes, brain damage, and black spots on the skin (Simaremare., 2019). Boundary regulations according to Indonesian National Standard (SNI) the maximum amount of mercury (Hg) contamination in shellfish is 1.0 ppm (*parts per million*). Research related to Hg content in green mussels in Suranenggala and Losari was 0,01 ppm (Andayani, et al., 2020). Another researh showed that the levels of Hg in green mussels at Krueng Cut market, Kec. Syiah Kuala, Banda Aceh City, were obtained at 0.0098 ppb (*parts per billion) (Hazmizal and Bhayu, 2020).* Green mussels were discovered on Tangerang Regency's estuary coast, according to recent research. Samples A, B, and C had concentrations of mercury and other heavy metals of 0.1387 ppm, 0.4543 ppm, and 0.3227 ppm, respectively (Putri and Rina, 2023). In addition to Suranenggala, Losari, Aceh, Tangerang and It does not completely rule out the chance that green mussels in the Muncar Banyuwangi waters are contaminated with heavy metals like Hg.

Compounds are broken down into their constituent elements during the digestion process so that they can be examined. In general, chemistry recognizes two types of digestion: wet digestion (also known as wet oxide) and dry digestion (also known as dry oxide). The conventional closed wet destruction method can be used for mercury analysis in samples. Reflux is one of the methods used in the destruction method to identify the type of volatile mercury metal and the amount of metal present in the sample. Reflux's closed system and condenser contribute to a reduction in analyte loss in the form of volatile mercury. Temperature variations are required to determine the ideal temperature for the reflux destruction of mercury metal in samples. A temperature was used in this investigation, specifically 60^{0}C, 80^{0}C, and 100^{0}C. The composition of HNO_3 and H_2O_2 (1:0; 2:1; 4: 1; 10:3) were employed in a number of studies.

Using cold vapour atomic absorption spectrophotometry, the contents of the samples were examined for mercury (CV-AAS). SnCl2 is the reducing agent (Li, et al., 2020). Research on the mercury content in forage mussels with temperature variations and the composition of the destruction solution with CV-AAS was carried out based on the above description.

METHODS

Materials and Tools

The materials used were standard solutions of $Hg(NO_3)_2$, $SnCl_2$, and HNO_3 pa. 65 %, H_2O_2 p.a 30%, HCl p.a 37%, aquabidest, aquades, green mussel meat, Whatman Paper Size 42. The Tools used in this study were Cold Vapour Atomic Absorption Spectrophotometry variant type AA 240 spectra with an Hg cathode lamp, a set of glassware, Kern-type analytical balance, a set of reflux tools, and a fume hood.

Methods

Determining of Mercury Standard Curve

One milliliter of $Hg(NO_3)_2$ solution is added to a 100 milliliter measuring flask to create a standard solution of 10 parts per million (ppm) of Hg. Next, 2.5 mL of a typical 10 ppm Hg solution is transferred into a 250 mL measuring flask to create a 100 parts per billion (ppb) Hg solution. A range of Hg solutions with concentrations of 10 ppb, 20 ppb, 30 ppb, 40 ppb, and 50 ppb were created from a standard solution of Hg 100 ppb.

Determining of the Best Temperature Digestion Process

One-gram samples of blended green mussel meat were taken, and after adding HNO_3 destruction solution, the meat was reflux digested for three hours at temperatures ranging from 60^{0}C to 100^{0}C until it became clear. Three repetitions of the Whatman 42 paper were used to filter the analyte. Following that, it was examined at a wavelength of 253.7 nanometers (nm) using CV-AAS. The optimal composition of the destructor was ascertained by analyzing the temperature results. The obtained absorbance value is translated into a standard curve with the absorbance on the y-axis and the concentration on the x-axis.

Determining the Composition of the Best Destructive Solutions

A sample of one-gram green mussels was dissolved in the destruction solution using reflux digestion for three hours with the first destructor (HNO_3), followed by the addition of a second destructor (H_2O_2) and one hour of re-digestion, in accordance with the composition in Table 1. Three repetitions of the Whatman 42 paper were used to filter the analyte. Following that, it was examined at a wavelength of 253.7 nanometers (nm) using CV-AAS. The obtained absorbance value is translated into a standard curve with the absorbance on the y-axis and the concentration on the x-axis.

Determining Mercury on the Green Mussels

Using reflux digestion, a sample of one gram of green mussels was dissolved for three hours at the optimal temperature and composition of the destructor. Three repetitions of the Whatman 42 paper were used to filter the analyte. Following that, it was examined at a wavelength of 253.7 nanometers (nm) using CV-AAS. The obtained absorbance value is translated into a standard curve with the absorbance on the y-axis and the concentration on the x-axis.

Table 1. Composition of the Destructor

Compositions	Destructor	
	HNO_3 (ml)	H_2O_2 (ml)
1 : 0	30	0
2 : 1	20	10
4 : 1	24	6
10 : 3	23	7

Data Analyst

By entering measurement data from the relationship between concentration (C) and absorbance (A), subsequently known as the value of slope and intercept, one can determine the level of mercury metal (Hg) at the optimal temperature and destruction. The Lambert-Beer Law can then be used to enter the sample concentration value into the linear regression equation, which is as follows:

$$Y = BX + A \quad (1)$$

Where X represented the sample's concentration, B represented the slope, A represented the intercept, and Y represented the absorbance of the sample. The following formula can be used to find the mercury metal levels:

$$\text{Hg} \left(\frac{mg}{kg}\right) = \frac{V\ x\ b}{m} \quad (2)$$

Where V stood for the solution's volume, b for the instrument's read levels, and m for the sample's mass.

Through data analysis using the statistical test t-Test with SPSS software, differences in the influence of temperature and variations in the composition of the destruction on the results of mercury metal can be determined.

RESULT AND DISCUSSION

Standard curve of mercury (Hg)

Cold vapour atomic absorption spectrophotometry is used to determine the mercury standard curve due to its quick processing time, high selectivity, accuracy, and sensitivity. An atom's electrons are present at different energy levels. The atom can absorb the energy (photons) and electrons transition from a ground state to an excited state when it is exposed to its own wavelength. The transition that takes place during this process is directly related to the radiant energy absorbed by the electrons. Moreover, since each element has a distinct electronic structure, the radiation absorbed is a unique attribute that can be measured for each element (Visser, 2021). Mercury metal absorbs light at a wavelength of 253.7 nm. These light wavelengths are strong enough to change the electronic level of the mercury atom, producing maximum intensity sharp spectral lines and a decrease in interference at ineffective wavelengths. The blanks used are a mixture of 5% HNO_3 and 5% HCl.

$$Hg^{2+} + SnCl_2 \rightarrow Hg^0 + Sn^{4+} + 2Cl^- \quad (3)$$

The atomic absorption spectrometry with cold vapor generation (CV AAS) method is widely employed due to its high level of sensitivity and ease of use (Boneman, et al, 2021). The optimum conditions of mercury (Hg) analysis with CV-AAS can be seen in **Table 2**. Following the tool's optimization, a standard mercury curve with concentration was created, and the measurement results are displayed in **Figure 1.** The correlation coefficient obtained from the curve is 0.999174, indicating that the outcome satisfies the predefined parameters. R^2 is greater than 0.99.

Table 2. Hg metal measurement parameters by CV-AAS

Parameters	Optimum Conditions
Wavelength	253,7 nm
Gap width	0,5 nm
Strong current cathode lamp	4,0 mA
Air flow rate	Asethilene
Asethilene flow rate	NO
Smoothing	7 point
Gain	40 %

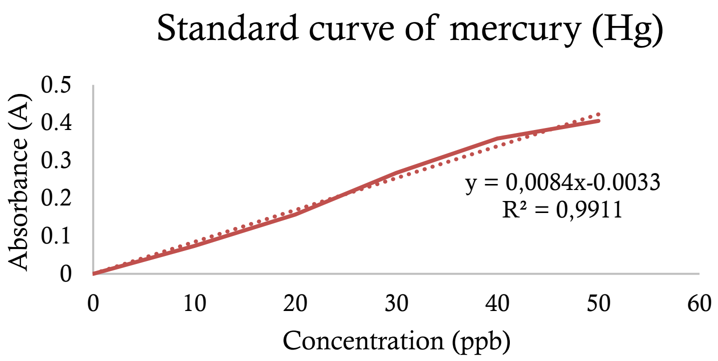

Figure 1. Mercury (Hg) calibration curve graph

Determining the Best Temperature Digestion Process

Three different temperatures were used in this study: 60^0, 80^0, and 100^0 degrees Celsius. The goal of using temperature variations is to compare the three temperature variations with the measured Hg levels in order to determine the ideal temperature of destruction to Hg levels in the sample. The solution turns from white to a brownish-yellow color during the destruction process, and brown bubbles that indicate NO_2 gas are present around the flask with a circular bottom are visible. This suggests that the sample has undergone oxidation as a result of heating. Heat is released from the system as a result of the heating. There is an exothermic reaction in the system. The condenser will then take in and cool the released heat. An endothermic reaction occurs in the condenser system. Different values are obtained at different temperatures due to variations in digestion; Table 3 provides further information on this.

Table 3. Absorption (y) result through CV-AAS

Variation of Temperature	Repetitions	Sample Weight (kg)	Adsorption (y)	Concentration (ppm)
60^0C	I	0.0010025	0.225	0.745
	II	0.001003	0.3667	1.1091
	III	0.0010033	0.313	0.7149
80^0C	I	0.0010029	0.3076	1.0537
	II	0.0010029	0.2591	0.8568
	III	0.001003	0.2368	0.8393
100^0C	I	0.0010421	0.2183	0.769
	II	0.0010013	0.1896	0.6509
	III	0.0010369	0.1569	0.8002

The collected data are backed up by statistical analyses to ascertain how temperature affects mercury metal concentrations. Based on Table 4 data, the p-value (0.759) > α (0.05) indicates that the temperature variation employed had no effect on the levels of mercury produced; however, in this instance, the optimal temperature determination is derived from the quantity of mercury absorbed by the instrument at 80^0C. In addition, digestion temperature has a significant impact on digestion efficiency. Because a higher temperature will result in a flatter average kinetic energy (Ek), the impact event between the acid and the sample will be greater. Elevating the temperature of digestion will lead to a rise in mercury levels. There will be more samples because the reagent and sample will collide more frequently as the temperature rises. A lot of destroyed sediments change the sample matrix into a specific matrix. Sediment and other inorganic samples may contain sufficient amounts of organic material that can be dissolved by treating them with acid. Since it's essentially a process Destruction is the process of disassembling compounds into their constituent parts for analysis, as well as the dissolution of compounds from organic metals into metallic and inorganic forms.

Table 4. Paired sample test

	Pair Differences					t	df	Sig (2-tailed)
	Mean	Std. Deviation	Std. error mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 60^0C and 80^0C	-6.00300000E-2	.2975146	.1717701	-.7993672	.6787672	-.351	2	.759

Determining the Composition of the Best Destructive Solutions

To find the ideal composition for destroying the mercury metal in the sample, the best destruction composition is determined. After adding H_2O_2, the solution's color changed from yellow to clear. This is because H_2O_2 was used as bleaching in a variety of industries, including the paper and textile industries. The following response takes place:

$$Hg + H_2O_2 + 2H^+ \rightarrow Hg^{2+} + 2H_2O \quad (4)$$

When H_2O_2 is added drop by drop, it will break down into H_2O and O2 gases, and when NO_2 reacts with HNO_3, it will produce NO2 gas. The resulting NO_2 gas will slowly react with H_2O_2 to break down any remaining organic compounds, and HNO_2 will then break down again into NO_2 and NO. Until all organic compounds are broken down, this process will keep happening [Vogel, 1985]. Different values are obtained at each variation due to variations in the composition of the destructive solutions; Table 5 provides more information on this.

Table 5. Absorption (y) result through CV-AAS

Variation of Destructive solutions	Repetitions	Sample Weight (kg)	Adsorption (y)	Concentration (ppm)
HNO_3 : H_2O_2 (1:0)	I	0.0010029	0.3076	1.0537
	II	0.0010029	0.2591	0.8568
	III	0.001003	0.2368	0.8393
HNO_3 : H_2O_2 (2:1)	I	0.0010254	0.0941	0.3153
	II	0.0010834	0.0934	0.296
	III	0.0010571	0.0933	0.3026
HNO_3 : H_2O_2 (4:1)	I	0.0010221	0.1637	0.5249
	II	0.0010226	0.1586	0.5125
	III	0.0010228	0.1607	0.5212

Variation of Destructive solutions	Repetitions	Sample Weight (kg)	Adsorption (y)	Concentration (ppm)
$HNO_3 : H_2O_2$ (10:3)	I	0.0010298	0.1708	0.550
	II	0.0010294	0.1626	0.5429
	III	0.0010287	0.1507	0.5035

Table 6. Paired sample test

	Pair Differences					T	df	Sig (2-tailed)
	Mean	Std. Deviation	Std. error mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 $HNO_3 : H_2O_2$ (1:0) and $HNO_3 : H_2O_2$ (10:3)	.3844667	.1038381	.0599509	.1265186	.6424147	6.413	2	.023

The optimal composition of HNO_3 pa H_2O_2 1:0 is demonstrated in Table 5. It is believed that HNO_3 has the strength to completely dissolve mercury metal in the HNO_3 conductor by damaging and breaking the mercury metal compound. The analysis results show which kind of destruction is most effective in causing the highest concentration of mercury to dissolve in perfect phytate by inflicting damage to the matrix. Therefore, using hydrogen peroxide as an auxiliary reagent in place of diluted nitric acid is a good way to increase the efficiency of digestion [Bizzi et al., 2014]. Table 6 indicates that there is an influence on the composition of the destruction used, as indicated by the p-value (0.023) < (0.05).

Determining Mercury on the Green Mussels

Using cold vapour atomic absorption spectrophotometry (CV-AAS) reflux destruction with 80^0C and HNO_3 pa H_2O_2 1:0, green mussel samples can have mercury levels of 0.354 ppm, 0.374 ppm, and 0.350 ppm, respectively. Since green mussels are good at absorbing metals, it stands to reason that the more green-mussels used in a sample, the more mercury the mussels will have taken up. The results, nevertheless, are still far short of the government-set 1.0 ppm threshold.

CONCLUSION

The best temperature was 80^0C, and temperature variations had no discernible effect on the amount of mercury present, according to the research that was done. and the best destruction solution's composition yielded HNO_3 and H_2O_2 at a ratio of 1:0, indicating that the best destruction solution can only be produced with HNO_3. The composition of the destruction solution has a major impact on the amount of mercury metal present.

REFERENCES

Andayani, A., Koesharyani, I., Fayumi, U., Rasidi., & Sugama, K. (2020). Akumulasi Logam Berat Pada Kerang Hijau di Perairan Pesisir Jawa. *Oseanologi dan Limnologi di Indonesia*. 5(2). https://doi.org/135-144. 10.14203/oldi.2020.v5i2.279

Bizzi, C. A., Flores, E. L. M., Nóbrega, J. A., Oliveira, J. S. S., Schmidt, L., & Mortari, R. S. (2014). Evaluation of a digestion procedure based on the use of diluted nitric acid solutions and H_2O_2 for the multielement determination of whole milk powder and bovine liver by ICP-based techniques. *Journal of Analytical Atomic Spectrometry*. 29.

332. https://doi.org/10.1039/C3JA50330E

Bonemann, D. H., Ossanes de Souza, A., Pereira, C. C., Oreste, E. Q., Ribeiro, A. S., & Vieira, M. A. (2021). Determination of Hg in xanthan gum by CV AAS after acid decomposition using reflux system. *Food Hydrocolloids*.118. https://doi.org/10.1016/j.foodhyd.2021.106802

Eshmat, M. E., Mahasri, G., & Rahardja, B. S. (2014). Analisis kandungan logam berat timbal (Pb) Cadmium (Cd) pada kerang hijau (perna viridis) di perairan Ngembok kabupaten gresik jawa timur. *Jurnal Ilmiah Perikanan dan Kelautan*. 6(1). 101-108. https://dx.doi.org/10.20473/jipk.v6i1.11387

Hadi, A., , Muthmainah, D., & Wardhana, M. G. (2020). Analisis Data Hasil Penangkapan Ikan di Unit Pelaksana Teknis Pelabuhan Perikanan Pantai Muncar. *Jurnal Ilmu Perikanan dan Kelautan*. 2(1). 1-5. https://doi.org/10.36526/lemuru.v2i1.1226

Hasmizal, H. & Bhernama, B. G. (2020). Analisis Kadar Logam Hg Pada Sampel Perna Viridis L Dengan Menggunakan Atomic Absorption Spectrophotometer. *Amina*. 1(3). 120-125. https://doi.org/10.22373/amina.v1i3.487

Li, S., Hao, C., Xing, P., Xia, X., Liu, T. & Maoa, X. (2020). Rapid Screening Analysis of Methylmercury in Fish Samples Using Stannous Chloride Reduction and Direct Sampling Electrothermal Vaporization Atomic Absorption Spectrometry. *At Spectrosc*. 41(5). 211-21. http://dx.doi.org/10.46770/AS.2020.05.006

Putri, U. R. P., & Rismaya, R. (2023). Determination Of Mercury (Hg) Levels in Green Mussels (Perna Viridis) In Serpong Market Using a Mercury Analyzer with Direct Thermal Decomposition Method. *Jurnal Food Science and Technology*. 3(1). 27-39. https://doi.org/10.33830/fsj.v3i1.4911.2023

Simaremare, E. S. (2019). Analisis Merkuri Dan Hidrokuinon Pada Krim Pemutih Yang Beredar Di Jayapura. *Jurnal Sains dan Teknologi*. 8(1). 1-11. https://doi.org/10.23887/jstundiksha.v8i1.11813

Visser, D. (2021). Atomic Absorption Spectroscopy, Principles and Applications. Article. https://www.technologynetworks.com/analysis/articles/atomic-absorption-spectroscopy-principles-and-applications-356829