

DETERMINATION OF LEAD AND CADMIUM LEVELS IN CANNED SARDINES USING ATOMIC ABSORPTION SPECTROPHOTOMETRY

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Artikel submitted: March 17th, 2024; Accepted: March 26th, 2024

DOI: <https://doi.org/10.36387/jiis.v9i1.1669>

ABSTRAK

Ikan sarden kalengan dikemas tertutup secara hermetik dan diproses untuk menjamin sterilitas serta memberikan kemudahan dalam mengolah ikan secara praktis. Semakin lamanya kontak makanan pada kemasan kaleng menyebabkan terjadinya migrasi unsur-unsur logam di dalamnya. Tingginya kadar logam, khususnya timbal (Pb) dan kadmium (Cd), dalam makanan kemasan kaleng, berdampak buruk terhadap kesehatan. Penelitian ini bertujuan untuk menetapkan kadar Pb dan Cd pada ikan sarden kalengan serta mengidentifikasi pemenuhan parameter validasi terhadap metode Spektroskopi Serapan Atom (SSA). Ikan sarden ditimbang, didestruksi dengan HNO₃ pekat:30% H₂O₂ dan dianalisis dengan SSA. Logam Pb dan Cd pada ikan sarden kalengan tidak dapat terdeteksi dengan SSA. Metode ini memenuhi persyaratan parameter validasi, Pb dan Cd, diantaranya selektif pada panjang gelombang 283,3 nm dan 228,3 nm, kurva baku linear dengan nilai proses relatif standar deviasi (V_{x_0}) < 5% dan nilai X_p lebih kecil dari konsentrasi terendah dalam kurva baku, 0,69 mg/l dan 2,31 mg/l adalah nilai batas deteksi (*Limit of Detection/LOD*) dan kuantitasi (*Limit of Quantitation/LOQ*) logam Pb, logam Cd 0,20 mg/l dan 0,67 mg/l, 80-120% adalah nilai % perolehan kembali (*%recovery*) pada parameter akurasi dan nilai standar deviasi relatif (*Relative Standard Deviation/RSD*) < 20% adalah parameter presisi. Metode SSA dapat digunakan untuk menetapkan kadar Pb dan Cd pada ikan sarden kalengan secara kuantitatif.

Kata kunci: Ikan sarden kaleng, Kadmium, Spektrofotometri, Timbal

ABSTRACT

Canned sardines are hermetically sealed and processed to ensure sterility and provide practical convenience in processing fish. Contact between food and canned causes the migration of metal elements into them. The high content of metals, lead (Pb) and cadmium (Cd) in canned food has a negative impact on health. This study aims to determine both metal levels in canned sardines and to identify the fulfillment of several validation parameters against Atomic Absorption Spectrophotometry (AAS). Sardines were weighed, digested with concentrated HNO₃:30% H₂O₂ and analyzed using AAS. Pb and Cd levels in canned sardines were not detected by AAS. This method meets the requirements of validation parameters, Pb and Cd, selective on specific wavelengths 283.3 nm and 228.3 nm, the standard curve is linear with a relative process standard deviation (V_{x_0}) value < 5% and the X_p value is smaller

than the lowest concentration in the standard curve, 0.69 mg/l and 2.31 mg/l are the results of the Limit of Detection (LOD) and Limit of Quantitation (LOQ) values of Pb while Cd 0.20 mg/l and 0.67 mg/l, 80-120% is %recovery of accurate parameters and a relative standard deviation (RSD) value < 2% is precision parameters. The SSA method can be used to quantitatively determine Pb and Cd levels in canned sardines.

Keywords: *Canned sardines, Cadmium, Spectrophotometry, Lead*

INTRODUCTION

National fish consumption in 2019 in Indonesia was 55.95 kg/capita per year and will be increasing in 2024, according to the 2020 data of the Indonesian Ministry of Maritime Affairs and Fisheries (Suherman, 2020). Increasing fish consumption makes the food industry produce fish products that are ready to eat (RTE), namely canned sardines, consisting of sardines, tomato paste, salt, and preservatives (Mütamirah, 2018).

Fish canning is widely recommended for long-term preservation (Cruz et al., 2022). The packaging, which takes place in tightly closed containers and is sterilized at high temperatures, aims to inhibit the growth of microorganisms, causing food to expire easily (Indraswati, 2017). Heavy metals, such as As, Fe, Ni, Pb, Sn, Zn, Cd, Cr, and Cu (Massadeh et

al., 2018; Mütamirah, 2018; Perdana, 2019; Refilda et al., 2021), Co, Ag, Sb, Hg, V, Mn, and U (Kowalska et al., 2020), are found in canned food.

High amounts of Pb and Cd in canned food, cause increased blood pressure and heart disease, reducing the development of cognitive and intelligence, and damaging the kidneys, muscles also reproductive organs (Lahdimawan et al., 2022; Massadeh et al., 2018; Nuryanti, 2018). According to Standar Nasional Indonesia (2016), the maximum Pb level is 0.3 mg/kg, while Cd is 0.1 mg/kg in canned sardines. Pb and Cu levels increased 30-60% in canned sarden during 6, 18, and 36 months of storage time after production (Refilda et al., 2020). Meanwhile, the increasing level of Zn in canned condensed milk at the expiration date was higher than before the expiration date (Go et al., 2019). Heavy metal contamination is caused by migrating

the elements in canned food during the storage period due to the long interaction between food and packaging components (Perdana, 2019; Sheeladevi and Ramanathan, 2011; Tehubijuluw et al., 2013).

Atomic Absorption Spectroscopy (AAS) can be used to determine the presence of metal and metalloid elements in canned sardines (Kusnadi, 2016). Quantitative analysis of AAS is measured by the Lambert-Beer law, which is a linear relationship between concentration and absorbance (Lagalante, 2004). This method is widely used because it is selective, specific, sensitive, relatively cheap, fast, and easy to do (Kusnadi, 2016).

The preparation was done by wet destruction method to obtain the elements in canned sardines. By utilizing hydrochloric acid (HCl), nitric acid (HNO₃), sulfuric acid (H₂SO₄), or a combination of these acids to decompose the organic and inorganic matrix in a sample (Bader, 2011; Idera et al., 2015). In fish tissues (bones, skin, meat, and tail), heavy metals were detected using

AAS (Fonge et al., 2011; Khalifa et al., 2010; Uzairu et al., 2009).

As a result, research is required to identify the safety of food products, especially Pb and Cd in canned sardines. The levels of both metals are determined, besides the validation parameters, such as selectivity, linearity, limit of detection/LOD, limit of quantification/LOQ, accuracy, and precision, were previously conducted before analyzing the heavy metals quantitatively.

METHOD

Materials

Standard solutions of Pb and Cd 1000 mg/l (*Merck*), concentrated nitric acid (HNO₃ p.a) (*Merck*), 30% hydrogen peroxide (H₂O₂) (*Smart*), filter paper ashless grade 41 and nylon filter membrane (0.45 µm porosity) (*Whatman*) and aqua bidestilata (*Otsuka*). Canned sardines with different brands (X, Y and Z) and different expired dates (2021, 2022 and 2023) were collected from 3 traditional markets. Atomic Absorption Spectroscopy (*Agilent 240FS AA*), analytical balance

(Ohaus), oven (Mettler), hot plate (Ceramag) and laboratory glassware.

Analysis of Heavy Metals

Canned sardines from each brand were mashed and weighed ± 5 grams, dried ($105 \pm 1^\circ\text{C}$, 3 hours), cooled, and weighed until constant weight (Departemen Kesehatan Republik Indonesia, 1979; Massadeh et al., 2018; Refilda et al., 2020). 30 ml concentrated HNO_3 at $90 \pm 1^\circ\text{C}$ was used to digest the sardines until the volume was ± 5 ml, then the digestion was completed by adding 10 ml of 30% H_2O_2 and heating until it produced a clear solution. Afterward, it was immediately transferred to a volumetric flask 50.0 ml after it was filtered using filter paper No. 41. 4% HNO_3 was added to a volumetric flask, homogenized, and filtered using a nylon filter membrane. The sample solution was prepared for AAS measurement (Refilda et al., 2020; Sumiyani et al., 2021).

Analytical method of validation

1. Selectivity

A specific wavelength of Pb and Cd could be determined by the hollow

cathode lamps, which can be used to demonstrate selectivity.

2. Linearity

The calibration curve of Pb and Cd came from 1000 ppm, each metal was diluted into 3, 5, 7, 8, 9, 10, and 12 mg/l for Pb, while 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, and 2.4 mg/l for Cd. A linear regression curve ($y = a + bx$) was obtained through the regression of concentration (x) and absorbance (y). A linear correlation value ($r > 0.999$), a relative process standard deviation ($V_{x0} < 5\%$), and the lowest value of the calibration standard must be greater than the X_p value (Indrayanto, 2018).

3. Limit of Detection and Limit of Quantification

The LOD and LOQ parameter could be calculated as follows (Fatimah et al., 2020; Musiam and Alfian, 2017):

$$LOD = \frac{3s_{y/x}}{s} \quad (1)$$

$$LOQ = \frac{10s_{y/x}}{s} \quad (2)$$

Standard deviation of blank is notated as s and standard deviation residual is $s_{y/x}$.

4. Accuracy

The accuracy was determined by digesting ± 5 grams canned sardines and each metal with concentrated HNO_3 , then 30% H_2O_2 until a clear solution. Afterward, it was immediately transferred to a volumetric flask 50.0 ml and filtered using filter paper No. 41. 4% HNO_3 was added to a volumetric flask, homogenized, and filtered using a nylon filter membrane, then analyzed with AAS (Refilda et al., 2020; Sumiyani et al., 2021).

This method is defined as spike recovery method, which could be calculated as follows Indrayanto (2018):

$$\% \text{Recovery} = \frac{C_f - C_u}{C_a} \times 100 \quad (3)$$

The concentration of unspiked matrix (original concentration) is notated as C_u , the concentration of spiked matrix is C_f , and the concentration of added compound (not analyzed) is C_a .

5. Precision

The precision was measured through series of measurements from multiple data of %recovery and the absorbance of the spike recovery method 5 times. This parameter was

calculated as follows (Musiam and Alfian, 2017; Sumiyani et al., 2021):

$$RSD = \frac{SD}{\bar{x}} \times 100 \quad (4)$$

While SD is standard deviation and \bar{x} is average level.

Data Analysis

Descriptive data analysis by presenting Pb and Cd levels in canned sardines based on validation parameters (selectivity, linearity, LOD, LOQ, accuracy, and precision).

RESULT AND DISCUSSION

Analysis of Heavy Metals

The water content of canned sardines from each brand is 64.85-67.17% shown in **Table 1**. It was verified that canned sardines had the highest water content because of the type of sauce, whether water or oil, the basis for making sauce for canned sardines which is tomato, the compositional variation of fish, which is depends on season or gender, and also technological processing (Cruz et al., 2022).

Table 1. Water content in canned sardines

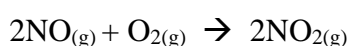
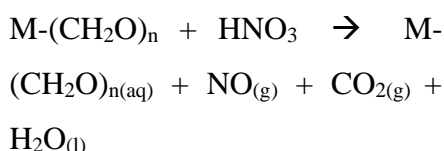
Merck	Water content (% w/w)
X	67.17 \pm 0.69
Y	64.85 \pm 5.01
Z	65.57 \pm 0.07

Wet destruction was used to determine the elements with low

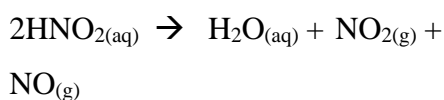
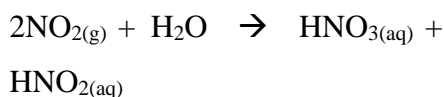
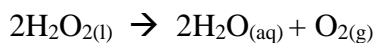
concentrations by decomposing the organic matter such as carbohydrates, proteins and fats to free metals (Go et al., 2019; Tehubijuluw et al., 2013). HNO₃ was used as a strong oxidizer while the oxidizing ability and dissolution performance could increase by using H₂O₂. This destruction was heated to complete the process (Refilda et al., 2020; Tehubijuluw et al., 2013).

The reactions were shown below (Go et al., 2019):

- Organic compounds with nitric acid



- Hydrogen peroxide



Based on **Table 2**, Pb and Cd levels in canned sardines are not detected because the concentrations are lower than limit of detection of the instrument. Not detecting heavy metals does not mean that their

presence is not available in canned sardines. In order to determine relatively small levels of Pb and Cd, an instrument with a sensitivity of up to ppb is needed (Sharma, 2020).

In addition, the expiration date which is still valid also properly packaging processes and manufacturing canned processes, can extend the shelf life of its product. Metallurgical factors are one of the contamination causes against canned packaging, the type of metal alloy and the homogeneity of manufacturing canned processes (Tehubijuluw et al., 2013).

Table 2. Pb and Cd levels in canned sardines

Heavy Metals	Merck	Replication		
		1	2	3
Pb	X	Nd	Nd	Nd
	Y	Nd	Nd	Nd
	Z	Nd	Nd	Nd
Cd	X	Nd	Nd	Nd
	Y	Nd	Nd	Nd
	Z	Nd	Nd	Nd

Nd: Not detected

Pb: LOD: 0.69 mg/L, LOQ: 2.31 mg/L

Cd: LOD: 0.20 mg/L, LOQ: 0.67 mg/L

Analytical method of validation

1. Selectivity

The selectivity parameter can be used to identify certain analytes in mixtures or matrices without interference from other components (Indrayanto, 2018). Pb was observed

at 283.3 nm while Cd was at 228.3 nm.

2. Linearity

Pb calibration curve was performed with concentrations ranging from 3 to 12 mg/l, while Cd was in the concentration range of 0.6 to 2.4 mg/l (**Figure 1**).

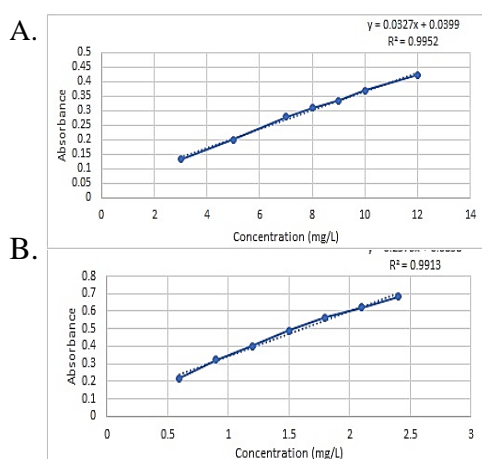


Figure 1. Calibration curve of Pb (A) and Cd (B)

The regression equation of Pb was $y = 0.0327x + 0.0399$ ($r = 0.9976$), while Cd was $y = 0.2573x + 0.0858$ ($r = 0.9956$), respectively. It was shown that the r values for Pb and Cd were less than 0.999. However, the result did not meet the requirements for expressing linearity. The linearity of a calibration curve was demonstrated by other parameters, such as V_{xo} and X_p (Indrayanto, 2018). The result showed the parameters complied with

the standard, V_{xo} and X_p for Pb were 2.99% and 1.67 mg/l, respectively, while 4.44% and 0.46 mg/l for Cd. The relationship between the response of absorbance (y) and concentration (x) for both heavy metals is linear.

3. Limit of Detection and Limit of Quantification

The smallest concentration of an analyte that is not necessarily quantified as a true value but can be detected is expressed as the limit of detection (LOD). While the limit of quantification (LOQ) is the lowest amount of analyte that can be determined quantitatively with adequate accuracy and precision (ICH, 2005).

Low concentration calibration curve of the target compound can be detected using the detection limit parameters. It develops a linear regression curve with relatively low concentrations of the target compound in a suitable solvent (Indrayanto, 2018). **Table 3** describes the limit of detection (LOD) and quantification (LOQ) of Pb and Cd using equations (1) and (2).

Table 3. LOD and LOQ of Pb and Cd

Heavy metals	LOD (mg/L)	LOQ (mg/L)
Lead (Pb)	0.69	2.31
Cadmium (Cd)	0.20	0.67

4. Accuracy

Accuracy is the test results that are close to the true or accepted value (an individual value, an average of a set of values, the average of averages, or an assigned value) (Emawati et al., 2022; Indrayanto, 2018). %Recovery can be calculated using equation (3), which shows the accuracy parameters of both metals (**Table 4**). The accuracy parameter requirements are 80 to 120% (Yuwono and Indrayanto, 2005). The accuracy of Pb was 84.67-108.50% while Cd gave a 106.00-119.00%. Hence, the accuracy both of Pb and Cd meet the requirements.

5. Precision

Repeatability precision is the closeness of agreement between a

series of measurements from multiple data of the same homogeneous sample under the the same operating condition over a short interval of time (Emawati et al., 2022; ICH, 2005). The system suitability test is a test to ensure that the testing system include instrument, reagents and analyst, is suitable for intended application (Ata et al., 2015).

Table 5 shows the precision of both heavy metals, which has been calculated using equation (4). The method analysis was called precise if the precision parameter is less than 20% (Food and Drug Administration, 2018). Meanwhile, the precision parameter of system suitability test is less than 2% (Ata et al., 2015). Hence, these parameters of each metal meet the requirements.

Table 4. Percentage recovery and precision method of Pb and Cd

Heavy metals	Merck	Added concentration of standard compound (mg/l)	Total concentration (mg/l)	Percentage Recovery (%)	Average (%)	SD	RSD (%)
Pb	X	6	6.26	104.33	103.03	3.50	3.39
			6.06	101.00			
			6.51	108.50			
			6.10	101.67			
			5.98	99.67			
	Y	3	3.18	106.00	101.33	9.40	9.28
			3.13	104.33			
			3.13	104.33			

Heavy metals	Merck	Added concentration of standard compound (mg/l)	Total concentration (mg/l)	Percentage Recovery (%)	Average (%)	SD	RSD (%)
Cd	Z	6	3.22	107.33	99.80	4.87	4.88
			2.54	84.67			
			5.87	97.83			
			5.87	97.83			
			6.51	108.50			
			5.85	97.50			
	5.84	97.33					
	X	1	1.12	112.00	110.00	1.58	1.44
			1.08	108.00			
			1.09	109.00			
1.10			110.00				
1.11			111.00				
Y		1	1.19	119.00	118.20	1.10	0.93
			1.19	119.00			
			1.19	119.00			
			1.17	117.00			
			1.17	117.00			
Z		1	1.12	112.00	108.20	2.39	2.21
			1.07	107.00			
	1.09		109.00				
	1.07		107.00				
	1.06		106.00				

Table 5. Precision system suitable test of Pb and Cd

Heavy metals	Merck	Replication	Absorbance	Average	Standar deviation	Relative standard deviation (%)
Pb	X	1	0.2282	0.2289	0.0017	0.72
		2	0.2291			
		3	0.2275			
		4	0.2281			
		5	0.2317			
	Y	1	0.1303	0.1295	0.0008	.64
		2	0.1297			
		3	0.1298			
		4	0.1296			
		5	0.1281			
	Z	1	0.2244	0.2248	0.0004	0.20
		2	0.2242			
		3	0.2251			
		4	0.2252			
		5	0.2249			
Cd	X	1	0.3608	0.3597	0.0014	0.39
		2	0.3578			
		3	0.3606			
		4	0.3607			
		5	0.3586			
	Y	1	0.3803	0.3789	0.0013	0.34
		2	0.3794			
		3	0.3797			

Heavy metals	Merck	Replication	Absorbance	Average	Standar deviation	Relative standard deviation (%)
		4	0.3777			
		5	0.3774			
	Z	1	0.3431	0.3451	0.0015	0.42
		2	0.3457			
		3	0.3464			
		4	0.3462			
		5	0.3440			

CONCLUSION

Atomic Absorption Spectroscopy (AAS) can be used to analyze lead (Pb) and cadmium (Cd) in canned sardines. The validation parameters such as selectivity, linearity, limit of detection, limit of quantification, accuracy, and precision were meet all the validation requirements. Additional research is needed to quantify both heavy metals in canned sardines using more sensitive instrument, Inductively Coupled Plasma Spectrometer (ICPS).

ACKNOWLEDGEMENT

Lembaga Penelitian dan Pengabdian kepada Masyarakat Universitas Surabaya (LPPM UBAYA) by Penelitian Pemula No. 115/SP-Lit/LPPM-01/FF/XI/2022 funded this research.

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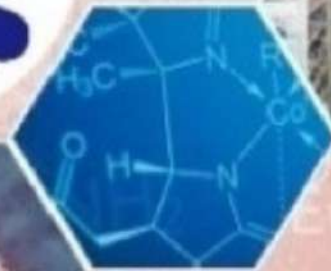
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ISSN : 2502-647X
eISSN : 2503-1902

JURNAL ILMIAH IBNU SINA

Ilmu Farmasi dan Kesehatan



**DITERBITKAN OLEH :
SEKOLAH TINGGI ILMU KESEHATAN ISFI
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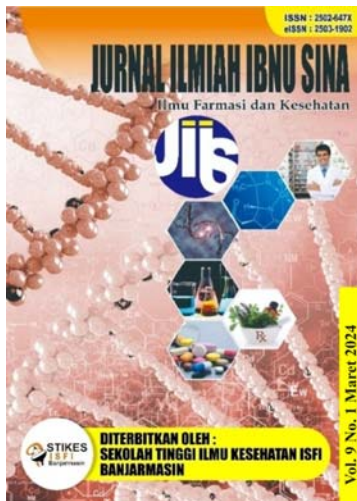
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Vol. 9 No. 1 (2024): JIIS



DOI: <https://doi.org/10.36387/jiis.v9i1>

Published: 2024-03-31

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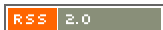
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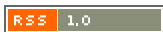
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Nomor 85/M/KPT/2020
Peringkat Akreditasi Jurnal Ilmiah Periode 1 Tahun 2020
Nama Jurnal Ilmiah

Jurnal Ilmiah Ibnu Sina (JIIS): Ilmu Farmasi dan Kesehatan

E-ISSN: 25031902

Penerbit: Akademi Farmasi ISFI Banjarmasin

Ditetapkan sebagai Jurnal Ilmiah

TERAKREDITASI PERINGKAT 3

Akreditasi Berlaku selama 5 (lima) Tahun, yaitu
Volume 4 Nomor 2 Tahun 2019 sampai Volume 9 Nomor 1 Tahun 2024

Jakarta, 01 April 2020

Menteri Riset dan Teknologi/
Kepala Badan Riset dan Inovasi Nasional
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p-ISSN: 2502-647X; e-ISSN: 2503-1902

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