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Article

Analysis Of Calcium Levels in Wet and Dried Anchovies at Traditional Markets in Bekasi City

Fadliyah Rani Safitri, Elfira Maya Sari*, Siti Nurfajriah

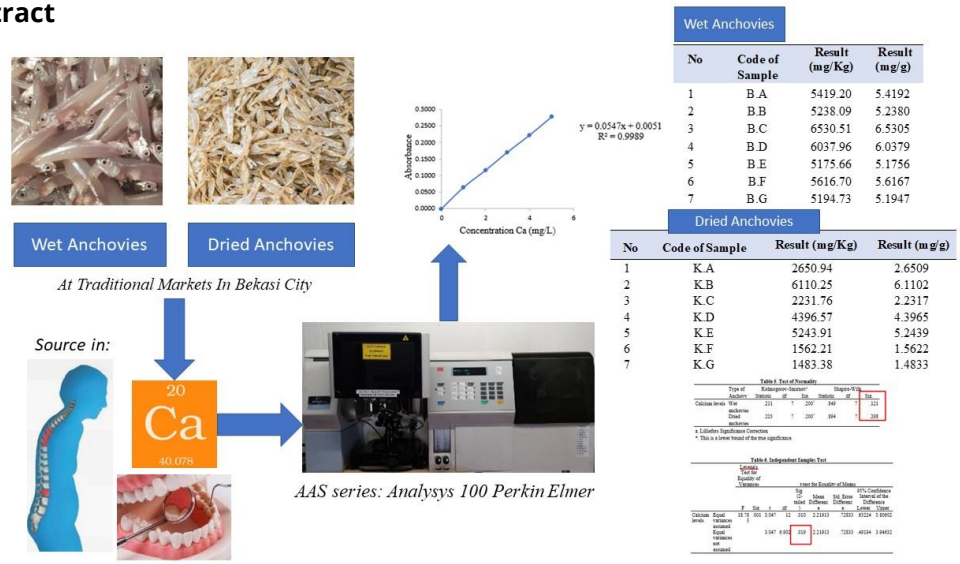
Department of Medical Laboratory Technology, STIKes Mitra Keluarga, Bekasi 17115, West Java, Indonesia

Abstract

Anchovy is a food that is widely consumed by the people of Indonesia because it has high quality and affordable prices for the middle and lower economic class. Anchovy as a source of calcium is very good for preventing osteoporosis, the formation and maintenance of bones and teeth, smooth muscle function, and the nervous system. The purpose of this study was to determine the calcium levels of wet anchovies and dried anchovies sold in traditional markets in the city of Bekasi. Determination of calcium content of wet anchovies and dried anchovies can be determined using atomic absorption spectrophotometry (AAS). Data were analyzed using descriptive analysis. The results of the analysis of the highest calcium levels in wet anchovies were 653.05 mg/100g and the lowest was 517.56 mg/100g. The highest calcium level in dried anchovies is 611.02 mg/100g and the lowest is 148.33 mg/100g. These results indicate that there are differences in calcium levels in wet anchovies and dried anchovies. This occurs due to the presence of water content and salt content in anchovy which affects the high and low calcium results in the analysis.

Keywords: *wet anchovies, dried anchovies, calcium, atomic absorption spectrophotometry (AAS)*

Graphical Abstract



* Corresponding author
Email addresses: elfira.mayasari.0808@gmail.com

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Introduction

Indonesia is a country that has vast seas. One of the marine potentials is anchovies. Anchovies are widely consumed by people from the middle to lower economic classes because they are affordable and easy to get on the market. Anchovy is a type of food that has high quality because all parts of its body can be consumed [1].

Anchovy bones contain lots of protein and calcium. The source of calcium from anchovy bones is very good for preventing osteoporosis. Because the bones in anchovies are relatively small and soft compared to other types of fish [2]. The types of anchovies that are usually traded are fine anchovies, jengki anchovies, rice anchovies. The nutritional content in 100 grams of fresh anchovies contains 77 kcal of energy, 16 grams of protein, 1.0 grams of fat, 500 mg calcium, 500 mg phosphorus, 1.0 mg iron, 47 vitamin A and 0.1 mg vitamin B. The nutritional content of fresh and dried anchovies is higher than other fish [3].

Calcium has many benefits for the body, namely for the formation and maintenance of bones and teeth, preventing osteoporosis, storing glycogen, improving muscle, brain and nervous system function. The body needs the greatest amount of calcium during growth and is still needed further into adulthood. The daily requirement for calcium is 800 mg for adults over 25 years and 1000 mg after the age of 50 years [4].

Based on the description above, research is needed on the analysis of calcium levels in wet anchovies and dried anchovies sold in traditional markets in the city of Bekasi. This research needs to be carried out because researchers see that there are still many people who do not know that the calcium levels contained in anchovies are higher than other types of fish. The method used is atomic absorption spectrophotometry because atomic absorption spectrophotometry is very appropriate for the analysis of metal elements at low concentrations with quite high accuracy, namely 0.002 ppm compared to the complexometric method [5].

Experimental Section

Materials

The materials used in this research were samples of wet anchovies and dried anchovies, standard Calcium solution ($CaCO_3$), 3 M HCl, distilled water, concentrated HNO_3 solution, $HClO_4$ 70-72% solution [6].

Instrumentation

The tools used in this research were analytical balance, Erlenmeyer 250 mL, bulb, calibrated 10 mL and 25 mL volumetric pipettes, glass stirring rod, electric heater, 100 mL measuring cup, calibrated 100 mL measuring flask, glass funnel, Whatman filter paper No. 40, dropper pipette, test sample bottle, air compressor, acetylene gas, Atomic Absorption Spectrophotometer (AAS) series: Analysys 100 Perkin Elmer.

Procedure

Preparation of samples

Samples of dried anchovies and wet anchovies were ground with a blender then weighed 2.5 grams using an analytical balance and then placed in a porcelain cup. Then it is digested by adding 25 ml of concentrated HNO_3 and boiling on a hot plate slowly for 30-45 minutes to remove all compounds that are easily oxidized. The sample solution was cooled and 10 ml of 70-72% $HClO_4$ solution was added, boiled slowly until the solution was clear, cooled and added 50 ml of H_2O_2, heated until all the NO_2 gas came out. The clear sample was diluted and filtered with Whatman paper into a 100 ml volumetric flask, diluted to the mark, and homogenized [7].

Preparation of calcium stock solution

Preparation of calcium stock solution with a concentration of 1000 ppm (2.497 g in 1000 mL) and 100 ppm based on the stock solution. The standard solution of 100 ppm is made into variations in concentration ranging from 0, 1, 2, 4 and 5 ppm.

Background setting

There are two causes of background absorption, namely scattering of the cathode hallow light by gas particles in the flame, and absorption of

molecules that are thermally unstable in the cathode hallow light by molecules in the flame.

Determination of standard curves

The blank solution was inserted into the AAS apparatus through the capillary tube, then the standard solution was added, and the absorbance was measured at a wavelength of 422.7 nm. A standard curve is created by plotting the absorption value against the solution concentration (ppm). Then the examination results will appear on the computer.

Testing of Sample

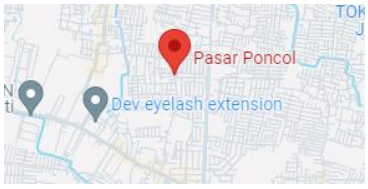
The sample is inserted into an atomic absorption spectrophotometry instrument through a capillary tube and then the sample is measured at a wavelength of 422.7nm.

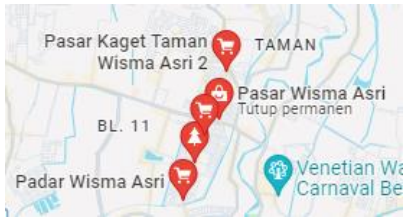
Results and Discussions

Research on the analysis of calcium levels in wet anchovies and dried anchovies sold in traditional markets in the Bekasi city area has been carried out at the Calibration and Certification Testing Services Laboratory of the Bogor Agricultural Institute. The samples analyzed were wet anchovies and dried anchovies which were sold in several traditional markets in the city of Bekasi. Anchovy samples were taken from 8 markets, namely the Poncol Baru market, Kranji market, Bekasi Baru market, Taman Wisma Asri market, Mango market, Pintu Air market, Pulo Galaxy market and Pondok Gede market like the Figure 1 below. The sampling location can be seen in the following Table 1 from 8 markets. The distance between samples is separated from the distance between sub-districts spread across Bekasi city.

Figure 1. (a) samples of wet anchovies; (b) samples of dried anchovies.

Table 1. Market location for sampling of wet and dried anchovies.

No.	Market	Location	Code of Sample	Specific of sample
1.	New Poncol	 Corridor Baru Street of Nusa Indah No.014, RT.006/RW.018, Middle Kaliabang, Subdistrict North Bekasi, Bekasi City, West Java, 17125	K.A	dried anchovies
			B.A	wet anchovies

No.	Market	Location	Code of Sample	Specific of sample
2.	Kranji Bekasi	 RT 001/RW.013, Kranji, Subdistrict West Bekasi, Bekasi City, West Java	K.B	dried anchovies
			B.B	wet anchovies
3.	Taman Wisma Asri	 RT.005/RW.005, Pucung Bay, Subdistrict North Bekasi, Bekasi City, West Java 17121	K.C	dried anchovies
4.	Mangga	 Street of Mangga Raya No.61, RT.004/RW.007, Kranji, Subdistrict West Bekasi, Bekasi City, West Java 17135	B.C	wet anchovies
5.	New Bekasi	 Street of Insinyur H. Juanda No.47, RT.006/RW.001, Duren Jaya, Subdistrict East Bekasi, Bekasi City, West Java, 17111	K.D	dried anchovies
			B.D	wet anchovies

No.	Market	Location	Code of Sample	Specific of sample
6.	Pintu Air	 Street of Pintu Air, RT.002/RW.001, Marga Mulya, Subdistrict North Bekasi, Bekasi City, West Java, 17142	K.F	dried anchovies
			B.F	wet anchovies
7.	Pulo Galaxy	 Street of Pulo Sirih Bar. Raya, RT.006/RW.003, Jaka Setia, Subdistrict South Bekasi, Bekasi City, West Java, 17147	K.E	dried anchovies
			B.E	wet anchovies
8.	Pondok Gede	 Street of Jati Makmur No.15 Jatiwaringin Pondok gede, RT No.003, RT.001/RW.002, Jatirahayu, Subdistrict Jasmine cottage, Bekasi City, West Java, 17411	K.G	dried anchovies
			B.G	wet anchovies

Sampling was carried out at 14 different distributors selling anchovies in several traditional markets in the Bekasi City area. The sample consisted of 7 wet and 7 dried anchovies with predetermined inclusion and exclusion criteria. The samples were characterized by fresh wet anchovies and complete body structure and dried anchovies which had intact bodies, brownish yellow and dry.

Determining the quality of wet and dry anchovies

Good sample selection can determine good sample results. The characteristics of the selected wet and dry anchovies were tested based on the criteria of aroma, size and texture. The content of anchovies is influenced by the quality of the raw materials, quantity and purity

of salt during drying [8]. The results obtained can be seen in Table 2.

Based on Table 2, determining the quality characteristics of wet anchovies and dry anchovies can be seen from smell, size and texture. On average, dry and wet anchovies have a distinctive smell and some are odorless, namely codes K.B and K.G. The size of the wet and dry anchovies obtained ranged from 3 – 5 cm. The texture of wet anchovies is soft and the texture of dry anchovies is rather hard. This is in accordance with research by Imelda, 2018 which states that the quality of dried anchovies has better characteristics [9]. Good anchovies also have the appearance of being intact, clean and fresh [10].

Anchovy samples were taken to the Bogor Agricultural Institute's Calibration and Certification Testing Services Laboratory. The preparation stage carried out was using wet digestion and the method used was the Atomic Absorption Spectrophotometer (AAS). The wet digestion method uses an HNO_3 solution which functions as an oxidizer which can reduce the digestion temperature. After digestion, a lanthanum oxide solution is added to the sample solution to prevent disturbances that can affect the number or number of atoms that occur in the flame. This is caused by the formation of compounds that are refractory (difficult to be broken down in a flame), namely phosphate, potassium and sodium ions, which will reduce the calcium content [11].

Table 2. Determination of anchovy characteristics test.

Code of Sample	Specific of sample	Characteristic test
K.A	dried anchovies	Smell: typical Size: 4 cm Texture: little hard
B.A	wet anchovies	Smell: typical Size: 5 cm Texture: soft
K.B	dried anchovies	Smell: No smell Size: 3 cm Texture: little hard
B.B	wet anchovies	Smell: typical Size: 4 cm Texture: soft
K.C	dried anchovies	Smell: Khas Size: 3 cm Texture: hard
B.C	wet anchovies	Smell: typical Size: 5 cm Texture: soft
K.D	dried anchovies	Smell: typical Size: 4 cm Texture: little hard
B.D	wet anchovies	Smell: typical Size: 4 cm Texture: soft
K.F	dried anchovies	Smell: typical Size: 4 cm Texture: little hard
B.F	wet anchovies	Smell: typical Size: 4 cm Texture: soft

Code of Sample	Specific of sample	Characteristic test
K.E	dried anchovies	Smell: typical Size: 5 cm Texture: little hard
B.E	wet anchovies	Smell: typical Size: 3 cm Texture: soft
K.G	dried anchovies	Smell: no smell Size: 4 cm Texture: little hard
B.G	wet anchovies	Smell: typical Size: 5 cm Texture: soft

Determination of the calibration curve

The calibration curve is a statistical method used to determine the comparison of the effect of analyte levels with the response of the tool (instrument). The calibration curve consists of a standard calcium carbonate ($CaCO_3$) solution without the addition of wet anchovies samples and dried anchovies samples in the standard solution. The Ca calibration curve was made with 6 variations concentrations namely 0 ppm, 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm obtained from dilution of standard solutions. After making a series of standard solutions, the absorbance of each concentration was measured at a wavelength of 422.7 nm. This is in accordance with the theoretical concept that Calcium can be measured at a wavelength of 422.51 nm using the acetylene air flame AAS method. [12]. Based on the data in Table 3, the following linear regression equation can be obtained.

Based on measurements between the absorbance value and calcium concentration, the regression equation y = 0.0547x + 0.0051 was obtained with a correlation coefficient (R^2) of 0.9989. An R^2 value close to one indicates that there is a close correlation and good linearity between calcium concentration and absorbance. This is in accordance with the Lambert-Beer law, absorbance is directly proportional to the length of the flame through which the light passes and the concentration of atoms. The Y value is the absorbance and the X value is the concentration. So the Y value will be substituted for the absorbance to become the calcium concentration. The value of a is the slope and the value of b is the intercept. Absorbance and concentration are directly proportional, that is, the greater the absorbance, the greater the concentration [13].

Tabel 3. Results of calibration curve measurements.

Concentration of Ca (mg/L)	Absorbance
0	0.0000
1	0.0644
2	0.1162
3	0.1709
4	0.2222
5	0.2771

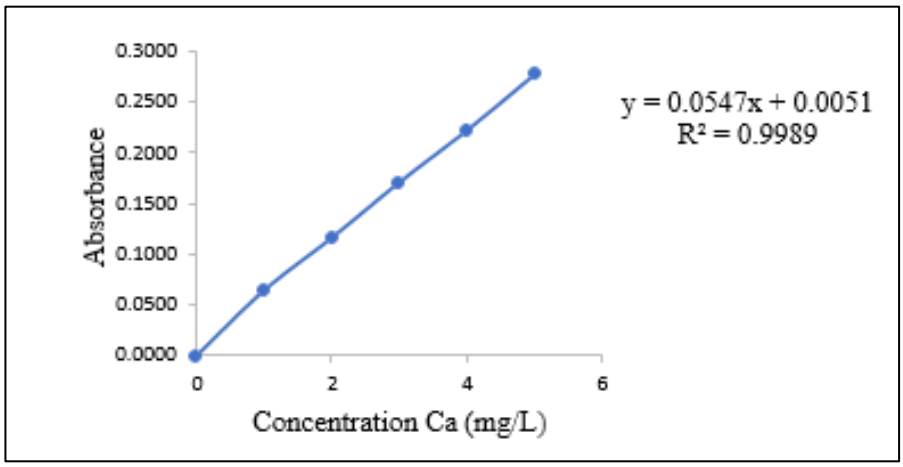


Figure 1. Calibration curve of calcium.

The R^2 value shows that there is a linear correlation between concentration and absorbance with other points on the line whose gradient is positive because this value is in the range $-1 \leq R^2 \leq 1$. A good R^2 value is in the range $0.9 \leq R^2 \leq 1$. Apart from that, by getting an R^2 result of 0.9989, this is a very strong result [14].

Analysis of calcium levels using atomic absorption spectrophotometry

Analysis of calcium levels in wet and dried anchovies was carried out using atomic absorption spectrophotometry. The calcium concentration in the sample is determined based on the regression line equation of the calibration curve of the standard calcium solution.

The sample solution was measured using an atomic absorption spectrophotometer at a wavelength of 422.7 nm by being put into a flame as an aerosol, namely a mist in the form of fine water droplets. The sample solution will approach the flame, the solvent evaporates and produces fine spots in the form of particles, then this substance will dissociate to produce metal atoms. Then it is reacted with a cathode lamp in the form of an SR lamp so that the atoms in their ground state require a large amount of energy and will absorb energy from the light source, so that they can be read by the detector [15].

From the results of tables 4 and 5, it shows that wet anchovies and dried anchovies have different calcium mineral contents. The highest calcium levels in wet anchovies were in sample B.C at 653.05 mg/100g and the lowest in sample B.E 517.56 mg/100g. The highest calcium level in dried anchovies was in sample K.B 611.02 mg/100g and the lowest in sample K.G 148.33 mg/100g.

Table 4. Results of analysis of calcium levels in wet anchovies.

No	Code of Sample	Result (mg/Kg)	Result (mg/g)
1	B.A	5419.20	5.4192
2	B.B	5238.09	5.2380
3	B.C	6530.51	6.5305
4	B.D	6037.96	6.0379
5	B.E	5175.66	5.1756
6	B.F	5616.70	5.6167
7	B.G	5194.73	5.1947

Table 5. Results of analysis of calcium levels in dried anchovies.

No	Code of Sample	Result (mg/Kg)	Result (mg/g)
1	K.A	2650.94	2.6509
2	K.B	6110.25	6.1102
3	K.C	2231.76	2.2317
4	K.D	4396.57	4.3965
5	K.E	5243.91	5.2439
6	K.F	1562.21	1.5622
7	K.G	1483.38	1.4833

Based on these results, it shows that the calcium content value in wet anchovies is higher than the calcium content in dried anchovies. However, in Khairunnisa's (2018) research, regarding the comparison of calcium levels in dried rice anchovies and wet rice anchovies using the AAS method, the calcium levels in dried rice anchovies were found to be 284.14 mg/100g while the calcium in wet rice anchovies was 223.87 mg/100g.

The difference in calcium levels in dried and wet anchovies can be caused by the water content in the anchovies' bodies. Wet anchovies contain a lot of water which influences the high and low calcium results that are analyzed. Most of the changes in food ingredients occur in the medium of water that is added or comes from the ingredients themselves, because the high and low levels of calcium are influenced by the water content contained in wet anchovies. whereas dried anchovies have undergone a process of reducing water content [13].

The process of drying the water content is by adding salt to make salted anchovies. The addition of salt by producers will affect the water content in the fish's body. The water in the fish's body will undergo an osmosis process due to differences in solvent concentration. The high-water content in fish flesh will be drawn out by the NaCl solution which is more concentrated or has a lower solvent (water). However, the osmosis process that occurs will stop if it reaches the balance point (isotonic). So the amount of NaCl used in the process of making salted anchovies greatly influences the water content of the fish [16].

The process of salting dried anchovies reduces the water content in the anchovies and has an effect on increasing the calcium levels in the anchovies. The NaCl used in the process of making salted fish is not of good quality, you can see from the color that it is yellow or dirty brown, stiff and tastes bitter. The Indonesian National Standard (SNI) requires NaCl levels in salted fish not to exceed 20% because high NaCl levels can trigger hypertension if consumed in excess [16].

The calcium consumption limit should not exceed 2500 mg per day. Because if there is excess calcium it can cause kidney stones and can cause constipation (difficulty defecating). However, calcium deficiency can also cause problems with bone growth, such as brittle bones or what is commonly called osteoporosis. Therefore, dried anchovies and wet anchovies can be alternative food sources that can meet the calcium needs of the body [17].

Statistical results for wet and dry anchovy samples

Analysis of calcium levels in wet and dried Analysis of data results on wet anchovies and dried anchovies was carried out using the SPSS 2019 application. The SPSS application helps in managing research data results to make them more systematic and quantitative. Data was entered in table and numerical [18]. The first test carried out was the normality test. The distribution of SPSS data results shows that from 7 data each data is normally distributed because

according to the Shapiro Wilk test, the data is less than 50 samples. The p-value for each sample can be seen in table 6, namely 0.121 and 0.298 > from α 0.05. This data can be subjected to an ANOVA test [19].

After carrying out the Normality test, the next test is the T-test with independent samples because it has 2 different variants but are not related to each other and the variables that are linked are numbers. [20]. The mean values for wet and dry anchovies respectively are 5.6018 and 3.3827. The independent sample T-test results obtained can be seen in table 7.

Based on table 7 above, a significant value of 0.019 (sig. ≤ 0.05) was obtained. These results show that there is a real difference between wet anchovies and dry anchovies and the mean value resulting from the differences between the two groups is smaller than the calculated T-test mean. [19].

Conclusions

The calcium levels obtained in wet anchovies and dried anchovies sold in several traditional markets in the city of Bekasi gave the highest calcium levels in wet anchovies, namely in the B.C sample of 653.05 mg/100g and the lowest in the B.E sample of 517.56. mg/100g. The highest calcium level in dried anchovies was in sample K.B 611.02 mg/100g and the lowest in sample K.G 148.33 mg/100g.

Table 6. Test of normality

	Type of Anchovy	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Calcium levels	Wet anchovies	.211	7	.200*	.849	7	.121
	Dried anchovies	.225	7	.200*	.894	7	.298

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

Table 7. Independent samples test.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference Lower	Upper
Calcium levels	Equal variances assumed	18.783	.001	3.047	12	.010	2.21913	.72833	.63224	3.80602
	Equal variances not assumed			3.047	6.902	.019	2.21913	.72833	.49194	3.94632

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