

# Analysis of Calcium, Iron, Ash, Fat, and Water Content in Fresh and Processed Cow's Milk Using Atomic Absorption Spectrophotometry Methods

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## Abstract

Milk is a source of nutrition for the body's growth, containing calcium and iron minerals. This study analyzes the optimal calcium, iron, ash, water, and fat levels in fresh and processed cow's milk. This study used five samples: pure milk (S<sub>1</sub>), pasteurized pure milk (S<sub>2</sub>), pasteurized milk with sugar (S<sub>3</sub>), pasteurized milk with paste coloring and citric acid (S<sub>4</sub>), and pasteurized milk with powder coloring (S<sub>5</sub>). The results showed that the calcium content of milk 4 (S<sub>4</sub>) treated with pasteurized milk added with sugar and paste coloring and citric acid had a higher calcium content than the other four samples by 98.61 mg/kg, at levels of milk iron 5 (S<sub>5</sub>) treated with pasteurized milk added with sugar and powdered dyes had a higher iron content than the other four samples by 73.81 mg/kg. The ash content of pure milk was higher at 0.96%. The fat content of pasteurized milk, the taste of coloring paste and powder, was higher than the other three samples at 2.80%. The water content of pasteurized added sugar milk was higher than the other four samples at 88.58%. Pure milk had the most optimal calcium (Ca) and iron (Fe) levels from the other four samples.

**Keywords:** ash content; cow's milk; calcium; fat content; iron; moisture content

## Data of article

Received : 25 Jun 2023

Reviewed : 12 Jul 2023

Accepted : 18 Jul 2023

## DOI

10.18196/jfaps.v4i1.18938

## Type of article:

Research

## INTRODUCTION

According to the Central Statistics Agency (BPS), in 2019, Indonesia's milk consumption was 16.23 liters/capita/year. This amount has increased from the previous year to 0.20 liters/capita/year. Although said to increase, the amount of milk consumption by Indonesian people is still relatively low compared to other Southeast Asian countries because people still ignore the fact that milk is a top priority for children and toddlers to fulfill a source of nutrition for growth.<sup>1</sup> There is a close relationship between nutritional

status and food consumption. The level of optimal nutritional status will be achieved if optimal nutritional needs are met. Nutritional needs come from daily food, such as carbohydrates, proteins, fats, vitamins, minerals, and milk, necessary to support health.<sup>2</sup> However, in everyday life, not all Indonesians drink unprocessed milk because they are not used to smelling fresh or raw milk. Processed milk is in great demand by the general public because the taste and smell are more acceptable to the public.<sup>3</sup> In addition to having a high nutritional value, cow's milk is beneficial for health, preventing heart disease and

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vascular disorders, goiter, lightening the work of the cerebrum, good for anemia sufferers, maintaining healthy skin, relaxing and calm,<sup>4</sup> helps grow teeth and bones, maintain health, accelerates healing, sharpens vision, as a substance neutralizer, prevents osteoporosis,<sup>5</sup> as energy reserves, reduces the risk of type 2 diabetes, inhibits the growth of colon cancer, and reduces the risk of breast cancer in women.<sup>3</sup>

Fresh cow's milk is a liquid that comes from the milking of dairy cows, the natural content of which is not reduced or added to anything and has not received any treatment. Milk is a good food source because it contains many nutrients. Fresh milk is perishable and cannot be stored long if improperly handled. Fresh milk taken directly from the farm still contains microorganisms. Therefore, fresh milk must be processed through a heating process (pasteurization) first. The heating aims to avoid disease transmission and milk spoilage.<sup>6</sup> Milk is a source of energy because it contains lots of lactose and fat. It is also called a source of building blocks because it contains lots of protein, minerals, and vitamins. Chemically, normal milk has a composition of 87.20% water, 3.70% fat, 3.50% protein, 4.90% lactose, and 0.07% minerals.<sup>7</sup> Milk is a food ingredient with high nutritional value, and high levels of one of the nutrients found in milk are minerals.<sup>8</sup>

Calcium is necessary for tooth formation. Lack of calcium during teeth formation leads to tooth decay susceptibility.<sup>9</sup> Iron in the body plays an important role in many biochemical reactions, including the production of red blood cells. Iron acts as a carrier of oxygen, inhaling oxygen to tissues and tissues or cells.<sup>10</sup> When the body needs iron (Fe) in large quantities, for example, in children who are still growing

(toddlers), menstruating women, and pregnant women, savings amounts are usually low.<sup>11</sup>

There are various methods for analyzing Ca and Fe. These methods include Gravimetry, Compleximetry, and Atomic Absorption Spectrophotometry (AAS). This study used the Atomic Absorption Spectrophotometry (AAS) method. SSA has the advantage that the analysis is sensitive, thorough, and fast. The process is relatively simple, and it is unnecessary to separate metal elements in its implementation.<sup>12</sup> This study analyzes the optimal calcium, iron, ash, water, and fat levels in fresh and processed cow's milk.

## METHOD

### Tools

The tools used in this study included Atomic Absorption Spectrophotometer (AAS) (Thermo Scientific iCE 3300), Silica disk, Karl Fischer, Desiccator, Furnace, Drying oven, Analytical balance scale, Babcock bottle, Erlenmeyer 250 mL, 100 mL measuring cup, 100 mL volumetric flask, Bulp, Pipette volume of 10 mL and 25 mL, Glass stir bar, Glass funnel, Dropper pipette, Hot plate, and Whatman filter paper No 40.

### Materials

The material used in this research was fresh cow's milk, Aquadest, Nitric acid (HNO<sub>3</sub>) Merck, Perchloric acid (HClO<sub>4</sub> 70-72%) Merck, Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) Merck, and Ethyne (C<sub>2</sub>H<sub>2</sub>).

### Procedure

#### *Wet Destruction Process*

Weigh 2-3 grams of each milk and put it in the digestion tube. Then add 25 mL of concentrated HNO<sub>3</sub> and boil slowly for 30-45 minutes, then cool the solution and add 10 mL of 70-72% HClO<sub>4</sub>. Continue heating

with the hot plate until the solution looks colorless. Cool the sample solution, add 50 mL H<sub>2</sub>O, and boil until all the NO<sub>2</sub> gas comes out. Next, cool the sample solution again, filter it using Whatman paper No. 40 into a 100 mL volumetric flask, dilute to mark, and then homogenize.

#### ***Making a Calcium Calibration Curve***

Prepare Ca concentration series solutions by diluting 100 ppm standard solution taken as much as 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL, then put into a 100 mL volumetric flask and diluted with distilled water up to the mark then homogenized. After that, a serial solution of Ca concentration was obtained with concentrations of 0 ppm, 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm and then measured at a wavelength of 422.7 nm.

#### ***Preparation of Iron Calibration Curves***

Prepare Fe concentration series solutions by diluting 100 ppm standard solution taken as much as 0.4 mL, 0.8 mL, 1.2 mL, and 1.6 mL. Then, put into a 100 mL volumetric flask and diluted with distilled water up to the mark mark. After that, a serial solution of Fe concentration was obtained with concentrations of 0 ppm, 0.4 ppm, 0.8 ppm, 1.2 ppm, and 1.6 ppm and then measured at a wavelength of 248.3 nm.

#### ***Determination of Ash Content***

Accurately weigh 2 – 5 g of the sample into a porcelain (or platinum) cup of known weight for the vaporized liquid sample in a water bath until dry. Charcoal over the burner flame then ashes in an electric furnace at a maximum temperature of 550°C until complete ashing. Cool in a desiccator, then weigh until the weight remains.

#### ***Determination of Fat Content***

Add 17.6 mL of milk, then put it into the Babcock bottle, then mix 17.5 mL of milk and sulfuric acid, shake until

homogeneous, and centrifuge (rpm 700 – 1000, T = 60°C, for 5 minutes), then add hot distilled water T = 60°C to the lowest scale and centrifuged for 2 minutes, T = 60°C, after that add hot distilled water T = 60°C to below the upper limit and centrifuged for 1 minute, T = 60°C.

#### ***Determination of Water Content***

A total of 0.5 grams of sample was weighed and put into the Karl Fischer tool, and the results were obtained.

#### ***Determination of Calcium and Iron Levels in Samples***

The digested solution was analyzed to measure the Fe and Ca levels in fresh and processed cow's milk, and the absorbance was measured using a conditioned and regulated atomic absorption spectrophotometer, where the Fe content was determined at a wavelength of 248.3 nm. In comparison, the Ca content was carried out at a wavelength of 422.7 nm with an air-acetylene flame. The absorbance value obtained must be within the range of the iron standard solution calibration curve. The concentration of iron in the sample was determined based on the regression line equation of the calibration curve.

## **RESULTS AND DISCUSSION**

#### ***Linearity Test of Standard Solution Ca and Fe***

The main solution for Fe was made from Fe (NO<sub>3</sub>)<sub>2</sub> 1000 ppm, while Ca was made from CaCO<sub>3</sub> 1000 ppm, then diluted to 100 ppm. Then, the 100 ppm standard solution was diluted to 0 ppm, 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm into a 100 mL volumetric flask. Fe was diluted to 0 ppm, 0.4 ppm, 0.8 ppm, 1.2 ppm, and 1.6 ppm in a 100 mL volumetric flask. Absorbance measurement of concentration series solutions using a flame Atomic Absorption Spectrophotometer can be seen in Pictures 1 and 2.

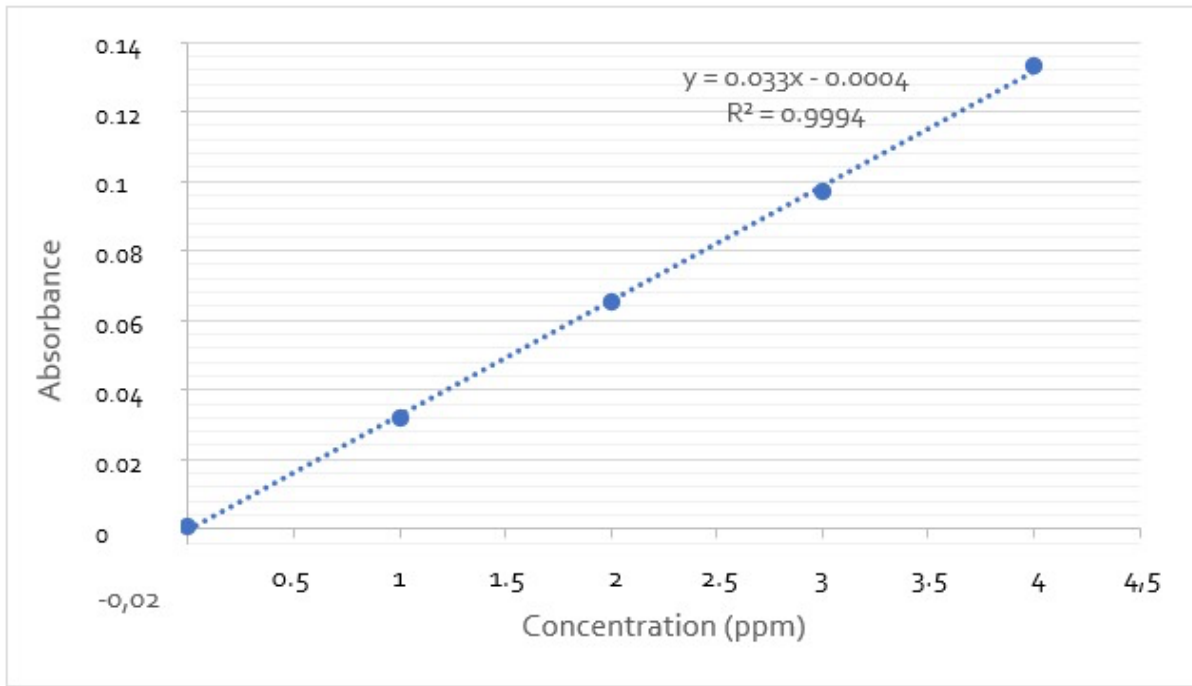


Figure 1. Graph of Ca Metal Calibration Curve

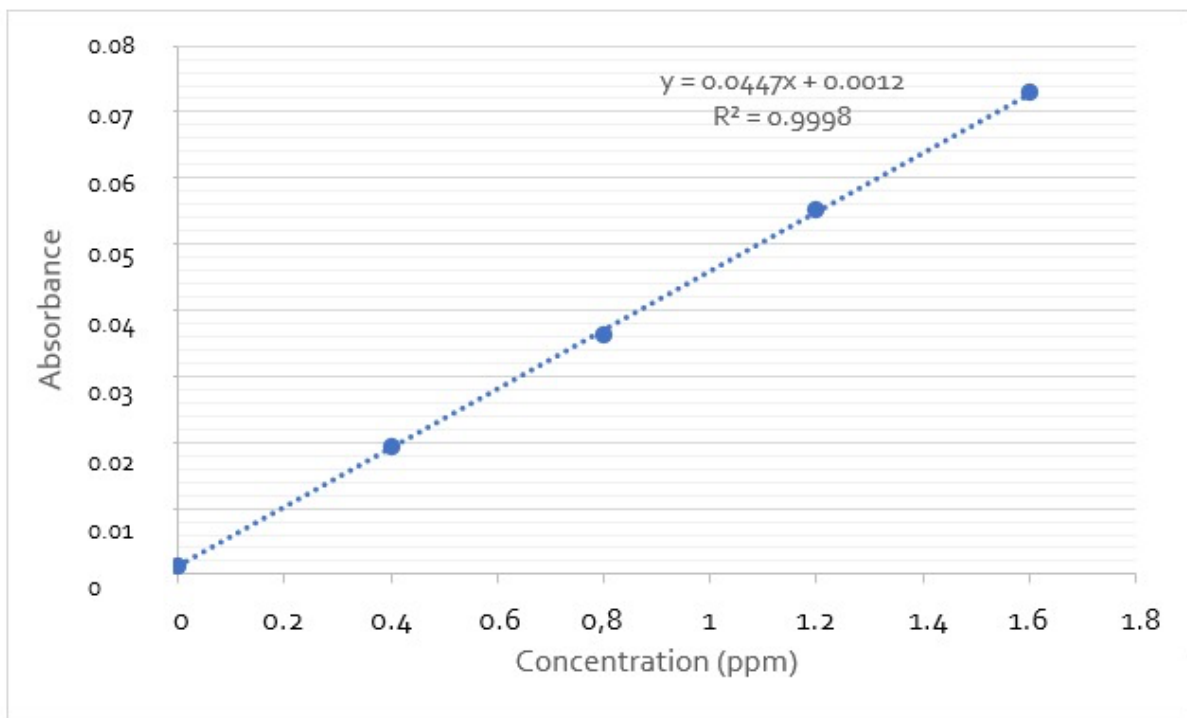


Figure 2. Fe Metal Calibration Curve

Figures 1 and 2 show the relationship between the serial values of the standard solution concentrations and the absorbance values expressed in a straight graph (linear). The equation  $y = bx + a$  will be obtained using linear regression. The

graph shows that the higher the standard solution concentration value, the higher the absorbance value produced, similar to Lambert Beer's law. So, the equation of the linear regression line for Ca is  $y = 0.0327x - 0.00006$ , while for Fe, the

equation for the linear regression line is  $y = 0.0447x + 0.00122$ . From the linear regression equation obtained from calculating the calibration curve of the Ca and Fe standard solution, the calibration curve correlation coefficient is 0.9998. The correlation value serves as quality control for monitoring, checking, and controlling data from analysis results to ensure testing quality is within statistical control limits. The quality control applied by SNI is the correlation coefficient value

( $r \geq 0.995$ ), and the correlation coefficient value is acceptable if the value ( $r \leq 1$ ).<sup>13</sup> So, the calibration curve of Fe metal already has an ideal linear relationship.

### Test Results for Metal Content of Ca and Fe

Based on the results of Ca metal content in the five milk samples with different treatments tested using an atomic absorption spectrophotometer, can be seen in Table 1.

**Table 1.** Results of Analysis of Ca and Fe Metal Levels in Cow's Milk and Processed Products

Sample Name	Actual Average Ca Levels mg/kg	Actual Average Fe Levels mg/kg
Pure milk	62.76	6.22
Pasteurized milk	91.03	9.07
Pasteurized milk + sugar syrup	76.25	4.12
Pasteurized milk + sugar syrup + paste coloring + citric acid	98.61	11.47
Pasteurized milk + sugar syrup + powder dye	84.11	73.81

The analysis of Ca and Fe metal content in Table 1 used an atomic absorption spectrophotometer. Ca levels with a wavelength of 422.7 nm and Fe with 248.3 nm were obtained at the maximum wavelength to obtain the maximum absorbance value. This research determines the wavelength—maximum for Ca 422.7 nm and Fe 248.3 nm.<sup>14</sup> Sample testing was replicated two times (duplo) experiments for analyzing Ca and Fe levels in samples in this study used five milk samples with different treatments: pure milk, pasteurized milk, pasteurized milk with sugar, pasteurized milk with paste coloring and citric acid, and pasteurized milk with powder coloring.

The five milk samples studied show that the mineral content will increase with more treatment and adding food ingredients to milk. The 2nd milk with pasteurized pure milk and the 4th milk with the pasteurized milk treatment with

added sugar and pasta coloring have a higher calcium and iron content than the other three samples due to the addition of citric acid to the strawberry paste coloring, as sour fruity taste. Citric acid is a tricarboxylic acid in which each molecule contains a carboxyl group and one hydroxyl group attached to a carbon atom. Citric acid is effective as a metal-chelating agent.<sup>15</sup> Excess iron can cause vomiting, diarrhea, increased heart rate, pain in the head, and passing out.<sup>16</sup>

In pasteurized milk, the pasteurization method was carried out with an effect on nutritional content. This study for milk pasteurization used a temperature of 72° for 15 seconds is graded more effective and less damage to nutrient content.<sup>17</sup> However, in the 3rd and 5th, milk levels decreased calcium and iron. In the 3rd milk with pasteurized milk treatment, which added sugar decreased calcium and iron levels because at the time of adding sugar

syrup, the temperature of the sugar syrup was still high, so the heat treatment of milk could cause some changes in the properties of milk determined by the combination of time and temperature used. One of the chemical changes in milk is a change in the content of minerals found in milk.<sup>18</sup> Then, the raw materials are added to the milk, which will be easily oxidized with air, while pure cow's milk is

not. A mixture of additional (natural) raw materials makes milk with added raw materials have a higher iron content than natural milk that is not mixed with anything.<sup>19</sup> Iron contained in food will be damaged in most processing processes because it is sensitive to pH, light, or heat.<sup>20</sup>

**Table 2.** Results of analysis of ash, fat, and water content in cow's milk and Processed

Sample Name	Average Ash Content (%)	Average Fat Content (%)	Average Water Content (%)
Pure milk	0.96	1.31	79.80
Pasteurized milk	0.66	1.28	69.55
Pasteurized milk + sugar syrup	0.67	1.28	88.58
Pasteurized milk + sugar syrup + paste coloring + citric acid	0.70	2.80	77.60
Pasteurized milk + sugar syrup + powder dye	0.75	2.80	81.2

The analysis of ash, fat, and water content in Table 2 proves that pure milk has a higher ash content than the other four samples. In comparison, the pasteurized milk has a stronger flavor than the other three samples, and the water content of pasteurized milk with added sugar was higher than the other four samples because it is influenced by handling, storage, and processing, which often causes changes in nutritional value.<sup>11</sup>

## CONCLUSION

In the five samples of fresh cow's milk with different treatments, namely pure milk, pasteurized milk, pasteurized milk added with sugar, pasteurized milk colored with paste coloring, and pasteurized milk given powdered coloring, pure milk had levels of calcium, iron, ash, optimum fat, and water.

## ACKNOWLEDGMENTS

We would like to thank the Faculty of Pharmacy, Universitas Muhammadiyah A.R Fachruddin Tangerang, for providing support and the LPPM for completing the journal.

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