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PHOSPHORUS ANALYSIS IN MEAT USING UV-VIS SPECTROPHOTOMETRY WITH $SnCl_2$ AND HYDRAZINE SULFATE REDUCTION

Yussi Pratiwi*, Rizky Amelia, and Aulia Natasya

Chemistry Program Study, Faculty of Mathematics and Natural Science, Universitas Negeri Jakarta, Jakarta, Indonesia

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**Corresponding Author*
Email:yussipratiwi@unj.ac.id

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ABSTRACT

This study aimed to optimize phosphorus analysis in meat using a molybdenum blue reaction involving $SnCl_2$ and hydrazine sulfate as reducing agents to establish the most effective conditions for phosphorus detection. Meat, an essential source of nutrients like phosphorus, plays a vital role in human health, particularly bone and tooth strength. However, overconsumption of phosphorus can lead to health issues such as hyperphosphatemia, making regular monitoring of phosphorus levels in food necessary. The experiment used $SnCl_2$ and hydrazine sulfate under varying acidic conditions to produce a stable blue complex indicative of phosphorus presence. The complex exhibited maximum absorbance in the 689–729 nm wavelength range. Validation of the method showed high linearity with R^2 values of 0.9983 and 0.9984 for $SnCl_2$ and hydrazine sulfate. The molar absorptivity for $SnCl_2$ was 2.093 x 105 L mol^{-1} cm^{-1} and 7.92 x 104 L mol^{-1} cm^{-1} for hydrazine sulfate. Detection and quantification limits were established, and the %RSD values in each sample ranged from 1.08% to 1.93%, conforming to standard requirements. Upon analysis of meat samples, including duck, chicken, and beef, the phosphorus levels did not meet the Indonesian Nutritionist Association (PERSAGI) standards. This result emphasizes the need for regular phosphorus analysis in meat products to prevent health risks associated with excessive phosphorus intake, such as hyperphosphatemia.

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INTRODUCTION

Nutritious food constitutes a fundamental human need, with phosphorus playing a critical role in bone and tooth formation. The human body requires a balanced intake of carbohydrates, proteins, fats, vitamins, minerals, and water. Animal meat, a popular choice among various societal segments, is renowned for its delectable taste and rich nutritional profile. For instance, 100 grams of beef contains approximately 22 grams of fat, 17.50 grams of protein, 10 mg of calcium, and 150 mg of phosphorus, offering substantial nutritional benefits [1].

Meat, the soft tissue of animals excluding the skin and attached to bones, is a primary diet component in many cultures, including Indonesia. The content consumption is titularly prevalent due to its comprehensive nutritional content and health benefits. Meat provides essential nutrients such as calories, protein, iron, and the Vitamin B complex. Additionally, it is a

significant source of macro minerals like calcium (Ca), phosphorus (P), and potassium (K), which are crucial for various bodily functions [2]. Meat satisfies dietary preferences and contributes significantly to fulfilling the human body's nutritional requirements positions; fats, vitamins, and minerals, especially phosphorus, underscore its importance in a balanced diet. The comprehensive nutritional profile of meat makes it an essential component of a healthy diet, contributing to overall wellness and physical development.

The composition of feed ingredients influences the phosphorus content in animal meat. Adequate mineral intake is essential for optimizing animal reproduction, as it directly affects the availability of these minerals in the bloodstream. Inadequate daily mineral intake can impact the physiological functions of animals [3]. Phosphorus, the second most abundant mineral in the body after calcium, is present in lower concentrations in hard tissues than calcium but higher in soft tissues. One of the key roles of phosphorus in the body is forming bones and teeth [4]. Common dietary sources of phosphorus include milk, meat, and nuts [5].

Hyperphosphatemia, caused by excessive phosphorus levels in the body, is a concern, particularly in the context of abnormal phosphate metabolism, a common issue in chronic kidney disease. The kidneys play a crucial role in eliminating excess fluids, and dysfunction in these organs can lead to hyperphosphatemia, where blood phosphate levels become excessively high [6,7]. This condition can precipitate further complications, including kidney damage and increased risks of heart attacks and strokes. The normal blood phosphate levels for individuals aged 18 and above range from 2.4 mg dL^{-1} to 6.5 mg dL^{-1} [8-10].

Given the potential health risks associated with phosphorus, routine and accurate analytical methods are necessary for phosphorus detection to ensure food safety and quality. Most analytical methods for phosphorus determination require the samples to be in or introduced into a solution. The complexity of phosphorus analysis in samples stems from the fact that phosphorus can exist in various inorganic and organic forms. Therefore, developing robust analytical techniques is vital for effective phosphorus monitoring in food products

The analysis of phosphate anions can be conducted using various instruments such as chromatography, electrophoresis, X-ray fluorescence (XRF), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and Nuclear Magnetic Resonance (NMR) [11]. However, these methods often entail expensive operational costs and complex usage [12]. An alternative approach for quantitative phosphorus determination is UV-visible (UV-Vis) spectrophotometry. This technique offers several advantages, including analyzing a wide range of organic and inorganic substances, selectivity, and high accuracy with a relative error of 1% to 3%. Additionally, UV-Vis spectrophotometry allows for rapid and precise analysis and can detect minuscule quantities of substances [13]. The results obtained are immediately recorded digitally, ensuring accuracy.

In this study, spectrophotometry with ammonium molybdate was employed for quantitative analysis. This method is simple,

easy to use, and cost-effective, making it a suitable sample alternative. The determination is based on the reaction of orthophosphate with ammonium molybdate in an acidic medium, followed by reduction with various reducing agents, resulting in a blue molybdenum complex with maximum absorbance at around 800 nm. The intensity of this blue color is directly proportional to the phosphate content in the sample [14].

Phosphorus (P) analysis methods have evolved from qualitative analysis using silver nitrate ($AgNO_3$) and zinc sulfate ($ZnSO_4$) to quantitative techniques involving instrumentation. For accurate phosphorus analysis, metaphosphate and pyrophosphate must be converted to orthophosphate using acid solvents. Ammonium molybdate is a popular method in phosphorus analysis, with high phosphorus content leading to the rapid dissolution of yellow crystal ammonium phosphomolybdate in alkaline conditions, which can be converted to $(NH_4)_3[PMo_{12}O_{40}]$ in acidic environments. Low phosphorus concentrations result in a yellow solution measurable via UV-Vis spectrophotometry when treated with excess reagents. However, this yellow solution's stability can be affected by iron ions and colored organic substances. Therefore, reducing agents like chlorine, ascorbic acid (with or without tartrate), and bismuth form more stable blue molybdenum complexes with stronger light absorption at longer wavelengths of about 600 nm to 800 nm [4] .

The "molybdenum blue method" fundamentally relies on the formation of heteropolyphosphoric acid molybdate, which, upon reduction, yields a blue color whose intensity correlates with the orthophosphate ion quantity in the complex. Over time, various modifications to the analytical procedure have been proposed, with the most frequently used modification involving the addition of hydrazine sulfate and $SnCl_2$ as reducing agents. This produces a phosphoantimonylolybdenum blue complex with maximum absorption at approximately 880 nm [11,15,16]. In this research, the determination of phosphate content in meat was performed using a UV-Vis spectrophotometer. The developed method underwent validation testing, including linearity assessments, Limit of Detection (LOD), Limit of Quantification (LOQ), precision, accuracy, and sensitivity. This validated method can be effectively applied to analyze phosphate content in meat, thereby contributing to food safety assurance.

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METHODS

1. Chemicals and Materials

The chemicals and materials used included ammonium heptamolybdate tetrahydrate, potassium dihydrogen phosphate (KH_2PO_4), sulfuric acid 96% p.a, hydrochloric acid 37% p.a, nitric acid 65% p.a, $SnCl_2.2H_2O$, hydrazine sulfate, H_2O_2 30%, aquabidest, and various meat samples such as duck, chicken, and beef.

2. Instrumentation

The instruments used include KERN ABS 2204 GERMANY Analytical Balance and Shimadzu UV-Visible spectrophotometry UV-1240 UV-Vis. The advantage of the UV-Vis spectrophotometry instrument is that it can analyze many organic and inorganic substances, is selective, and has high accuracy with a relative error of 1–3%. In addition, this analysis can be carried out quickly and precisely and can be

used to determine tiny amounts of substances [13].

3. Optimization Of Hydrazine Sulfate

For the optimization of hydrazine sulfate, several experiments were performed. First, we determined the maximum wavelength by combining 1 mL of phosphate solution with various concentrations (0.3, 0.4, 0.5) ppm, 2 mL of ammonium heptamolybdate tetrahydrate, 2 mL of sulfuric acid, and 1 mL of hydrazine sulfate in a 50 mL volumetric flask and dissolved with distilled water. The absorbance was measured in the 500–800 nm wavelength range against a blank solution.

Second, we observe the effect of variation ammonium heptamolybdate tetrahydrate solution concentration by combining 1 mL of phosphate solution, 2 mL of ammonium heptamolybdate tetrahydrate in various volumes (0.006, 0.007, 0.008, 0.009, 0.010, 0.020, 0.025) M, 2 mL of acid sulfate and 1 mL of 0.0076 M hydrazine sulfate was put into 50 mL volumetric flasks. The solution was then diluted with distilled water, homogenized, and left for 30 minutes. The solution was read with a UV-Vis spectrophotometer at the maximum wavelength.

Third, we observe the effect of hydrazine sulfate optimum concentration as a reducing agent by combining 1 mL of standard phosphate solution 0.9 ppm into a 50 mL volumetric flask and then 2 mL of 0.010 M, ammonium heptamolybdate tetrahydrate, 2 mL of sulfuric acid 0.9 M and 1 mL of hydrazine sulfate solution with various concentrations (0.001; 0.003; 0.005; 0.007; 0.009; 0.01; 0.03; 0.05; 0.1; 0.3; 0.5) M was added and diluted with distilled water. The mixture was homogenized and left for 30 minutes. Placed in a cuvette and scanned with a UV-Vis spectrophotometer at the maximum wavelength.

4. Optimization Of Tin (II) Chloride

First, we determined the maximum wavelength by adding a standard solution of (0.3; 0.4; 0.5) ppm into the volumetric flask and added the reagent solution of ammonium heptamolybdate tetrahydrate, sulfuric acid solution, and tin (II) chloride solution, shaken and allowed for 10 minutes and then the absorption was measured by visible spectrophotometry at 400–800 nm to obtain the maximum wavelength.

Second, we observe the effect of ammonium heptamolybdate tetrahydrate solution concentration by combining a sulfuric acid solution, a standard phosphate solution, and a tin (II) chloride solution in several volumetric flasks. Variations in concentration (0.0008, 0.0016, 0.0024, 0.0032, 0.0040, and 0.0048) M, ammonium heptamolybdate tetrahydrate solution was diluted to the mark with aquabidest and homogenized and then allowed for 10 minutes. Inserted into a cuvette and scanned with a UV-Vis spectrophotometer at the maximum wavelength.

Third, we observed the effect of tin (II) chloride optimum concentration as a reducing agent by combining phosphate standard solution, ammonium heptamolybdate tetrahydrate solution, sulfuric acid solution, tin (II) chloride solution was put into a volumetric flask, then the concentration of $SnCl_2$ solution was added with variations (0.0006; 0.0012; 0.0018; 0.0024 0.0030; 0.0036; 0.0042; 0.0048 and 0.0054) M and diluted to mark limits. The mixture was homogenized and allowed for 10 minutes. Inserted into a cuvette and scanned with a UV-Vis spectrophotometer at the maximum wavelength.

The research utilized several validated instruments, including student learning activity observation sheets, student learning activity questionnaires, pretest-posttest questions, chemistry comics, and teaching modules. These

instruments underwent validation by two panelists, and their feasibility was assessed using the Gregory formula. Additionally, the reliability of the pretest-posttest questions was tested through the KR-20 formula, while item analysis and question difficulty were conducted using SPSS version 26.

5. Sample Preparation

First, we prepare some samples such as beef, chicken, and duck meat. The samples were washed, cut into small pieces, and weighed as much as 10 grams. Then, the sample was agitated at 600 for 12 hours until it was carbon-free and cooled. The ash was put into a 250 mL beaker, and 40 mL HCl and 4 drops of concentrated HNO_3 were added. It was then heated and cooled in a water bath for 30 minutes at 70°C.

Second, we prepared some hydrazine sulfate samples: beef, chicken, and duck meat. Before analysis, the meat samples were chopped, and then 5 grams of meat were demineralized using 7.0 mL HNO_3 (65%) and left for 24 hours in a Kjeldahl flask. After that, it was added to the mixture and heated at 100℃ for 60 minutes, and 2.0 mL H_2O_2 (30%) was heated at 200 °C for 120 minutes, resulting in a clear yellow solution, which was transferred to a 50.0 mL measuring flask and filled to the mark.

6. Sample Analysis Used Hydrazine Sulfate Method with UV-Vis Spectrophotometer

Furthermore, phosphorus analysis of meat samples using UV-Vis Spectrophotometry was carried out by taking 1 mL of demineralized samples, then transferring them into a 50 mL volumetric flask, then adding 2.0 mL of ammonium heptamolybdate tetrahydrate reagent, 2 mL of sulfuric acid and added 1 mL of hydrazine sulfate then the solution was diluted to the mark absorbance of the solution was calculated against the blank reagent at 689 nm.

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RESULTS AND DISCUSSION

1. Optimization Condition Analysis

Phosphate analysis via UV-visible (UV-Vis) spectrophotometry utilizes the reaction between phosphate and ammonium molybdate under acidic conditions. The environmental pH in phosphate determination is maintained in an acidic medium to stabilize the resultant complex's color (absorbance signal). A reducing agent then reduces this complex to form a blue phosphomolybdate compound [17]. The reaction mechanism is represented as follows (1):

PO_4^{3-} (aq) + $12MoO_4^{2-}$ (aq) + $12H^+ \rightarrow [PMo_{12}O_{40}]^{3-} + 12H_2O$ (aq) $\xrightarrow[H+]{Reductor} [PMo_{12}O_{40}]^{6-}$…..**(1)**

Phosphate with ammonium molybdate in an acidic environment will react to form $[PMo_{12}O_{40}]^{3-}$ a form of phosphomolybdate with a Keggin structure. Then, this molybdate heteropoly ion is reduced with $SnCl_2$ or hydrazine sulfate to produce a complex compound $[PMo_{12}O_{40}]^{6-}$ which is a reduced form of phosphomolybdate with a Keggin structure [18].

In this study, orthophosphate formed a yellow complex with molybdate ions, but the yellow complex was not very stable, and iron ions and other organic substances could influence its color. Therefore, a reducing agent was used to form a blue molybdenum complex $[PMo_{12}O_{40}]^{6-}$ with stronger light absorption than the yellow form at a longer wavelength [19].

2. Maximum Wavelength Of The Phosphomolybdate Complex With $SnCl_2$ and Hydrazine Sulphate

The wavelength that gives the maximum absorption is required for spectrophotometric quantitative analysis because the compound's absorption measured at the maximum absorption wavelength will provide good sensitivity and accuracy [20]. UV-Vis spectrophotometry was measured in the 500–800 nm wavelength range. This aims to see whether the different concentrations change the wavelength at the maximum absorption. This study used three different concentrations of phosphate. The phosphate concentration used was (0.3, 0.4, 0.5) ppm. The results of the maximum wavelength for the phosphomolybdate complex compound are presented in Figure 1 and 2.

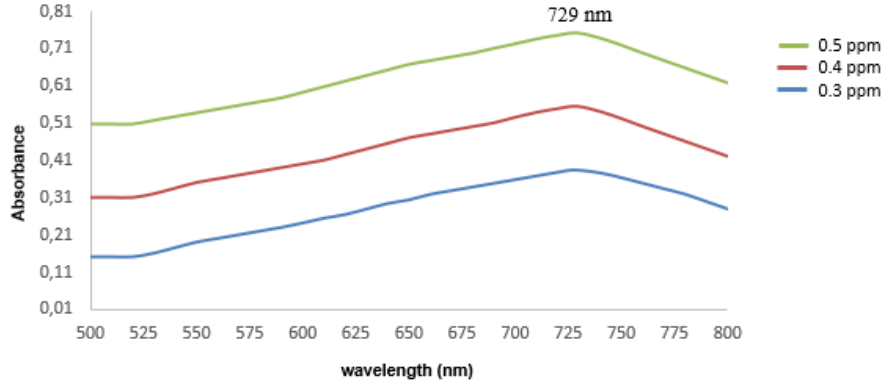


Figure 1. Maximum wavelength spectrum of phosphomolybdate compound with $SnCl_2$.

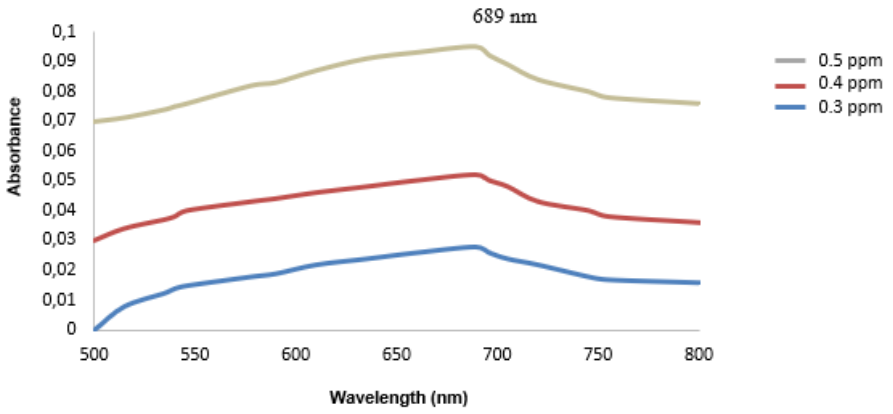


Figure 2. Maximum wavelength spectrum of phosphomolybdate compound with hydrazine sulfate.

The maximum absorption wavelength of the phosphomolybdate complex, a critical parameter in UV-visible spectrophotometric analysis, was determined to be within the range of 689–729 nm. A potassium dihydrogen phosphate solution is combined with ammonium molybdate in this analytical process. Under acidic conditions, this mixture is reduced to form a blue-colored ammonium phosphomolybdate complex. The resultant blue hue is attributed to the complex anion formed by the reduced phosphomolybdate hetero-poly ion. The spectral absorption characteristics of the phosphomolybdate complex solution are predominantly in the blue-green region, encompassing a wavelength range of approximately 650–780 nm [10].

This observation is integral to understanding the optical properties of the phosphomolybdate complex and its application in quantitative analysis. The ability to pinpoint the maximum absorption wavelength enhances the accuracy and reliability of phosphate detection using UV-visible spectrophotometry, a widely employed technique in various fields of analytical science.

3. Optimization Concentration of Ammonium Molybdate With $SnCl_2$

The addition of ammonium molybdate concentration affects the optimization of the reaction process of phosphate into a phosphomolybdate complex because the concentration of ammonium molybdate acts as a phosphorus-binding agent. The concentration of ammonium molybdate added will be directly proportional to the formation of phosphate as the product to be produced. Therefore, optimizing the concentration of ammonium molybdate solution is necessary to make the maximum amount of phosphate. Optimization was done with various concentrations (0.0008, 0.0016, 0.0024, 0.0032, 0.0040, 0.0048, 0.0056, 0.0064, and 0.0072) M. The results of the optimization process of ammonium molybdate solution concentration are shown in Figure 3.

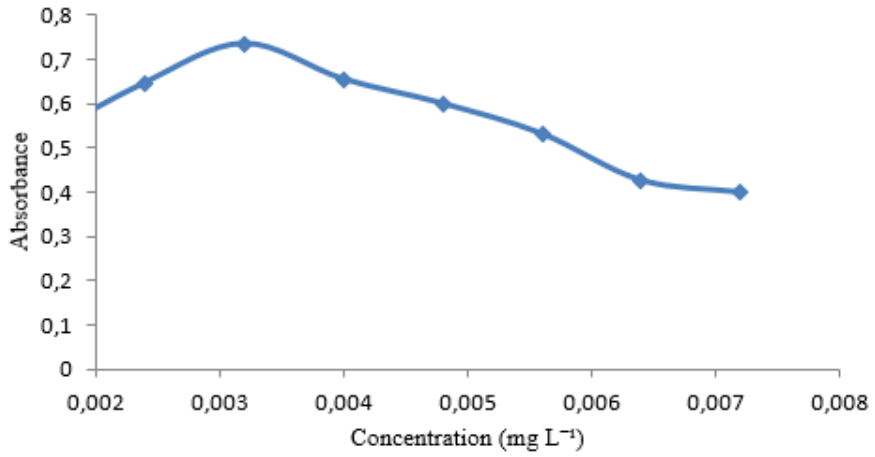


Figure 3. Graph of the relationship between ammonium molybdate concentration and the absorbance of complex compounds phosphomolybdate.

Based on the results, when the concentration is less than 0.0032 M, the amount of complex $(PMo_{12}O_{40})^{3-}$ formed decreases. This is indicated by complex absorption $(PMo_{12}O_{40})^{3-}$ lower. This occurs due to an excess of phosphoric acid and a lack of molybdate so that only part of the complex is formed $(PMo_{12}O_{40})^{3-}$ while others are only formed intermediate compounds $(PMo_{11}O_{39})^{7-}$ is presented in equation (2).

H_3PO_4 (aq) + MoO_3 (aq) + $2H_2O_9$ (l) ⇌ $(PMo_{11}O_{39})^{7-}$ (aq) + $7H^+$ ……**(2)**

Plus, its complex $(PMo_{11}O_{39})^{7-}$ can also be formed from complex hydrolysis $(PMo_{12}O_{40})^{3-}$ according to reactions (3) and (4).

$(PMo_{12}O_{40})^{3-}$ (aq) + $2H_2O$ (l) ⇌ $(PMo_{11}O_{39})^{7-}$ (aq) + $(HMoO_3)$ (aq) + $3H^+$ ……**(3)**

$(PMo_{12}O_{40})^{3-}$ (aq) + $2H_2O$ (l) ⇌ $(PMo_{11}O_{39})^{7-}$ (aq) + $(H_2Mo_2O_6)^{2+}$ (aq) + $3H^+$ ……**(4)**

If the concentration of molybdate is excessive, the amount of complex $(PMo_{12}O_{40})^{3-}$ also decreases; the absorption is seen in Figure 3. Under very acidic conditions and the molybdate is too excessive, the complex

$(PMo_{12}O_{40})^{3-}$ formed reacts with phosphoric and molybdic acid, creating a new equilibrium reaction with the complex $(P_2Mo_{18}O_{62})^{6-}$ on the reaction (5).

$(PMo_{12}O_{40})^{3-}$ (aq) + H_3PO_4 (aq) + $6(HMoO_3)^+$ (aq) ⇌ $(P_2Mo_{18}O_{62})^{6-}$ (aq) + $9H^+$ ……(5)

Based on the study's results, the most optimum complexing concentration was 0.0032 M; this indicates that the heptamolybdate ion with a concentration of 0.010 M could form complexes with phosphate completely.

4. Optimization Concentration of Ammonium Molybdate with Hydrazine Sulphate

Concentration variations of ammonium molybdate with hydrazine sulfate (0.006, 0.007, 0.008; 0.009, 0.010, 0.020, and 0.025) M. The results of the concentration optimization process of ammonium molybdate solution are presented in Figure 4.

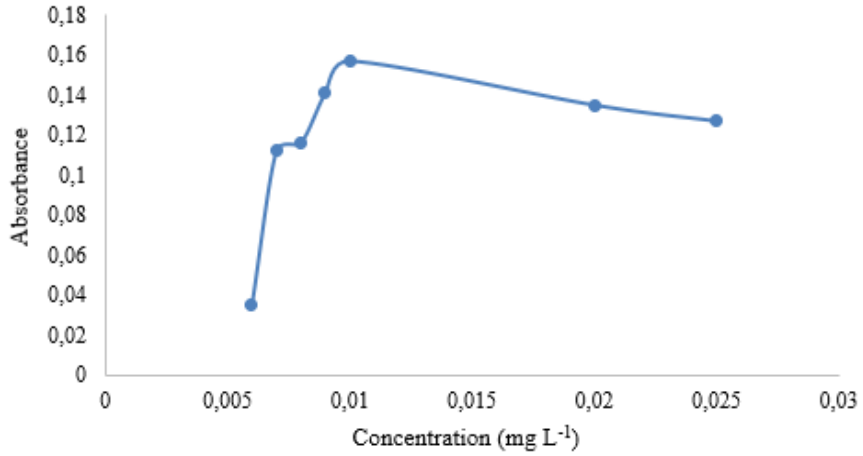


Figure 4. Graph of the relationship between ammonium molybdate concentration and the absorbance of complex compounds phosphomolybdate.

Based on Figure 4, at a concentration of 0.006–0.010 M, there was an increase in absorbance, which indicated that a blue molybdenum complex had formed, which was supported by the formation of a blue color in the blue molybdenum complex. The higher concentration of ammonium molybdate solution was directly proportional to the amount of phosphomolybdate formed, with the optimum concentration of ammonium molybdate solution being 0.010 M. The concentration of ammonium molybdate solution was then 0.010–0.025 M, the absorbance decreased.

Under very acidic pH conditions and excess molybdate concentration, the molybdophosphate complex formed is $(PMo_{12}O_{40})^{3-}$ [21]. Equilibrium reaction equation adalah $(PMo_{12}O_{40})^{3-}$:

H_3PO_4 (aq) + $12(HMoO_3)^+$ (aq) ⇌ $(PMo_{12}O_{40})^{3-}$ (aq) + $15H^+$ (aq) ……..……...**(6)**

If the concentration of molybdate used is too excessive, the complex formed is $(P_2Mo_{18}O_{62})^{6-}$ according to the reaction:

$(PMo_{12}O_{40})^{3-}$ (aq) + H_3PO_4 (aq) + $6(HMoO_3)^+$(aq) ⇌ $(P_2Mo_{18}O_{62})^{6-}$ (aq) + $9H^+$...**(7)**

But if the phosphate concentration is excessive and the molybdate concentration is reduced, then the molybdophosphate complex formed is $(PMo_{11}O_{39})^{7-}$,

H_3PO_4 (aq) + MoO_3 (aq) + $2H_2O$ (l) ⇌ $(PMo_{11}O_{39})^{7-}$ (aq) + $7H^+$ ……**(8)**

This complex can also be formed from the hydrolysis of the complex $(PMo_{12}O_{40})^{3-}$, according to the following reaction:

$(PMo_{12}O_{40})^{3-}$ (aq) + $2H_2O$ (l) ⇌ $(PMo_{11}O_{39})^{7-}$ (aq) + $(HMoO_3)^+$ (aq) + $3H^+$……..……**(9)**

$(PMo_{12}O_{40})^{3-}$ (aq) + $2H_2O$ (l) ⇌ $(PMo_{11}O_{39})^{7-}$ (aq) + $(H_2Mo_2O_6)^{2+}$ (aq) + $3H^+$ ……..**(10)**

Based on the equilibrium reaction, adding molybdate dramatically affects the formation of the molybdophosphate complex. Thus, the concentration of complexing used at the most optimum condition was 0.010 M, because the molybdophosphate complex was completely formed under optimum conditions.

When the concentration is less than 0.010 M, the amount of $(PMo_{12}O_{40})^{3-}$ complex formed decreases. This is indicated by the lower absorption of the $(PMo_{12}O_{40})^{3-}$ complex. This is due to the excess of phosphoric acid and lack of molybdate, so only part of the complex $(PMo_{12}O_{40})^{3-}$ is formed, while, the other part only forms the intermediate compound $(PMo_{11}O_{39})^{7-}$.

5. Optimization Concentration Reductor of $SnCl_2$

The reducing concentration affects the process of reducing ammonium molybdate into a complex phosphomolybdate because the concentration of the added reducing agent will be directly proportional to the formation of the phosphomolybdate complex as the product to be produced. Therefore, it is necessary to optimize the concentration of the reducing agent so that the phosphomolybdate complex is produced in the optimum amount. The reducing agent used is $SnCl_2$ in an acidic environment because $SnCl_2$ has a reduction potential of +0.151 Volt, which can reduce ammonium molybdate to a phosphomolybdate complex—the process of reducing ammonium molybdate to a phosphomolybdate complex with $SnCl_2$ in an acidic environment. The equation for the reduction reaction that occurs during the reduction of ammonium molybdate by thiourea is presented in equation (11).

Optimization of concentration reduction of $SnCl_2$ was carried out by varying the concentration (0.0006, 0.0012, 0.0018, 0.0024, 0.0030, 0.0036, 0.0042, 0.0048 and 0.0054) M. Then set at the optimum stability time and measured absorbance at a maximum wavelength of 729 nm. The results of the optimization process for $SnCl_2$ concentration are presented in Figure 5.

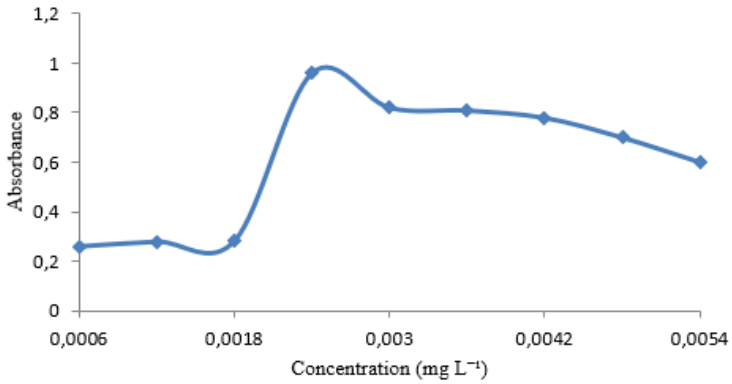


Figure 5. Graph the relationship between thiourea concentration and the absorbance of the phosphomolybdate complex compound.

Based on the results of optimization of thiourea concentration, at a concentration of 0.0006–0.0024 M, there was an increase in absorbance, which indicated that the phosphomolybdate complex resulted from the ammonium molybdate oxidation process had been formed. The higher $SnCl_2$ concentration was directly proportional to the number of

phosphomolybdate complexes formed, with the optimum concentration of $SnCl_2$ being 0.0024 M. The further increase in $SnCl_2$ concentration from 0.0030 to 0.0054 M resulted in a decrease in absorbance. Excessive $SnCl_2$ will react further with the intensity of the color of the solution found to decrease so that the concentration of $SnCl_2$ added should not exceed its optimum conditions.

6. Optimization Concentration Reductor of Hydrazine Sulphate

The reduction of ammonium molybdate to a phosphomolybdate complex occurs when hydrazine sulfate transfers electrons directly to ammonium molybdate. Therefore, optimizing the concentration of oxidizing agents is necessary to produce the maximum amount of phosphomolybdate complex. In this study, the reducing agent used was hydrazine sulfate in acid because hydrazine sulfate has a reduction potential energy capable of reducing ammonium molybdate to a phosphomolybdate complex.

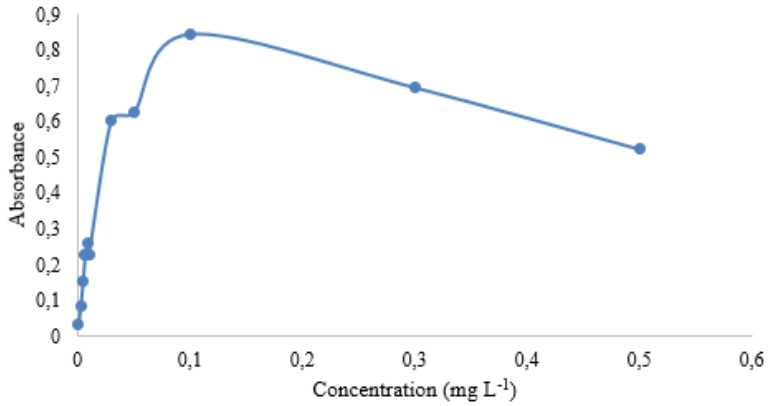


Figure 6. Graph of the effect of hydrazine sulfate concentration on the absorbance of phosphomolybdate complex.

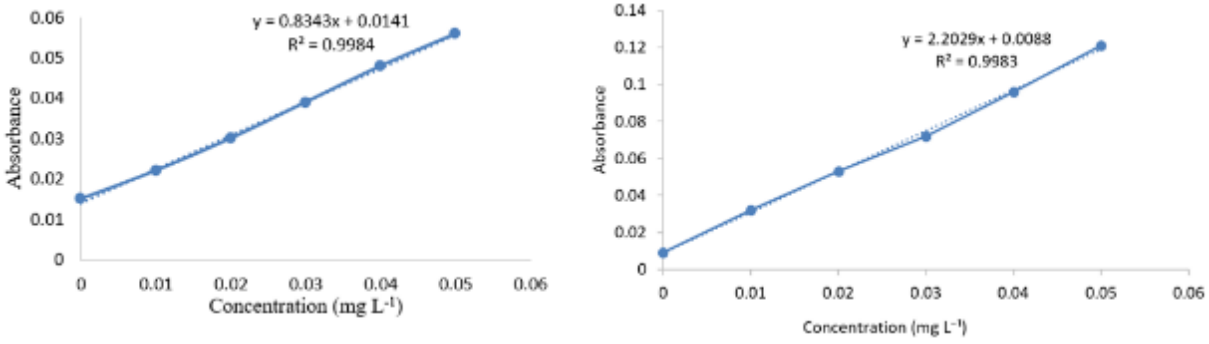
Optimizing the concentration of hydrazine sulfate using concentrations of (0.001, 0.003, 0.005, 0.007, 0.009, 0.01, 0.03, 0.05, 0.1, 0.3, 0.5) M. Then the absorbance was measured using UV-Vis spectrophotometry at a maximum wavelength of 689 nm. Reducing ammonium molybdate to a phosphomolybdate complex occurs in an acidic environment. The equation for the reduction reaction that occurs during the reduction of ammonium molybdate by hydrazine sulfate is presented in equation (12).

Based on the results of the study in Figure 6. The concentration of hydrazine sulfate at a concentration of 0.001–0.1 M increased absorbance. The concentration of hydrazine sulfate is directly proportional to the amount of phosphomolybdate concentration formed; this indicates that the phosphomolybdate complex resulting from the ammonium molybdate oxidation process has been formed. Furthermore, the absorbance value decreased at the concentration of hydrazine sulfate 0.1–0.5 M; this indicates that hydrazine sulfate reduces completely and is stable. If there is an excess of hydrazine sulfate, the hydrazine compound does not function as a hydrogen group donor that can completely reduce it.

7. Method Validation

In this study, the linearity test was carried out by measuring the absorbance of the standard solution. Standard series solutions were prepared with variations in concentration

(0, 0.01, 0.02, 0.03, 0.04, 0.05) ppm taken from the phosphate solution with a concentration of 1 ppm. After the blank solution was measured, the standard solution was measured using UV-Vis spectrophotometry with a wavelength of 689 nm.



(a) **(b)**

Figure 7. A phosphate calibration curve with (a) hydrazine sulfate and (b) $SnCl_2$.

$Mo_7O_{24}^{6-}{}_{(aq)} + 48H^+{}_{(aq)} + 42e^- \rightleftharpoons 7Mo_{(aq)} + 24H_2O_{(aq)}$ $E^o = +0.075$ V

$Sn^{2+}{}_{(aq)} \rightleftharpoons Sn^{4+}{}_{(aq)} + 2e^-$ $E^o = +0.151$ V +

$Mo_7O_{24}^{6-}{}_{(aq)} + 21Sn^{2+}{}_{(aq)} + 48H^+{}_{(aq)} \leftrightarrow 7Mo_{(aq)} + 21Sn^{4+} + 24H_2O_{(aq)}$ $E^0 = +0.233$ V **(11)**

$Mo_7O_{24}^{6-}{}_{(aq)} + 48\ H^+{}_{(aq)} + 42e^-{}_{(aq)} \rightleftharpoons 7Mo_{(aq)} + 24\ H_2O_{(aq)}$ $E° = +0.082$ V

$2NH_4OH_{(aq)} + 2OH^-{}_{(aq)} \rightleftharpoons N_2H_{4(aq)} + 4H_2O_{(aq)} + 2e^-{}_{(aq)}$ $E° = -0.11$ V +

$Mo_7O_{24}^{6-} + 6H^+ + 42NH_4OH \rightleftharpoons 21N_2H_4 + 66H_2O + 7Mo$ $E° = 0.192$ V **(12)**

Based on the results of the calibration curve using the spectrophotometric method that relates the concentration to the absorbance, the linear equation for hydrazine sulfate y = 0.8343x + 0.0141 with the correlation coefficient (R^2) obtained is 0.9984 and for $SnCl_2$ y = 2.2029x + 0.0088 is obtained with the correlation coefficient (R^2) obtained is 0.9983. According to [22], the linearity requirement for the validation of the analytical method can be accepted if the coefficient of determination (R^2) value is greater than or equal to 0.995. In this study, the value (R^2) obtained has met the specified linearity requirements.

Lambert Beer's law can determine the relationship between absorbance and concentration, and the light used is monochromatic. The sensitivity value is expressed as molar absorptivity (ε), sensitivity depends on the monochromatic light of radiation. In the presence of monochromatic light at a bandwidth very close to ʎ max, a molar absorbivity can be obtained. The greater the value of the molar absorptivity of a substance, the more light it absorbs, or in other words, the greater the absorption value [23]. In Figure 7, the slope for hydrazine sulfate is 0.8343x so that the sensitivity obtained is 7.9259 x 105 L mol^{-1} cm^{-1}, and the slope for $SnCl_2$ is 2.2029x so that the sensitivity obtained is 2.093 x 10^5 L mol^{-1} cm^{-1}.

Determining the LOD and LOQ is done by measuring the absorbance of the blank solution without analyte 10 times. The results of

calculating LOD and LOQ in the phosphomolybdate complex with hydrazine sulfate as a reducing agent were 5.62 x 10^{-4} ppm and 1.7 x 10^{-3} ppm. The result of calculating LOD and LOQ in the phosphomolybdate complex with $SnCl_2$ reducing agent was 1.41 x 10^{-3} ppm and 4.28 x 10^{-3} ppm. LOD and LOQ values were calculated statistically through linear regression lines obtained from the calibration curve. These two values indicate the smallest limit of analyte that can still be detected by a UV-Vis spectrophotometer [24]. The lower the measurement result, the better the analysis method used.

In this study, the test was carried out by testing the sample at the maximum wavelength of phosphate for 5 repetitions on the same day. % RSD expresses repeatability to see the accuracy of the method. From the calculation data, it was found that the %RSD with hydrazine sulfate in the samples of duck, chicken, and cow was 1.33%, 1.34%, and 1.08%, and %RSD for $SnCl_2$ in the samples of duck, chicken, and cow was 1.93%, 1.37%, and 1.25%, respectively.

In this study, accuracy was determined using the standard addition method, namely by adding several samples to be analyzed with a standard solution of analyte whose concentration was known and then analyzed. From the calculation data, the average %recovery value for hydrazine sulfate obtained from the duck sample was 102.66%, the chicken sample was 112.16%, and the cow sample was 95.18%. From the research conducted, it was found that the %recovery with thiourea in the samples of duck, chicken, and cow were 107.12%, 108.14%, and 105.83%, respectively.

8. Phosphorus Analysis in Meat

After the analytical method is validated with specific parameters, then the analytical method can be applied to determine the phosphate content in the sample. Determination of phosphate content was carried out by the standard addition method. Standard addition is an analytical technique carried out by adding a standard solution of known concentration to the research sample to equate the matrix between the sample and the standard [25]. The standard addition method was chosen in phosphorus analysis because it can minimize analytical errors caused by matrix differences. Measurements were made at a maximum wavelength of 689 nm and then calculated using the standard addition curve regression equation. The results of the calculation of the sample content are presented in Table 1.

Table 1. Determination of phosphorus levels

Sample	$[PO_4^{3-}]$ spike (ppm)	Average	$[PO_4^{3-}]$ (mg g^{-1})
Duck	0	0.340	1.493
	0.01	0.354	
	0.02	0.364	
	0.03	0.371	
	0.04	0.385	
	0.05	0.395	
Chicken	0	0.403	0.206
	0.01	0.416	
	0.02	0.425	
	0.03	0.435	
	0.04	0.444	
	0.05	0.452	
Cow	0	0.250	0.522
	0.01	0.263	
	0.02	0.273	
	0.03	0.280	
	0.04	0.289	
	0.05	0.302	

After calculating the sample in Table 1, the phosphate content obtained in the duck sample was 1.493 mg g^{-1}, phosphorus levels in chicken was 0.206 mg g^{-1}, and phosphorus

levels in cow obtained 0.522 mg g^{-1}. According to the Indonesian Nutritionist Association (PERSAGI), the level of phosphorus allowed in the body in duck meat sample is 1.88 mg g^{-1}, for chicken meat is 2 mg g^{-1}, and the cow sample is 1.7 mg g^{-1}. Thus, the measurement results of phosphorus in legume samples do not exceed food safety quality standards.

CONCLUSION

The optimal conditions for the phosphomolybdate complex in the reductant $SnCl_2$ and hydrazine sulfate obtained maximum wavelengths of 729 and 689 nm. The acidic conditions and the reductant concentration determine optimal conditions in phosphomolybdate complexes. Method validation is a step in the assessment to verify that the data obtained is valid by previous guidelines. In research [26] using the spectrophotometric-colorimetric method to determine P in food, RSD precision was obtained at 1.1% for 0.96 grams of phosphorus/100 grams and 5.4% for 0.29 grams of phosphorus/100 grams. Also obtained was an RSD accuracy of 3.6% for 0.96 grams of phosphorus/100 grams and 7.7% for 0.23 grams of phosphorus/100 grams. The phosphorus analysis method using hydrazine sulfate and $SnCl_2$ reductant using UV-Vis spectrophotometry meets the requirements for analytical method validation. Hydrazine sulfate reductant has better sensitivity, LOD, LOQ, precision, and accuracy in reducing phosphomolybdate complex compounds. The analysis results of phosphate content analysis in duck samples were 1.493 mg g^{-1}, chicken samples were 0.206 mg g^{-1}, and cow samples were 0.522 mg g^{-1}. So that the phosphorus content did not exceed the food safety quality standards according to the Association of Indonesian Nutritionists (PERSAGI) in 2009; in the future, fast food will undoubtedly increase rapidly; therefore, it is necessary to develop phosphorus analysis in fast food for the sake of health sustainability.

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