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Monosodium Glutamate Analysis in Meatballs Soup

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Abstract. The analysis of monosodium glutamate (MSG) in meatball soup using Cu^{2+} ion as a MSG complex by UV-Vis spectrophotometry has carried out. Reaction of MSG with Cu^{2+} ions have formed complex compounds $[Cu(C_5H_8NO_4)^2]^{2+}$ characterized by the color change of Cu^{2+} ion solution from light blue to dark blue. Maximum of complex absorbance $[Cu(C_5H_8NO_4)^2]^{2+}$ is at 621 nm wavelength. The results showed that, the greatest condition of complex $[Cu(C_5H_8NO_4)^2]^{2+}$ was at pH 10, concentration of Cu^{2+} 0.01 M, complex time is a 30 minute and stable for 170 minutes. Linear response and detection limit of MSG analysis with Cu^{2+} ions are 0.0005-0.025 M (R^2 = 0.994) and (LOD) 0.0003 M. repeatability and recovery method is quite good (% RSD = 0.89% and %recovery = 93%). The analysis of MSG content in meatball soup with MSG complex method was 0.00372 M in sample A and 0.00370 M in sample B.

1. Introduction

Flavouring is a food additive that can add flavour and aroma to the food. The need for food that tastes good and delicious causes the use of flavouring agents to increase over time such as monosodium glutamate (MSG). MSG was first isolated in the form of crystals from seaweed (Laminaria japonica) and identified as glutamic acid [1]. Excessive use of MSG can interfere with health such as dizziness and accelerate the growth of cancer cells [2]. The maximum dose of MSG allowed in humans is 60 mg / kilogram of body weight [3]. The results of a survey by the Indonesian Consumers Foundation (YLKI) found that the traders of meatball noodles, dumpling noodles and boiled noodles in Jakarta using MSG as much as 1840-3400 mg/bowl. Food Additive Organization (FAO) and World Health Organization (WHO) classified MSG as food additive with acceptable daily intake of 60 mg/kg body weight/day [3].

Methods that have used for MSG analysis are with HPLC, fluorescence, gas chromatography, and paper chromatography [4]. In this research has done MSG analysis by UV-Vis spectrophotometry because this method is simple, fast, economical and have good sensitivity and selectivity. MSG solution is a colourless solution, which has carboxyl and amino groups that cannot absorb visible light radiation, so it cannot measure absorbance in visible light areas. Condition of the compounds that can measured absorbance by UV-Vis spectrophotometer is a compound that can give coloured uptake. Therefore, we need a reagent that can give a coloured uptake spectrum with MSG. In this study used Cu^{2+} ion solution as MSG complex. Glutamate reacting with Cu^{2+} ions in aqueous solution will form a blue coloured complex compared to the colour of the Cu^{2+} ion solution before forming the complex, such as the following reaction.

$$Cu^{2+} + 2C_5H_7NO_4 \rightarrow [Cu(C_5H_7NO_4)_2]^{2+}$$

2. Experiment

2.1. Chemical

Copper (II) Nitrate [$Cu(NO_3)_2$], Sodium hydroxide (NaOH) and hydrogen phosphate (Na_2HPO_4) are produced by Emsure. L-Monosodium glutamate (MSG) and Sodium dihydrogen phospate (NaH_2PO_4) were obtained from Sigma Aldrich. All the chemical solutions were prepared in aquades.

2.2. Complexing MSG with Cu^{2+} Ion and Maximum Wavelength Determination

Solution of Cu^{2+} 0.01 M 1 ml mixed with 2 ml of MSG solution 0.01 M and then stirred until homogeneous. Furthermore, absorbance of complex $[Cu(C_5H_7NO_4)_2]^{2+}$ was tested with a UV-Vis spectrophotometer at a wavelength of 400-700 nm. Maximum absorbance wavelength $[Cu(C_5H_7NO_4)_2]^{2+}$ is the highest absorbance.

2.3. Complex MSG with Cu2 + Ion and Maximum Wavelength Determination

Solution of Cu^{2+} 0.01 M 1 ml mixed with 2 ml of MSG solution 0.01 M and then stirred until homogeneous. Furthermore, absorbance of complex $[Cu(C_5H_7NO_4)_2]^{2+}$ was tested with a UV-Vis spectrophotometer at a wavelength of 400-700 nm. Maximum absorbance wavelength $[Cu(C_5H_7NO_4)_2]^{2+}$ is the highest absorbance.

2.4. Complex optimization

The effect of pH on complex formation $[Cu(C_5H_7NO_4)_2]^{2+}$ was carried out by introducing 2 ml of MSG solution (0.01 M) into the test tube separately at varying pH (6-12), then into the test tubes added 1 mL of Cu^{2+} (0.01 M) ion solution and homogenized. Furthermore, absorbance of each solution measured at maximum wavelength with UV-Vis Spectrophotometer. The highest absorbance is the optimum pH of a more complete complex reaction between MSG and Cu^{2+} ions. While, influence of concentration of Cu^{2+} Ion Solution on complex formation $[Cu(C_5H_7NO_4)_2]^{2+}$ is done by mixing 2 ml of Cu^{2+} ion solution with varying concentration (0.0001; 0.001; 0.002; 0.004; 0006; 0.008; 0.01; 0.02; 0.06; 0.08 M) into the test tube separately, then into the Cu^{2+} ion solutions add 2 ml of MSG 0.05 M solution at optimum pH. Absorbance Each of these mixtures measured at maximum wavelength with a UV-Vis Spectrophotometer. Time and stability of complex done by mixing 2 ml of MSG solution (0.01 M) at optimum pH with 1 mL of Cu^{2+} 0.01 M (optimum concentration) solution. Complex absorbance measured at a maximum wavelength with time periods 0-180 minutes.

2.5. Analysis of MSG in meatball soup

2.5.1. Preparation of Calibration Curve and Limit of detection. The calibration curve prepared by mixing 2 mL of MSG solution (0.0005-0.025M) with 1 ml of Cu^{2+} 0.01 M ion solution separately at optimum pH and homogenized. Each of these mixtures measured its absorbance at a wavelength of 621 nm. The calibration curve prepared with a graph between MSG concentration versus absorbance value, then the linear regression equation determined. While the limit of detection (LOD) calculated based on calibration curve [5].

2.5.2. Reproducibility and Recovery. The Reproducibility test carried out by mixing 2 ml of MSG solution (0.01 M) with 1 mL of Cu^{2+} (0.01 M deep) ion solution in the reaction tube at optimum pH. Measure absorption at maximum wavelength with UV-Vis Spectrophotometer (n=7). Then calculate the percentage of relative standard deviation (% RSD) [6]. While the precision test performed by taking 5 mL of meatball extract and added with 5 mL of phosphate buffer pH 10. Then added 0.0253 gr MSG (MSG level 0.015 M) into solution, so on 2 mL the solution mixed with 1 mL Cu^{2+} ion solution (0.01 M and allowed to stand for 30 minutes. The absorption measured at a wavelength of 621 nm with a UV-Vis Spectrophotometer. Percent recovery (% R) calculated by comparing the concentrations obtained with real concentrations. The% recovery value (% R) can be calculated by the

following equation [7], while, Cf = the total concentration of the sample obtained from the measurement, Ca = the actual sample concentration and C = concentration of the analyte added.

$$\% \, R = \frac{Cf - Ca}{C} \, X \, 100 \, \%$$

MSG analysis in the meatball soup done by filtering 50 mL of meatball sample. The meatball soup filtrate was centrifuges for 10 min, then 5 mL of meatball soup extract added 5 mL of phosphate buffer pH 10. Then 2 mL mixed with 1 mL of Cu^{2+} 0.01 M ion solution and allowed to stand for 30 min. The solution measured uptake at a wavelength of 621 nm with a UV-Vis Spectrophotometer. The MSG concentration in the meatball soup calculated by the linear regression equation obtained from the calibration curve with the formula y = ax + b with, while y = UV-Vis absorbance of complex, a = slope of the calibration curve x = MSG concentration in sample and b = intercept.

3. Result and Discussion

3.1. Complex MSG with Cu^{2+} Ion and Maximum Wavelength Determination

Compound complexes $[Cu(C_5H_8NO_4)_2]^{2+}$ produced from MSG and Cu^{2+} ions form a planar quadrilateral geometry. One common phenomenon that arises when formation of complex compounds is the colour change. Glutamate is a non-essential natural amino acid that reacts with Cu^{2+} ions to form a deep blue complex [8]. Interaction of glutamate with Cu^{2+} ions will form a covalent bond of coordination between metal and ligand. Figure 2 shows the absorbance and wavelength of MSG Cu^{2+}ions before and after forming complexes. Presence of differences in absorbance before and after the reaction shows that there has been a complex reaction between MSG and Cu^{2+} ions, this is accordance with previously reported [8]. The results of this study found that the maximum absorbance complex $[Cu(C_5H_8NO_4)_2]^{2+}$ is at 621 nm wavelength.

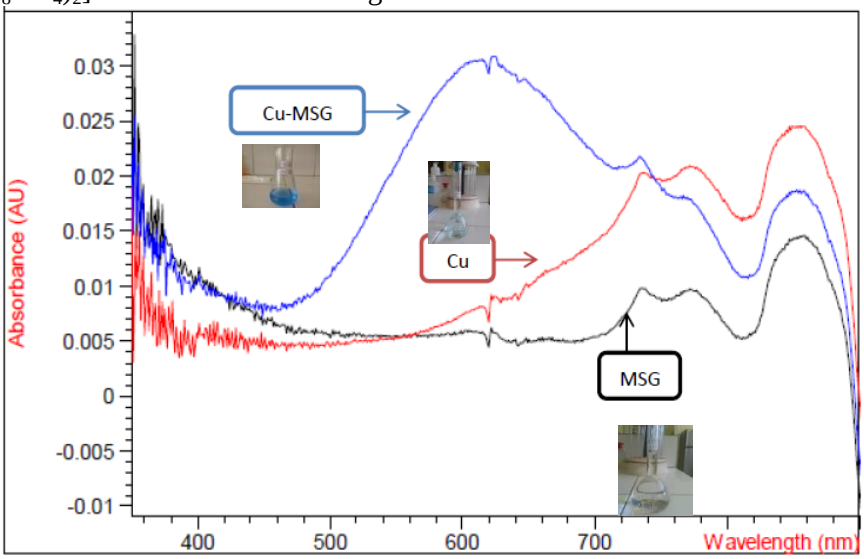

Figure 1. Absorbance and wavelength of MSG, Cu^{2+} ion and $[Cu(C_5H_8NO_4)_2]^{2+}$complex

3.2. MSG Complex Optimization

The degree of acidity (pH) is very influential on formation of complex $[Cu(C_5H_8NO_4)_2]^{2+}$, the greater the number of complexes formed the more intense color [8]. Figure 3 shows the effect of pH on complex formation $[Cu(C_5H_8NO_4)_2]^{2+}$. The results of the study found that formation of the complex $[Cu(C_5H_8NO_4)_2]^{2+}$ was more if the pH of the MSG solution increased from pH 6 to pH 10 and decreased after pH 10. In an acidic condition MSG solution contained more H + ions which could

interfere Cu^{2+} activity to react with MSG so that the little $[Cu(C_5H_8NO_4)_2]^{2+}$ complexes formed. While the MSG solution is in an alkaline condition, interruption of H + ions decreases to activity of Cu^{2+} ions and formation of $[Cu(C_5H_8NO_4)_2]^{2+}$ complexes increase, but if excessive MSG solution conditions at pH>10, OH- ion can interact with Cu^{2+} ions and decrease the amount of complex $[Cu(C_5H_8NO_4)_2]^{2+}$formed. So on, means that at pH 10 condition the complex $[Cu(C_5H_8NO_4)_2]^{2+}$ formed is the most widely compared to the other pH.

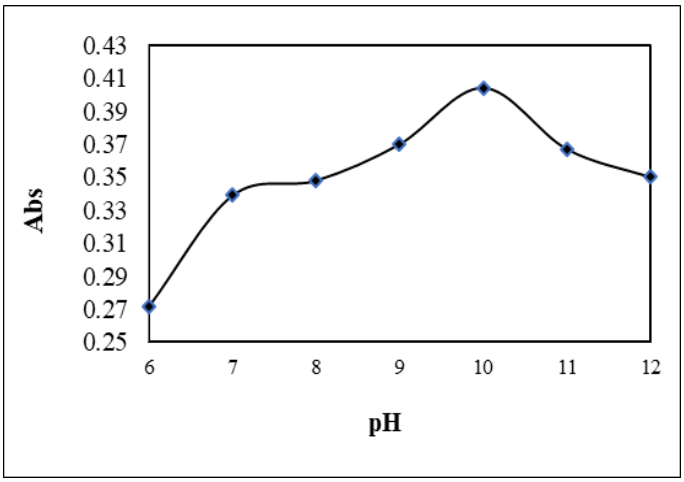

Figure 2. pH effect to complex of $[Cu(C_5H_8NO_4)_2]^{2+}$ formation

Influence of Cu2+ ion concentration to formation of complex compound [Cu(C5H8NO4)2]2+ is as shown in Figure 4. Higher complex absorbance with increasing Cu2+ ion concentration, indicates that addition of Cu2+ ion concentration can increase the amount of complex [Cu(C5H8NO4)2]2+ formed. While, the number of complexes [Cu(C5H8NO4)2]2+ does not increase if Cu2+ is more than 0.01 M ions, because it estimated that the all Cu2+ ion was complexed with MSG.

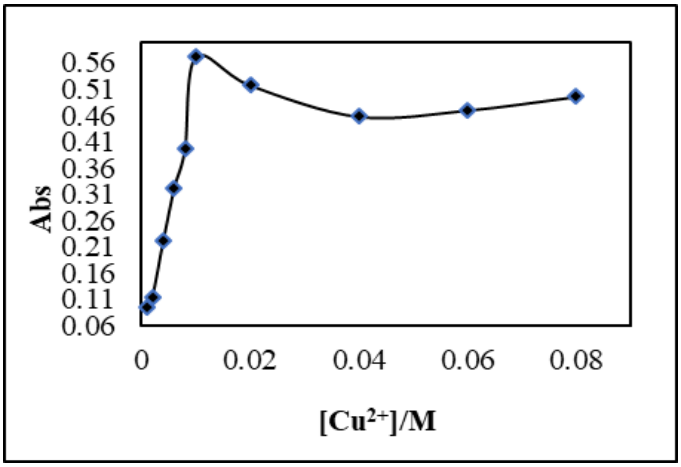

Figure 3. Concentration of Cu^{2+} ion effect to complex formed of $[Cu(C_5H_8NO_4)_2]^{2+}$

The time effects of complex formation $[Cu(C_5H_8NO_4)_2]^{2+}$ are as shown in Figure 5. The complex absorption correlates with the reaction time in which the number of complexes $[Cu(C_5H_8NO_4)_2]^{2+}$ formed increases until the reaction time reaches 30 minutes and remained until the reaction time of 170 minutes, subsequently decreased after reaction time of 170 minutes. This means that the time of complex forming of $[Cu(C_5H_8NO_4)_2]^{2+}$ is perfectly within 30 minutes, stability of the complex is up to

170 minutes. This study is accordance with what reported before that time greatly affects the formation and complex stability [9].

3.3. Analysis of MSG in meatball soup

3.3.1. Calibration Curve and Limit of detection. Figure 6 is an MSG calibration curve showing the relationship between MSG concentration and the complex $[Cu(C_5H_7NO_4)_2]^{2+}$ absorbance value. There is excellent linear stimulation at concentration of MSG 0.0005-0.025 M (R^2 = 0.9941) with the equation y = 11.259x - 0.0012. The value generated by the calibration curve is good if the value of the correlation coefficient (R^2) approaches 1.0. This means that the increase in MSG absorbance value is linear and significant with increasing concentration [8]. While the detection limits value (LOD) is 0.0003M.

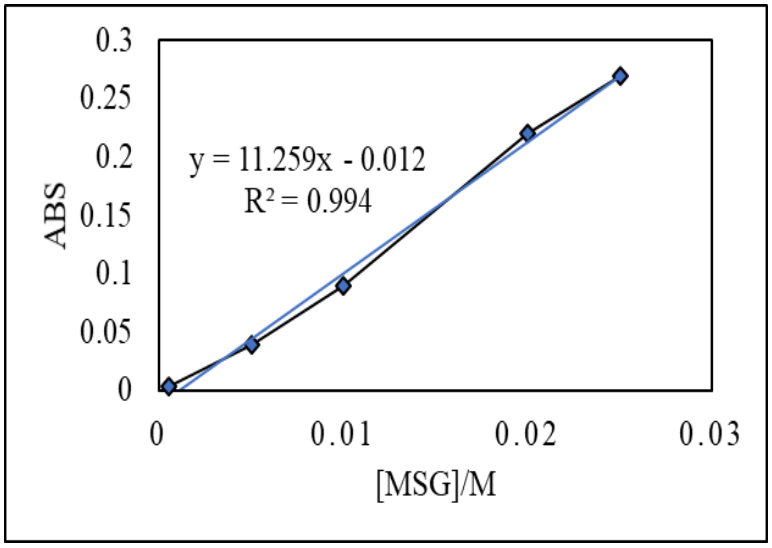

Figure 4. Calibration curve of MSG

3.3.2. Reproducibility and Recovery. From the research results obtained %RSD is 0.89% (n = 7) with standard deviation (SD) 0.005. The results show that the reproducibility value is eligible and the method used is valid, so this method can be used for MSG analysis. Further, the accuracy expressed as the retrieval test with the percent unit (%recovery) of the analyte added [7] . The results obtained a recovery value of 82% for sample A and 93% for sample B. According to Rahayu et al, (2010) A method has good accuracy if the acquisition price is 80-120%. Based on the% recovery value, this method can give the right results for MSG analysis. Next, the analysis of MSG content in meatball soup is 0.00372 M in sample A and 0.00370 M in sample B.

4. Conclusion

MSG analysis in meatball soup can be done by spectrophotometric method and using Cu^{2+} ion as complexing. The color change of Cu^{2+} ion solution from light blue to dark blue after added MSG showed a positive response for MSG analysis. The results of this study indicate that from some samples of meatball saup shows MSG levels that do not exceed the permitted threshold.

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