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Protein Analysis of Canned Legumes by using Visible Spectrophotometry and Kjeldahl Method

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Abstract: Legumes are one of dietary protein source. Protein is usually measured as crude protein which is called as the total protein in food and is determined using Kjeldahl method. But, along with advances in bioanalysis, many methods are being developed to facilitate the substance analysis. One of the method which is commonly used is spectrophotometry methods. This method is commonly used because its simplicity and rapidity. The purpose of this study is to determine the protein concentration of canned legumes using visible spectrophotometry and to recognize the difference between spectrophotometry and Kjeldahl method.

Five canned legumes which are green peas, peanuts, flageolet beans, ginkgo nuts and kidney beans were used**.** Protein determination was applied by using visible spectrophotometry with Biuret as the reagent at maximum wavelength 553.36 nm and Kjeldahl method.

The result show that the protein contents obtained using visible spectrophotometry with Biuret reagent are: 1.37 ± 0,0308 g/100 g in green peas; 1.36 ± 0.0247 g/100 g in peanuts; 2.77 ± 0.0263 g/100 g in flageolet beans; 1.44 ± 0.0728 g/100 g in ginkgo nuts; and 2.26 ± 0.0126 g/100 g in kidney beans. Protein contents obtained using Kjeldahl method are: 3.98 ± 0.4691 g/100 g in green peas; 3.30 ± 0.2249 g/100 g in peanuts; 5.41 ± 0.5156 g/100 g in flageolet beans; 3.56 ± 0.3332 g/100 g in ginkgo nuts; and 5.73 ± 0.2458 g/100 g in kidney beans. Among all five canned legumes that have been analyzed using visible spectrophotometry with Biuret reagent, green peas and peanuts have almost the same amount of protein content, but the contents are lower than in ginkgo nuts, kidney beans and flageolet beans.

Keywords: canned legumes, protein, visible spectrophotometry, Biuret, Kjeldahl.

Introduction

Dietary protein is required for growth, tissue maintenance and repair, synthesis of enzyme proteins, nutrient transport proteins, and protein required for the immune processes or defense mechanisms of the body. Legumes deposit large amounts of protein in their seeds with the help of bacteria that live in the plant roots. Although the protein of legumes is not as high-quality as animal protein, it is an adequate substitute when eaten in combination with a mixed diet, particularly if it contains wheat or corn products[1,2].

The great demand for high quality foods triggers the development of food preservation methods, for example canning, which is a more recent technology but has proven to be one of the most effective methods. Canning serves the purposes of preserving food product, maintaining (and in some cases increasing) its nutritional value, and facilitating its storage and transport[3,4].

Protein content of organic matters can be determined by two ways: (1) directly by using certain specific chemical or physical properties unique to proteins, or (2) indirectly by determining their nitrogen content. The Kjeldahl method is one of the nitrogen determination method commonly used, however, protein quantification using spectrophotometry also has been used widely because of its faster, simpler and less laborious procedures[5,6].

The purpose of this study is to determine the protein concentration of canned legumes using visible spectrophotometry and to recognize the difference between spectrophotometry and Kjeldahl method.

The methods used for the protein determination in this study were visible spectrophotometry and Kjeldahl method.

Experimental

Apparatus

Apparatus used in this study were spectrophotometer Shimadzu UV-1800, analytical balance, centrifuge, mortar and pestle, graduated cylinder, volumetric flask, Mohr pipette, beaker glass, erlenmeyer flask, Kjeldahl flask, hot plate, magnetic stirrer, water hose, liebig condenser, burette, stative, clamps, stirring rod, propipetter, pasteur pipette, blender, filter paper, spatula and funnel.

Materials

The samples of green peas (Hosen), peanuts (Ayam Brand), flageolet beans (Daucy), ginkgo nuts (Mili) and kidney beans (SW) used were canned legumes. All samples were obtained from Brastagi Supermarket, Medan. Chemical materials used in this study were E. Merck pro analytic quality unless otherwise stated: Bovine Serum Albumine (BDH Chemicals), distilled water, $CuSO_4$, Na K Tartrate, KI and NaOH.

Samples Preparation

Samples were rinsed with distilled water and drained in room temperature, which then were weighed about 25 g, homogenized using waring blender and the volume were added to 50 ml. The solution obtained were filtered and centrifuged at 11000 rpm for 10 minutes. The supernatant from centrifugation were decanted and used for analysis (proteins in the supernatant were soluble proteins)[7].

Standard Solution Preparation

For the first standard solution, 250 mg Bovine Serum Albumin were weighed and placed in 50 ml volumetric flask, then distilled water was added and homogenized (C = 5000 mcg/ml). For the second standard solution, 1000 mg Bovine Serum Albumin were weighed and placed in 50 ml volumetric flask, then distilled water was added and homogenized (C = 20000 mcg/ml).

Absorption Curve

3 ml of the first standard solution (C = 5000 mcg/ml) were placed in 10 ml volumetric flask. 6 ml of Biuret reagent (mixture of $CuSO_4$, K Na Tartrate, KI in NaOH) were added. Distilled water were then added until the graduation marking and homogenized. The solution was allowed to stand for approximately 30 minutes and measured using spectrophotometer at 553.36 nm.

Operating Time

3 ml of first standard solution (C = 5000 mcg/ml) were placed in 10 ml volumetric flask. 6 ml of Biuret reagent were added. Distilled water were then added until the graduation marking and homogenized. After 1 minute, the solution was measured using spectrophotometer at 553.36 nm for 60 minutes.

Bovine Serum Albumin Calibration Curve

0.5, 0.75, 1, 1.25, 1.5, 1.75, 2 and 2.25 ml of standard solution (C = 20000 mcg/ml) were placed in 10 ml volumetric flasks to prepare solutions in following concentrations: 1000, 1500, 2000, 2500, 3000, 3500, 4000, and 4500 mcg/ml respectively. 6 ml of Biuret reagent were added to each volumetric flasks. Distilled water were then added until the graduation marking and homogenized. The solutions were allowed to stand for 17-22 minutes and the absorbances were measured at 553.36 nm. Absorbances and concentrations obtained

from the assays were used for calculating regression equation which then was used for calculating the protein concentration in samples.

Protein Content Determination

3 ml of sample solution were placed in 10 ml volumetric flask. 6 ml of Biuret reagent were added. Distilled water were then added until the graduation marking and homogenized. The solution was allowed to stand for 17-22 minutes and the absorbance was measured at 553.36 nm[7].

Concentration was measured by substituting sample's absorbance obtained to Y in the regression equation[8]:

$Y = ax + b$

where, Y = sample's absorbance
X = sample's concentration
a = slope
b = intercept

Protein content in sample was calculated using the following expression[8]:

$$\text{Protein content (C)} = \frac{X \times V \times Fp}{BS}$$

where, K = total protein content in sample (mg/g)
X = concentration after dilution (mcg/ml)
V = sample's volume (ml)
Fp = dilution factor
BS = sample's weight

Analysis Method Verification

Accuracy

In this study, accuracy test was done using standard addition method. Sample preparation was done the same way as for protein content determination. Before distilled water was added to the volumetric flask, Bovine Serum Albumin standard equivalent to 15 mg/g sample's weight was added. Next procedure was done the same way as in protein content determination procedure. Accuracy test result was expressed as percent recovery.

Percent recovery was calculated using the following expression[9]:

$$\text{Percent Recovery (\%)} = \frac{(CF - CA)}{C^*A} \times 100\%$$

where, CF = concentration obtained from measurement (mg/g)
CA = real concentration of the sample (mg/g)
C*A = concentration of standard added (mg/g)

Precision

Precision degree was calculated by using the following expression[9]:

$$CV = \frac{SD}{\bar{X}} \times 100\%$$

Criteria of precision was given if the method gives relative standard deviation (RSD) or coefficient of variation (CV) ≤ 2%.

Limit of Detection and Limit of Quantitation

Limit of detection and limit of quantitation were calculated from standard curve regression equation. Limit of detection (LOD) and limit of quantitation (LOQ) can be calculated using following expressions[9]:

$$LOD = \frac{3\,Sy/x}{slope}$$

$$LOQ = \frac{10\,Sy/x}{slope}$$

where, Sy/x = standard deviation
slope = gradient of the standard curve

Sy/x was calculated using following expression:

$$Sy/x = \sqrt{\frac{\sum(y-yi)^2}{n-2}}$$

Protein Content Determination using Kjeldahl Method

Approximately 5 g of sample were weighed and homogenized using mortar and pestle. Then, the sample were dried in the oven at 60°C for 1 hour. About 0,2 g of dried sample were weighed and placed into the Kjeldahl flask. 2 g of $CuSO_4$: K_2SO_4 mixture (1:1) and 3 ml of concentrated H_2SO_4 were added. Digestion was done for approximately 2 hours until the color of the digest turned to clear green. After the digest was cooled, 10 ml of distilled water were added and the digest were transferred into erlenmeyer flask. 0.02623 N NaOH were added until the color of the solution turned to black (± 5 ml) and then the solution was distilled. 25 mL of 0.02 N H_2SO_4 and 3 drops of mixed indicator were added into the receiving flask. The distillate was titrated with 0.02623 N NaOH until the distillate's color changed to green[10].

Protein content in sample was calculated using the following expression:

$$\text{Protein content} = \frac{(\text{V blank-V sample}) \times \text{N NaOH} \times 0.014 \times \text{CF}}{\text{SW}} \times 100\%$$

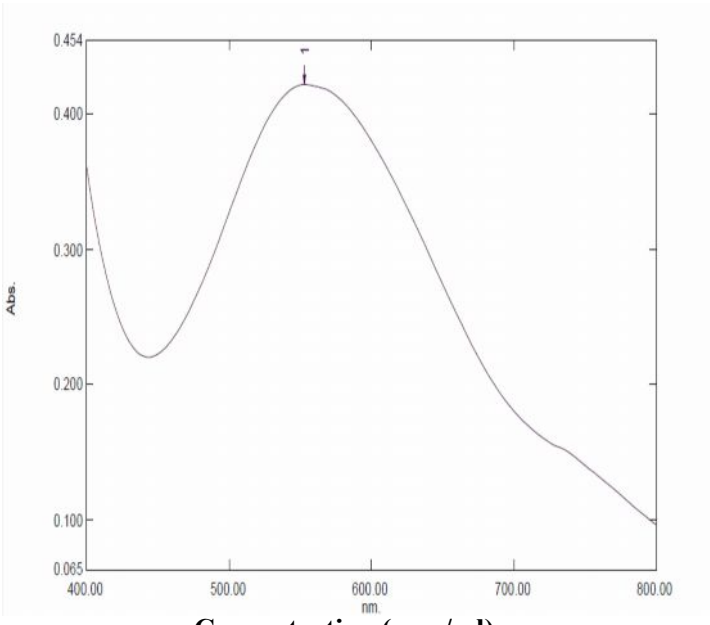
where, V = Titration volume
N = Normality of NaOH
CF = Conversion factor (6,25)
SW = Sample's weight (g)

Results and Discussion

Absorption Curve

Bovine Serum Albumin maximum wavelength obtained from measurement is at 553.36 nm. According to Gornall et al., (1948), determination using Biuret reagent, maximum wavelength of Bovine Serum Albumin standard solution will be obtained in the range of 450 – 650 nm. Thus, the maximum wavelength of Bovine Serum Albumin in this study is in the teority range[11].

Absorption curve of Bovine Serum Albumin standard is shown in Figure 1 below.



Concentration (mcg/ml)

Figure 1. Absorption curve of Bovine Serum Albumine Standard

Operating Time

Operating time of Bovine Serum Albumin was obtained from 17th to 22nd minutes.

Bovine Serum Albumin Calibration Curve

Calibration curve of bovine serum albumin standard is shown in figure 2 below.

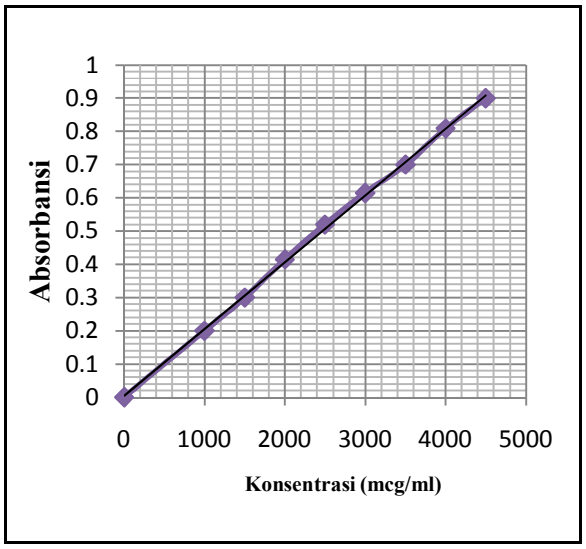


Figure 2. Bovine Serum Albumin calibration curve

Calibration curve is obtained from concentrations range from 0 mcg/ml to 4500 mcg/ml. The result shows that there is a linear correlation between absorbance and concentration with the coefficient of relationship (r) = 0.9996, which meets the acceptance limit of r = 0.999.[12]

Regression equation obtained from calculation is Y = 0.00020097 X + 0.00413.

Protein Content

Protein contents of the samples determined using visible spectrophotometry, Kjeldahl method and protein contents listed on the packaging labels are shown in table 1 below.

Table 1. Protein content determined using visible spectrophotometry, Kjeldahl method and protein content listed on the packaging label

No.	Sample	Protein content determined using visible spectrophotometry (g/100g)	Protein content determined using Kjeldahl method (g/100g)	Protein content listed on the packaging label (g/100g)
1.	Green Peas	1,37 ± 0,0308	3.98 ± 0.4691	3.2
2.	Peanuts	1,36 ± 0,0247	3.30 ± 0.2249	3.0
3.	Flageolet beans	2,77 ± 0,0276	5.41 ± 0.5156	4.9
4.	Ginkgo nuts	1,44 ± 0,0728	3.56 ± 0.3332	1.5
5.	Kidney beans	2,26 ± 0,0126	5.73 ± 0.2458	5.3

The value of protein content analyzed in this study represent an average of six determinations

Protein content determined using visible spectrophotometry in the five samples show that protein content in green peas and peanuts are not significantly different, but their protein contents are lower than in ginkgo nuts, kidney beans and flageolet beans. Protein contents obtained from fresh samples are 5.45 g/100g (green peas), 25.80 g/100g (peanuts), 22.51 g/100g (flageolet beans), 4.32 g/100g (ginkgo nuts) and 22.55 g/100g (kidney beans), protein contents obtained show a drastic decline. This is probably caused by heating

process in canning method that breaks peptide bonds in proteins, thus reducing the peptide bonds which interact with Biuret reagent to form complex and protein content analyzed is lower[2,13].

Protein contents obtained using Kjeldahl method are 3.98 g/100g (green peas), 3.30 g/100g (peanuts), 5.41 g/100g (flageolet beans), 3.56 g/100g (ginkgo nuts) and 5.73 g/100g (kidney beans). In Kjeldahl method, the sample is heated in sulfuric acid and digested till the carbon and hydrogen are oxidized and the protein nitrogen is reduced and transformed into ammonium sulfate. Then concentrated sodium hydroxide is added, and the digest heated to drive off the liberated ammonia into a known volume of a standard acid solution. The unreacted acid is determined and the results are transformed, by calculation, into a percentage of protein in the organic sample[14].

Compared to protein contents obtained using visible spectrophotometry, protein contents obtained using Kjeldahl are higher. This is caused by the different principle of both methods. In protein content determination using visible spectrophotometry with Biuret reagent, Cu^{2+} from $CuSO_4$ in alkali conditions will form a complex with peptide bonds from protein (-CO-NH-), this complex will form a color which can be measured using visible spectrophotometry. In Kjeldahl method, all nitrogen from samples are measured as protein, thus, this protein content obtained from this method is called crude protein. This causes protein content determined using Kjeldahl method is higher than the spectrophotometry methods[7].

Protein contents obtained using visible spectrophotometry with Biuret reagent are different from the protein contents showed on the packaging labels. Protein contents showed on the packaging labels are 3.2 g/100g (green peas), 3.0 g/100g (peanuts), 4.9 g/100g (flageolet beans), 1.5 g/100g (ginkgo beans) and 5.3 g/100g (kidney beans). This is probably caused by the different analysis method used. The most widely used method of determining protein is the analysis of nitrogen. The usual method employed for the determination of nitrogen in foods is the Kjeldahl method[15].

Analysis Method Verification

The purpose of verification test in this study is to assure if the method used is accurate for the protein content determination in the canned sample. Verification test were done on canned green peas. The verification test results are shown in table 2 below.

Table 2. Verification test results done on green peas

No.	Concentration before Bovine Serum Albumin standard addition (mg/g)(CA)	Concentration of Bovine Serum Albumin standard added (mg/g) (C*A)	Concentration after Bovine Serum Albumin standard addition (mg/g) (CF)	Percent recovery (%)
1.	13,7026	15,0078	28,6539	99,62
2.	13,7026	15,0039	28,6211	99,43
3.	13,7026	15,0023	28,4600	98,37
4.	13,7026	15,0078	28,5778	99,12
5.	13,7026	15,0000	28,4884	98,57
6.	13,7026	15,0000	28,6539	99,67
Average of percent recovery (%)				99,13
Standard Deviation (SD)				0,5493
Coefficient of Variation (CV) (%)				0,55
Limit of Detection (mcg/ml)				129,6313
Limit of Quantitation (mcg/ml)				432,1043

Accuracy

Accuracy test result is expressed as percent recovery (%). Percent recovery of protein determination using visible spectrophotometry is 99.13% with standard deviation (SD) of 0.5493. This result is accepted for accuracy test[9].

Precision

Precision test result is expressed as coefficient of variation (CV). From precision test done in this study, coefficient of variation obtained is 0.55%. The result shows that the method used in this study has a good precision because the coefficient of variation (CV) ≤ 2.[9]

Limit of Detection and Limit of Quantitation

Limit of detection and limit of quantitation obtained are 129.6313 mcg/ml and 432.1043 mcg/ml respectively.

Conclusion

In this study, protein contents obtained in canned legumes using visible spectrophotometry with Biuret reagent are 1.37 ± 0.0308 g/100 g (green peas), 1.36 ± 0.0247 g/100 g (peanuts), 2.77 ± 0.0276 g/100 g (flageolet beans), 1.44 ± 0.0728 g/ 100g (ginkgo nuts) and 2.26 ± 0.0126 g/100 g (kidney beans). Protein contents obtained using Kjeldahl method are 3.98 ± 0.4691 g/100 g (green peas), 3.30 ± 0.2249 g/100 g (peanuts), 5.41 ± 0.5156 g/100 g (flageolet beans), 3.56 ± 0.3332 g/100 g (ginkgo nuts) and 5.73 ± 0.2458 g/100 g (kidney beans).

The results in this study show that protein contents in green peas and peanuts are not significantly different, but their protein contents are lower than in ginkgo nuts, kidney beans and flageolet beans.

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