# A COMPARISON OF BIURET, LOWRY AND BRADFORD METHODS FOR MEASURING THE EGG'S PROTEINS

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*Abstract:* Quantitation of the total protein content in a sample is a critical step in protein analysis. Molecular UV-Vis absorption spectroscopy is very efficient in quantitative analysis such as protein quantitation and has extensive applications in chemical and biochemical laboratories, medicine and food industry. Traditional spectroscopic methods are cheap, easy-working and the most common way to quantitate protein concentrations. This study compares Biuret, Lowry and Bradford methods for measuring hen albumen and egg yolk as protein samples. These methods are commonly used for determination proteins. The Biuret test uses as a reagent: Biuret reagent. For Lowry assay are used four reagents: reagent A, reagent B, reagent C and reagent D. For last method, Bradford, is used as a reagent Coomassie Brilliant Blue G-250. The absorbance was measured at a wavelength of 750 nm for Lowry, 540 nm for Biuret and 595 nm for Bradford assay. The lowest content of proteins was analysed in albumen (0.706 $mg \cdot ml^{-1}$) and egg yolk (0.996 $mg \cdot ml^{-1}$) for Biuret method. According to the Lowry assay, was content of proteins in albumen 0.908 $mg \cdot ml^{-1}$ and content in egg yolk was 1.003 $mg \cdot ml^{-1}$. The highest content of proteins, which was analysed using method Bradford, was content of protein in albumen 1.125 $mg \cdot ml^{-1}$ and content for egg yolk was 1.369 $mg \cdot ml^{-1}$.

*Key Words:* protein, Lowry method, Biuret method, Bradford method, albumen, egg yolk

## INTRODUCTION

The quantitation of protein content is important and has many applications in food industry practices and in research especially in the field of biochemistry. The exact monitoring of protein content in samples is a critical step in protein analysis. The different protein assay techniques have been developed for the assessment of the protein concentration in a sample (Okutucu et al. 2007). Modern instrumental methods such as mass spectrometry, absorption spectroscopy, chromatography etc. are expensive, difficult for manipulation and time-challenging. Traditional spectrophotometric methods are cheap, fast, easy-working and the most common way to quantitate protein concentrations. Spectrophotometric protein quantitation assays are methods that use UV and visible spectroscopy to rapidly determine the concentration of protein, relative to a standard or using an assigned extinction coefficient. Methods are described to provide information on how to analyse protein concentration using UV protein spectroscopy measurements, traditional and common dye-based absorbance measurements: Biuret, Lowry and Bradford assays and the fluorescent dye-based assays: amine derivatization and detergent partition assays.

The Biuret method is based on the reaction $Cu^{2+}$ with functional groups in the protein's peptide bonds. The formation of a $Cu^{2+}$ protein complex requires two peptide bonds and produces a violet-coloured chelate product.

Lowry method is very sensitive, but on the other hand, two-stage and requires a minimum incubation time around 40 minutes. It is based on a biuret reaction that includes the use of Folin-Ciocalteu reagent for enhanced colour development. Proteins are firstly treated with alkaline copper sulphate in the presence of tartrate. This step is then followed by addition of the Folin-Ciocalteu reagent. The enhancement of the colour reaction in the Lowry procedure occurs when the tetradentate copper complexes transfer electrons to Folin-Ciocaulteu (phosphomolybdic/phosphotungstic acid blue

complex). Reduction of the Folin-Ciocalteu reagent is measured as a blue colour at 750 nm. (Noble, Bailey 2009). Colour is caused by electronic transitions involving the valence electrons to another.

The Bradford method is very favourite because the results are already known after 5 minutes, however, for proteins, with a very low content of arginine, is useless. It is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250 in which under acidic conditions the red form of the dye is converted into its blue form to bind to the protein being assayed (Noble, Bailey 2009). One disadvantage of this test is that interferes with many compounds. Interference, the production of colour by substances other that the analyte of interest, is a common problem with indirect colorimetric assays.

It is necessary to choose the appropriate technique from the available methods. Several criteria such as the nature of the protein (sample), the presence of interfering substances and the preferred speed, accuracy and sensitivity of assay are considered. Many of the dye-based assays have unique chemical mechanisms that are prone to interference from chemicals prevalent in many biological buffer preparations. It is also good to know which particular range of protein concentration an assay is sensitive to (see Table 1).

In the ideal test, the most preferred calibration curve generates a linear response to the standard solutions that covers the range of the concentration of the unknown. As the linearity range for the calibration curve is known, it will give the assay more accurate, time efficient and cost effective.

*Table 1 Overview of methods.*

| Method | Sensitivity | Accuracy | Interference |
|---|---|---|---|
| Lowry | 0−0.1 mg | Partially dependent on amino acid composition | Acids, EDTA, DTT, phenol, $(NH_4)_2SO_4$ |
| Biuret | 0−1 mg | High, no depend on amino acid composition | Amino-group [$(NH_4)_2SO_4$] |
| Bradford | 0−0.01 mg | Dependent on amino acid composition | Detergents (soap, SDS, Triton X-100) |

The main scope of this paper is to performance of the three methods (Biuret, Lowry and Bradford) for determining the concentration of protein in albumen and egg yolk, compare the methods and evaluation of proteins in egg using these methods.

## MATERIAL AND METHODS

Hen eggs 10-pack, which is commercially distributed. For one sample one egg was used. The albumen and egg yolk were separated, after both were diluted twenty times with distilled water, filtered through gauze and used for determination of protein.

### Determination of protein in albumen and egg yolk

The Biuret method

• Solution of bovine serum albumin.

• **Biuret reagent**: copper sulfate pentahydrate $CuSO_4 \cdot 5\ H_2O$ c = 13.0 $mmol \cdot l^{-1}$, potassium sodium tartrate $KNaC_4H_4O_6 \cdot 4\ H_2O$ c = 32·0 $mmol \cdot l^{-1}$, NaOH c = 0.6 $mol \cdot l^{-1}$.

Further 0.5 ml diluted sample was added into 3 tubes, 3 ml of Biuret reagent and the tubes were allowed 30 minutes at room temperature. After 30 minutes the absorbance was measured at 540 nm. Blank contains water instead of the protein (Coleland 1994).

The Lowry method

• Solution of bovine serum albumin.

• **Reagent A** consists of 2% $Na_2CO_3$ (20 g/1 l), 0.05%, sodium potassium tartrate x 4 $H_20$ (0.05g/1000 ml), 0.1 M NaOH (4 g/1 l).

• **Reagent B** consists of 0.1% $CuSO_4 \cdot 5H_2O$ (1 g/1 l).

• **Reagent C** consists of 45 ml of solution A + 5 ml solution B (newly diluted in proportion 9:1).

• **Reagent D** consists of 1 vol Folin-Ciocalteau reagent diluted with 1.6 vols water.

The reaction mixture consists of 0.5 ml supernatant and reagent C was incubated for 30 minutes in laboratory temperature. Further, 0.5 ml of reagent C was added to reaction mixture and additional 30 minutes of incubation proceed. The absorbance was measured at a wavelength of 750 nm. Sample was replaced water for determination blank (Lowry et al. 1951, Waterborg 2009).

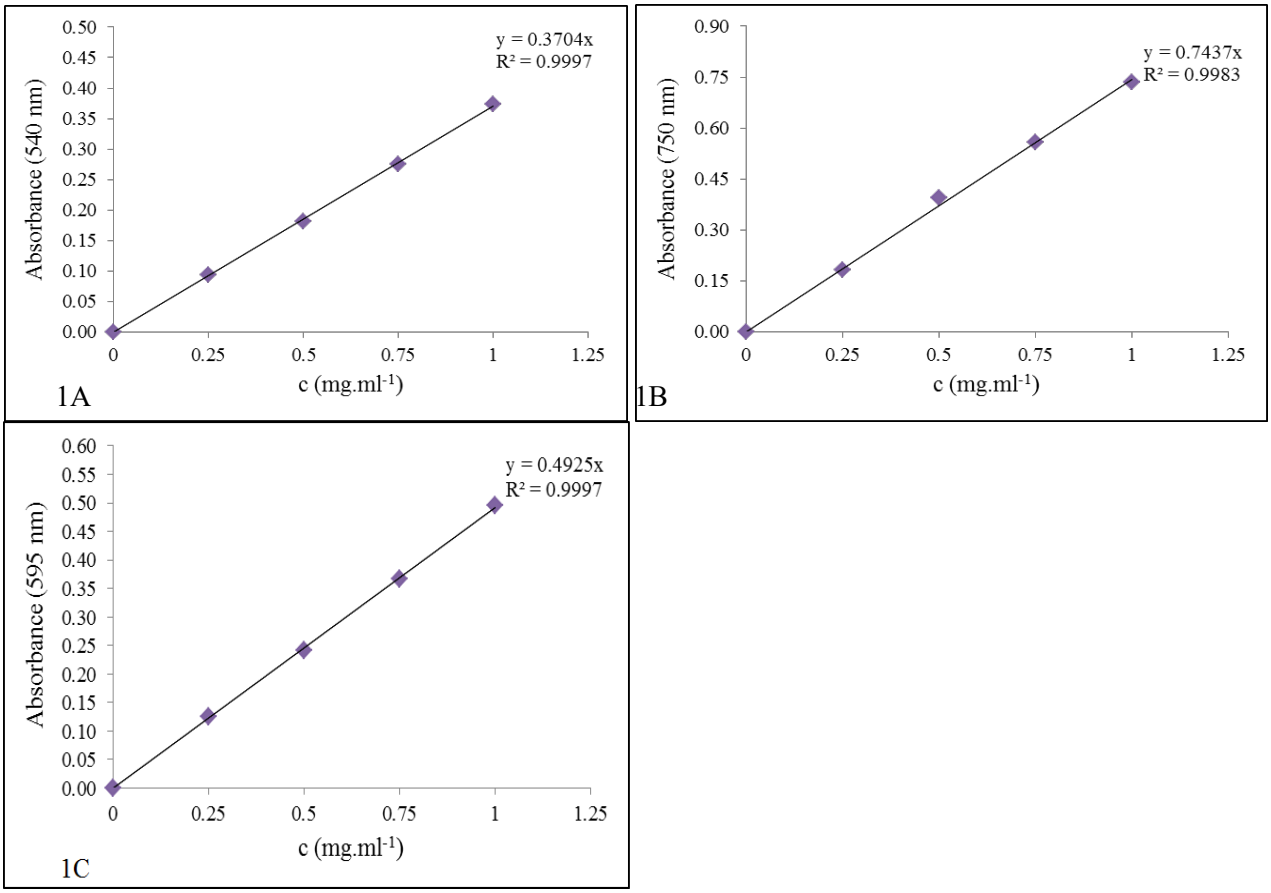
The Bradford method

• Solution of bovine serum albumin.

• **Coomassie Brilliant Blue G-250**: 10 mg of Coomassie G-250 was dissolved in 5 ml of ethanol, after concentrated phosphoric acid (10 ml) and 85 ml distilled water were added. Solution was filtered.
200 µl sample was added into 1.8 ml Coomassie Brilliant Blue G-250. Blank contained 200 µl of distilled water and 1.8 ml Coomassie Brilliant Blue G-250. After 5 minutes the absorbance was measured at 595 nm (Kruger 1994).

The proteins were determined by the UV/VIS spectrometry using a UV/VIS Lambda 25 Spectrophotometer (Perkin-Elmer). The protein assays were always performed in triplicate for verification result.

*Figure 1 Calibration curves - Biuret method (1A), Lowry (1B) and Bradford (1C)*




Calculation of the concentration of proteins was based on linear regression equation obtained by evaluation of standard curves of bovine serum albumin (see Figure 1A–C).

Formula for calculation of the concentration of proteins eq. (1):

$$c = \frac{A}{x} \qquad (1)$$

c concentration of protein

A absorbance

x value based on linear regression equation

## RESULTS AND DISCUSSION

### A comparison of methods

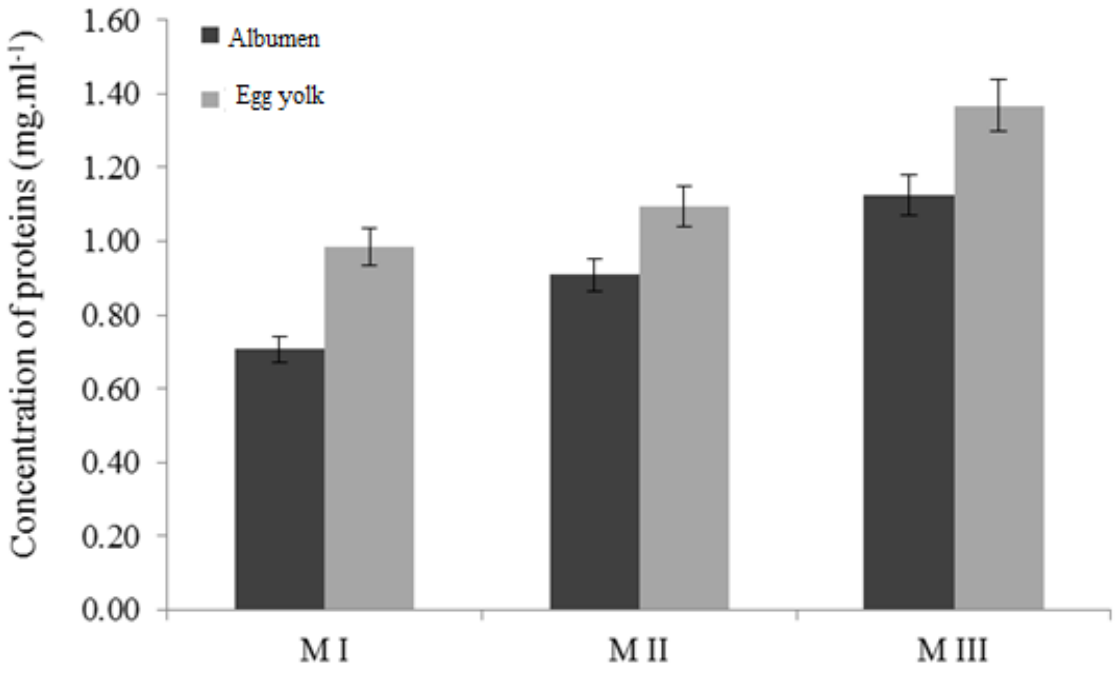
Methods for the determination of protein concentration are based on the quantity and nature of the protein to be analysed, the presence of interfering substances and sensitivity requirements. In this work were used three common assays that use UV/VIS spectroscopy to rapidly determine the concentration of protein were tested on albumen and egg yolk and further the methods were compared.

Biuret method indicated the lowest content of proteins in albumen (0.706 $mg \cdot ml^{-1}$) and egg yolk (0.996 $mg \cdot ml^{-1}$) which was probably due to low sensitivity of this method (see Figure 2). Our results agree with work of Janairo et al. (2011), who tested sensitivity of Biuret method. The Biuret assay is not much good for protein concentrations below 5 mg/ml. By using the Folin-Ciocalteu reagent to detect reduced copper makes the Lowry assay nearly 100 times more sensitive than Biuret reaction alone. Our results (see Figure 2) show high concentration of proteins using Lowry method (0.908 $mg \cdot ml^{-1}$ for albumen and 1.003 $mg \cdot ml^{-1}$ for egg yolk). Similar results were observed in the study of Malin and Ridzuan (2010), which observed that Lowry method is more sensitive than the Biuret method. Similar results gave work of Anggun (2013), who determined higher sensitivity of protein concentration in albumen through Lowry method.

The Bradford assay showed the highest values of proteins (1.125 $mg \cdot ml^{-1}$ for albumen and 1.369 $mg \cdot ml^{-1}$ for egg yolk). But this assay is not without errors, it is sensitive to interference by many other compounds (basic conditions and detergents-SDS). However, there are detergent-compatible Bradford reagents. The Bradford assay depends on the sequence of the protein. If the protein doesn't contain a decent number of arginine and/or aromatic residues, then the dye will not bind to the protein as efficiently, resulting in an underestimation of the protein concentration. Our observation agrees with study of Lu et al. (2010), who studied differences between Bradford and Lowry techniques and they observed significant variations in protein concentrations following assessment with the Lowry versus Bradford methods, using identical egg samples.

Quantitation of total protein content is a measurement common to many applications in basic science research and routine clinical laboratory practice. Most biochemical studies that involve the measurement of a biological activity require the normalization of that activity to the protein content. Given the importance of protein assay, it is significant to choose the appropriate technique from the available methods.

*Figure 2 Comparison of Biuret, Lowry and Bradford methods*




*Legend: M I – Biuret method, M II – Lowry method, M III – Bradford method*

## CONCLUSION

As the conclusion, the Lowry technique seems to be the best method in determining the protein concentration of hen egg, because Biuret assay is not much sensitive and Bradford can be inhibited by the presence of many compounds.

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