National University of Science and Technology

FACULTY OF APPLIED SCIENCES
DEPARTMENT OF APPLIED BIOLOGY AND BIOCHEMISTRY

PROJECT TITLE :

A QUALITATIVE AND QUANTITATIVE COMPARISON OF THREE METHODS OF PROTEIN DETERMINATION IN YEAST AND COWPEAS

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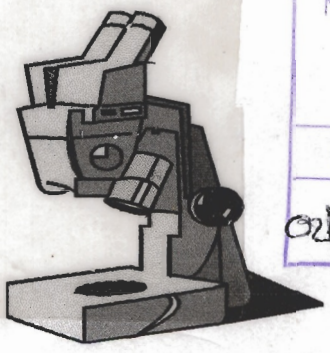

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ABSTRACT

Protein determination is important in various field of study which include among others medicine, nutrition, industry process control, Agriculture and Toxicology. Various methods of protein determination can be used in these studies but a highly precise, accurate and sensitive method that is relatively simple is usually preferred. Some methods used for protein determination include the Kjeldahl, Thermal combustion, formal titration, Dye binding methods for example Bradford and other chemical methods such as the Biuret and Lowry. The Bradford, Biuret and Lowry are the methods commonly used by research biochemists due to their simplicity, speed, low cost and ease of operation.

A comparison of the Biuret, Lowry and Bradford was conducted so that the best method that can be used for routine protein determination is established. The Biuret method involves complexation of Nitrogen of the peptide bond with copper ions and the colour is proportional to protein content. The Lowry works on some principle as Biuret but in addition phosphomolybidic tungstate also binds groups of a phenolic nature for example tryrosine thus increasing sensitivity. The Bradford involves the binding of coomassie G250 on to proteins and colour developed is proportional to protein content.

Standard curves were prepared for each method by using bovine serum albumin (BSA) of a specified concentration. A linear relationship between absorbances and protein concentration in range of BSA concentration used was obtained. For protein determination yeast and cowpeas samples were used. These were homogenized by using a pestle and mortar, suspended in buffers PH5 and 9, left to extract at 0°C for 40 mins, centrifuged, and the supernatant collected and used as the samples. Appropriate reagents for each method were then added to each sample amount and absorbance read after colour development. The protein content by each method was then read off the standard curve.

The data for protein content obtained by the three different methods were not significantly different from each other. The Lowry method was found to be the most accurate and precise method followed by Bradford then lastly Biuret. The lowest limits of detection obtained by each method was 20 g/ml for Bradford, 5 g/ml for Lowry and 100 g/ml for Biuret. The Lowry method was therefore the most sensitive of the three. The Bradford was found to be the simplest and fastest of the three followed by Biuret then Lowry.

In conclusion all the three methods were found to be suitable for protein determination but the choice of which one to choose depends on the problem at hand.