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Analysis of riboflavin in green leafy vegetables by fluorescence spectroscopy

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GRAPHICAL ABSTRACT

Riboflavin sample in cuvette

ABSTRACT

Green leafy vegetables are good source of vitamins and also rich in antioxidant. Riboflavin (vitamin B_2) is one of the antioxidant that helps to convert free radicals into non-toxic forms. This study was conducted to determine the content of riboflavin in green leaves of vegetables. The samples of green leafy vegetables such as spinach (*Spinacia oleracea*), mustard green (*Brassica juncea*), water morning glory (*Ipomoea aquatic*) and lettuce (*Lactuca sativa*) were digested using wet digestion technique. Effects of several digestion parameters such as types of solvent, concentration of solvent and sample weight have been optimized. Determination of riboflavin in green leafy vegetables were performed by dissolving 1.0 g of the sample in 0.2 M acetic acid and then measured directly by spectrofluorometer. Results showed that riboflavin content in spinach, mustard green and water morning glory were 4.2421 µg/g, 2.7450 µg/g and 2.2784 µg/g while riboflavin not detected in lettuce. It is proven that green leafy vegetables can act as natural source of riboflavin by taking it in adequate amount during meals.

Keywords: Green leafy vegetables, riboflavin, spectrofluorometer, wet digestion.

1. INTRODUCTION

Green leafy vegetables are rich source of essential minerals and vitamins that can help to detoxify our body and regular intake of this vegetable helps to keep away the serious diseases. Perhaps one of the most appealing benefits of green leafy vegetables is their low calorie and carbohydrate contents. These features make them an ideal food to facilitate achieving and maintaining a healthy body weight. Besides, almost every part of the green vegetables like their leaves, fruits, barks and so on is being used for food supplement production. A comparative study showed that the strongest antioxidant capacity displayed by leaves compared to fruits [1]. They are consumed in both rural and urban areas as a main food, food ingredient or traditional medicine [2]. Moreover, the consumption of green leafy vegetables which have the highest nutritional value will enhance the nutritional status of both poor rural and urban households who may not be in a position to consume enough vegetables because of affordability [3].

Although riboflavin is present in many common human foods, dark green vegetables constitute the major dietary sources [4]. Riboflavin has the chemical formula $C_{17}H_{20}N_4O_6$ while the chemical structure of this compound is shown in Figure 1. Riboflavin is converted to 2 coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are necessary for normal tissue respiration [5]. The content of riboflavin in human body fluids and organs relevant for functions of riboflavin as a photosensitizer in human [6]. Riboflavin also plays an important role in how your body functions. It helps lower homocysteine levels which can help protect against heart disease [7]. The antioxidant content and nutritional value are important for human consumption.

Noteworthy, different instrumental methods have been used for the determination of vitamin B_2 including electrochemical methods, spectrophotometry, derivative UV spectrophotometry, spectrofluorimetry, HPLC as well as capillary electrophoresis [8]. Considering the importance of the minerals, phytochemicals and antioxidants in human health and their presence in these indigenous green leafy vegetables, efforts to promote their consumption should be implemented.

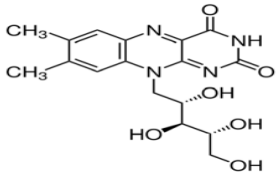

Figure 1 Chemical structure of riboflavin

Green leafy vegetables is widely used in the world and despite this widespread use of the vegetable, information is lacking on the effect of preservation method on nutrient contents and the best methods for its preservation. Many countries have poor eating habits that result in too many calories and not enough nutrients increases risk for chronic diseases. Despite this, more research into these green leafy vegetables will help address such problems. In order to reduce this trend, it is necessary to determine the possible nutrient content especially riboflavin in green leafy vegetables.

2. EXPERIMENTAL

2.1. Sample Collection

Samples used for this study were green leafy vegetables such as spinach (*Spinacia oleracea*), mustard green (*Brassica juncea*), water morning glory (*Ipomoea aquatic*) and also lettuce (*Lactuca sativa*) which were obtained from supermarket in Skudai, Johor. The leaves were separated from the stalks and rinsed with deionized water. Then, the leaves were dried in the oven overnight at 110°C for removing the moisture content. The dried leaves were ground to very fine powder and stored in an airtight container for further used. The container was also wrapped with aluminium foil to protect it from light in order to prevent the analyte from being destroyed.

2.2. Samples Preparation

Samples solution were prepared by dissolving the powdered green leafy vegetables in two beakers labeled as A and B containing sulphuric acid and acetic acid. The solution was heated on a hot plate for 1 hr and stirred using a magnetic stirrer. After 1 hr the solution were left to cool to room temperature and then filtered by using an Advantec filter paper to obtain a clear solution. The solution was diluted to 25 mL in a volumetric flask with 0.2 M acetic acid.

2.3. Optimization of Types of Solvent

The sample used for optimization analysis was spinach sample. The spinach samples were digested in two different solvents which were 0.2 M sulphuric acid and 0.2 M acetic acid and then were heated on a hot plate for 1 hr and stirred using a magnetic stirrer. The samples were gravitationally filtered into 25 mL volumetric flask and the concentration of riboflavin was determined by using fluorescence spectroscopy. Riboflavin has λ excitation at 466.57 nm and λ emission at 525.07 nm. The solvent that gave highest reading of riboflavin was selected as the best solvent and used for next parameter.

2.4. Optimization of Concentration of Solvent

The optimum solvent was prepared in three different concentrations which were 0.1 M, 0.2 M, and 0.3 M. The samples were taken and dissolved in the solvents of varied concentration in different beakers. Then, the concentration of riboflavin was measured by using fluorescence spectroscopy. The solvent of concentration that gave highest reading of riboflavin was selected for the next parameter.

2.5. Optimization of Sample Weight

The spinach samples used were 0.8 g, 1.0 g and 1.2 g. Then the samples were dissolved in the best solvent which is acetic acid with concentrations of 0.2 M. Next, the concentration of riboflavin was measured by using fluorescence spectroscopy. The ideal sample's weight for digestion was determined in such that it gave the reasonable reading of the riboflavin.

2.6. Method Validation

Validation of an analytical procedure is the process of defining the analytical requirements and confirming that the method under consideration has performance capabilities with what the application requires by which is established by the laboratory studies. In this study, a total of three types of method validation were used which include reproducibility, limit of detection and percentage recovery.

3. RESULTS AND DISCUSSION

3.1. Optimization of Types of Solvent

One of the objectives of this study is to find the best solvent used to determine the riboflavin content in green leafy vegetable samples. The best solvent was selected based on the extraction that can give the highest intensity by spectrofluorometer. There were various ways in extracting riboflavin. One of them is the pretreatment which is acid digestion with autoclaving to completely liberate riboflavin from protein bound forms and to hydrolyze phosphoric ester bonds. Riboflavin is initiated with acid hydrolysis by HCl to convert co-enzyme forms into free riboflavin [9]. Previous study indicated that riboflavin is more stable in acidic medium compared to basic medium. Therefore, both standard and sample solutions were prepared in acidic medium during the experiment.

Two different solvents were used for this purpose, which are sulphuric acid, H_2SO_4 and acetic acid, CH_3COOH. The concentration of each solvents used were fixed at 0.2 M. From this study, the best solvent used in extracting the riboflavin was acetic acid. Figure 2 shows that acetic acid gave the best result for the analysis of series of standard solution. As can be seen on Figure 3, sulphuric acid concentration only showed a small influence on the fluorescent intensity of series of standard solution. There are only small different on the readings of fluorescent intensity obtained. Therefore, in this study, acetic acid is the most suitable to use as a digesting solvent.

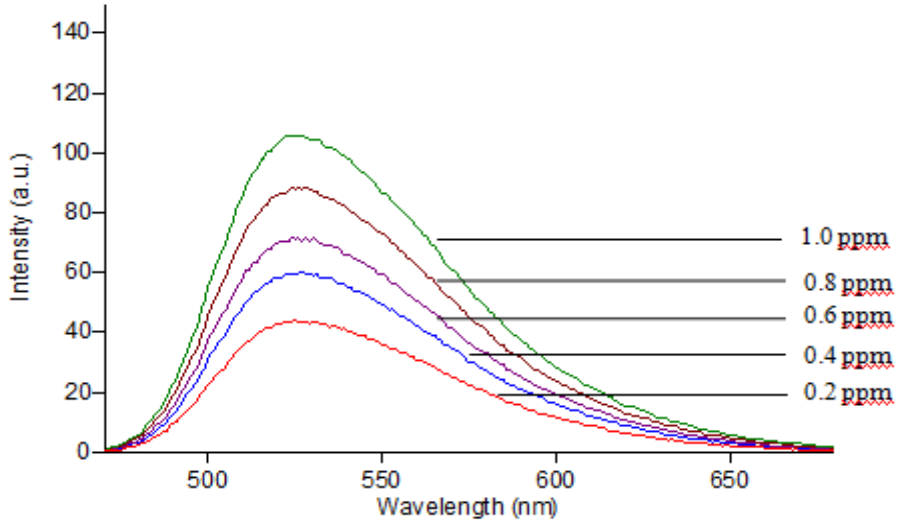

Figure 2 Spectrum of series of standard riboflavin solutions in acetic acid.

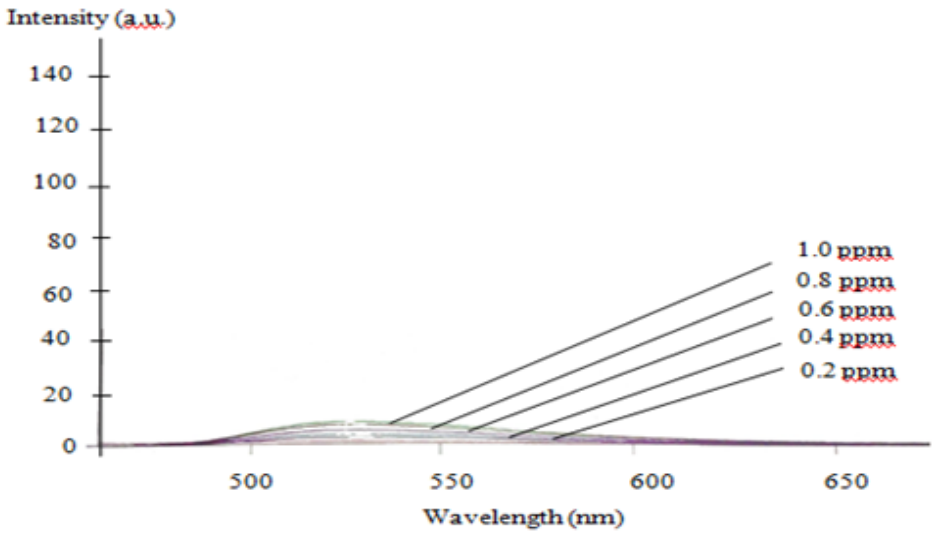

Figure 3 Spectrum of series of standard riboflavin solutions in sulphuric acid.

3.2. Optimization of Concentration of Solvent

Acetic acid was found to be the best solvent in extracting riboflavin from the green leafy vegetable samples. The concentration of acetic acid was varied from 0.1 M, 0.2 M and 0.3 M. The best concentration was based on the extraction that can give the highest intensity of fluorescence spectroscopy. Figure 4 show that 0.2 M of acetic acid is giving the best result for the digestion of riboflavin. Acetic acid concentration only showed a small influence on the digestion of the riboflavin. Result shows that there are only small different on the readings of riboflavin concentration obtained. Therefore, 0.2 M acetic acid has the higher concentration that lead to higher efficiency. In this study, the optimum condition in term of concentration of solvent was 0.2 M acetic acid.

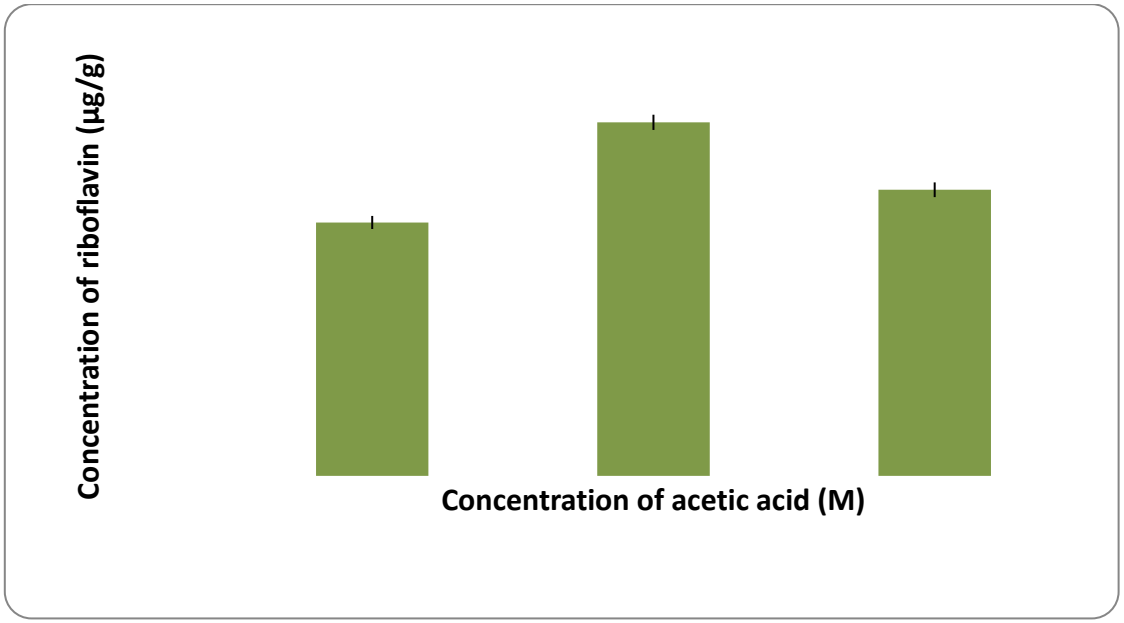

Figure 4 Concentration of riboflavin in varied concentration of acetic acid.

3.3. Optimization of Sample Weight.

The best solvent with optimum concentration, 0.2 M acetic acid was chosen to optimize the third parameter. Three different weights of green leafy vegetable samples have been used which were 0.8 g, 1.0 g and 1.2 g. Figure 5 shows results obtained from the different weight of green leafy vegetables sample varied in the experiment. The highest intensity of riboflavin have been detected from the sample that having weight of 1.2 g. As the weight of sample was increased and thus the compound was sufficiently extracted from the sample as can be seen on the sample of 1.2 g. The result shows that a 1.0 g of sample used gave a reasonable intensity of riboflavin compared to 0.8 g. The concentration given for 1.2 g is slightly higher as the weight of sample was increased. This might due to the amount of the compound extracted from samples were increased when higher sample weight was used. As in result, 1.2 g is the best sample weight but 1.0 g was chosen as the optimum condition because there is only slightly changed in riboflavin concentration with moderate amount of sample between 1.0 g and 1.2 g.

3.4. Analysis of Riboflavin by Using Optimized Conditions

In this study, three parameters of wet digestion for determination of riboflavin content in green leafy vegetables were being studied. 1.0 g of sample was digested in 0.2 M of acetic acid. Riboflavin content was then being analyzed by using fluorescence spectroscopy and the concentration of riboflavin is given in the Table 1. The spinach had the highest riboflavin content followed by mustard green and water morning glory. However, riboflavin content in lettuce was not detected. This might be suggested by the loss of analyte with prolonged exposure to light. Other than that, the reaction might be disrupted during digestion process due to increase of temperature. Besides, the concentration of target analytes in the lettuce sample was low enough to be detected by spectrofluorometer.

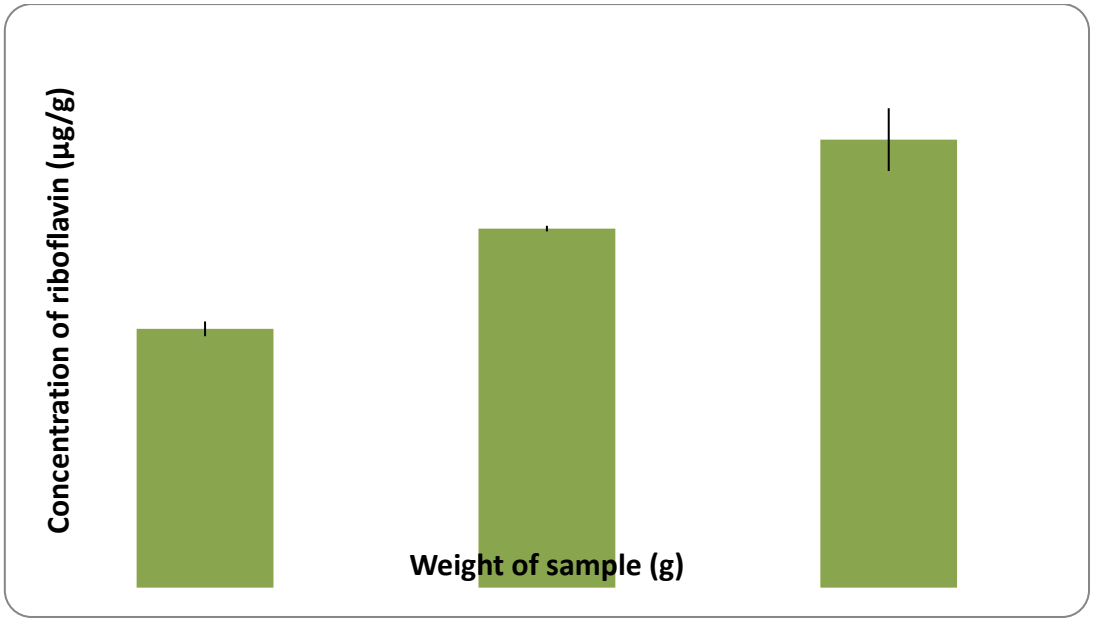

Figure 5 Concentration of riboflavin in varied weight of sample.

Table 1 Concentration of riboflavin in four different green leafy vegetable samples.

Green Leafy Vegetables	Concentration of riboflavin (µg/g ± s.d.)
Spinach	4.2421 ± 0.1911
Mustard Green	2.7450 ± 0.0341
Water Morning Glory	2.2784 ± 0.0857
Lettuce	ND

*ND – Not detected

3.5 Method Validation

Reproducibility

In this experiment, reproducibility was done in order to identify the precision of the method by comparing two measurements. Both results obtained in intraday and interday were compared to determine the closeness of the measurements with each other. The concentration of riboflavin obtained in intraday was 2.6907 ± 0.0750 µg/g while for interday was 2.6330 ± 0.0645 µg/g. By referring both results obtained, there is slight different in concentration of riboflavin in mustard green samples. Therefore, the result for both methods can be considered as close to each other. This result shows that both methods gave slightly different result for riboflavin concentration and can be considered as good precision.

Limit of Detection

Limit of detection is defined as three times of the standard deviation of 10 measurements of a reagent blank. In this study, the reagent blank was prepared by using digesting solution which is 0.2 M acetic acid (CH_3COOH). The limit of detection of riboflavin is 0.0632 µg/g which is can be considered as low detection limit.

Percentage of Recovery

In this study, the percentage recovery of riboflavin was calculated by using an equation. The percentage of recovery of riboflavin in mustard green is 34.87 %. This shows that the experimental has very poor recovery as the percentage recovery achieved was below than 50%. This might be suggested by the loss of analyte due to

increase of temperature and thus lead to the reaction inefficiency. Other than that, the reactions are incomplete and the reactants are not completely converted to products. Besides spills and other experimental errors, there are usually losses due to undesirable side reactions. Losses occur in the digestion and extraction of the desired product from the reaction mixture.

4. CONCLUSION

It was found that green leafy vegetables have high potential to be produced commercially as supplements due to its nutrient content. The digestion of solid sample by using wet digestion method was successfully investigated. The method was simple to be used as it does not require any special equipment, low cost and easily available. The analysis of riboflavin in green leafy vegetables sample was carried out by using Fluorescence spectroscopy. 0.2 M of acetic acid was a suitable extraction solution used to obtain efficient digestion. A 1.0 g of sample was suitable to provide a good intensity of the extracted riboflavin. The result showed that concentration of riboflavin in spinach, mustard green and water morning glory were 4.2421 μg/g, 2.7450 μg/g and 2.2784 μg/g while riboflavin not detected in lettuce. This finding was similar to a report published by Malaysian Journal of Nutrition which found no major differences in riboflavin content of green leafy vegetables [10]. The proposed procedure can be applied for the determination of riboflavin in green leafy vegetables sample with good precision and low detection limit but poor recovery. Therefore, the proposed method should be re-optimized and re-validated for the future study.

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