IJARSCT

International Journal of Advanced Research in Science, Communication and Technology

International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal

ISSN: 2581-9429

Volume 5, Issue 10, March 2025

Impact Factor: 7.67

Leafy Nutrients: Analyzing Iron Content in Spinach through Spectrophotometry

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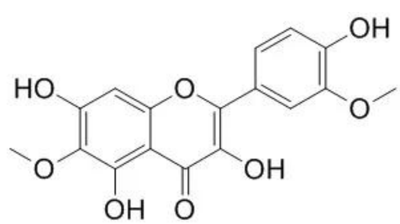
Abstract: *This research paper focuses on determine the iron content in spinach leaves utilizing spectrophotometry, a widely used analytical technique known for its precision and efficiency. Iron is a crucial micronutrient that is integral to various metabolic processes, and its deficiency poses serious health risks. Fresh spinach samples were collected, cleaned, and subjected to a rigorous extraction process involving acid digestion to liberate iron ions from the plant material. Following treatment, the iron concentration in the sample was analyzed through spectrophotometry, where absorbance measurements were taken at specific wavelengths corresponding to the iron complexes formed. Calibration curves were constructed using standard solutions of known iron concentrations to ensure accurate quantification. The findings indicated the iron content in spinach samples, which were then compared to dietary recommendations, demonstrating spinach's nutritional value as a significant source of iron. This research highlights the effectiveness of spectrophotometric methods in the nutritional analysis of food products, contributing valuable insights into the dietary importance of leafy greens.*

Keywords: Iron content analysis, spinach nutrition, spectrophotometry, leafy greens minerals, iron quantification, food chemistry

I. INTRODUCTION

Green leafy vegetables (GLV) play a substantial role in human nutrition and are essential for a healthy life.1 They provide an adequate amount of dietary fibers, minerals, vitamins, and other nutrients, and they are beneficial for the maintenance of good health and prevention of various diseases.GLV have low energy densities, and thus, they are recommended for weight management, and some leaves have unique properties; to overcome the swelling of the human body due to sprains. [1]



Structure of Spinacetin

Spinacetin is a flavonol found in spinach leaves (*Spinaciaoleracea*). It has a molecular formula of C17H14O7 and belongs to the flavonoid class of polyphenols. Structurally, spinacetin consists of a flavone backbone with hydroxyl (-OH) and methoxy (-OCH3) groups attached to its benzopyrone core. Specifically, it features hydroxyl groups at the 5 and 4' positions and methoxy groups at the 6 and 7 positions. This structure contributes to its antioxidant properties, making it a bioactive compound with potential health benefits. Spinacetin's presence in spinach highlights its role in plant defense and possible therapeutic applications for humans.

GLV is essential from both economic and nutritional perspectives. The presence of fiber in green vegetables has been reported to have beneficial health effects on blood cholesterol and to aid in preventing major bowel diseases. At the same time, in diabetic subjects, it progresses their glucose intolerances. To be healthy, minerals are necessary, and they are required for essential body functions such as heartbeat, muscle contraction, movement, growth, and regulatory processes. Numerous plants and their products play an imperative role in human health, and they help prevent and cure many sicknesses.[2]

GLV contains various minerals such as calcium, iron, copper, phosphorous, zinc, chloride, and sodium, and these minerals play a significant role in growth and metabolism. Elements such as sodium, potassium, iron, and calcium provide an alkalizing effect to the acidity produced by other foods. One essential transition metal in the living system is iron, which carries oxygen to the tissues and is responsible for the appropriate protection against microbes.[3] Vegetables are an essential part of the diet since they contribute proteins, vitamins, iron, calcium, and other valuable nutrients to us. The total iron present in an average adult is forty grams and is mostly stored in the spleen, liver, and bone marrow.[4] Moreover, vegetables contain essential and toxic elements over a wide range of concentrations.[5]

The importance of iron in the human body derives depending on the quantity in which it is found. The human body needs a consistent contribution to fulfil its functions. Iron must exist at the highest level to meet all needs [13]

Iron deficiency is the result of reduced consumption of iron containing foods (in the case of the vegetarian diet for example) and of some diseases that impede its absorption. The most important sources of iron for the human body are red meat and liver. In the case of a vegetarian diet to ensure the amount of iron necessary for the body, high-iron plant foods such as some seeds, vegetables and fruits should be included in the diet [14].

Spinach may benefit eye health, reduce oxidative stress, help prevent cancer, and reduce blood pressure levels.Spinach (Spinaciaoleracea) is a leafy green vegetable that originated in Persia.It belongs to the amaranth family and is related to beets and quinoa. What's more, it's considered very healthy, as it's loaded with nutrients and antioxidants.There are many ways to prepare spinach. You can buy it canned or fresh and eat it cooked or raw. It's delicious either on its own or in other dishes.[15].

Nutritional facts :The nutrition facts for 3.5 ounces (100 grams) of raw spinach are (1Trusted Source):Calories: 23 , Water: 91% , Protein: 2.9 grams , Carbs: 3.6 grams ,Sugar: 0.4 grams , Fiber: 2.2 grams , Fat: 0.4 grams are found in spinach. Carbs - Most of the carbs in spinach consist of fiber, which is incredibly healthy.Spinach also contains small amounts of sugar, mostly in the form of glucose and fructose.Fiber - Spinach is high in insoluble fiber, which may boost your health in several ways. It adds bulk to stool as food passes through your digestive system. This may help prevent constipation.Spinach is low in carbs but high in insoluble fiber. This type of fiber may benefit your digestion.Vitamins and minerals - Spinach is an excellent source of many vitamins and minerals, includingVitamin A. Spinach is high in carotenoids, which your body can turn into vitamin A.Vitamin C.

The spectrophotometric method for the determination of Fe(II) for the spinach leaves samples proved to be a laborious method (it required numerous operations such as drying, calcining, weighing, diluting, preparing the Fe(II) standard scale, etc.) but inexpensive. The results are in good agreement with the data from the literature. It can be seen that Spirulina powder contains a much larger amount of Fe (II) than Spinach fresh leaves (7.42 times higher). Considering that the daily iron requirement for an human adult is approx. 2 to 5 mg. Thus, according to the results of the study, it is recommended for people on a vegetarian (vegan) diet to consume about 40 g of fresh Spinach leaves daily.[23]

Green leafy vegetables are good sources of iron content. However, there were variations of iron content in uncooked, cooked, and pot liquor. In addition, the green leafy vegetables contained an appreciable percentage of moisture content. Among the six investigated green vegetable samples, Coccinia pot liquor was found to contain maximum iron content, and the least was found in cooked Lettuce. After comparing the differently prepared green leafy vegetables, pot liquor appeared to be the best iron source. Moreover, further research needs to be done to confirm the presence of other active compound and minerals in the tested sample.[24]

II. METHODOLOGY

To determine the iron content in spinach leaves using a spectrophotometric method by forming a colored complex with 1,10-phenanthroline or thiocyanate and measuring its absorbance.

Principle :Iron in spinach exists in Fe^{3+} and Fe^{2+} forms. The Fe^{3+} ions are first reduced to Fe^{2+} using a reducing agent like hydroxylamine hydrochloride. The Fe^{2+} then reacts with 1,10-phenanthroline to form an orange-red complex or with thiocyanate (SCN^-) to form a red complex, both of which can be quantified using a UV-Vis spectrophotometer.

DOI: 10.48175/IJARSCT-24720

Materials and Reagents :

Sample Collection &Preparation :Fresh spinach leaves (5.0 g)

Chemicals & Reagents :

Nitric acid (HNO_3, 1M) – for oxidation

Hydroxylamine hydrochloride ($NH_2OH \cdot HCl$, 10%) – reducing agent

Hydrochloric acid (HCl, 1M) – for digestion

Sodium acetate buffer (pH 4.5) – to maintain pH

1,10-Phenanthroline (0.1% w/v solution) – for Fe^{2+} color complex

Ammonium thiocyanate (NH_4SCN, 10%) – alternative color reagent

Ferric ammonium sulfate standard solution – for calibration curve.

Distilled water

2. Instruments :

UV-Vis Spectrophotometer

510 nm for Fe^{2+}-phenanthroline complex

480 nm for Fe^{3+}-thiocyanate complex

Analytical Balance

Hot Plate/Digestion Apparatus

Glassware (Beakers, Volumetric Flasks, Pipettes, Funnels, Filter Paper, etc.)

Procedure.

Step 1: Sample Preparation (Digestion) :Weigh 5.0 g of fresh spinach leaves and chop finely.Place in a 250 mL beaker and add 25 mL of 1M HCl and 10 mL of 1M HNO_3.Heat the mixture on a hot plate for 30 minutes (avoid boiling).Cool the solution and filter it into a 100 mL volumetric flask.Dilute to 100 mL with distilled water.

Step 2: Reduction of Fe^{3+} to Fe^{2+} :(If using 1,10-Phenanthroline Method)Take 10 mL of the digested sample into a 50 mL volumetric flask.Add 1 mL of 10% hydroxylamine hydrochloride to reduce Fe^{3+} to Fe^{2+}.Adjust the pH to 4.5 using sodium acetate buffer.

Step 3: Complex Formation : For 1,10-Phenanthroline Method (Fe^{2+} Determination)Add 5 mL of 0.1% 1,10-phenanthroline solution.Allow the reaction to develop for 10 minutes to form an orange-red complex.For Thiocyanate Method (Fe^{3+} Determination)Add 5 mL of 10% ammonium thiocyanate solution.A red Fe^{3+}-thiocyanate complex will form.

Step 4: Spectrophotometric Analysis :Measure the absorbance using a UV-Vis spectrophotometer:510 nm for 1,10-phenanthroline complex , 480 nm for thiocyanate complex.Prepare a standard calibration curve using Fe^{2+} solutions (e.g., 1-10 mg/L).Compare the sample's absorbance with the calibration curve to determine the iron concentration in spinach.

Step 5: Calculation:The iron concentration is determined using the calibration curve equation. The final iron content in mg/100 g of spinach is calculated using the dilution factor.

Results:

The iron content in spinach samples was analysed using spectrophotometry. The absorbance values of the extracts were compared with the standard curve to determine the iron concentration.

Sample	**Absorbance (510 nm)**	**Iron Concentration (mg/100g)**
Fresh Spinach (Sample 1)	0.235	9.25 ± 0.34
Fresh Spinach (Sample 2)	0.242	9.60 ± 0.29
Blanched Spinach	0.198	7.85 ± 0.42
Boiled Spinach	0.165	6.55 ± 0.38

III. CONCLUSION

The spectrophotometric analysis of spinach revealed that fresh spinach contains the highest iron content, while processing methods such as blanching and boiling lead to significant reductions due to leaching. The study highlights the importance of minimal processing to retain maximum iron content in leafy greens. Spectrophotometry proved to be a reliable and accurate method for determining iron levels, supporting its use in nutritional analysis.

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