

IRON (II) SPECTROFOTOMETRIC DETERMINATION IN SPINACH (*SPINACIA OLERACEA*) FRESH LEAVES AND SPIRULINA (*ARTHROSPIRA PLATENSIS*) POWDER

Alexandrina FODOR¹, Anda Ioana Grațîela PETREHELE¹, Claudia MORGOVAN¹, Teodora SOCA²

¹ University of Oradea, Romania, Oradea, 418070, Str. Universității no. 1, afodor@uoradea.ro

² University of Oradea, Faculty of Informatics and Sciences, Master CSA I -Student

Abstract: The determination of the iron content in two important components of the vegetarian diet (fresh Spinach leaves and Spirulina powder) were performed using spectrophotometrical method, using as colour indicator o-o'-phenanthroline ($C_{12}H_8N_2$), at pH = 4-5 and 510 nm. The results indicated that Spirulina contains a much larger amount of Fe (II) than spinach (7.42 times higher).

Key words: *Spinacia Oleracea* fresh leaves, *Arthrospira Platensis* powder, UV-VIS Spectrophotometric determination, o-o'-phenanthroline

INTRODUCTION

Iron plays an important role in biochemistry, forming molecular oxygen compounds in hemoglobin and myoglobin; two compounds which are common proteins that play role in the transport of oxygen in vertebrates body's. Iron is also the metal most commonly used by the most important redox enzymes, which are involved in cellular respiration, oxidation and reduction processes in plant and animal organisms [1].

Iron is an essential mineral for the human body. The iron source for animal organisms is the intake food and is lost through the continuous exfoliation of the cells of the digestive tract, urinary tract and skin cells. It is stored in the form of ferritin in the cells of the spleen, liver and bone marrow, and another part circulates in blood bounded to transferrin (a transport protein specific to iron) [2].

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 [2].

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 [3].

Two important components of the vegetarian diet, used as sources of Fe, fresh Spinach (*Spinacia Oleracea*) fresh leaves and Spirulina (*Arthrospira Platensis*) powder, were investigated in terms of the Fe (II) content.

The determination of the iron content was performed using spectrophotometrical method, using as colour indicator o-o'-phenanthroline ($C_{12}H_8N_2$), at pH = 4-5 and 510 nm, based on the reaction [4]:

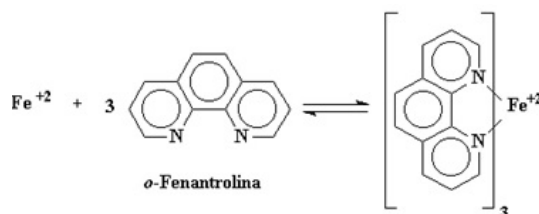


Figure 1. Fe(II) reaction with o-o'-phenanthroline

MATERIALS AND METHOD

The used method was the spectrophotometric determination using the etalon scale method.

The used reagents were: deionised water solution of: 25% sodium acetate, 10%, hidroxilamine clorohidrat, 0,5 % ethanol alcoholic solution of o-o'-phenanthroline, H₂SO₄, Fe (II) etalon solution for the etalon scale preparation (1-5 mg Fe (II)/L) 25 mg/L Fe(II) (prepared by dissolving 67.96 mg FeSO₄ in 25 mL distilled water acidified with H₂SO₄).

For the spectrophotometric analysis a Spectrophotometer Vis Jenway 6320D, 320 - 1000 nm were used.

Sample preparation

The spinach (*Spinacia Oleracea*) fresh leaves were placed in an oven (at 1000 °C) for 1 hour in order to determine the humidity. The humidity of the sampled spinach leaves was 76,84%. The dried leaves were wetted to be subsequently treated for Fe (II) extraction.

Spirulina (*Arthrospira Platensis*) was purchased in powder form therefore does not require drying and soaking.

5 g of samples powders (Spinach and Spirulina), were place in porcelain capsules and place in a calcining oven at 500 °C for one hour. The obtained powders were moistened with 5 drops of sulphuric acid and taken with distilled water in small portions, filtered and passed into 50 ml graduated flasks and brought to the mark with distilled water.

For the Spinach sample: 10 ml of sample solution were taken from the flask and pass into a 25 ml flask and brought to the mark with distilled water.

For the Spirulina sample: 1 ml of sample solution was taken and passed into a 25 ml graduated flask and brought to the mark with distilled water.

Higher dilution is practiced in the case of Spirulina due to the fact that in the literature it is stated that Spirulina contains higher quantities of Fe (II) than Spinach [8]

The following reagents (same reagents as for the standard scale) are added to the two samples:

- 5 ml 25% sodium acetate
- 1 ml 10% hydroxylamine chlorhydrat,
- 1 ml 0,5 % ethanol alcoholic solution of o-o'-phenanthroline.

The added solutions were stirred well, distilled water was added to the mark and then the obtained mixture was leave to stand for 1 hour.

The blank solution was prepared from:

- 5 ml 25% sodium acetate
- 1 ml 10% hydroxylamine chlorhydrat,
- 1 ml 0,5 % ethanol alcoholic solution of o-o'-phenanthroline.

The added solutions were stirred well, distilled water was added to the mark and then the obtained mixture was leave to stand for 1 hour.

The standard solutions (standard scale), consisting in 10 Fe (II) solutions with concentrations between 1-5 mg/L are prepared as follows:

In 10 25 ml graduated flasks were added:

- 5 ml 25% sodium acetate
- 1 ml 100,5 % ethanol alcoholic solution of o-o'-phenanthroline.
- 1,2,3,4 and respectively 5 ml Fe (II) etalon solution (25 mg/L)

The added solutions were stirred well, distilled water was added to the mark and then the obtained mixture was leave to stand for 1 hour.

All the spectrophotometric determination was performed at 510 nm.

RESULTS

By graphically representing the result of the measurement of the absorbance for the standard scale, the right of calibration is obtained:

The measured absorbance's for the investigated samples were presented in figure 2.

The amounts of Fe(II) determined for spinach and spirulin samples from the interpolation on the calibration curve are:

Spinach: 3,6245 mg/L

Spirulin: 3,5021 mg/L

Calculus:Spinach

1000 ml.....3,6245 mg/L mg Fe(II)

10 ml.....x

x= 0,036245 mg Fe(II)

taking into account the dilution: 0,036245 · 50 = 1,81225 mg Fe(II) în dried leaves

taking into account the moisture of the Spinach leaves: 1,81225 · 100/76,84= **2,35847 mg Fe(II) in 100 g fresh Spinach leaves**

Calculus:Spirulin

1000 ml.....3,5021 mg/L mg Fe⁺²

10 ml.....x

x= 0,035021 mg Fe(II)

taking into account the dilution: 1,75105·

500= 17,5105 mg Fe(II) in 100g Spirulina

powder

The results are in good agreement with the data from the literature (3,8 mg Fe (II) in 100 g fresh Spinach leaves and respectively 28 mg Fe(II) in 100 g Spirulina powder)[4].

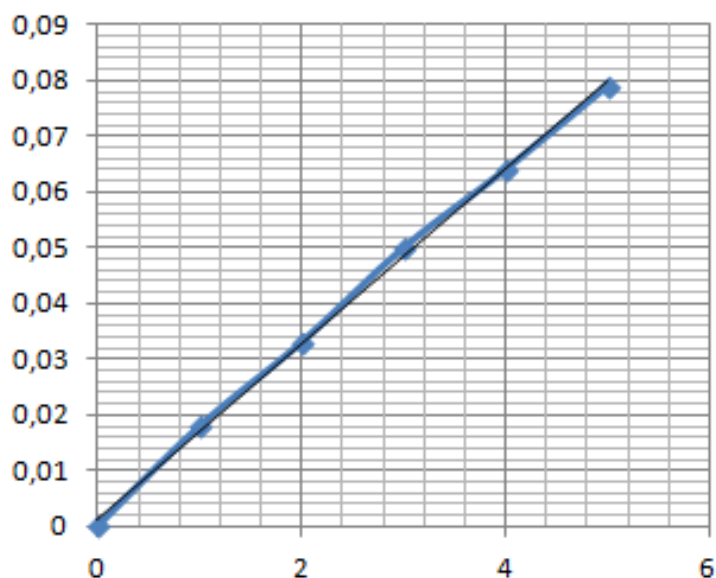


Figure 2. Calibration curve for the determination of the Fe (II)

Table 1. The absorbance's measured for the spinach leaves samples and for the spiruline samples

	Absorbance Determination 1	Absorbance Determination 2	Absorbance Determination 3	Absorbance Average
Spinach (<i>Spinacia Oleracea</i>)	0,0563	0,0564	0,0561	0,0562
Spirulina (<i>Arthrospira Platensis</i>)	0,0498	0,0497	0,0499	0,0498

mg Fe(II) / 100 g

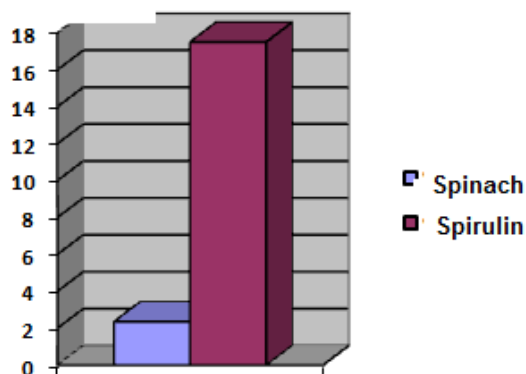


Figure 3. Fe (II) content in the samples of fresh Spinach leaves and Spirulina powder

CONCLUSIONS

The spectrophotometric method for the determination of Fe(II) for the two plant 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 [4].

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 or about 4 g of Spirulina powder.

REFERENCES

- [1] L., Ghizdavu, Chimie bioanorganică, Editura Poliam Cluj-Napoca, 2000
- [2] A., Găman, G., Găman, Explorarea metabolismul fierului, INFOMedica 1998, (8), pp. 54
- [3] R. Eliliot, The Vegetarian Low-Carb Diet, Piatkus Book, UK, 2005
- [3] L. D. Harvey, Modern Analytical Chemistry, McGraw-Hill, 2000
- [4] USDA Food Composition Databases-<https://ndb.nal.usda.gov/ndb/h>, 08.01.2020