# ANALYTICAL CHEMISTRY FOR CHEMICAL ENGINEERS: A HANDS-ON ACTIVITY FOR DISCOVERING COOKING EFFECTS ON MICRONUTRIENT QUANTITIES IN RAW AND COOKED VEGETABLES

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## INTRODUCTION

Hands-on activities (in which students have to observe and collect experimental data) become a useful aid to encourage the understanding and learning of new concepts and scientific procedures.[1] In addition, they are optimal tools to improve emotional satisfaction, intellectual accessibility, and usefulness of the laboratory and laboratory equipment.[2]

As previously discussed in the literature, professors employ different strategies to involve undergraduate students in research in the early stages of their studies. For example, Piunno et al. [3] present "Launching Your Research" as a non-traditional learning environment in which students take field samples and design laboratory experiments to investigate them at the University of Toronto Mississauga (UTM, Canada). Franco-Mariscal [4] combines four powerful teaching strategies (context-based teaching, inquiry-based teaching, murals, and augmented reality) to discover chemical elements in food, and Chowdhury [5] proposes to explore an industrial process to increase students' motivation and engagement based on classroom and laboratory practices.

In the above-mentioned publications, hands-on activities, field-based teaching strategies, and self-guided research are used to establish connections between chemistry principles and daily life. By following these principles, the Chemical Engineering Laboratory (ChEnLab) at the Universidad Nacional del Sur (UNS, Bahía Blanca, Argentina) proposes a space in which the students play the starring role in learning. The main characteristics of the course are described below.

ChEnLab is a second-year course in the Chemical Engineering degree program at the UNS.[6] Each year, an average of 50 students attend this one-semester-long course. Throughout the semester, students learn the basic principles to develop their own research projects. As previously discussed,[7] the main purpose of these projects is to improve abilities and skills, such as self-initiative, teamwork, technical drafting, oral presentations, and technical writing.[8-10]

According to the academic program, the ChEnLab course is divided into two parts. The first part (approximately 1½ months long) corresponds to the performance of guided practical activities by which students become familiar with conventional laboratory activities such as titrations, solubility tests, viscosity measurements, and spectrophotometric

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analysis. Improving data analysis and communication skills is essential at this stage. In the second part (the rest of the semester), students are divided into working groups (with a maximum of 2 or 3 students per team), and each group develops its own research project selected according to their own interests. Throughout this period, students collect data on the topic and then choose and carry out the appropriate experiments to meet their own objectives while complying with the schedule proposed by the instructors. At the end of this stage, students write a report on the activities and main results of their project and present its content in a short lecture (15 minutes). The final grade considers a student's performance at the different stages of the course.

ChEnLab creates an empowering environment in which self-learning and self-assessment are promoted.[11] In addition, students and professors encourage an atmosphere in which all members of the ChEnLab community are respected and accepted, promoting inclusion, diversity, equity, and integrity.[12,13] Over the years, participating students have developed many alternative/novel laboratory activities as part of the self-guided research projects begun in the ChEnLab course. Many of these activivities were tested and improved during the subsequent years, and these enhanced versions were subsequently published.[14,15] In this paper we present the results of one of these activities that was taken from a research project developed by students who attended the ChEnLab course.

## RESEARCH DESIGN

Plant growth depends on the absorption of specific chemical elements. Apart from macronutrients (P, N, S, or K, among others), micronutrients represent a family of chemical elements that are required in much smaller amounts for vegetables to grow. Cationic metals such as $Mg^{2+}$, $Fe^{2+}$, $Ca^{2+}$, and $Zn^{2+}$ are well-recognized micronutrients essential for plant growth.[16]

Aside from vegetable growth, micronutrients also play a crucial role in human health. Scientific literature reports a list of essential micronutrients in the human diet, which includes minerals (such as Fe, Mg, and Ca) and vitamins (water- and fat-soluble ones).[17] In this sense a great deal of research has been performed over the years on different ways of increasing micronutrient uptake from vegetables, such as plant breeding or molecular genetic approaches.[18] The main target of these strategies is to improve the nutritional quality of plants and vegetables in order to ensure adequate nutrition.

A practical activity with the aim to determine micronutrient amounts in raw and processed vegetables is reported herein. The activity was proposed by a team of three students during the ChEnLab course in 2018 and was carried out without sophisticated analytical devices or automatic analyzers.[19,20] The main purpose of this activity was the detection of Ca, Mg, and Fe losses before and after cooking the vegetables, using conventional chemical procedures such as titration and spectrophotometry. Results were obtained in (at least) triplicate and statistically analyzed.

### Learning Outcomes

The main learning outcomes from the activity are as follows:

- Explain different analytical techniques to measure micronutrients
- Design experiments in order to study the effect of cooking on a micronutrient level
- Use statistical techniques (ANOVA) to correlate cooking effects on micronutrient availability

The activity resembles previous work already published,[21-23] and it is presented as an easy, economically feasible, and environmentally friendly procedure that enables an alternative approach to conventional laboratory analyses, relating chemistry topics from the curriculum with daily issues. By using this approach, creativity and critical thinking are expected to be promoted.[24]

### Materials and Methods

Spinach, sugar beets, and carrots were obtained from local groceries. They were chosen as materials sources because of their easy handling and proven uses as pedagogical tools, as was already reported by literature.[25] Raw and clean samples from vegetables (square chips or small pieces from leaves) were placed in boiling water or in a conventional steamer and cooked for 15 minutes. Eriochrome Black T (Anedra), murexide (Anedra), and ethylenediaminetetraacetic acid sodium salt dihydrate (EDTA, Anedra) were used for the titration of Ca and Mg. Munsey´s method[26] was applied for the quantification of Fe, using o-phenanthroline (Anedra) and hydroxylamine hydrochloride (Anedra) as reagents. Concentrated sulfuric acid ($H_2SO_4$, Cicarelli), sodium hydroxide pellets (NaOH, Anedra), concentrated ammonia solution (Cicarelli), and ammonium chloride (Anedra) were employed as received. The free version of InfoStat® software[27] was used to perform the statistical analysis. Statistical significance was set at $p < 0.05$. Then, the post hoc Fisher's Least Significant Difference (LSD) test was used to compare populations. (For those readers interested in further details regarding ANOVA analysis, and supplementary material and information for students or professors, please send an email to Dr. Andrés E. Ciolino at aciolino@plapiqui.edu.ar).

## RESULTS

### Quantification of Calcium and Magnesium

Quantifications of Ca and Mg using standard EDTA solutions and titrations are widely employed in the analysis of water or aqueous solutions. Conventional procedures and classical laboratory activities regarding this technique are widespread.[28-31] In the particular case of this activity, it was applied to determine micronutrient content in the studied vegetable samples after dry ashing procedures.

It is worth mentioning before presenting results that Ca and Mg quantification in vegetables offers an alternative to the conventional method for assessing water hardness. In this sense, the use of this technique adapted to vegetables provides a different approach to different concepts already discussed by the specific literature and in previous courses, such as metal complexes, complexometric techniques, stability of complexes versus pH, and precision and accuracy of complexometric titrations, among others.[32-35] As major outcomes, students were expected to be able to explain not only quantitative differences between samples but also other important aspects such as pH values, the use of different titration indicators, and the selectivity of the different methods.[36]

Figure 1 shows the results obtained for Ca (dark grey) and Mg (light grey) contents in spinach samples.

As an example of the ANOVA results obtained by students, Tables 1 and 2 and Figure 2 show the results for Mg contents in spinach for the raw sample and the samples obtained after cooking (from InfoStat® software report). The ANOVA tables give "p-value" (Table 1) and results from the Fisher LSD Test (Table 2). A "p-value" lower than 0.05 means statistical differences between samples. The tables illustrate that the Mg amount obtained from samples were statistically different according to the analysis.

### Quantification of Iron

Figure 3 shows the calibration curve obtained for standard $Fe^{2+}_{(aq)}$ solutions using Munsey´s method and solution standards ($\lambda$ = 510 nm). The experimental data present a good linear fit for the standard samples prepared. From this fitting, the amounts of Fe in the studied vegetables (sugar beets and carrots), both raw and cooked, were calculated.

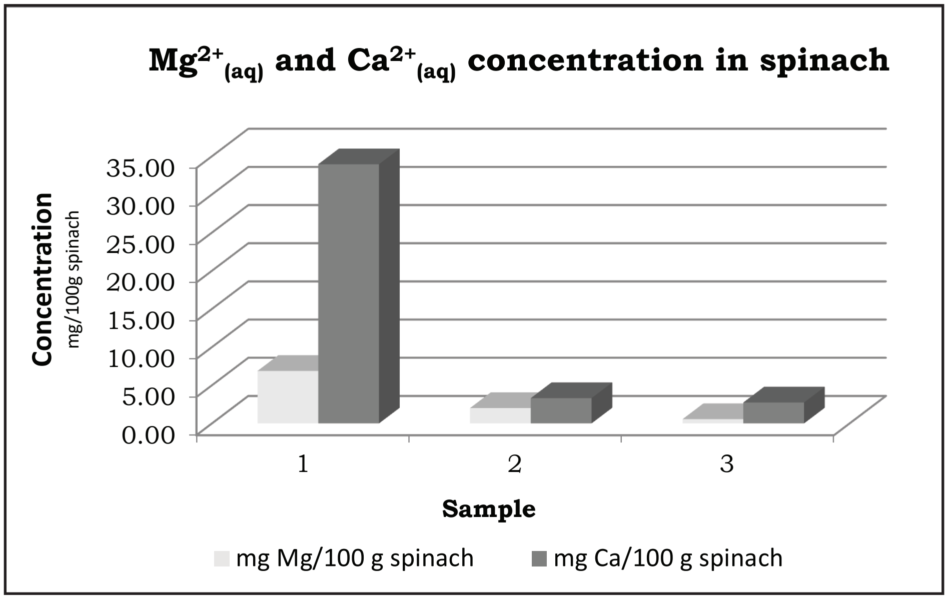


*Figure 1. Calcium (Ca, dark grey) and magnesium (Mg, light grey) concentrations in raw (1), steamed (2), and boiled (3) spinach samples (cooking time: 15 min). Values reported are an average of three titrations.*

**TABLE 1**
**Analysis of Variance (partial SS) for Mg in spinach.**

| S.V. | SS | df | MS | F | p-value |
|---|---|---|---|---|---|
| Model | 65.59 | 2 | 32.79 | 164.36 | < 0.0001 |
| Sample | 65.59 | 2 | 32.79 | 164.36 | < 0.0001 |
| Error | 1.20 | 6 | 0.20 | | |
| Total | 66.79 | 8 | | | |

**TABLE 2**
**Mg (mg)/100 g spinach. Fisher LSD test ($\alpha$ = 0.05, LSD = 0.8924).**

Error = 0.195. df = 6

| Sample of Spinach | Mean | n | S.E |
|---|---|---|---|
| Boiled | 0.55 | 3 | 0.26 |
| Steamed | 2.01 | 3 | 0.26 |
| Raw | 6.87 | 3 | 0.26 |

Before discussing the results obtained on the studied samples taken from vegetables, it is worth mentioning that the absorption of Fe in the human body occurs in its low oxidation state, $Fe^{2+}_{(aq)}$.[37] This point (often referred to as speciation in toxicological, environmental, and nutritional chemistry) should be clearly understood at the beginning of this test, since it is important not only for the analysis of results but also for the justification of the use of hydroxylamine hydrochloride to reduce $Fe^{3+}_{(aq)}$ to $Fe^{2+}_{(aq)}$ ions in the tested samples.

The obtained results are presented in Figure 4.

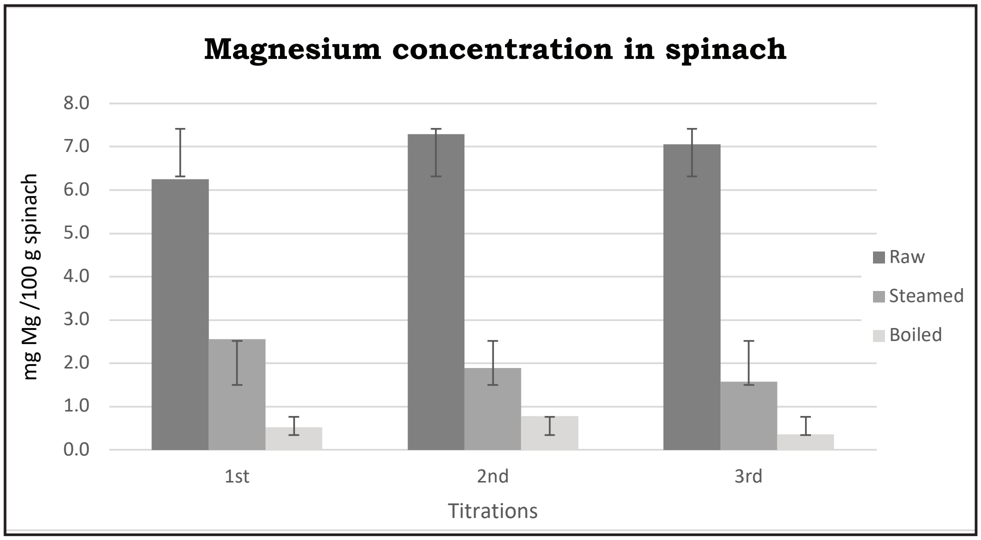


***Figure 2.*** *Mg concentrations in spinach (mg/100 g) for raw and cooked samples.*

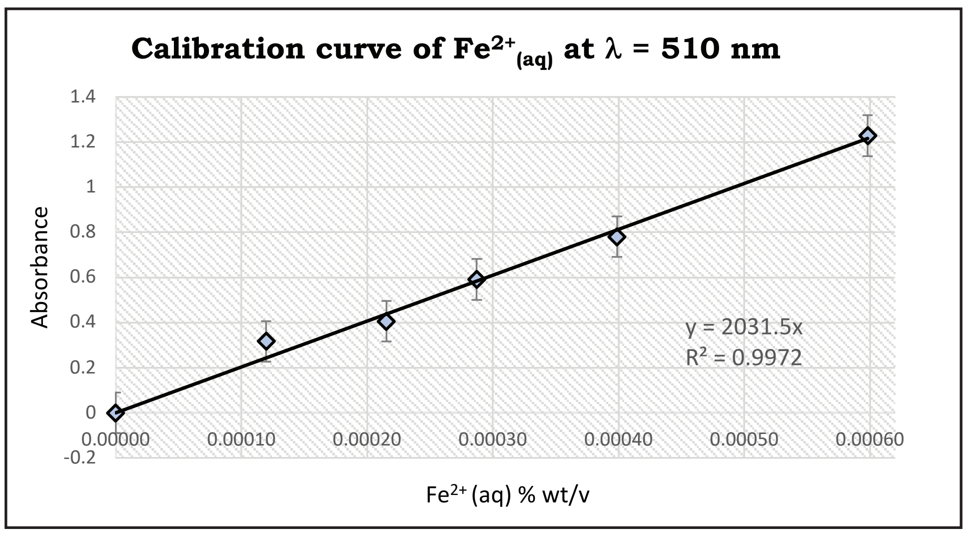


***Figure 3.*** *Calibration fitting for standard $Fe^{2+}(aq)$ solutions (Munsey´s method).*

## DISCUSSION

As it is reported by literature [38] each raw vegetable contains specified quantities of micronutrients in its composition. Different cooking methods change these initial quantities. For example, a higher content of Ca and Mg are observed in an unprocessed sample. After cooking, the concentration of both micronutrients (Ca and Mg) decreases regardless of the cooking method. However, boiling for 15 minutes results in the lowest amounts of micronutrients. Since micronutrients are water-soluable, the use of boiled water to cook the vegetables promotes the micrionutrients' dissolution, therefore yielding a cooked sample with lower quantities of Ca and Mg than both a raw sample and a sample cooked using steam.[39] Besides learning the fundamentals of titration techniques, students were able to note several physicochemical differences between the two cooking methods, namely those related to heat transfer, mass transport, and solubility mechanisms. From the obtained results, they were able to deduce that these phenomena are completely different if one uses steam or boiled water. Additional information on these topics was later discussed with professors and presented in a written project report.

Regarding Fe analysis, although its concentration decreases after cooking, the remaining amount is important for both cooking methods. These results can be compared with the ones for Ca and Mg, in which the final concentration of both micronutrients decreases. ANOVA analysis was performed to check the differences between the cooking methods of both vegetables, sugar beets and carrots. According to the analysis (Fisher´s LSD test), the final $Fe^{2+}_{(aq)}$ amounts show no differences between cooking methods for both vegetables. It is inferred that the material nature of the vegetable has the same influence on the dissolution phenomenon, regardless of the cooking method.

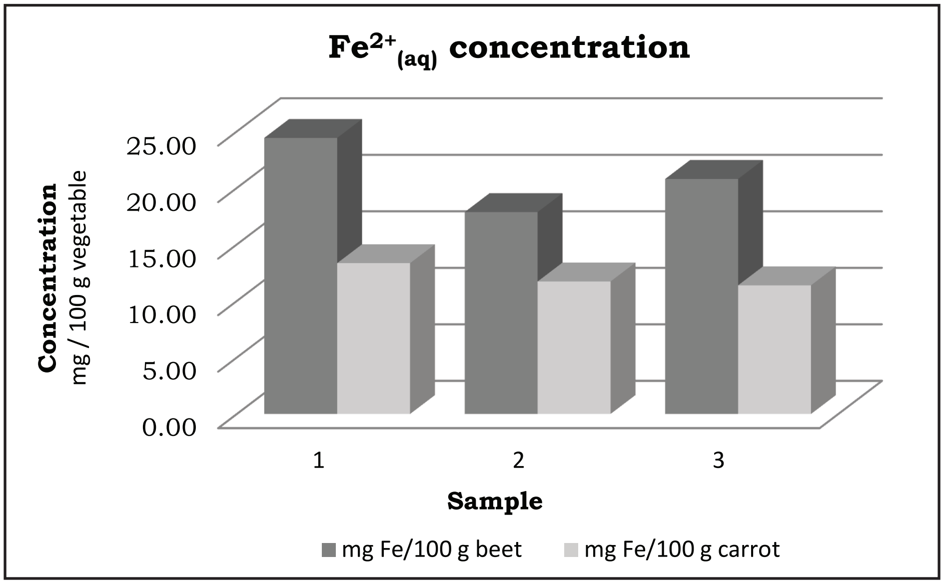


***Figure 4.*** *Average $Fe^{2+}$(aq) concentration in: raw (1), steamed (2), and boiled (3) sugar beets (dark grey) and carrots (light grey).*

undergraduate courses (titration and spectrophotometric analysis). Micronutrient losses were analyzed using statistical analysis (ANOVA). In the particular case of spinach, the amounts of Ca and Mg decreased in the processed samples when compared to the raw sample. For sugar beets and carrots, Fe losses were less substantial, although greater differences were observed in sugar beets. Although simple in nature, the activity proposed in this work provides a good linkage between daily activities and chemistry, and it can be thought of as a good introduction to several basics from the curriculum, such as separation processes, solubility, spectrophotometry, statistics, and data analysis.

After finishing the activity, the three students were able to explain the use of the different analytical techniques to determine the amounts of micronutrients in the studied vegetable samples and to correlate them with the different cooking methods. In addition, they were able to relate a daily activity such as cooking with a physicochemical phenomenon (the dissolution of difference micronutrients). Regarding the main learning outcomes of the activity (bulleted in the Research Design section), the observation of the students' behavior at the lab, their answers at given queries, and their analysis of the obtained experimental data (particularly, by using ANOVA tools) were employed as evaluation sources. In this sense, students were able to present their own research (written report and oral presentation at the end of the course) by designing an experimental activity schedule together with a proper analysis of the data obtained. In their own words, *"(…) From this activity, I could really realize the importance of Chemistry to provide answers to daily issues, such as food quality from cooking methods. We had some assumptions and hypothesis about it, but after this activity we really tested them in a scientific way (…)"*.

## CONCLUSIONS

The quantification of Ca, Mg, and Fe as micronutrients in selected vegetables before and after boiling or steaming was used as a hands-on activity at CheEnLab. The organization and development of this activity was performed by the students themselves as part of the academic goals of the course. They used chemical procedures conventional for

## ACKNOWLEDGMENTS


The authors would like to thank Departamento de Ingeniería Química (DIQ) and Universidad Nacional del Sur (UNS, Bahía Blanca, Argentina) for their financial support. The technical assistance at the laboratory provided by Gabriela Martínez Ipucha and Miguel Nievas and the grammar check performed by Alicia Comelli are also acknowledged.


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