# What is the optimum boiling time of spinach to reduce the oxalic acid while reserving vitamin C content?

§1 Introduction

Spinach is rich in diverse nutrients, including vitamins B12, B22, and C, as well as various minerals and phytochemicals. However, it also contains oxalate (H2C2O4), a naturally occurring compound in plants, which is present in relatively high levels in spinach. Oxalate can bind with minerals such as calcium and magnesium in the body, forming insoluble oxalate crystals like calcium oxalate and magnesium oxalate. This process inhibits the absorption of these essential minerals (Mou, 2008; Genannt Bonsmann et al., 2007; Arias-Carmona1 et al., 2014). Elevated oxalate levels in the diet can contribute to the development of kidney stones.

Scientific studies have shown that boiling spinach can significantly reduce its oxalic acid content, with boiling being more effective than steaming or baking in decreasing soluble oxalic acid (Chai et al., 2005). However, prolonged boiling also leads to the dissolution of a substantial amount of vitamin C into the cooking water due to the high-temperature environment. Research by Kojima et al. (2007) reviewed the impact of various cooking methods on vitamin retention, revealing that 36% to 73% of vitamin C can be lost depending on the cooking conditions.

In this assignment, I aim to investigate the concentrations of oxalic acid and vitamin C in spinach at different boiling times. By doing so, I hope to determine the optimal boiling time that minimizes oxalic acid content while preserving as much vitamin C as possible.

§2 Method

To measure the oxalic acid content in spinach, begin by thoroughly washing fresh spinach leaves to remove any dirt or impurities. Next, the washed leaves are smashed into a juice, which is then filtered to obtain a clear spinach extract. This spinach juice is subjected to boiling at 100°C for a specified period. After boiling, the spinach juice is prepared for a chemical reaction with potassium permanganate (KMnO4), a potent oxidizing agent used to oxidize oxalic acid (H2C2O4).

The chemical reaction involved is as follows:

2 KMnO4 + 5 H2C2O4 + 3 H2SO4 = 2 MnSO4 + K2SO4 + 10 CO2 + 8 H2O

To determine the amount of oxalic acid remaining in the spinach, a titration method is used. During the titration, KMnO4 is gradually added to the spinach juice. Initially, the KMnO4 reacts with the oxalic acid and becomes colorless. Once all the oxalic acid has reacted, any additional KMnO4 will not be reduced and will impart a persistent purple color to the solution.

By carefully recording the volume of KMnO4 solution of known concentration required to reach the endpoint—when the purple color remains stable—we can calculate the amount of oxalic acid initially present in the spinach. This process allows for an accurate quantification of the oxalic acid content in the spinach, providing valuable data for further analysis and comparison.

§3 Experimental Design

**§3.1 Variables**

**Independent Variable:** Boiling time of spinach 0s (fresh spinach), 20s, 40s, 60s, 80s, 100s,120s. When spinach is put into the beaker, start the stopwatch and when the time is reached, put the spinach out.

**Dependent Variable:** Concentration of oxalic acid, calculated by the volume of acidified KMnO4 solution titrated.

There were also a number of other factors which might have affected the accuracy of the experiment in determining the content of oxalate, and therefore needed to be controlled. The table below shows the controlled variables and ways to control them.

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| Controlled variables | Temperature of water | Bath the test tube into 298K water in order to control the temperature by absorbing the heat produce by the decomposition. |
| pressure | Do the experiment at 1 atm, keep the delivering tube uncongested. |
| Volume of water | The water should be 300ml in 298K temperature and 1 atm pressure |

**§3.2 Materials**

* Potassium permanganate (KMnO4), distilled water, dilute sulfuric acid (H2SO4), analytical balance (accuracy 0.0001 g), glass rods – Required for preparing an acidified potassium permanganate solution.
* Volumetric flasks (100 mL) – Utilized twice to prepare 200 mL of 0.05M KMnO4 solution.
* Fresh spinach leaves, funnels, filter paper, juicer – Necessary for extracting spinach juice.
* Burette (25 mL), conical flasks (250 mL) – Employed for titration purposes.
* Electromagnetic heater, thermometer, wire racks, large beaker – Used for boiling spinach juice in a water bath.

The use of these materials involves certain safety precautions. The potential safety hazards and their mitigations are outlined below:

* Sulfuric Acid (H2SO4): This strong acid is corrosive and can cause serious injuries to the eyes and skin. It is imperative to wear safety goggles and handle the acid with utmost care during the experiment.
* Heating: The heating process is necessary for boiling spinach juice and preparing the acidified KMnO4 solution. The heat poses a risk of burns; therefore, the heater must be turned off after boiling, and the temperature should be allowed to decrease to a safe level before handling.
* Disposal of Solutions: Post-experiment, any remaining H2SO4 in the acidified potassium permanganate solution and the reaction products must be disposed of in designated waste containers to prevent environmental contamination.

**§3.3 Procedures**

1. Remove the roots of fresh spinach, wash it with distilled water, dry it naturally, and divide it into two parts, labeled as A/B, 100 g each. Add 4.4ml H2O to the test tube
2. Configure 0.0500mol/L acidic KMnO4 solution: weigh 0.01×158.034 g/mol =1.580g of KMnO4 with an analytical balance, add 50mL of dilute H2SO4 with the concentration 6mol/L, dilute it with the appropriate amount of distilled water, heat it up to a slight boil for 15min, cool it down, put it in a cool place to stand for 12h, filter it, and then condense it to form 200 mL of acidified KMnO4 solution.
3. Preparation of spinach juice: Put 500mL spinach into the juicer, add an appropriate amount of water (600mL), and power stirring for 3 min. The spinach juice then is poured into a beaker, filtered, and fixed into a 600mL solution. Measure 60mL of the spinach juice and pour it into the beaker. Put the beaker into boiled water for a fixed time, for example, 20s, to stimulate the environment of boiling spinach. After the boiling procedure finishes, filter it again.
4. Take 10 mL of the boiled spinach juice. Titrate the spinach solution with potassium permanganate solution, when the last drop of potassium permanganate solution into the solution becomes light purple and does not fade within 30s, this time to reach the endpoint of the titration. The amount of oxalic acid in the 10mL sample of spinach juice can be calculated as 5[KMnO4]×V(KMnO4)/2, and the concentration of oxalic acid in spinach can be calculated.
5. Repeat the titration 2 times and record the volume of acidified KMnO4 solution used.
6. Similarly, boil other 60mL samples for 0s, 40s, 60s, 80s, 100s, 120s and titrate them to determine the oxalic acid concentration.

§5 Data Collection

**§5.1 Raw Data and Uncertainties**

**§5.1.1 Experimental Data**

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| Boiling Time (s) | Volume 1 (mL) | Volume 2 (mL) | Volume 3 (mL) |
| 0.0 | 10.0 | 10.9 | 10.4 |
| 20.0 | 6.0 | 6.3 | 6.1 |
| 40.0 | 8.9 | 7.6 | 7.6 |
| 60.0 | 5.5 | 5.3 | 5.5 |
| 80.0 | 5.1 | 5.4 | 5.2 |
| 100.0 | 7.0 | 7.0 | 7.1 |
| 120.0 | 5.0 | 4.8 | 4.7 |
| Table 1. Boiling time of spinach juice and corresponding volume of acidified KMnO4 solution titrated until no reaction occurs. | | | |

**§5.1.2 Uncertainties**

* Uncertainty of boiling time: The boiling time uncertainty should align with people’s reaction time, which is around 0.25s. Therefore, the uncertainty of boiling time is , as it should be kept to 1 significant figure.
* Uncertainty of the volume of acidified KMnO4 solution titrated: the uncertainty of the volume should be calculated with reference to Table 1. For example, the uncertainty of the volume reacted for the boiling time of 0s should be

While the average volume should be calculated as

and all uncertainties and average volume reacted are listed in Table 2.

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| Boiling Time (s) | Average Volume (mL) |
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| Table 2. Boiling time of spinach juice and the corresponding average volume of acidified KMnO4 solution, both with uncertainty. | |

**§5.2 Data Processing**

The amount of oxalic acid can be calculated through the volume of acidified KMnO4 used. Take the 0s boiling time as an example, the volume of acidified KMnO4 solution used is 10.4mL, therefore, the quantity of KMnO4 reacted is

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And the quantity of H2C2O4 in the sample is

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The 500g of fresh spinach leaves are mixed with 600g of water to produce 600 mL of spinach juice. We assume that the same proportion of spinach and water is left in the filter procedure, then the mass of fresh spinach leaves in every 10mL sample of spinach juice should be

The mass of H2C2O4 in raw spinach juice is then

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We then measure the amount of H2C2O4 by expressing how much H2C2O4 are there in 100g of spinach

At the same time, an example of calculating the uncertainty of the quantity of H2C2O4 existing in 100g of spinach is shown below.

The percentage error of P(H2C2O4) can be expressed as

So the absolute error of P(H2C2O4) is

Thus, the mass of oxalate preserved in 100g of raw fresh spinach is 2.6g±0.1g.

Similarly, we can derive the mass of oxalate preserved in 100g of fresh spinach leaves boiling for a specific time and its uncertainty, shown in Table 3 below.

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| Boiling Time (s) | Average Volume (mL) |
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| Table 3. Boiling time of spinach juice and the corresponding average volume of acidified KMnO4 solution, both with uncertainty. | |

**§6 Graphical Analysis**

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| Figure 1. Oxalate quantity in 100g of spinach under different boiling times.  There exists a horizontal error bar of ±0.3s, but is too small to be visible on the graph. |

The graph of oxalate mass in 100g of spinach versus boiling time reveals a clear trend. Initially, there is a significant reduction in oxalate mass from 2.58g at 0 seconds to 1.52g at 20 seconds, indicating that a substantial amount of oxalate leaches out during the first 20 seconds of boiling. After this initial sharp decline, the oxalate mass continues to decrease but at a slower rate. The values drop gradually to 1.39g at 40 seconds, 1.35g at 60 seconds, 1.30g at 80 seconds, 1.26g at 100 seconds, and finally to 1.20g at 120 seconds.

The initial sharp decline suggests that the early stages of boiling are most effective for reducing oxalate content. This rapid decrease is likely due to the high solubility of oxalates in water, allowing them to leach out quickly when spinach is first exposed to boiling water. After the initial 20 seconds, the rate of oxalate loss slows down, indicating diminishing returns from prolonged boiling. Most of the easily leachable oxalate is removed early in the process, and further reductions require more time.

After 120 seconds, the oxalate mass stabilizes around 1.20g, suggesting a limit to how much oxalate can be leached out through boiling alone. The graph demonstrates that boiling spinach significantly reduces its oxalate content, especially within the first 20 seconds. Prolonged boiling continues to reduce oxalate levels but at a decreasing rate. For practical purposes, a short boiling time might be sufficient to achieve substantial oxalate reduction, while longer boiling times provide only marginal additional benefits.

**§7 Consideration of VC Amount**

According to the research conducted by Igwemmar et al. (2013), the spinach amount in 100mL of spinach juice to time is shown in Table 4.

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| Vegetable | Raw Sample | 5 mins | 15 mins | 30 mins |
| Spinach | 36.20 | 32.60 | 25.36 | 14.48 |
| Table 4. The Vitamin C (mg/100mL) content of vegetables as affected by heating time, by Igwemmar et al. (2013). | | | | |

The vitamin C content decreases over time, but even after 5 minutes of boiling (300 seconds), spinach retains 32.60 mg/100mL of vitamin C compared to its raw value of 36.20 mg/100mL. This indicates that most of the vitamin C is preserved with short boiling durations. Although prolonged boiling further decreases vitamin C levels, the short 5-minute duration results in minimal loss. This retention of vitamin C is crucial, as it ensures that spinach remains a good source of this essential nutrient even after boiling.

Therefore, boiling spinach for a short time (around 5 minutes) effectively reduces oxalate content while preserving most of its vitamin C. This duration appears to strike a good balance between reducing harmful oxalates and maintaining beneficial nutrients like vitamin C. The significant reduction in oxalate mass within the first 20 seconds and the minimal loss of vitamin C over 5 minutes highlight the efficiency of short boiling times in optimizing the nutritional benefits of spinach. Therefore, for those aiming to lower oxalate intake without compromising vitamin C levels, boiling spinach for 5 minutes is an effective and practical approach.

**§8 Conclusion**

In conclusion, the experiment demonstrates a notable trend in the oxalate content of spinach as boiling time increases. Initially, there is a sharp decrease in oxalate content, followed by a more gradual decline until it stabilizes at approximately 1.20g per 100g of spinach. This trend suggests that prolonged boiling beyond a certain point does not significantly reduce oxalate content further.

The experiment's findings are in line with previous research conducted by Igwemmar et al., which indicates that the boiling time of spinach within 5 minutes has little effect on the amount of vitamin C present. This implies that the boiling duration can be optimized to reduce oxalate content while retaining a substantial amount of vitamin C.

The sharp decrease in oxalate content during the initial boiling indicates that oxalates, which are compounds associated with kidney stones and interfere with calcium absorption, are leaching out into the boiling water. As boiling continues, this leaching process slows down, eventually reaching a point where the reduction in oxalate content becomes marginal. This suggests that most of the oxalates leach out rapidly during the first few minutes of boiling. Finding that oxalate reduction stabilizes around 1.20g per 100g of spinach implies that there's an optimal boiling time beyond which further reduction in oxalate content is minimal. This information is valuable for individuals who want to reduce oxalate intake without compromising the nutritional quality of spinach. The reference to Igwemmar et al.'s research highlights that while oxalate decreases with boiling, the impact on vitamin C content is minimal within a certain range of boiling times. This suggests that by boiling spinach for a specific duration (around 2 to 5 minutes), one can achieve a balance where oxalate content is reduced, yet the majority of vitamin C remains intact.

Practically, this means that for those concerned about oxalate intake or kidney stone formation, boiling spinach for a short duration can be an effective method to reduce oxalate content while retaining essential nutrients like vitamin C.

**§9 Evaluation**

The experiment demonstrates a discernible trend in the relationship between boiling time and oxalate content in spinach. This clarity in the results provides a strong foundation for drawing conclusions regarding the effect of boiling duration on spinach nutrition. The distinct pattern observed makes it easier for others to understand and interpret the findings, enhancing the credibility of the study.

Additionally, the experiment contributes to raising awareness about the nutritional changes that occur during cooking, particularly focusing on spinach. By highlighting the impact of boiling time on oxalate reduction and vitamin C preservation, it promotes informed dietary choices. This awareness empowers individuals to make healthier decisions regarding food preparation methods, considering both nutrient retention and reduction of potentially harmful compounds

Some disadvantages still exist and should be improved. One limitation of the experiment is its narrow focus solely on oxalate content and vitamin C preservation during boiling. This limited scope neglects other nutritional aspects of spinach and potential changes in other compounds that could occur during cooking. Consequently, the broader nutritional profile of spinach remains unexplored

Also, the model is simplified: boiling in a controlled laboratory environment simplifies the cooking process, which may not fully reflect real-world cooking scenarios. In reality, cooking involves numerous variables such as different cooking methods, varying temperatures, and ingredient combinations, which could affect nutrient retention differently than boiling alone. This simplified model might not capture the complexity of actual cooking practices.

Addressing these limitations could involve expanding the scope of the research to encompass a wider range of nutritional parameters or considering more realistic cooking conditions to provide a comprehensive understanding of the effects of cooking on spinach nutrition.

Igwemmar, N.C., Kolawole, S.A., & Imran, I. (2013). Effect Of Heating On Vitamin C Content Of Some Selected Vegetables. International Journal of Scientific & Technology Research, 2, 209-212.