What is the relation between boiling time ($t = 0, 20, 40, 60, 80, 100, 120$s) and oxalic acid concentration in spinach?

1. Introduction

Spinach is rich in diverse nutrients, including vitamins B12, B22, and C, as well as various minerals and phytochemicals. Growing up, spinach salad was a regular dish at our family table. While my family members enjoy its fresh, crisp taste, I have always disliked the bitter flavor that comes with it, produced by the high-level presented oxalic acid ($H_2C_2O_4$) in spinach. What's more, 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 [1, 2, 3], and elevated oxalate levels in the diet can contribute to the development of kidney stones.

At the same time, I discovered that boiled spinach does not have the bitterness favor, making it much more palatable for me. This led me to wonder about the underlying chemistry involved in this change in concentration of oxalic acid within time.

2. Background

Spinach 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 [4]. This is primarily due to the fact that oxalic acid allows a substantial portion of it to leach out into the cooking water during the boiling process. In contrast, steaming or baking retains more of the oxalic acid within the spinach, as there is less water contact. As a result, boiling can generally enhances the overall nutritional profile of the spinach by reducing the anti-nutrient content.

Extracting Spinach Juice 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 juice by a juicer and then filtered to obtain a clear spinach extract. This spinach juice is then put in the beaker and boiled at 100°C for the given time recorded by the stopwatch. After boiling

for a specific length of time, it is then filtered again to remove the precipitates.

Measuring Oxalic Acid After boiling, the spinach juice is prepared for a chemical reaction with potassium permanganate ($KMnO_4$), a potent oxidizing agent used to oxidize oxalic acid ($H_2C_2O_4$).

The chemical reaction involved is as follows:

$$2\,KMnO_4 + 5\,H_2C_2O_4 + 3\,H_2SO_4 \longrightarrow 2\,MnSO_4 + K_2SO_4 + 10\,CO_2 + 8\,H_2O$$

To determine the amount of oxalic acid remaining in the spinach, a titration method is used. During the titration, potassium permanganate $KMnO_4$ is gradually added to the spinach juice. $KMnO_4$ is originally in purple due to $MnO_4{}^-$ ion, but it is then reduced by oxalic acid to form $Mn_2{}^+$, making the titrated mixture colorless. Once all the oxalic acid has reacted, any additional $MnO_4{}^-$ will not be reduced and will impart a persistent purple color to the solution.

By carefully recording the volume of $KMnO_4$ 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.

Research Question: In what extent does boiling time t ($t = 0, 20, 40, 60, 80, 100, 120$s) affect oxalic acid concentration by measuring the volume of $KMnO_4$ titrated?

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. The time upper boundary is set as 120s because cooking receipt [5] suggests that the

optimum cooking time for spinach is 120s.

Dependent Variable: Concentration of oxalic acid, calculated by the volume of acidified $KMnO_4$ 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. Table 1 shows the controlled variables and ways to control them.

Controlled Variable	**Reason**	**Approach**
Spinach Part Taken	Different parts of spinach may preserve different concentrations of oxalic acid.	Take spinach leaves, since the part consumed in salad is mainly the leaf.
Concentration of Spinach Juice	The fundamental water amount in spinach is not enough to get juice, and extra water is needed. The amount of solution in each sample is fixed, so the concentration of juice should also be the same.	Get spinach juice in one turn with a fixed amount of water, and split into different groups of solutions to be boiled later.
Concentration of $KMnO_4$	The amount of oxalic acid is calculated by the volume of acidified $KMnO_4$ titrated, so the concentration of $KMnO_4$ should be controlled.	Prepare the solution in one go and record the concentration of $KMnO_4$ in the solution.
Concentration of H_2SO_4	The redox reaction for titration involves H^+ as the reactant, so the concentration of H_2SO_4 must remain in excess.	Prepare the solution in one go and record the concentration of H_2SO_4, ensuring it remains in excess by calculation.
The temperature of boiling	The temperature of boiling is determined by the pressure and can affect the speed of decomposition of oxalic acid in spinach juice, therefore should be controlled	Bath the beakers containing spinach juice into boiling water, and the experiment is conducted in a short time so that the water pressure is relatively constant and resulting in same boiling temperature.

Table 1: Controlled Variables and Approaches to Control

3.2. Materials and Apparatus

Material	**Amount Required**
Potassium Permanganate ($KMnO_4$)	2g
Distilled Water	1L
Dilute Sulfuric Acid (H_2SO_4)	50mL (6mol/L)
Fresh Spinach Leaves	500g

Table 2: Materials and required amounts for the experiment.

Apparatus	**Number Required**	**Scale / Capacity**	**Uncertainty (Typical)**
Analytic Balance	1	0.0001g to 200g	± 0.0001g
Glass Rods	3	N/A	N/A
Volumetric Flasks	2	200mL	± 0.05mL, ± 0.12mL
Funnels	2	N/A	N/A
Filter Paper	20	N/A	N/A
Juicer	1	1L	N/A
Burette	1	25mL	± 0.05mL
Conical Flasks	3	250mL	N/A
Electromagnetic Heater	1	N/A	N/A
Thermometer	1	−10°C to 110°C	± 0.5°C
Wire Racks	2	N/A	N/A
Large Beaker	1	1000mL	N/A
Small Beakers	6	100mL	N/A
Large Graduated Cylinder	1	100mL	±0.5mL
Small Graduated Cylinders	2	25mL	±0.05mL

Table 3: Apparatus names, numbers, scales, and uncertainties.

3.3. Procedures

Preperation of Spinach Juice

1. Take the leaves of fresh spinach, wash it with distilled water, dry it naturally

2. Put 500g spinach into the juicer, add an appropriate amount of water, and power stirring for 3 mins.

3. Filter the spinach juice (Figure 1(a)), fix it into 600mL solution, record the total amount of water used (600mL).

4. Measure 60mL of the spinach juice and pour it into small beakers

5. Bath the beaker for a fixed time $t = 20$s to stimulate the environment of boiling spinach

6. Filter the solution after boiled (Figure 1(b)).

7. Repeat step 5 and 6 for $t = 0, 40, 60, 80, 100, 120$s.

(a) (b)

Figure 1: Procedures for preparing the spinach juice: (a) filter the fresh spinach juice; (b) boil the spinach juice.

Configure 0.0500mol/L acidic $KMnO_4$ solution

1. Weigh 0.01×158.034g/mol =1.580g of $KMnO_4$ with an analytical balance

2. Add 50mL of dilute H_2SO_4 with the concentration 6mol/L
3. Dilute it with the appropriate amount of distilled water
4. Heat it up to a slight boil for 15min, cool it down, and put it in a cool place to stand for 12h
5. Filter the solution and condense it to form 200mL of acidified $KMnO_4$ solution.

Titration for Oxalic Acid Content Determining

1. Fill burette with acidified $KMnO_4$ solution, record the initial volume V_i.
2. Take 10mL of the boiled for $t = 20$s spinach juice.
3. Titrate the spinach solution with $KMnO_4$ 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, record the volume remained in the burette V_f.
4. The amount of acidified $KMnO_4$ solution consumed can be calculated by $V(KMnO_4) = V_f - V_i$. The amount of oxalic acid in the 10 mL sample of spinach juice can be calculated as $5[KMnO_4] \times V(KMnO_4)/2$, and the concentration of oxalic acid in spinach can be calculated.
5. Repeat the titration for 2 more times and record the volume of acidified $KMnO_4$ solution used.
6. Repeat steps 1-5 for spinach juice boiled for time duration $t = 0, 40, 60, 80, 100, 120$s to determine their oxalic acid concentration.

3.4. Safety Precautions and Environmental Consideration

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

Safety Precautions Listed in Table 4.

Potential Dangers	Safety Precautions
$KMnO_4$ is a strong oxidizing agent and may cause harm to the body	Wear safety goggles and nitrile gloves to prevent contact with eyes or skin. If the solution splashes onto the skin during heating, wash immediately with cold water.
H_2SO_4 is a strong acid and can be corrosive, also when diluting H_2SO_4 large amount of heat is released	Handled with care to avoid contact with skin and eyes. Ensure proper ventilation when working with this substance.
Heating the solution may cause burns	Exercise caution when heating. Use a towel or heat-resistant pad to avoid direct contact with the beaker.

Table 4: Potential dangers and corresponding safety precautions

Environmental Considerations Certain materials used in this experiment can have adverse environmental effects if not disposed of properly:

- $KMnO_4$: Contains $MnO_4{}^-$ ions, which act as strong oxidizing agents. Manganese (Mn) is a heavy metal that can be harmful to the environment if not handled correctly.
- H_2SO_4: Can lead to soil acidification, which may disrupt soil health and affect plant growth.
- Leftover reacted solution: Contains $Mn_2{}^+$ ions, which are heavy metal ions that can damage soil if released untreated. The solution may also contain excess acid and unreacted $MnO_4{}^-$ ions, which can further contribute to environmental harm.

Therefore, the materials used in this experiment should be disposed into designated waste containers to prevent environmental contamination.

4. Data Collection

4.1. Raw Data and Uncertainties

Experimental Data

Boiling Time / s ± 0.3s	**Burette (Acidified $KMnO_4$ Solution) Reading** / mL ± 0.05mL					
	Trial 1		**Trial 2**		**Trial 3**	
	V_i	V_f	V_i	V_f	V_i	V_f
0.0	2.41	12.40	12.40	23.31	14.41	24.43
20.0	4.92	10.91	10.91	17.23	17.23	23.31
40.0	0.89	6.82	6.82	12.51	12.51	17.80
60.0	1.51	7.01	7.01	12.32	12.32	17.82
80.0	1.74	6.81	6.81	12.21	12.21	17.41
100.0	2.91	7.91	7.91	13.09	13.09	18.22
120.0	1.02	6.03	6.03	10.78	10.78	15.53
Room Temperature: 300K; **Air Pressure:** 1.010×10^6Pa						

Table 5: Boiling time and reading of the burette containing acidified $KMnO_4$ solution over the 3 different trials

Qualitative Observations

- The color change in the titration process is notable: the solution was originally light yellow, and when titrated, it first became purple, following the color of the acidified $KMnO_4$ solution. When the beaker was stirred, the color changed gradually from light purple to grey to white/nearly no color.

- There are bubbles coming out of the beaker; also when stirred, the solution seemed very easily to form bubbles on the surface of the solution.

4.1.1. 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 $\pm 0.25\text{s} \approx \pm 0.3\text{s}$, as it should be kept to 1 significant figure.

Uncertainty of the volume of acidified $KMnO_4$ solution titrated The uncertainty of the volume should be calculated with reference to the uncertainty in burette (±0.05mL). Therefore,

the fixed uncertainty caused by the apparatus should be $\pm(0.05 + 0.05) = \pm 0.1$mL.

4.2. Data Processing

The amount of oxalic acid can be calculated through the volume of acidified $KMnO_4$ used, which is calculated by $V_f - V_i$, all the volumes of acidified $KMnO_4$ used are listed in Table 7 with uncertainties in boiling time and volume reacted.

Boiling Time / s $\pm$ 0.3s	**Volume of Acidified $KMnO_4$ Reacted** / mL $\pm$ 0.1mL		
	Trial 1 (V_1)	**Trial 2** (V_2)	**Trial 3** (V_3)
0.0	10.0	10.9	10.0
20.0	6.0	6.3	6.1
40.0	5.9	5.7	5.3
60.0	5.5	5.3	5.5
80.0	5.1	5.4	5.2
100.0	5.0	5.2	5.1
120.0	5.0	4.8	4.8

Table 6: Boiling time and volume of acidified $KMnO_4$ reacted in the 3 different trials.

Take the Trial 1 for $t = 0$s boiling time as an example, the volume of acidified $KMnO_4$ solution used is $V_1 = V_f - V_i = 12.40 - 2.41 = 9.99 \approx 10.0$mL. Therefore, the quantity of $KMnO_4$ reacted is

$$n(KMnO_4) = 10.0 \times 10^{-3} \times 0.0500 = 5.00 \times 10^{-4}\text{mol}.$$

And the quantity of $H_2C_2O_4$ in the sample is

$$n(H_2C_2O_4) = 5.00 \times 10^{-4} \times 5/2 = 1.25 \times 10^{-3}\text{mol}.$$

The 500g of fresh spinach leaves are mixed with 600g of water to produce 600mL 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

$$10 \times 500/(500 + 600) = 4.545\text{g}$$

The mass of $H_2C_2O_4$ in raw spinach juice is then

$$m_0(H_2C_2O_4) = 1.25 \times 10^{-3} \cdot 90.03 \approx 0.113\text{g}$$

We then measure the amount of $H_2C_2O_4$ by expressing how much $H_2C_2O_4$ are there in 100g of spinach

$$m(H_2C_2O_4) = 0.113/4.545 \cdot 100 = 2.48\text{g}.$$

At the same time, an example of calculating the uncertainty of the quantity of $H_2C_2O_4$ existing in 100g of spinach is shown below.

The percentage error of $m(H_2C_2O_4)$ can be expressed as

$$\Delta m(H_2C_2O_4)/m(H_2C_2O_4) = \Delta V(KMnO_4)/V(KMnO_4)$$

So the absolute error of $m(H_2C_2O_4)$ is

$$\begin{aligned}\Delta m(H_2C_2O_4)/m(H_2C_2O_4) &= \Delta V(KMnO_4)/V(KMnO_4)\\ \Delta m(H_2C_2O_4) &= \pm 0.1/10.0 \cdot 2.48 = \pm 0.0248 \approx \pm 0.02\text{g}\end{aligned}$$

Thus, the mass of oxalate preserved in 100g of raw fresh spinach is (2.48 ± 0.02)g.

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 7 below.

Boiling Time / s $\pm$ 0.3s	**$H_2C_2O_4$ Content in 100g of Spinach Leaves** / g $\pm$ 0.02g		
	Trial 1 (m_1)	**Trial 2** (m_2)	**Trial 3** (m_3)
0.0	2.47	2.70	2.48
20.0	1.48	1.56	1.51
40.0	1.47	1.41	1.31
60.0	1.36	1.31	1.36
80.0	1.26	1.34	1.29
100.0	1.24	1.28	1.27
120.0	1.24	1.18	1.18

Table 7: Boiling time and content of oxalic acid obtained in 100g of spinach over the 3 different trials

Average Take $t = 0$s as example, we can calculate the average of the three trials' values,

$$\begin{aligned}\bar{m} &= \frac{m_1 + m_2 + m_3}{3} \\ &= \frac{2.47 + 2.70 + 2.48}{3} \\ &= 2.55\text{g}\end{aligned}$$

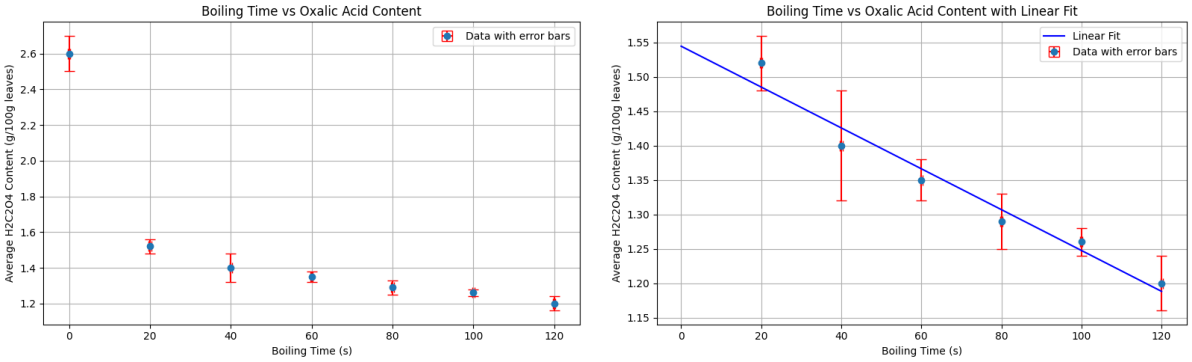
Standard Deviation To calculate the error drawn by different trials, the standard deviation is calculated for different boiling times t. Comparing the standard deviation and the error drawn by the apparatus uncertainty, we derive the average mass of oxalic acid presented in 100g of spinach leaves boiled for $t = 0, 20, 40, 60, 80, 100, 120$s:

5. Graphical Analysis

The plot of the scattered points is shown in Figure 2(a), and in Figure 2(b) the first point $t = 0$s is excluded because there exists a rapid decrease in the duration from $t = 0$s to $t = 20$s. Other points excluding $t = 0$s form a linear relationship with a linear best-fit line passing through all their error bars.

Boiling Time / s $\pm$ 0.3s	**$H_2C_2O_4$ Content in 100g of Spinach Leaves (g)**			
	Trial 1 (m_1)	**Trial 2** (m_2)	**Trial 3** (m_3)	**Average** ($\bar{m}$)
0.0	2.47	2.70	2.48	2.6 ± 0.1
20.0	1.48	1.56	1.51	1.52 ± 0.04
40.0	1.47	1.41	1.31	1.40 ± 0.08
60.0	1.36	1.31	1.36	1.35 ± 0.03
80.0	1.26	1.34	1.29	1.29 ± 0.04
100.0	1.24	1.28	1.27	1.26 ± 0.02
120.0	1.24	1.18	1.18	1.20 ± 0.04

Table 8: Boiling time and content of oxalic acid obtained in 100 g of spinach over the 3 different trials and average with errors calculated from standard deviation



(a) $t = 0$s included

(b) $t = 0$s excluded

Figure 2: Oxalate quantity in 100g of spinach under different boiling times. (a) with $t = 0$s included; (b) excluded, with linear fit.

Figure 2 reveals a clear trend of decreasing in oxalic acid content in spinach after being boiled. Initially, there is a significant reduction in oxalate mass from (2.6 ± 0.1)g at 0 seconds to (1.52 ± 0.04)g 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 relatively constant rate. The values drop gradually to 1.40g at 40 seconds, 1.35g at 60 seconds, 1.29g at 80 seconds, 1.26g at 100 seconds, and 1.20g at 120 seconds.

The initial sharp decline suggests that the early stages of boiling are most effective for reducing oxalate content. Most of the easily leachable oxalate is removed early in the process, and further reductions require more time. After 120 seconds, the oxalate mass may still decrease, but at a slower rate.

6. Conclusion

In conclusion, the increase in boiling time t ($t = 0, 20, 40, 60, 80, 100, 120$s) can result in a decrease in oxalic acid concentration in spinach.

This follows the chemical explanation: oxalic acid content is reduced in the procedure of boiling because first, soluble oxalic acid may leach into the water when heated [6]; and additionally, oxalic acid can decompose when heated under high temperature [7], undergoing the reaction

$$(\mathrm{COOH})_2 \longrightarrow \mathrm{CO_2} + \mathrm{CO} + \mathrm{H_2O}$$

Initially, from 0-20s the oxalic acid content shows a sharp decrease and after 20s the decreasing trend is more gradual, approximately linear, which may be a result of most soluble oxalic acid leaching to the water and/or most oxalic acid undergoing the decomposition procedure from 0 to 20s.

Compared with other experiments, [6] states that in spinach the oxalic acid content in 100g of spinach is 1.76g and after boiled reduced to 1.32g; [4] stated that before boiling, the oxalic

acid content in spinach is measured to be 1.145g and after boiling for 5 minutes, it decreases to 0.460g. Both results align with the decreasing trend demonstrated in this experiment, showing the reliability.

The **systematic errors** are contributed by the limitations of the apparatus' least counts and human reaction time when boiling water. However, the data of the experiment has a relatively low percentage error of about 2% contributed by the systematic error, showing that the apparatus used in the experiment is of high accuracy.

The **random errors** contributed to the uncertainty including the color change in the titration may be subtle or missed, leading to over- or under-result. However, the uncertainties presented by the standard deviations all within ±6% means that the experiments are repeatable and the results are hopefully precise and consistent.

From our experiment, the conclusion can be drawn that for spinach consumption, boiling would be recommended as it can effectively reduce the amount of oxalic acid in spinach.

However, the length of the boiling time can be taken less into consideration, as after 20s the change in oxalic acid content within time is slower. While other nutrition preserved in spinach content decreases as the boiling time increases. Research conducted in [8] indicates that the boiling time of spinach within 5 minutes reduced vitamin C content from 15.34mg/100g to 5.94mg/100g.

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.

7. Evaluation

7.1. Strength

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 concluding

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.

In the experiment, a juicer is first used to extract all spinach juice and later on boil them separately with different time set for boiling. The use of juicer and extracting the spinach juice together not only improve the convenience, but also eliminates the error brought by the difference of oxalic content caused by different spinach leaves, ensuring consistent oxalic acid content in the juice.

7.2. Limitation

The endpoint of the titration may be hard to detect accurately, leading to inconsistent results. In the titration, the color of the solution (boiled and filtered) is originally yellow and while titrating, the color changed to colorless and then after passing the end point of titration, the color changed to pink/purple. The gradual change in color results in a less accurate result.

Also, for the titration, the influence of other substances obtained in spinach is overlooked in titration. Some other substances for example ascorbic acid may also get oxidized by $KMnO_4$ and therefore result in an overestimated result in the content of oxalic acid measurement.

A corresponding improvement for these two drawbacks may be taking other approaches for measurement, for example, measuring the calcium in the precipitate by atomic absorption and calculating it as oxalic acid [9].

The oxalic acid may not be fully extracted from the spinach leaves due to inefficient extraction methods by using the filter funnel. The oxalic acid content in spinach also exists in both soluble and insoluble forms, so no guarantee that all oxalic acid content is included in the spinach juice extracted. Instead, oxalic acid may also exist in the filter residue. To improve, we may use a more effective solvent and ensure that the extraction process is carried out for a longer time with proper agitation. Additionally, repeating the extraction several times can help recover more oxalic acid.

Also, during experiment, oxalic acid in the spinach juice sample may degrade when stored for

a long period in ideal conditions. An action that can be taken to improve the result of experiment is storing the samples in airtight containers and protecting them from light and heat to minimize decomposition.

7.3. Further Research

The experiment only focuses on oxalate content 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 only discussed the scenario when spinach is cooked in boiling water, and might not capture the complexity of actual cooking practices.

In further research, different methods of cooking such as steaming and baking can be further studied. Other nutritional substances including iron (Fe), vitamin C, and fiber obtained in spinach can also be taken into consideration.

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.

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