

Effect of Stir-Frying on Calcium Inside Terrestrial Water Spinach (*Ipomoea Reptans Poir*) with Water Spinach (*Ipomoea Aquatica Forks*) with Complexometric Titration Method

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Abstract. Water spinach is a variant of vegetables containing a lot of nutrients, including calcium. Inappropriate cooking will affect nutrients inside the foods. This research aimed to determine the effect of stir-frying on calcium inside terrestrial water spinach and water spinach using complexometric titration. This research used terrestrial water spinach (*Ipomoea Reptans Poir*) and water spinach (*Ipomoea Aquatica Forks*). Researchers chose stir-frying as a cooking process as commonly popular and known to affecting calcium inside foods. The research used laboratory experiments. Results on qualitative research showed that samples positively contained calcium, marked with a white sediment. On quantitative, levels of calcium inside terrestrial water spinach before stir-frying was 0,0540% w/v while 0,0715% w/v after the cooking process. Meanwhile, levels of calcium on water spinach before stir-frying was 0,0549% w/v while 0,0746% w/v after the cooking process. The research had concluded that there were differences on the levels of calcium, before and after stir-frying, $p = 0,000$ (terrestrial water spinach) and $0,018 < 0,05$ (water spinach). In contrast, there were no differences on the effect of stir-frying on calcium levels on the terrestrial water spinach and water spinach, with *independent t-test* $p = 0,310 > 0,05$.

1. Introduction

Vegetables are a source of vitamins and minerals for the body and are needed by human as an intake of healthy and fresh foods. One vegetable that has a beneficial nutrients is water spinach. There are two types of water spinach available in Indonesia, namely terrestrial water spinach and water spinach¹³. The differences on environment where the plants grow affect nutrients inside both water spinach types¹⁶.

Water spinaches have complete nutrients, one of them is calcium. Calcium is a mineral needed by human body in large enough amounts. It plays an important role for the body to regulating cell function as well as a growth factor. It is formed from 1.5% to 2% of adult bodyweight or about 1 kg and 99% found in bones²

The levels of calcium can either be reduced or increased after undergoing cooking processes. Calcium will reduce when using cooking media, such as oil and water, and food surfaces in contact with air¹⁹. Stir-frying will cause the outer skin to constrict due to dehydrating process. Water inside foods will evaporate because of the heat from oils so the foods get constricted⁹. Empty spaces previously filled with water and other components will be filled mostly by the oils during the cooking process. It is thought to cause calcium inside vegetables gets decreased¹⁵. This mineral has a boiling



point of 1484°C, with a density of 1.6 g/cm³ at 20°C. It can increase due to the presence of oil, and calcium reacts quickly to the open air can react with water vapor and oxygen⁴.

Researchers used complexometric titration methods with EDTA reagents as they are faster, cheaper with a high precision and accuracy to form a calcium complex¹⁷. The reagents are also easily soluble in water, its stability in forming chelates is very constant so the reaction goes perfectly and can react quickly with calcium metal¹².

Research by Aisyah¹ confirmed that heating process can affect antioxidants inside vegetables, while the results of study by Setyopratiwi¹⁴ showed that foods high in calcium are generally not resistant to heating process. The results of the study are supported by a research conducted by Burhanuddin⁵ which showed that there were significant differences on the interaction between processing and reheating variations in the calcium inside Moringa leaves. In contrast, a research by Hidayat⁷ showed that there was no difference before and after boiling with high temperatures.

Researchers, based on all descriptions above, were interested to conduct a scientific research on the effect of stir-frying to calcium inside terrestrial water spinach leaves and water spinach. Researchers chose both terrestrial water spinach and water spinach as there were differences in the environments where the plants grow that can affect calcium. This research was intended to find out whether cooking process can affect calcium or not, so people can still meet their daily calcium needs after processing, especially in the stir-frying process.

2. Materials

Laboratory apparatuses used in this research included: analytical balance, 125 ml Erlenmeyer(Pyrex), volume pipette, measuring flask, weighing bottle, measuring cup, filter paper, funnel, 50 ml burette, blender, beaker glass, dropper, reaction, stative clamps, watch glass, thermometers, fire spirits, clamp bars, stoves and gas cylinders, pans, and stirring rods.

Materials used in this research included leaves of terrestrial water spinach and water spinach, 0.01 M EDTA reagents, CaCO₃ solution, 1% EBT indicator, HCl, Methyl Red, 1 M NaOH solution, ammonium oxalate solution, and aquadest.

3. Methods

The method of this research was laboratory experimental, while the design was One Group Pre-test Post-test. In this study, samples were treated with a stir-fry cooking process¹⁰. Populations in this study were terrestrial water spinach taken from a vegetable garden at Tegalsari while water spinach taken from a paddy pond at Tegalsari, Kemudo, Prambanan, Klaten. It was because people in the locations cultivate the plants.

Samples in this study were water spinach leaves taken when still fresh, colored green or dark green, and having long-form with pointed leaves for terrestrial water spinach while rounded or having wider-looking for water spinach. All samples were taken as many as one kilogram.

After collection, identification of calcium inside water spinach was immediately conducted using ammonium oxalate to form a white precipitate.

Quantitative test for samples before stir-frying was conducted by titrating samples namely the leaves of fresh terrestrial water spinach and water spinach. Titration was added with Eriochrom Black T indicator and titrated with 0.01 M EDTA solution until the red color turned into blue³. Samples were then given the effect of processing in the form of stir frying by heating leaves of terrestrial water spinach and water spinach in a pan filled with a little cooking oil (30 ml), which had been heated with a temperature of 80-100°C, then sautéed for 5 minutes on medium heat, while stir-frying for several times. Determination of calcium levels after stir-frying was also done by complexometric titration method using 3-time replication¹¹.

Data analysis used descriptive analysis of percentages with mean and SD and then proceeded with Kolmogorov-Smirnov analysis, Paired T-Test and Independent T-Test⁸.

4. Result and Discussion

4.1. Plant Determination

Samples were taken in Tegalsari, Kemudo, Prambanan, Klaten. Terrestrial water spinaches were obtained from the cultivation of vegetable garden, while water spinach came from ponds in rice fields. They were then determined in the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Yogyakarta. The result of the determination showed that samples used in this study were actually terrestrial water spinach and water spinach with species name known as *Ipomoea aquatica* Forssk.

4.2. Qualitative Calcium Test

Qualitative tests using ammonium oxalate showed that samples positively contained calcium characterized by the formation of white sediments.

Table 1. Identification of Calcium Inside Terrestrial Water Spinach

Replication	Result	Note
1	White sediment	+
2	White sediment	+
3	White sediment	+

Table 2. Identification of Calcium Inside Water Spinach

Replication	Result	Note
1	White sediment	+
2	White sediment	+
3	White sediment	+

Note :

+ : Showing calcium inside samples

- : Not showing calcium inside samples

4.3. Standardization of Na-EDTA Solution

Prior to qualitative testing of samples, standardization of 0.01 M EDTA solution was carried out. The following table was the result of the Na-EDTA standardization.

Table 3. Results of Titration and 0,01 M EDTA Standardization Score

Titration	EDTA volume (ml)
Replication I	39,40
Replication II	39,80
Replication III	41,00
Mean (\bar{x}) \pm SD	40,06 ml \pm 0,83
Coefficient of Variation (CV)	2,07 %
Molarity (M)	0,02 M

Based on the table, it was obtained molarity (M) of EDTA solution as many as 0.02M. The molarity was close to the desired standardized molarity.

4.4. Determination of Calcium Inside Samples

Calcium inside samples was analyzed using the complexometric titration method with 3-times replication or repetition.

Table 4. Result of Qualitative Analysis on Calcium Inside Leaves of Terrestrial Water Spinach and water Spinach.

Treatment	Replication	Volume Titrant (ml)	Level Calcium (% w/v)	\bar{X} Level (% w/v)	SD	CV (%)
Terrestrial Water Spinach Before Stir-Frying (O_1)	I	5,25	0,0525	0,0540	0,0015	2,77
	II	5,40	0,0540			
	III	5,55	0,0555			
Terrestrial Water Spinach After Stir-Frying (O_1^1)	I	6,95	0,0695	0,0715	0,0018	2,51
	II	7,20	0,0720			
	III	7,30	0,0730			
Water Spinach Before Stir-Frying (O_2)	I	5,48	0,0548	0,0549	0,0012	2,29
	II	5,63	0,0563			
	III	5,38	0,0538			
Water Spinach After Stir-Frying (O_2^2)	I	7,95	0,0795	0,0746	0,0042	5,67
	II	7,18	0,0718			
	III	7,25	0,0725			

Based on the tables above, calcium obtained from terrestrial water spinach prior to stir-frying was averagely $0.0540\% \text{ w/v} \pm 0.0015$, while after stir-frying was $0.0715\% \text{ w/v} \pm 0.0018$. Calcium obtained from water spinach before stir-frying was $0.0549\% \text{ w/v} \pm 0.0012$, while after stir-frying was $0.0746\% \text{ w/v} \pm 0.0042$.

4.5. Analyze Data of Sample Testing Results

Calcium inside samples that were stir-fried and not stir-fried was analyzed using statistical analysis *paired t-test*.

Table 5. Data Analysis of Samples With *Paired T-Test*

Samples	Significance
Terrestrial water spinach before – after stir-frying	0,000
Water spinach before – after stir-frying	0,018

Based on table 5, the significance value (p) of stir-fried terrestrial water spinach and water spinach samples were 0,000 and 0.018, both of which have $p < 0.05$ so that H_0 was rejected, then H_a was accepted. It means that there are significant differences in calcium levels inside the samples before and after stir-frying.

Table 6. Data Analysis of the Effect on Stir-Frying to Samples With *Independent T-Test*

Effects of Stir-Frying	\bar{X} Level (% w/v)	Significance
Terrestrial Water Spinach	0,0715	

Water Spinach

0,0746

0,310

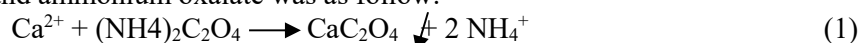
Based on table 6, the significance value (p) of terrestrial water spinach and water spinach samples after stir-frying was 0.310, both of which have $p > 0.05$ so that H_0 is accepted, then H_a is accepted. It means that there is no difference in the effect of stir-frying on calcium inside the leaves of terrestrial water spinach and water spinach.

4.6. Discussion

This study aimed to analyze the effect of stir-frying on differences in calcium levels inside the leaves of terrestrial water spinach and water spinach. Water spinaches were chosen because they are one of the high calcium vegetables. People generally consume water spinach on the leaves. In this study, water spinach samples have been proven through determination in the Plant Systematic Laboratory, Faculty of Biology, Gadjah Mada University, Yogyakarta. The results of the determination showed that samples used in this study were actually terrestrial water spinach and water spinach with species name known as *Ipomoea aquatica* Forssk.

The study was conducted with two tests, namely qualitative and quantitative test. The qualitative test was conducted at the Analysis Laboratory of STIKES Muhammadiyah Klaten. Based on tests using ammonium oxalate, it was showed that the sample solutions contained calcium, known from the formation of white deposits which are calcium oxalate deposits (CaC_2O_4)¹².

Reaction between calcium and ammonium oxalate was as follow:



Standardization of EDTA solutions was replicated 3 times. The aim of this process was to standardize because the sodium salt from EDTA did not meet the requirements as the main standard solution. Test of the titration results was obtained molarity of 0.02 M. It is in accordance with the literature and the desired molarity so that the solution is considered standard and can be used in complexometric titration.

After standardization, the EDTA solution can already be used to titrate the solution of terrestrial water spinach and water spinach with the complexometric titration method. In the titration, it is necessary to add 1.0 M NaOH which acts as a buffer to prevent pH changes in the sample solution¹². Of such titration, a calcium-EDTA complex compound was produced in the form of a solution. The reaction of the formation of complex compounds is commonly referred to as the Lewis Acid Base reaction¹².

Based on the results of quantitative tests, calcium inside terrestrial water spinach before stir-frying was 0.0540% w/v \pm 0.0015 and 0.0715% w/v \pm 0.0018 after stir-frying; meanwhile calcium inside water spinach before stir-frying was 0.0549% w/v \pm 0.0012 and 0.0746% w/v \pm 0.0042 after stir-frying. From these results, it was known that there are differences in calcium levels before and after stir-frying. In comparison of calcium levels in the terrestrial water spinach and water spinach, the results were calcium levels get higher after stir-frying.

Based on the literature, minerals inside foods can be lost during processing or can be influenced by cooking utensils used to process foods and differences in temperature exposure can affect food quality, one of which is in terms of calcium inside foods⁵.

The influence of heating also causes calcium to react with water to form a base solution of calcium hydroxide. Calcium hydroxide dissolves easily in water. If calcium hydroxide is made continuously, it will rise the concentration of the solution and a calcium hydroxide suspension will be formed thus causing high levels of calcium. In stir-frying, the oil used is coconut oil which contains calcium so that the oil will be hydrolyzed into water spinach thus causing increased calcium levels after stir-frying. Lipase enzymes in oil can hydrolyze triglycerides to produce free fatty acids and glycerol⁶.

From the results of research by Burhanuddin⁵ which showed the same results, there are differences between before and after heating. It is different from a research conducted by Hidayat⁷ where there was no difference in calcium before and after boiling.

Based on a quantitative test after normality test using Kolmogorov-Smirnov One Sample test, it was found that the data were normally distributed. Statistical analysis of Paired T-Test showed that there were significant differences in calcium before and after stir-frying for each type of water spinach. Differences in calcium before and after stir-frying due to high temperature food processing can cause the evaporation of water in these foods¹⁸.

Since the digestibility of human body is limited, it is recommended to consume water spinach after processing, namely by stir-frying because the results of the study show there is no difference in the effect of stir-frying on calcium inside terrestrial water spinach and water spinach. Based on statistical analysis, Independent T-Test, it was found that there was no difference in the effect of stir-frying on calcium inside both terrestrial water spinach and water spinach. It is caused by the duration of the heating process, type of cooking utensils, method of processing and same temperature.

5. Conclusion

- Calcium levels of terrestrial water spinach before stir-frying was $0.0540\% \text{ w/v} \pm 0.0015$ and after stir-frying was $0.0715\% \text{ w/v} \pm 0.0018$; meanwhile water spinach contains $0.0549\% \text{ w/v} \pm 0.0012$ before stir-frying and $0.0746\% \text{ w/v} \pm 0.0042$ after stir-frying.
- There are significant differences in calcium levels before and after stir-frying in both terrestrial water spinach and water spinach with significant values $p = 0,000 < 0.05$ for terrestrial water spinach and $0.018 < 0.05$ for water spinach.
- There is no difference in the effect of stir-frying on calcium levels of terrestrial water spinach and water spinach, independent t-test $p = 0.310 > 0.05$.

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