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Original Article



The Eeffect of Soaking on the Soluble Oxalic Acid Content of Spinach

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Oxalic acid is a primary risk factor of kidney stones. Leafy vegetables such as spinach are known to moderate amounts of soluble and insoluble oxalic acid. The effect of soaking practices on reduction of soluble oxalic acid content of spinach leaf was evaluated by measuring oxalic acid eluted into tap water using ion chromatography. There was a large effect of more than 70% on soaking for 60 min at 80%. Soaking spinach leaves at high temperature was an effective way of reducing the soluble oxalic acid content.

Key Words: Spinach, Soluble oxalic acid, Soaking, Ion chromatography

Introduction

Leafy vegetables such as spinach are known to moderate amounts of soluble and insoluble oxalic acid.

Approximately 75% of all kidney stones are composed primarily of calcium oxalate [1], and hyperoxaluria is a primary risk factor for this disorder [2, 3]. Because urinary oxalate originates from a combination of absorbed dietary oxalate and endogenously synthesized oxalate, restriction of dietary oxalate intake has been suggested as a treatment to prevent recurrent nephrolithiasis in some patients [4].

Soluble oxalic acid, when consumed, has the ability to bind to calcium in the spinach and any calcium in foods consumed with the spinach, reducing the absorption of soluble oxalic acid [5].

Absorptive or 'dietary' hyperoxaluria is generally thought to be a direct consequence of oxalic acid bioavailability. Therefore, people with an increased risk of calcium oxalic acid stone formation are commonly advised to avoid consuming oxalic acid rich foods.

A number of foods such as spinach, rhubarb, beets, nuts, chocolate, wheat bran, and strawberries are known to contain high oxalic acid levels [6]. These are foodstuffs that have a high ratio of oxalic acid to calcium and are thought to have a large effect on calcium availability from other foods consumed at the same time.

Until recently, there was little interest in food oxalic acid values because the dominant paradigm was that dietary oxalic acid contributed only 10% of daily oxalic acid excretion. This changed in 2001 when Holmes and colleagues showed that 24% to 53% of urinary oxalic acid originated from dietary oxalic acid at typical intakes of 10 to 250 mg per day [7].

The objective of the present study was to evaluate the effect of soaking practices on the reduction of soluble oxalic acid content of spinach leaf using ion chromatography.

Materials and Method

Samples and Chemicals

Spinach was purchased from local supermarkets. All chemicals were analytical grade and the water was

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deionized prior to distillation.

Procedure of soaking at room temperature

A 2g sample of spinach was cut in two different sizes ($1\,\mathrm{cm^2}$ or whole) with a kitchen knife. The sample of spinach was placed in 200 ml of tap water for the given experimental time. The tap water temperature was $15\,\mathrm{^{\circ}C}$.

Procedure of soaking at 80° C

A 2g sample of spinach was cut in two different sizes ($1\,\mathrm{cm^2}$ or whole) with a kitchen knife. The sample was placed in 200 ml of tap water for the given experimental time at $80\,\mathrm{^{\circ}C}$.

Determination on the reduction rate

One ml of tap water was taken from the flask experiment in progress. Soluble oxalic acid in the test solution was determination to be diluted as necessary by ion chromatography.

Determination of Soluble oxalic acid of spinach

Spinach was chopped into small sections with a kitchen knife. Portions of 5g each were weighed and homogenized with 45ml of water in a mortar. They were then placed in a water bath at 80°C for 3min and centrifuged for 15min at 3,000 rpm. The supernatant was filtered, diluted 100 times with water and subjected to ion chromatography.

Ion chromatography

The ion chromatography system used in this study consisted of a Dionex 2000i/SP ion chromatograph, a Dionex HPIC-AS4A column ($4\times250\,\mathrm{mm}$) with an IonPac AG4A guard column ($4\times50\,\mathrm{mm}$) and a Chromatocorder 21 integrator. The mobile phase was composed of 1.8 mM sodium carbonate, 1.7 mM sodium hydrogencarbonate at a flow rate of 1.0 ml/min at 30°C. Nitrate and oxalic acid content were

calculated from the standard curve prepared with standard sodium nitrate and oxalic acid solution.

Results and Discussion

Although there are other studies that have measured the reduction of the oxalic acid in spinach, the technique in this study was different from the conventional method. In the conventional method, spinach was wrung by hand to remove excess water after soaking, and the oxalic acid remaining in the spinach was measured. There is a disadvantage that the amount of water removed by hand is not constant in the conventional method and thus this method will affect the measurements' results.

In the technique used in this study, oxalic acid eluted into tap water was measured. This method compensates for the disadvantages of the conventional methods, and duration of elution can be observed.

A typical ion chromatogram of the test solution is shown in Fig. 1. Oxalic acid was eluted at 11.5 min and was completely separated from other peaks. This ion chromatography was a very useful method for the analysis of oxalic acid. It can be expected that another direct technique for analyzing oxalic acid, gas chromatography, would also be suitable for the analysis of oxalic acid, but it would require a derivatization step to make oxalic acid volatile, thus increasing the assay time.

Fig. 2 shows the time-dependent curve of the reduction rate of soluble oxalic acid eluted into tap water from 1cm² spinach at 15°C. Reduction of soluble oxalic acid increased gradually, with peak solubility observed at 18hours. The reduction rate

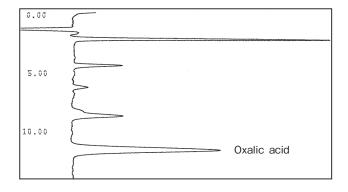


Fig. 1 A typical ion chromatogram of a spinach leaf

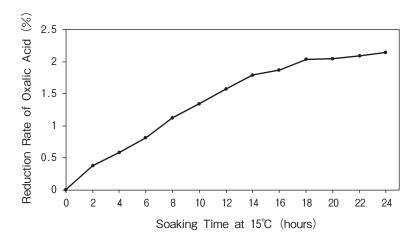


Fig. 2 Time-dependent curve of the reduction rate of soluble oxalic acid eluted into tap water from 1cm² spinach at 15°C

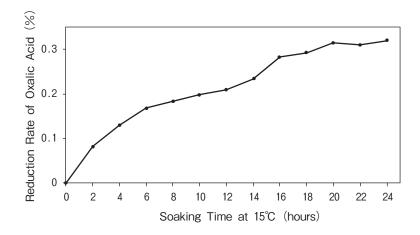


Fig. 3 Time-dependent curve of the reduction rate of soluble oxalic acid eluted into tap water from whole spinach at 15°C

was 2.0%.

Fig. 3 shows the time-dependent curve of the reduction rate of soluble oxalic acid eluted into tap water from whole spinach at 15° C. Reduction of soluble oxalic acid increased gradually, with peak solubility observed at 20hours. The reduction rate was 0.3%.

Fig. 4 shows the time-dependent curve of the reduction rate of soluble oxalic acid eluted into tap water from 1 cm² spinach at 80°C. Reduction of soluble oxalic acid increased gradually, with peak solubility observed at 60 min. The reduction rate was 77.5%.

Fig. 5 shows the time-dependent curve of the

reduction rate of soluble oxalic acid eluted into tap water from whole spinach at 80°C. Reduction of soluble oxalic acid increased gradually, with peak solubility observed at 80 min. The reduction rate was 71.7%.

These results are summarized in Table 1. Soaking the raw leaves in tap water at 15°C had no effect with only 2% reduction even after 18hours. Conversely, there was a large effect of more than 70% on soaking for 60min at 80°C. Soaking spinach leaves was an effective way of reducing the soluble oxalate content.

This result shows that much of the oxalic acid contained in spinach would leach into the soup of a

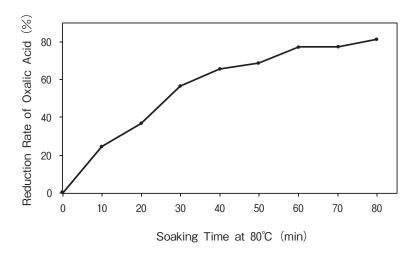


Fig. 4 Time-dependent curve of the reduction rate of soluble oxalic acid eluted into tap water from 1cm² spinach 80°C

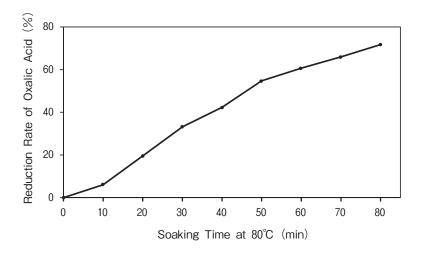


Fig. 5 Time-dependent curve of the reduction rate of soluble oxalic acid eluted into tap water from whole spinach at 80°C

Table 1 The reduction rate of soluble oxalic acid of spinach in various conditions

Size	Temperature	Soaking Time	Reduction Rate
whole	80°C	80min	71.7%
	15°C	20hours	0.3%
1cm²	80℃	60min	77.5%
	15°C	18hours	2.0%

Japanese casserole. In order to reduce the intake of oxalic acid, it is advised not to drink the soup.

High oxalic acid uptake from the diet is thought to play a role in hyperoxaluria, a documented risk factor in the formation of calcium oxalate kidney stones. Therefore, people with an increased risk of calcium oxalate stone formation would be advised to avoid or reduce their regular consumption of leafy vegetables such spinach or to follow the advice given by Brogren and Savage [8] to reduce the availability of ingested soluble oxalates by consuming a high-calcium-containing milk product at the same time. The

bioavailability of soluble oxalic acid can be reduced by eating the food in combination with calcium in the milk.

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