Correlation between the Bioavailability of Magnesium, Other Minerals and Oxalic Acid in Spinach

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Spinach was evaluated for the bioavailability of important dietary components. The contents of the analyzed substances in spinach were, for minerals (mg/100 g: Mg, 76; Ca, 67; Fe, 2.0; Zn, (0.9), oxalic acid (mg/100 g: total oxalic acid, 843; water-soluble oxalic acid, 459; water-insoluble oxalic acid, 384) and dietary fiber (mg/100 g: NDF, 748; ADF, 636; cellulose, 533; hemicellulose, 112; pectin, 307). The highest elution ratios of the minerals, oxalic acid and pectin in the several fractions during pectin extraction were 74% of magnesium, 39% of iron and 71% of oxalic acid in the aqueous fraction, 39% of zinc in the sodium hexametaphosphate fraction, and 67% of calcium and 75% of pectin in the HCl fraction. It is suggested that the greater part of calcium in spinach did not exist as Ca-oxalate. When the magnesium and/or calcium solution and the oxalic acid solution were mixed, the ratios of insoluble magnesium and calcium were about 20% and about 80% at the physiological pH level, respectively. The binding capacity to oxalic acid increased in accordance with rising pH value, the binding capacity for calcium being 3 to 5 times higher than that for magnesium. When both magnesium and calcium were present in the same solution, the binding capacity for magnesium and calcium to oxalic acid decreased by from 4 to 14% and from 1 to 7%, respectively. When spinach was boiled in 10 volumes of distilled and deionized water or in a 1% NaCl solution for 3 min, the elution ratio of the minerals from spinach was 77% of magnesium, 4.5% of calcium, 40% of iron and 41% of zinc with distilled and deionized water. Although there was no difference in the elution ratio of calcium, iron and zinc between water and the 1% salt solution, the elution ratio of magnesium decreased by about 10% with the 1% NaCl solution. When spinach was boiled in 5 or 10 volumes of distilled and deionized water for 1, 2, 3 or 4 min, the elution ratio of magnesium, calcium, iron, zinc and oxalic acid from spinach was from 40 to 84%, from 2 to 3%, from 26 to 51%, from 23 to 49% and from 40 to 82%, respectively. (Received November 29, 1993)

Keywords: spinach, cooking loss, magnesium and other minerals, oxalic acid, dietary fiber.

INTRODUCTION

Magnesium deficiency in humans can cause such diseases as atherosclerosis,¹⁾ myocardial damage,²⁾ arterial hypertension,³⁾ cardiac arrhythmia⁴⁾ and kidney stone.⁵⁾ The death rate from coronary heart disease⁶⁾⁷⁾ and ischemic heart disease⁸⁾⁹⁾ has been observed to correlate with the dietary calcim : magnesium ratio, the magnesium concentration in drinking water, and the content of exchangeable magnesium in the soil. Magnesium is necessary for the secretion and function of PTH,¹⁰⁾ the action of PTH on the bone, kidney and gut,¹¹⁾ and the renal 1 α -hydroxylation of vitamin D.¹²⁾ For these reasons just described, a safe and adequate daily dietary intake of magnesium by an adult in Japan has been

set at 300 mg.¹³⁾ Nuts, cereals (unrefined) and sea foods are the richest in magnesium, but the consumption of these foods per day is of small quantity when compared with green leaf vegetables which are also rich in magnesium. Among the green leaf vegetables, spinach in one of the most commonly consumed throughout the year. It is important to know the amount of magnesium in spinach that is absorbed. Spinach contains oxalic acid which can bind magnesium as well as calcium and other minerals. We have already reported that the oxalic acid concentration in spinach was the highest of those vegetables analyzed.14) Oxalic acid is well known to impair calcium absorption,15)16) and increasing calcium intake has been reported to depress the apparent absorption of magnesium in

rats,¹⁷⁾¹⁸⁾ because magnesium can form an insoluble complex with calcium and phosphorus.¹⁹⁾ The calcium content in spinach is not low among the vegetables we usually consume. There are several components in spinach such as oxalic acid, calcium, phosphorus and dietary fiber that can affect magnesium absorption. It is thought that these components and the interaction of minerals have a complicated action that affects the absorption of magnesium in spinach. Prior to animal experiments to investigate the absorption ratio of magnesium in spinach, the dietary factors which disturb magnesium absorption and the interaction between magnesium absorption and other minerals, we evaluated the contents of minerals (Mg, Ca, Fe and Zn), oxalic acid and dietary fiber, and the distribution of minerals, pectin and oxalic acid. The difference was then examined of mineral loss in spinach boiled in water with or without 1% salt, as well as the relationship among mineral loss, boiling time and the volume of boiling water. The binding capacity of magnesium and/or calcium to oxalic acid was also studied at several pH values in vitro.

MATERIALS AND METHODS

1. Materials

The spinach (smooth leaved) used for this study was obtained from a vegetable shop in Okayama City between May and July 1993. All experiments were conducted twice, and all samples were analyzed three times. The values for the analyzed substances in spinach are given as the means and standard deviations from 6 determinations. Distilled and deionized water was used in this work.

2. Analysis of minerals, oxalic acid and dietary fiber

Spinach was dried in a hot-air drier and was completely digested with a mixture of concentrated HNO_3 -concentrated $HClO_4$ (1 : 1, v/v) on a hot plate. The ash was dissolved in a small volume of 3 M HCl, and water was added to make a final concentration of 0.1 M HCl. This solution was used for determining the minerals (Mg, Ca, Fe and Zn) by an atomic absorption spectrophotometer as described previously.¹⁵

Total oxalic acid and water-soluble oxalic acid were each extracted with 3 M HCl and water, and were determined by isotachophoresis as described previously.¹⁴⁾ The amount of water-insoluble oxalic acid was calculated by subtracting the watersoluble oxalic acid from the total oxalic acid. Pectin was successively extracted from spinach (about 30 g) with four kinds of solution (ethanol adjusted to pH 4 with acetic acid, water, 0.5% sodium hexametaphosphate, and 0.05 M HCl) by the method of Fuchigami *et al.*²⁰⁾ and was determined by the carbazole method.²¹⁾ At the same time, the contents of minerals and oxalic acid in each fraction of pectin extracted with four reagents were determined to examine their distribution in spinach.

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were prepared according to the method of Van Soest *et al.*²²⁾ The contents of cellulose and hemicellulose were calculated from the values of NDF, and ADF and ADF-lignin (112 \pm 23 mg/100 g and 533 \pm 47 mg/100 g), respectively.

3. Evaluation of the binding capacity of magnesium and/or calcium to oxalic acid under various pH conditions *in vitro*

Twelve kinds of solution from pH 1 to 12 containing either 0.1 mM magnesium and/or calcium as a chloride or 0.1 mM oxalic acid were prepared. The pH of each solution which contained magnesium and/or calcium or oxalic acid was adjusted to the required pH with an HCl or NaOH solution. Ten ml of a 0.1 mm mineral (Mg and/or Ca) solution and 0.1 mm oxalic acid solution were mixed at room temperature, and the mixture shaken gently for 30 min. When necessary, the pH of this mixed solution was adjusted to the required value with the HCl or NaOH solution, and the mixture shaken for a further 60 min. This mixed solution was then centrifuged at $10,000 \times g$ for 20 min, after which the supernatant was passed through a millipore filter (0.22) μ m pore size). The concentration of magnesium and calcium in the filtered supernatant was determined by an atomic absorption spectrophotometer, the ratio of insoluble magnesium and calcium then being calculated from the concentration of magnesium and calcium in the filtrate.

4. Mineral loss from spinach boiled in different volumes of water for different times

When green-leaf vegetables are boiled, a small amount of salt is generally added to the boiling water to preserve the green color of the vegetables. The differences of the ratio of mineral (Mg, Ca, Fe and Zn) losses from boiled spinach was examined in 10 volumes of boiling water with or without 1% NaCl for 3 min.

To clarify the relationships between the mineral loss (Mg, Ca, Fe and Zn) and oxalic acid loss, the volume of boiling water and the boiling time, spinCorrelation between the Bioavailability of Magnesium, Other Minerals and Oxalic Acid in Spinach

ach (about 100 g) was boiled in 5 or 10 volumes of water for 1, 2, 3 or 4 min. The content of minerals and oxalic acid in the spinach was determined before and after boiling under each of these conditions.

RESULTS AND DISCUSSION

The contents of minerals, oxalic acid and dietary fiber are shown in Table 1. Spinach was rich in minerals (Mg, 76; Ca, 67; Fe, 2; Zn, 0.9 mg/100 g). The contents of minerals (mg/100 g) in spinach have previously been reported to be from 25 to 240 for calcium,¹⁷⁾²³⁾⁻²⁵⁾ from 32 to 57 for magnesium,²³⁾⁻²⁶⁾ from 1.7 to 5.0 for iron,²³⁾⁻²⁵⁾ and from 0.2 to 1.2 for zinc.²³⁾⁻²⁶⁾ Spinach contained a large amount of oxalic acid (total oxalic acid, 843; water-soluble oxalic acid, 459; water-insoluble oxalic acid, 384 mg/100 g) and dietary fiber (NDF, 748; ADF, 636; pectin, 307; hemicellulose, 112; cellulose, 533 mg/100 g) to impair the absorption of divalent minerals.²⁷⁾²⁸⁾ In a human study by Kelsay et al.,²⁹⁾ in which the effect of spinach in the diet on mineral balance was examined, the mean apparent balance of calcium, magnesium and zinc was negative, indicating that both oxalic acid and dietary fiber were bound to the minerals and these substances formed a dietary fiber-mineral-oxalate com-

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	Mg	76 ± 30
Minorala	Ca	67 ± 43
Millerais	Fe	2.0 ± 0.5
	Zn	0.9 ± 0.5
	T ox	843 ± 34
Oxalic acid	Ws ox	459 ± 12
	Wi ox	384 ± 35
	Pectin	307 ± 53
	NDF	$748\!\pm\!92$
Dietary fiber	ADF	636 ± 69
	Hc	112 ± 23
	Ce	533 ± 47

Table 1. Contents of minerals, oxalic acid and dietary fiber in spinach (mg/100 g)

The evaluation methods used were atomic absorption spectrophotometry for the minerals, isotachophoresis for oxalic acid, and the method of Van Soest for dietary fiber. T ox, total oxalic acid; Ws ox, water-soluble oxalic acid; Wi ox, water-insoluble oxalic acid; NDF, neutral detergent fiber; ADF, acid detergent fiber; Hc, hemicellulose; Ce, cellulose. plex. This mechanism may be one of several that impair the absorption of minerals in spinach.

Table 2 shows the elution ratios of minerals, pectin and oxalic acid in the several fractions of pectin extracted from spinach. The highest elution ratios for magnesium (74%), iron (39%) and oxalic acid (71%) were detected in the aqueous fraction. The elution ratio for zinc was highest in the sodium hexametaphosphate fraction at the level of 39%, while the highest elution ratios for calcium and pectin were 67 and 75% in the HCl fraction, respectively. It has previously been thought that calcium in green-leaf vegetables exists as calciumoxalate, which is not easily dissociated.³⁰⁾³¹⁾ The results in Table 2, however, indicate that most of the calcium and oxalic acid were eluted in different fractions. If oxalic acid and calcium form calciumoxalate in spinach, they would both have been eluted in the same fraction. In a human study by Heaney et al. in which the absorption of calcium from intrinsically labeled calcium-oxalate was measured and compared with the absorption of calcium from spinach, the absorbability of calcium from Ca-oxalate by the subjects was higher than that from spinach.³²⁾ We have previously reported the same result that was obtained from an animal experiment.¹⁵⁾ These results imply that a large part of the calcium in spinach is not bound to oxalic acid, and that most of the calcium is firmly bound to dietary fiber.

The greater part of the magnesium and oxalic acid, and about 40% of the iron in spinach were eluted in the same fraction (Table 2). The bioavailability of iron in spinach has been reported

Table 2.Elution ratios of minerals, pectin
and oxalic acid in each fraction

Fraction	Mg	Са	Fe	Zn	Pectin	Oxalic acid
Ethanol	2.6	0.9	9.7	16.1	ND	ND
Water	74.3	13.7	38.5	17.4	17.7	71.1
HMP	20.1	16.5	28.8	39.1	6.8	28.9
HC1	3.0	66.9	15.0	24.1	75.1	ND
Residue	ND	2.1	8.0	3.3	0.4	ND

Values represent the elution ratio (%) of each mineral, pectin and oxalic acid in each fraction of pectin extracted from spinach. Spinach was treated to extract pectin in the order of water, a sodium hexametaphosphate (HMP) solution and HCl. ND, not detected. to be lower than that in cereal.³³⁾ However spinach is a significant source of dietary iron, and dietary oxalic acid did not depress iron absorption.³⁴⁾ When rats fed with a zinc-deficient diet with or without added sodium oxalate were orally dosed once with either ⁶⁵Zn-labeled spinach leaves or ⁶⁵Znlabeled zinc sulfate, the dietary oxalate enhanced the availability of ⁶⁵Zn from zinc sulfate and had no effect on the absorption and retention from spinach leaves.³⁵⁾ These results suggest that there is no



Fig. 1. Binding capacity of Mg and Ca to oxalic acid under several pH conditions

Values represent the ratio (%) of insoluble Mg and Ca. a: An Mg or Ca solution and an oxalic acid solution were mixed and shaken gently. After centrifuging, the concentration of Mg and Ca in the supernatant was determined, and the ratio of insoluble Mg and Ca was calculated. b: A solution containing both Mg and Ca and an oxalic acid solution was treated in the same manner as that given for a. \bigcirc , Mg; \bigcirc , Ca.

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influence of oxalate in the absorption of iron and zinc in spinach.

The binding capacity of magnesium and/or calcium to oxalic acid was therefore examined under several pH conditions in vitro. The binding capacity of magnesium or calcium to oxalic acid and that of magnesium and calcium coexisting in the same solution to oxalic acid is presented in Fig. 1a and b. About 20% of the total magnesium and about 80% of the total calcium may have been respectively coprecipitated as Mg-oxalate and Ca-oxalate at a physiological pH level (Fig. 1a). The insoluble ratio of calcium increased from 58 to 94% with increasing pH, and was about 4 times higher than that of magnesium. When both magnesium and calcium were present in the same solution, the insoluble ratio of magnesium and calcium decreased by about 12% for magnesium and by from 1 to 2% for calcium at the physiological pH level. The effect of coexisting magnesium on the insoluble ratio of calcium could thus be negligible at the physiological pH level (Fig. 1b). Faboya has reported that the presence of Mg²⁺ in a solution inhibited the precipitation of calcium and Zn-oxalate.³⁶⁾ However, the presence of Mg²⁺ in the same solution with Ca²⁺ did not affect the binding capacity of calcium to oxalic acid in this study. It is thought that the intestinal absorption of magnesium in green-leaf vegetables high in oxalate content increases with the presence of calcium.

Table 3 shows the elution ratio of minerals from spinach boiled for 3 min in 10 volumes of water with or without a final concentration of 1% NaCl. There was no difference in the elution ratio of calcium, iron and zinc between plain water and the 1% salt solution. However, when spinach was boiled in plain water, the elution ratio of magnesium was higher by 10% than when boiled in the 1% salt solution. There was no macroscopic difference in the green color of boiled spinach in water with or without 1% NaCl. It has been reported that the best boiling conditions for spinach to preserve the green color were for 5 min with plain water,³⁷⁾ so it is not necessary to use a 1% salt solution to preserve the green color of boiled spinach.

Table 4 shows the ratios of magnesium, calcium, iron, zinc and oxalic acid eluted from spinach boiled in 5 or 10 volumes of plain water for 1, 2, 3 and 4 min. It is possible that the intestinal absorption of minerals from spinach increase when the water-soluble oxalic acid in them is removed by boiling.³⁸⁾

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Total (mg/100 g) -	Mg	Ca	Fe	Zn	
	110 ± 7	56 ± 11	$2.1 {\pm} 0.5$	0.68 ± 0.07	
Boiled in plain water Loss (%)	85±6 77	$2.5{\pm}0.5$ 4.5	$\begin{array}{c} 0.83 \pm 0.01 \\ 40 \end{array}$	0.28 ± 0.07 41	
Boiled in a 1% NaCl soln. Loss (%)	$73\pm 5\\66$	2.2 ± 0.3 3.9	0.82 ± 0.06 39	$\begin{array}{c} 0.24 {\pm} 0.03 \\ 37 \end{array}$	

Table 3. Amounts of eluted minerals and their ratios from spinach boiledin plain water or in a 1% NaCl solution

Each value is presented as the mean and standard deviation and as the ratio (%) of minerals eluted from spinach boiled in 10 volumes of plain or in a 1% NaCl solution for 3 min.

Table 4.	Elution ra	atios of	oxalic a	cid and	
	minerals	from	spinach	boiled	
	under several conditions				

Boiling time	Volumes of water	Oxalic acid	Mg	Ca	Fe	Zn
1 min	5	40.4	39.9	2.0	26.0	23.7
	10	45.6	45.5	2.3	30.0	23.7
2 min	5	40.0	71.6	2.4	43.2	22.9
	10	49.8	77.2	3.1	42.5	28.9
3 min	5	56.7	80.2	2.5	46.9	30.4
	10	69.1	83.9	2.8	51.1	42.2
4 min	5	75.1	79.2	2.3	45.4	29.2
	10	82.1	82.8	3.0	49.6	48.8

Values are presented as the ratio (%) of minerals eluted from spinach boiled in 5 or 10 volumes of water for 1, 2, 3 and 4 min.

The proportion of water-soluble oxalic acid in spinach was about 50% in this study (Table 1), and most of this was eluted by boiling for 3 min or more. When spinach was boiled in 10 volumes of plain water for 2 min, the elution ratio of the minerals was 77% for magnesium, 3% for calcium, 43% for iron and 29% for zinc. Little calcium was lost due to boiling, but the lost amount increased for iron and zinc by boiling for 3 min or more. Calcium absorption is inhibited dietary oxalic acid,¹⁵⁾¹⁶⁾ and it is desirable to remove the oxalic acid in spinach as much as possible to improve the quality of calcium. It is thought that spinach should be boiled in 5 or 10 volumes of plain water for 3 min or more to raise the calcium absorption ratio. On the other hand, dietary oxalic acid does not affect the absorption of iron³⁴⁾ and zinc.³⁵⁾ In this case, the loss of magnesium during boiling was large, so it is suggested that spinach should be boiled in a small quantity of plain water for only a short time (about 1 min) to prevent the elution of iron, zinc and magnesium.

From the foregoing results, it is suggested that spinach is not a good source of calcium due to its presence as a Ca-dietary fiber complex which is not easily dissociated. However, it is thought to be one of the most promising sources of magnesium, as the solubility of magnesium is not affected by oxalic acid. It is considered that the magnesium availability in green-leaf vegetables which are rich in oxalic acid is much higher than that of calicum.

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ほうれん草中のマグネシウムと他のミネラルの 利用性とシュウ酸との関連性

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ほうれん草 100g中のミネラル含量は Mg 76, Ca 67, Fe 2.0, Zn 0.9 mg, シュウ酸含量は 総シュウ酸 843, 水可溶性シュウ酸 459, 水不溶性シュウ酸 384 mg, 食物繊維の含量は NDF 748, ADF 636, セルロース 533, ヘミセルロース 112, ペクチン 307 mg であった. ペ クチンの抽出過程での各画分中のミネラル,シュウ酸, ペクチンの溶出率は, Mg, Fe,シュ ウ酸は水抽出画分で最も高く, Mg 74, Fe 39, シュウ酸 71%であった. また, Zn はヘキサメ タリン酸ナトリウム抽出画分で最も高く 39%, Ca, ペクチンは塩酸抽出画分で最も高く, Ca 67, ペクチン 75%であった. この結果から, ほうれん草中の Ca の大部分はシュウ酸カル シウムとして存在するのではないことが示唆された. Mg あるいは Ca 溶液, また Mg と Ca の共存液について,シュウ酸溶液を混合したときの Mg と Ca の不溶性の割合は,生理的な pH で Mg 約 20%, Ca 約 80%であった. Mg と Ca のシュウ酸に対する結合親和性は, pH の 上昇とともに増大した.また, Ca のシュウ酸への結合親和性は Mg よりも 3~5 倍高かった. Correlation between the Bioavailability of Magnesium, Other Minerals and Oxalic Acid in Spinach

Mg と Ca が共存する場合には、Mg と Ca のシュウ酸に対する結合親和性は、Mg で 4~14%、Ca で 1~7%低下した。ほうれん草を 10 倍量の脱イオン蒸留水または 1%食塩水で 3 分間ゆで た時のミネラルの溶出率は、脱イオン蒸留水で Mg 77、Ca 4.5、Fe 40、Zn 41%であった。し かしながら、1%食塩水では Ca、Fe、Zn の溶出率に変化はないが、Mg の溶出率は約 10%減 少した。また、ほうれん草を 5 あるいは 10 倍量の脱イオン蒸留水で 1、2、3、4 分間ゆでると、ほうれん草からの溶出率は Mg 40~84、Ca 2~3、Fe 26~51、Zn 23~49、シュウ酸 40~82% であった。

キーワード: ほうれん草, Mgと他のミネラルの調理による損失, シュウ酸, 食物繊維.