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## Effect of Different Cooking Methods on Vegetable Oxalate Content

WEIWEN CHAI AND MICHAEL LIEBMAN\*

Department of Family and Consumer Sciences (Nutrition), Department 3354, University of Wyoming, 1000 East University Avenue, Laramie, Wyoming 82071

Approximately 75% of all kidney stones are composed primarily of calcium oxalate, and hyperoxaluria is a primary risk factor for this disorder. Nine types of raw and cooked vegetables were analyzed for oxalate using an enzymatic method. There was a high proportion of water-soluble oxalate in most of the tested raw vegetables. Boiling markedly reduced soluble oxalate content by 30–87% and was more effective than steaming (5–53%) and baking (used only for potatoes, no oxalate loss). An assessment of the oxalate content of cooking water used for boiling and steaming revealed an approximately 100% recovery of oxalate losses. The losses of insoluble oxalate during cooking varied greatly, ranging from 0 to 74%. Because soluble sources of oxalate appear to be better absorbed than insoluble sources, employing cooking methods that significantly reduce soluble oxalate may be an effective strategy for decreasing oxaluria in individuals predisposed to the development of kidney stones.

#### KEYWORDS: Dietary oxalate; soluble oxalate; insoluble oxalate; vegetables

#### INTRODUCTION

Approximately 75% of all kidney stones are composed primarily of calcium oxalate (I), 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 (I), restriction of dietary oxalate intake has been suggested as a treatment to prevent recurrent nephrolithiasis in some patients (4).

Plants and plant products are the main sources of dietary oxalate (5). Oxalate in plant tissues is present as a combination of soluble oxalate sources such as sodium and potassium oxalate as well as insoluble oxalate salts such as calcium and magnesium oxalate (6). Oxalate bioavailability plays an important role in the determination of whether a food is a high risk for individuals with hyperoxaluria (7). The amount of soluble oxalate in a food item is also important because soluble oxalate is reported to be more bioavailable than insoluble oxalate (8).

The proportion of plant products in many individuals' diets is increasing due to health concerns associated with excessive animal product consumption and because of the purported benefits of increased consumption of phytochemical-rich fruits and vegetables, whole grains, and legumes (9). Vegetables are usually consumed raw or after various cooking methods. Total and soluble oxalate contents of some raw or boiled vegetables have been previously reported (6, 10). Savage et al. (6) found that boiling vegetables reduces oxalate content by leaching a significant amount of soluble oxalate into the cooking water. However, the oxalate content of vegetables cooked by other methods such as steaming has not been published. Thus, the purpose of the present study was to investigate the effect of different cooking methods on both the soluble and insoluble oxalate contents of some commonly consumed vegetables.

#### MATERIALS AND METHODS

**Sample Preparation.** The vegetables analyzed for oxalate were beet roots, broccoli, Brussels sprouts, carrots, potatoes, rhubarb stalks, spinach, and red and green Swiss chard leaves, all purchased from local establishments in Laramie, WY. Vegetables were chopped and cooked either by boiling in distilled deionized water or by steaming in a closed container for the same amount of time. The cooking times, computed from when the water began to boil or from when the steam became visible, were 12 min for beet roots, broccoli, Brussels sprouts, carrots, spinach, and red and green Swiss chard leaves, 15 min for rhubarb stalks, and 45 min for potatoes (without skin). Vegetables were covered with water during the boiling process, although the amount of water used for boiling or steaming was not measured. Potatoes were also baked in a 204 °C oven for 45 min, after which the skins were removed.

To determine whether the loss of oxalate with cooking could be recovered in the water, one serving of raw spinach and one serving of raw carrots were boiled or steamed for 12 min. Volumes of cooking water after cooking were recorded, and aliquots were saved for subsequent oxalate analyses.

**Total and Soluble Oxalate Extraction.** Total oxalate and soluble oxalate from all test vegetables were extracted according to the method described by Ohkawa (11). Representative samples of raw or cooked vegetables were cut into small pieces, weighed, and homogenized for 5 min with the same weight of distilled deionized water. A 10 g slurry (5 g of sample with 5 g of distilled deionized water) was homogenized with 20 mL of 2 M H<sub>3</sub>PO<sub>4</sub> (for total oxalate) or 20 mL of distilled

<sup>\*</sup> Corresponding author [telephone (307) 766-5597; fax (307) 766-5686; e-mail liebman@uwyo.edu].

 Table 1. Total and Soluble Oxalate Contents of Raw and Cooked

 Spinach Measured by Enzymatic and Capillary Electrophoresis (CE)

 Methods<sup>a</sup>

	oxalate content (mg/100 g of wet wt) (mean $\pm$ SD)						
sample	enzymatic method	CE method					
raw spinach total oxalate soluble oxalate steamed spinach	$\begin{array}{c} 1145\pm33\\ 803\pm9\end{array}$	1114 ± 4 791 ± 21					
soluble oxalate soluble oxalate boiled spinach total oxalate soluble oxalate	797 ± 12 468 ± 9 460 ± 9 107 ± 1	$794 \pm 8 \\ 489 \pm 35 \\ 454 \pm 1 \\ 114 \pm 2$					

<sup>*a*</sup> Sample n = 2 (two separate extractions) for raw and cooked spinach; wet weight refers to original store-bought weight for raw spinach or cooked weight for boiled (boiled and drained) and steamed spinach.

deionized water (for soluble oxalate), and the mixture was centrifuged at 10000 rpm for 5 min. The supernatant was transferred to a 100 mL volumetric flask. The extraction was repeated two additional times. The supernatant was collected and diluted to volume (100 mL) with distilled deionized water. To extract oxalate from the water after boiling or steaming of spinach and carrots, 10 mL aliquots of cooking water were diluted to 100 mL with 2 M H<sub>3</sub>PO<sub>4</sub> (for total oxalate) or distilled deionized water (for soluble oxalate). Two separate duplicate extractions were carried out for each vegetable or cooking water sample.

The use of  $H_3PO_4$  to extract total oxalate has been previously reported (*12*). The use of 2 M  $H_3PO_4$  in the present study rather than the 2 N HCl used by Ohkawa (*11*) was necessary to prevent distortion of the oxalate peak by Cl<sup>-</sup> ions in the capillary electrophoresis (CE) analysis of oxalate for spinach samples.

**Sample Analysis.** Each extract was analyzed for oxalate in duplicate using oxalate kits purchased from Trinity Biotech (Jamestown, NY). This method is based on the oxidation of oxalate by oxalate oxidase followed by detection of the  $H_2O_2$  produced during the reaction (13). Lyophilized (control) urine samples (Sigma Diagnostics, St. Louis, MO) providing predetermined oxalate levels of 20–30 mg/L were analyzed with each assay to ensure good quality control.

To confirm the validity of the results from the enzymatic method, oxalate from raw, boiled, and steamed spinach extracts was also measured using a CE method previously described by the current investigators (14). The extracts were diluted 10-fold with distilled deionized water, and a Biofocus (Bio-Rad Co., Richmond CA) 3000 CE system with a negative power supply was used to analyze oxalate content.

**Recovery Determination.** Oxalate recovery rates were determined by adding 4 mg of oxalic acid to 5 g of homogenized samples of raw Brussels sprouts. These samples were subsequently extracted using 2 M  $H_3PO_4$  (for total oxalate) or distilled deionized water (for soluble oxalate). Duplicate extractions were conducted for both soluble and insoluble oxalate determinations.

### **RESULTS AND DISCUSSION**

The mean ( $\pm$ SD) recoveries for the addition of 4 mg of oxalic acid to the homogenized raw Brussels sprout samples were 99.5  $\pm$  0.5% for total oxalate extraction and 99.3  $\pm$  1.1% for soluble oxalate extraction. This suggested the method used to extract total and soluble oxalate was effective. The validity of the enzymatic method for analyzing oxalate was suggested by a good agreement between the spinach sample results measured by the enzymatic and CE methods (**Table 1**).

Mean values for total, soluble, and insoluble oxalate contents of raw and cooked vegetables are given in **Table 2**. Oxalate levels were high in red Swiss chard leaves, green Swiss chard leaves, spinach, and rhubarb stalks, moderate in beet roots and carrots, and low in Brussels sprouts and broccoli. Total oxalate contents of raw spinach and rhubarb were within previously reported ranges: 320-1260 mg/100 g for spinach and 275-1336 mg/100 g for rhubarb (5). The total oxalate values reported by Savage et al. (6) for raw beet roots (45.6 mg/100 g), carrots (35.6 mg/100 g), and broccoli (16.1 mg/100 g) were similar to the values presently reported. The amount of total oxalate in red (1167 mg/ 100 g) and green (964 mg/100 g).

There was a significant loss of soluble oxalate in almost all test vegetables by boiling, ranging from 30 to 87%. The highest losses were observed in spinach (87%), red Swiss chard leaves (85%), and green Swiss chard leaves (84%), followed by Brussels sprouts (73%) and rhubarb stalks (61%). These findings were consistent with those of Savage et al. (6), who reported that boiling markedly reduced oxalate content of vegetables by leaching a high amount of soluble oxalate into the cooking water. These data suggested that the highest oxalate-containing vegetables exhibited the greatest boiling-induced losses in soluble oxalate.

With the exception of carrots (53%), green Swiss chard leaves (46%), and spinach (42%), the loss of soluble oxalate by steaming was only 6-19%. Although relatively high amounts of soluble oxalate were lost during steaming in green Swiss chard leaves and spinach, these were approximately only half of the losses that occurred with boiling (46 vs 84%, green Swiss chard leaves; 42 vs 87%, spinach).

The effects of cooking on insoluble oxalate varied. In some vegetables, such as spinach and beet roots, insoluble oxalate content was not affected by cooking, whereas in others, such as rhubarb stalks, red and green Swiss chard leaves, carrots, Brussels sprouts, and broccoli, boiling reduced the insoluble oxalate content by 15-74%. Overall, steaming was not effective in reducing insoluble oxalate content (i.e., 0-5%) with the exception of the 42 and 36% reductions observed in carrots and green Swiss chard leaves, respectively. There was a slight increase in insoluble oxalate content of steamed beet roots and boiled spinach as compared to their raw forms. This was most likely due to methodological variability, although an increase in insoluble oxalate content after cooking has been reported previously (6).

In addition to boiling and steaming, baking is also commonly used for cooking potatoes. Oxalate contents of raw, baked, steamed, and boiled potatoes are shown in **Table 3**. Boiling reduced soluble oxalate content by 34%, whereas the loss with steaming was only 5%. Total and soluble oxalate contents were slightly higher in baked potatoes than in raw potatoes. This could be explained by the moisture loss during baking as oxalate contents were expressed per 100 g of wet weight. The previously reported values by Hönow and Hesse (*10*) for raw (total oxalate, 17.1 mg/100 g; soluble oxalate, 13.0 mg/100 g) and baked potatoes (total oxalate 13.0 mg/100 g; soluble oxalate, 11.7 mg/ 100 g) supported the present finding that baking was not an effective method to reduce the soluble oxalate content of potatoes.

Additional carrot and spinach samples were used to assess whether losses of oxalate with boiling and steaming could be recovered in the cooking water (**Table 4**). The recoveries of the losses (total and soluble oxalate) in the cooking water were  $\sim 100\%$  for both spinach and carrot samples. Oxalate present in spinach cooking water was solely soluble oxalate. For carrot samples, approximately 78 and 94% of the total oxalate in the cooking water was recovered as soluble oxalate after boiling

Table 2. Total, Soluble, and Insoluble Oxalate Contents of Raw and Cooked Vegetables (Milligrams per 100 g of Wet Weight) (Mean ± SD)<sup>a</sup>

		total oxalate			soluble oxalate		insoluble oxalate <sup>b</sup>			
vegetable	raw	steamed	boiled	raw	steamed	boiled	raw	steamed	boiled	
red Swiss chard leaves	1167 (±5)	1052 (±2)	428 (±8)	806 (±19)	691 (±4)	121 (±10)	362 (±14)	360 (±2)	307 (±3)	
green Swiss chard leaves	964 (±8)	556 (±3)	335 (±4)	623 (±14)	338 (±3)	98 (±4)	341 (±6)	218 (±1)	238 (±1)	
spinach	1145 (±33)	797 (±12)	460 (±9)	803 (±9)	468 (±9)	107 (±1)	343 (±23)	329 (±4)	353 (±8)	
rhubarb stalks	532 (±8)	505 (±2)	309 (±7)	223 (±1)	210 (±2)	88 (±5)	309 (±7)	295 (±0)	221 (±2)	
beet roots	63.7 (±0.0)	62.5 (±0.2)	48.3 (±0.2)	48.8 (±0.4)	45.8 (±0.4)	34.0 (±0.2)	14.9 (±0.4)	16.7 (±0.2)	14.3 (±0.0)	
carrots	44.2 (±0.0)	22.3 (±0.3)	18.4 (±0.2)	31.1 (±1.3)	14.7 (±1.5)	13.6 (±1.1)	13.1 (±1.3)	7.6 (±1.2)	4.8 (±0.9)	
Brussels sprouts	15.2 (±0.2)	13.3 (±1.1)	6.1 (±0.2)	9.6 (±0.1)	7.8 (±0.0)	2.6 (±0.2)	5.6 (±0.1)	5.5 (±1.1)	3.5 (±0.0)	
broccoli	13.5 (±0.3)	12.4 (±0.2)	4.4 (±0.3)	5.3 (±0.1)	4.3 (±0.1)	2.3 (±0.4)	8.2 (±0.1)	8.1 (±0.1)	2.1 (±0.1)	

<sup>a</sup> Sample n = 2 (two separate extractions) for all raw and cooked vegetables; wet weight refers to original store-bought weight for raw vegetables or cooked weight for boiled (boiled and drained) and steamed vegetables. <sup>b</sup> Insoluble oxalate = total oxalate - soluble oxalate

Table 3.	Total.	Soluble	and	Insoluble	Oxalate	Contents o	of Raw	and	Cooked	Potatoes	(Milligrams	per	100	q of	Wet	Weigh	t) (l	Mean ± \$	SD	ĺ
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		total o	xalate			soluble	insoluble oxalate <sup>b</sup>					
	raw	baked	steamed	boiled	raw	baked	steamed	boiled	raw	baked	steamed	boiled
potato	30.9 (±0.8)	31.2 (±1.1)	29.7 (±0.5)	24.7 (±1.3)	25.5 (±0.8)	27.4 (±0.6)	24.1 (±0.1)	16.9 (±1.0)	5.4 (±1.7)	3.8 (±0.4)	5.6 (±0.5)	7.8 (±0.8)

<sup>a</sup> Sample n = 2 (two separate extractions) for all raw and cooked potatoes; wet weight refers to original store-bought weight for raw potatoes or cooked weight for boiled (boiled and drained), steamed, and baked potatoes. <sup>b</sup> Insoluble oxalate = total oxalate - soluble oxalate

Table 4.	Total and	Soluble	Oxalate	Cooking	Losses in	Spinach	and	Carrot	Samples	(Mean ±	SD)	۱â
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			oxala	ite					
	cooking	(mg/serving)	(mg/serving)	(mg) cook-	(mg) cook-	(mg/serving)	(mg/serving)	(mg) cook-	(mg) cook-
	method	before	after	ing loss	ing water	before	after	ing loss	ing water
spinach	boiling	602 (±6)	199 (±1)	404 (±5)	405 (±2)	437 (±8)	54 (±2)	383 (± 6)	400 (±0)
	steaming	564 (±5)	424 (±9)	140 (±4)	139 (±1)	407 (±8)	256 (±9)	151 (±2)	141 (±1)
carrots	boiling	28.2 (±0.6)	13.5 (± 0.3)	14.7 (±0.4)	14.2 (±0.5)	17.8 (±0.0)	6.7 (± 0.3)	11.1 (± 0.3)	11.1 (±1.5)
	steaming	28.9 (±0.6)	18.0 (±1.7)	10.9 (±1.1)	11.6 (±0.1)	18.5 (±0.5)	9.2 (±0.1)	9.4 (±0.5)	11.0 (± 0.3)

<sup>a</sup> Sample n = 2 (two separate extractions) for all raw and cooked spinach and carrot samples and for cooking water. Note: 73 g of raw spinach was used for boiling and 69 g of raw spinach was used for steaming (855 and 91 mL of cooking water were left after boiling and steaming, respectively); 71 g of raw carrots was used for boiling and 72 g of raw carrots was used for steaming (265 and 73 mL of cooking water were left after boiling and steaming, respectively).

and steaming, respectively. It is unclear why these latter percentages were not closer to 100%.

Soluble oxalate in foods appears to be more bioavailable than insoluble oxalate (8, 15). Chai and Liebman (8) suggested that the significantly higher oxalate absorption observed from almonds than from black beans could be attributed to the higher proportion of soluble oxalate in almonds (31%) than in black beans (5%). Most of the tested raw vegetables had high levels of soluble oxalate (39–83% of the total). The proportion of soluble oxalate exceeded 60% in carrots, beet roots, spinach, red and green Swiss chard leaves, Brussels sprouts, and potatoes. High proportions of soluble oxalate in some raw vegetables such as spinach, Swiss chard leaves, beet roots, carrots, and potatoes have been previously reported (6, 10). Ross et al. (16) also reported that oca (a New Zealand yam), a tuber crop, contained primarily soluble oxalate.

The present data suggested that boiling was most effective in reducing soluble oxalate content of vegetables compared to steaming and baking (used only for potatoes). The loss of soluble oxalate was 30-87% for boiling and only 5-19% (not including spinach, green Swiss chard leaves, and carrots) for steaming. Baking did not reduce soluble oxalate content of potatoes, whereas the loss through boiling was 34%. Brinkley et al. (7) defined spinach as a high-risk food for individuals susceptible to kidney stones due to its capability of increasing urinary oxalate by > 20 mg per load. The consumption of boiled spinach may constitute a lower risk because soluble oxalate levels were markedly reduced during cooking (from 803 to 107 mg/100 g). Brogren and Savage (17) reported that the ingestion of spinach with dietary sources of calcium significantly lowered oxalate bioavailability compared to the ingestion of spinach alone. Thus, dietary recommendations for individuals predisposed to kidney stones should consider the content of soluble oxalate, the methods used for cooking vegetables, and the presence of other dietary components such as calcium that have the ability to reduce oxalate absorption.

It should be acknowledged that boiling vegetables represents a tradeoff between the potentially beneficial loss of oxalate and the significant loss of many water-soluble vitamins and other nutrients into the cooking water. However, because the total oxalate content of the high-oxalate-containing vegetables such as spinach, Swiss chard leaves, and rhubarb was still high after boiling (309–460 mg/100 g), it is suggested that kidney stone patients should avoid these vegetables even after the use of cooking methods that maximize oxalate losses.

### LITERATURE CITED

- Williams, H. E.; Wandzilak, T. R. Oxalate synthesis, transport and the hyperoxaluric syndromes. J. Urol. 1989, 141, 742–747.
- (2) Goldfarb, S. Dietary factors in the pathogenesis and prophylaxis of calcium nephrolithiasis. *Kidney Int.* **1988**, *34*, 544–555.
- (3) Robertson, W. G.; Hughes, H. Importance of mild hyperoxaluria in the pathogenesis of urolithiasis—new evidence from studies in the Arabian peninsula. *Scanning Microsc.* 1993, 7, 391–401.

- (4) Massey, L. K.; Roman-Smith, H.; Sutton, R. A. L. Effect of dietary oxalate and calcium on urinary oxalate and risk of formation of calcium oxalate kidney stones. *J. Am. Diet. Assoc.* **1993**, *93*, 901–906.
- (5) Noonan, S. C.; Savage, G. P. Oxalate content of foods and its effect on humans. Asia Pacific J. Clin. Nutr. 1999, 8, 64–74.
- (6) Savage, G. P.; Vanhanen, L.; Mason, S. M.; Ross, A. B. Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. *J. Food Compos. Anal.* 2000, *13*, 201– 206.
- (7) Brinkley, L. J.; Gregory, J.; Pak, C. Y. C. A further study of oxalate bioavailability in foods. J. Urol. 1990, 144, 94–96.
- (8) Chai, W.; Liebman, M. Assessment of oxalate absorption from almonds and black beans with and without the use of an extrinsic label. J. Urol. 2004, 172, 953–957.
- (9) Rowland, I. Optimal nutrition: fibre and phytochemicals. *Proc. Nutr. Soc.* 1999, 58, 415–419.
- (10) Hönow, R.; Hesse, A. Comparison of extraction methods for the determination of soluble and total oxalate in foods by HPLCenzyme-reactor. *Food Chem.* 2002, *78*, 511–521.
- (11) Ohkawa, H. Gas chromatographic determination of oxalic acid in foods. J Assoc. Off. Anal. Chem. **1985**, 68, 108–111.

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- (12) Holmes, R. P.; Goodman, H. O.; Assimos, D. G. Dietary oxalate and its intestinal absorption. *Scanning Microsc.* **1995**, *9*, 1109– 1120.
- (13) Li, M. G.; Madappally, M. M. Rapid enzymatic determination of urinary oxalate. *Clin. Chem.* **1989**, *35*, 2330–2333.
- (14) Chai, W.; Liebman, M. Oxalate content of legumes, nuts, and grain-based flours. J. Food Compos. Anal. 2005, in press.
- (15) Albihn, P. B. E.; Savage, G. P. The bioavailability of oxalate from oca (*Oxalis tuberosa*). J. Urol. 2001, 166, 420–422.
- (16) Ross, A. B.; Savage, G. P.; Martin, R. J.; Vanhanen, L. Oxalate in oca (New Zealand yam) (*Oxalis tuberosa* Mol.). J. Agric. Food Chem. **1999**, 47, 5019–5022.
- (17) Brogren, M.; Savage, G. P. Bioavailability of soluble oxalate from spinach eaten with and without milk products. *Asia Pacific J. Clin. Nutr.* **2003**, *12*, 219–224.

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