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Original Article

Phylloquinone (vitamin K_1) content of vegetables

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Abstract

Assessment of vitamin K (VK) dietary intakes has been limited by incomplete VK food composition data for the US food supply. The phylloquinone (VK-1 or vitamin K_1) concentrations of a variety of geographically representative vegetables ($n = 218$) were determined by reversed-phase high performance liquid chromatography with fluorescent detection. Green leafy and flower vegetables including broccoli, broccoli raab, spinach, and certain lettuces, contained $>100\,\mu g$ phylloquinone/100 g vegetable. In contrast, raw tubers and roots contained $<10\,\mu g$ phylloquinone/100 g vegetable. Iceberg lettuce, a primary dietary source of phylloquinone, contained 24.1 μg phylloquinone/100 g vegetable, which is less than previously listed in nutrient databases. Potential factors affecting phylloquinone concentrations include processing and varietal type of leafy vegetables.

Keywords: Phylloquinone; Vegetables; HPLC; Vitamin K

1. Introduction

Vitamin K (VK) is a fat-soluble vitamin that functions as a coenzyme and is involved in the synthesis of a number of proteins involved in blood clotting and bone metabolism. It may also have a role in osteoporosis and atherosclerosis (Institute of Medicine, 2001). The predominant

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form of VK found in foods is phylloquinone or vitamin K_1. The National Academy of Sciences has established an Adequate Intake (AI) for phylloquinone of 90 μg/day for women and 120 μg/day for men (Institute of Medicine, 2001).

The last decade has shown major developments in the quality and availability of food composition data for phylloquinone. After the publication of US provisional tables of the phylloquinone content in select foods (Booth et al., 1993; United States Department of Agriculture, Agricultural Research Service, 1994), one US database and several European databases incorporated the phylloquinone contents of commonly consumed foods (Bolton–Smith et al., 2000; Deharveng et al., 1999; McKeown et al., 2000). However, as new foods and food products are introduced to the market and consumption patterns shift, the current food composition data are increasingly limited. An updated database would ideally include a large number of samples from an expansive geographical area, as well as a diversity of products to represent various processing techniques.

In 1993, composite samples of common foods in the US food supply were obtained from the Food and Drug Administration's (FDA) Total Diet Study (TDS) and analyzed for phylloquinone using high performance liquid chromatography (HPLC) procedures (Booth et al., 1995). Based on their phylloquinone content, it was concluded from these data that green, leafy vegetables were the primary dietary source of phylloquinone. This has been confirmed in dietary surveys and observational studies (Feskanich et al., 1999; Booth et al., 1996). It has been shown that geographical growth location, climate, soil condition, and stage of maturation can influence the phylloquinone content of green vegetables (Ferland and Sadowski, 1992). Since phylloquinone is primarily found in the photosynthetic tissue of plants, the phylloquinone concentration can also vary within a given vegetable. The outer leaves of cabbage and Brussels sprouts have been shown to contain more phylloquinone than the inner leaves (Bolton-Smith et al., 2000). Also seasonal variation has been reported to effect phylloquinone content of vegetables with summer months yielding more phylloquinone than winter months (Koivu et al., 1997).

Although much is known about factors influencing phylloquinone concentrations in plants, it is important to develop a current and comprehensive database of the average phylloquinone content of vegetables in the US food supply. The current database needs systematic sampling as well as expansion to include new additions in the food supply, such as broccoli raab and tomato products, and a greater variety of sampling within categories of key foods, such as lettuce.

The National Food and Nutrient Analysis Program (NFNAP), sponsored by the National Institutes of Health (NIH) and the US Department of Agriculture (USDA), is designed to provide a comprehensive update to the National Nutrient Databank (Pehrsson et al., 2000). This program will provide analytical nutrient estimates for approximately 1000 representative foods and selected ingredients, and add data for selected new components to the database, including phylloquinone. The phylloquinone concentrations of representative samples of select vegetables in the US food supply are presented in the current study.

2. Materials and methods

Food samples used in this study were obtained from the USDA Nutrient Data Laboratory (NDL) as part of the NFNAP, which has been described in detail elsewhere (Pehrsson et al., 2000). This

food-sampling plan provides aliquots of composited, homogenized samples that are representative of Key Foods consumed in the United States. The number of samples chosen for each product was based on the desired statistical results and the number of nutrient analyses NDL could afford to perform. Samples were collected from retail outlets in 12 cities and were shipped to the Food Analysis and Laboratory Control Center (FALCC) at Virginia Polytechnic Institute and State University, Blacksburg, Virginia where samples were processed as follows. Any refuse was removed and weighed, vegetables were cooked according to specified procedures (Haytowitz et al., 2002), if any, and then composited and homogenized according to standard operating procedures established at FALCC. Aliquots of the composited samples were shipped frozen to the Vitamin K Laboratory at Tufts University, Boston, Massachusetts where they were stored at −80 °C until analysis.

Phylloquinone content of the food samples was determined by reversed-phase high-performance liquid chromatography (HPLC), as described elsewhere (Booth and Sadowski, 1997). Samples were processed with an initial hexane extraction, followed by solid phase extraction (SPE) on silica columns. The extraction and chromatography solvents used were all of HPLC grade (Fisher Scientific Inc., Springfield, NJ). Phylloquinone, zinc chloride, and sodium acetate were purchased from Sigma Chemical Co (St. Louis, MO); zinc (−200 mesh) was purchased from Johnson Matthey Electronics (Ward Hill, MA); the internal standard, $K_{1(25)}$, was a gift from Hoffman La Roche (Basel, Switzerland). The chromatographic system consisted of a 2690 Separations Module (Waters, Milford, MA) equipped with a vacuum degasser and a model RF-10AXL Shimadzu Fluorescence Detector (Shimadzu Instruments, Columbia, MD). The C_{18} analytical column (150 × 3 mm) was packed with 5 μm BDS Hypersil C_{18} (Keystone Scientific, Bellefonte, PA). The mobile phase consisted of two solvents, as described elsewhere (Booth and Sadowski, 1997). Solvent A was methanol with 10 mM $ZnCl_2$, 5.0 mM CH_3COOH, and 5.0 mM CH_3COONa. Solvent B was methylene chloride. Peak integration and sample concentration calculations were performed using Waters Millennium[32] software, version 3.05.01.

All samples were analyzed in duplicate, so reported values for individual samples represent the mean of the duplicates. If the CV of duplicates was greater than 15% (for samples with phylloquinone concentrations >5 μg/100 g) the assay was repeated. The volume of hexane used in analysis was adjusted and dilutions were made to obtain a target of approximately 5 ng of phylloquinone for subsequent SPE on a silica column. A control sample of baby food peaches was run with each batch of foods. A compilation of results from this sample over a four-month period gave a mean ± S.D. result of 4.3 ± 0.7 μg/100 g. Over this same period, the average within-run CV for this sample was 7.8%. This method has a lower limit of detection (LOD) of 14 pg per injection (equivalent to 0.006 μg/100 g of sample).

Statistical analysis: To determine statistically significant differences in phylloquinone concentration due to cooking, two-tailed *t*-tests, assuming equal variances, were performed using Microsoft Excel 97. Comparisons were considered significant when $P < 0.05$.

3. Results and discussion

The phylloquinone data presented in Table 1 are from 166 samples obtained from the NFNAP (Pehrsson et al., 2000), and provide representative estimates of the phylloquinone content of selected vegetables in the US food supply. No 2′,3′-dihydrophylloquinine was detected in any of these samples.

Table 1
Phylloquinone content of selected vegetables (fresh weight basis)

Description	*n*	Phylloquinone (μg/100 g)		
		Mean	s.d.[a]	Range[b]
Artichokes	2	16.2		
Broccoli				
Raw	8	102	19.5	76.6–136
Cooked	4	141	31.7	103–180
Broccoli raab				
Raw	2	242		
Cooked	4	231	54.0	165–294
Carrots				
Raw	12	8.3	4.0	3.9–14.8
Cooked	4	13.7	3.2	10.8–18.2
Celery, raw	8	29.0	10.5	20.6–46.8
Cucumber				
With peel	4	16.4	4.5	11.5–22.2
Without peel	2	2.6		
Lettuce				
Butterhead (Boston)	8	102	26.5	75.5–159
Green leaf	8	127	14.7	111–154
Iceberg	8	24.1	6.9	15.6–36.2
Red leaf	8	123	11.9	103–140
Romaine	8	103	19.9	69.7–129
Onions, sweet	8	0.3	0.1	0.2–0.5
Onions, yellow				
Raw	2	0.2		
Cooked	2	21.7		
Peppers				
Green, raw	4	7.1	0.5	6.8–7.8
Green, cooked	4	21.4	8.1	13.8–32.9
Red, raw	4	4.9	1.2	3.3–6.0
Red, cooked	4	16.5	3.5	12.5–20.9
Potatoes				
Red, raw	4	3.2	1.0	1.6–3.7
Red, cooked	8	2.9	1.2	1.4–4.8
Russet, raw	4	1.8	1.1	0.8–2.7
Russet, cooked	8	2.0	0.9	1.1–3.7
White, raw	3	1.6	0.8	1.0–2.5
White, cooked	7	2.5	1.1	1.3–3.9
Radishes	8	1.4	0.9	0.5–3.1
Sweet potatoes				
Raw	2	1.8		
Cooked	4	2.2	0.7	1.6–3.0

[a]No s.d. reported for analyses of $\leqslant 2$ samples.
[b]No range reported for analyses of $\leqslant 2$ samples.

Consistent with previous studies (Booth et al., 1993; Bolton-Smith et al., 2000; Koivu et al., 1997), the highest phylloquinone concentrations in vegetables were found in green leaves (broccoli raab, spinach, and certain types of lettuces) and green flowers (broccoli). Of the root vegetables, only carrots contained $\geqslant 3.9\,\mu g/100\,g$ of phylloquinone. Other root vegetables were poor sources of phylloquinone. Broccoli (raw and cooked); carrots (raw); celery; butterhead, green leaf, iceberg, red leaf and romaine lettuces; sweet onions; red and russet potatoes (raw and cooked); radishes; and tomatoes (raw and cooked) were sampled at two different time points. Because there were no consistent trends when the phylloquinone concentrations were compared between the two time points, the values were aggregated within Table 1 with no reference to season. Overall, there was a trend toward higher concentrations of phylloquinone in cooked fresh vegetables than in their raw counterparts (Table 1), although this was not consistent across all comparisons. Cooked samples were not necessarily from the same lot of vegetables as the raw counterparts; therefore, the differences may in part be due to sampling variation, in addition to analytical variation. The seemingly large effect of cooking on red and green peppers and yellow onions is attributed to the use of phylloquinone-rich vegetable oils used for cooking. Vegetable oils have been shown to contribute significant amounts of phylloquinone to the US diet (Peterson et al., 2002).

Table 2 shows the effect of cooking on phylloquinone concentration of frozen carrots and spinach. A two-tailed *t*-test analysis showed a significantly higher concentration of phylloquinone in frozen spinach that had been boiled or microwaved as compared to frozen spinach that was not cooked ($P = 0.01$). Phylloquinone is located in the chloroplasts in plants. Cooking by boiling or microwaving may disrupt the plant cell wall, thereby releasing phylloquinone available for detection by HPLC. The sample preparation during analysis includes homogenization and sonication to disrupt cellular integrity. However, it is possible that these steps do not entirely extract all the phylloquinone in the sample prior to HPLC and fluorescent detection, especially among raw vegetables with an intact cell wall. In contrast, there was no apparent effect of cooking on the phylloquinone content of frozen carrots ($P = 0.32$).

Table 3 shows the amount of phylloquinone in tomatoes and tomato products. Tomatoes are typically low in phylloquinone; however, the amount of phylloquinone in the food increased as tomato products became increasingly concentrated (decreasing water content). Of those tomato products analyzed, tomato paste was the most concentrated tomato product and had on average

Table 2
Phylloquinone content of frozen carrots and spinach, before and after processing (fresh weight basis)

Description	*n*	Phylloquinone (μg/100 g)		
		Mean	S.D.	Range
Carrots, frozen whole, sliced, or crinkle cut				
Raw	4	16.4	4.7	9.8–20.6
Boiled	3	15.0	1.2	13.6–16.0
Microwaved	3	13.4	2.4	11.5–16.1
Spinach, frozen chopped or whole leaf				
Raw	5	369	53.9	293–441
Boiled	3	541	7.4	533–547
Microwaved	3	457	99.5	348–544

Table 3
Phylloquinone content of tomatoes and tomato products (fresh weight basis)

Description	*n*	Phylloquinone (μg/100 g)		
		Mean	S.D.[a]	Range[b]
Tomatoes, fresh	8	2.7	0.3	2.2–3.1
Tomatoes, fresh, cooked	8	3.2	0.5	2.6–3.9
Tomatoes, canned whole in tomato juice	2	0.9		
Tomato ketchup	2	2.8		
Tomato paste	3	9.9	2.0	8.5–12.1
Tomato puree	2	3.5		
Tomato sauce	2	2.8		
Salsa, bottled	2	4.5		
Pasta sauce (no meat), regular	2	13.9		

[a]No S.D. reported for analyses of $\leqslant 2$ samples.
[b]No range reported for analyses of $\leqslant 2$ samples.

9.9 μg phylloquinone/100 g. Tomatoes canned whole in tomato juice had a higher water content than the other products and had on average 0.9 μg phylloquinone/100 g. Tomato ketchup had an average of 2.8 μg phylloquinone/100 g. Both salsa, ketchup and pasta sauce are made with herbs and other ingredients, such as oils, that contribute more phylloquinone to the products. Salsa and meatless pasta sauce contained 4.5 and 13.9 μg phylloquinone/100 g, respectively. Given the popularity of tomato products in the diet, they may be a more important contributor of phylloquinone to the diet than previously considered.

The 1994 USDA provisional table reported the phylloquinone content of two classes of lettuce (Booth et al., 1993); however, differences in phylloquinone contents were observed in the current study when five types of lettuce were compared: butterhead (or Boston), green leaf, iceberg, red leaf, and romaine. While romaine lettuce contains 102.5 μg phylloquinone/100 g, iceberg lettuce contains only 24.1 μg phylloquinone/100 g. Lettuce has been reported to be the primary dietary source of phylloquinone in certain populations (Feskanich et al., 1999). Therefore, it is important to distinguish between lettuce varieties in dietary studies. This difference has important implications considering the risk of hip fracture has been shown to be inversely associated with lettuce consumption (Feskanich et al., 1999).

Although green, leafy vegetables are a rich dietary source of phylloquinone, information about the bioavailability of phylloquinone is currently limited. However, a comparison of absorption from vegetables and dietary supplements showed greater phylloquinone absorption from an oil-based supplement than from spinach (Garber et al., 1999; Gijsbers et al., 1996). The absorption of phylloquinone from fortified oil has been shown to be greater than absorption from broccoli (Booth et al., 2002).

These nationally representative food composition data for phylloquinone content of vegetables will update and expand upon those made available in Release 16 of the USDA National Nutrient Database for Standard Reference, which is available on the Nutrient Data Laboratory's Web site: http://www.nal.usda.gov/fnic/foodcomp.

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