Vitamin K_1 (Phylloquinone) Content of Green Vegetables: Effects of Plant Maturation and Geographical Growth Location†

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The vitamin K_1 (phylloquinone) content of five vegetables (cabbage, Swiss chard, leaf lettuce, spinach, and kale) was determined by reversed-phase high-performance liquid chromatography. The plants were grown from seed at two different locations (Boston, MA; Montreal, Canada) and analyzed with respect to maturation, soil conditions, and climatological effects. The green vegetables were found to be excellent sources of vitamin K_1 (5–16 $\mu g/g$). For most vegetables except cabbage, vitamin K_1 contents increased during maturation. Cabbage was found to contain 3–6 times more vitamin K_1 in the outer leaves compared to the inner leaves. The vitamin K_1 content of Montreal vegetables was higher than that of Boston vegetables ($p < 0.05$). The differences between the two locations suggest that climate, soil, and growing conditions may influence the vitamin K_1 content of green vegetables. The current required dietary allowance for vitamin K can be contained in as little as a 10-g portion of a green and leafy vegetable. From a nutritional point of view, these findings have their significance when dietary vitamin K_1 intake in humans is assessed.

INTRODUCTION

Of the four fat-soluble vitamins, vitamin K is probably the one that has received the least attention in dietary analysis. The low incidence of overt vitamin K deficiency in the general population and the belief that a significant part of the vitamin K requirement in humans is contributed by the productions of menaquinones by intestinal bacteria may explain why there was no recommended dietary allowance (RDA) for vitamin K until 1988 (National Research Council, 1989).

Discoveries made in the past two decades have shown that, in addition to its role in hemostasis, vitamin K is necessary for the synthesis of several proteins not involved in blood coagulation. Vitamin K-dependent proteins have been found in bone [bone gla protein or BGP (osteocalcin), matrix gla protein (MGP)] and various tissues (Vermeer, 1990). Although the precise physiological functions for these proteins are not known, the existence of these proteins signals the need to begin to reinvestigate methods for determining vitamin K nutritional status.

The predominant form of vitamin K in foods is vitamin K_1 or phylloquinone. For many years the determination of vitamin K_1 in foods was difficult due to cumbersome and unreliable methodologies. The recent application of high-performance liquid chromatography (HPLC) for the analysis of vitamin K_1 in blood has led to development of better methods for the routine determination of a wide range of vitamin K_1 concentrations in a variety of food matrices (Haroon et al., 1986, 1987a,b; Ferland et al., 1992). Although green and leafy vegetables are generally recognized as the richest source of dietary vitamin K_1, there is little information about nutrient variation during plant growth and maturation. In the experiments reported here, the vitamin K_1 content of five commonly consumed green vegetables (cabbage, Swiss chard, leaf lettuce, spinach, and kale) was determined at different stages of maturation. Also, the effects of geographical growth location, climate, and soil conditions were evaluated by performing the experiment simultaneously in two cities (Boston and Montreal).

EXPERIMENTAL PROCEDURES

Sampling Procedure. The effects of plant maturation and growth location on vitamin K_1 content were studied in five green vegetables: cabbage (inner and outer leaves), Swiss chard, leaf lettuce, spinach, and kale. Vegetables were seeded and grown simultaneously in randomly chosen communal outdoor garden plots located in Boston and Montreal. The experiments were performed during the months of June–August of 1989. Seeding homogeneity in both locations was achieved for each vegetable by using seeds that originated from common lots. The vegetables were harvested at either three (33, 66, 100%) or four (25, 50, 75, 100%) different developmental stages. Plant maturation or development was determined using plant size as criteria. Size of individual maturation stages was established with reference to the expected final vegetable size obtained from the seed package. At harvesting, the plants were frozen intact at −20 °C and protected from light until analysis. All samples were processed and analyzed in the Vitamin K Laboratory of the USDA Human Nutrition Research Center on Aging at Tufts University in Boston. For each maturation period, triplicate samples from three different plants of the sample species were obtained. The effects of climatological conditions on plant maturation and vitamin K_1 content were investigated by recording the daily average temperature (centigrade), precipitation (centimeters), and sunshine (hours) for both cities. Monthly meteorological summaries for Montreal and Boston were obtained, respectively, from the Canadian Atmospheric Environment Service and the American National Weather Service. Finally, to evaluate the possible effect of soil composition on plant maturation and vitamin K_1 content, a composite soil aliquot from each plot was collected and analyzed (Soil and Plant Tissue Laboratory, University of Massachusetts, Amherst, MA).

Analytical Procedure. The vitamin K_1 content of the vegetables was determined by reversed-phase high-performance liquid chromatography (HPLC) using postcolumn solid-phase

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Table I. Summary of Climatological Data Recorded in Montreal and Boston during the Experimental Period[a]

	av temp, °C		precipitation, cm		sunshine, h	
month	Boston	Montreal	Boston	Montreal	Boston	Montreal
June	20.3	17.2	3.3	7.2	311.5	257.3
July	23.2	22.5	19.4	3.1	260.9	236.9
Aug	24.2	20.5	2.8	15.9	298.4	202.2

[a] Data were obtained, respectively, from the Canadian Atmospheric Environment Service and the American National Weather Service.

chemical reduction for the conversion of the quinone to its hydroquinone derivative, which is subsequently detected in a fluorescence detector as previously described (Haroon et al., 1987a,b). Prior to HPLC, the lipid extracts were prepared from the vegetables and partially purified by solid-phase extraction on silica. All steps of the analytical procedure were carried out in subdued lighting or under yellow lights to prevent the deterioration of the vitamin K_1. Prior to extraction, the vegetables were allowed to thaw and blotted onto several layers of absorbent paper to remove excess water. After the nonedible portions were removed, the vegetables were cut into 1-in. pieces and blended in a commercial food processor (Waring, New Hartford, CT). Between 0.5 and 1.0 g of the resulting plant homogenate was weighed and the tissue further disrupted by grinding in a mortar and pestle with 10 times its weight in anhydrous sodium sulfate. After grinding, the sample was transferred to a commercial stainless steel blender (Waring) along with an appropriate amount of internal standard (dihydrovitamin K_1) and 250 mL of a mixture of 2-propanol–hexane (3:2 v/v). The lipids were extracted by homogenizing the combined mixture of solvent, salt, and tissue in the blender at full speed (setting 7) for 2 min. The resulting homogenate was filtered through a medium-porosity sintered glass funnel and the clear filtrate (lipid extract) concentrated to dryness in a rotary evaporator at 40 °C (Büchi, Brinkman Instruments, Inc., Chicago, IL). The residue remaining after evaporation was redissolved in 40 mL of hexane and an aliquot of the reconstituted lipid extract applied to a 3 mL solid-phase extraction silica column (J. T. Baker Inc., Phillipsburg, NJ) which had been preconditioned by washing with 8.0 mL of hexane. The solid-phase extraction column was washed with an additional 8.0 mL of hexane after application of the sample to remove the hydrocarbons. The vitamin K_1 containing fraction was eluted with 8.0 mL of a mixture of hexane–diethyl ether (97:3 v/v). The eluate was collected and evaporated to dryness under reduced pressure in a centrifugal evaporator (Savant Instrument Inc., Farmingdale, NY). The final residue was dissolved first in 0.045 mL of 100% methylene chloride with swirling to help dissolve the lipids, followed by 0.255 mL of methanol containing 10 mM zinc chloride, 5.0 mM acetic acid, and 5.0 mM sodium acetate. The reconstituted sample (150 μL) was injected into the chromatography system. The authenticity of the vitamin K_1 peak was confirmed by removing the postcolumn reactor and verifying that the suspected vitamin K_1 peak disappeared. The percentage recovery of added internal standard varied from 70 to 85% throughout the study.

Values were assessed for statistical significance using two-factor analysis of variance (SAS general linear procedure; Statistical Analysis System Institute, 1985). Differences between groups were determined using paired t test.

RESULTS AND DISCUSSION

Table I shows the climatological data recorded in Boston and Montreal during the experimental period. These meteorological summaries were obtained from the respective American and Canadian National Weather Services. As expected, average temperature and sunshine exposure were slightly lower in Montreal than in Boston. Total precipitation was comparable (Boston, 25.5 cm; Montreal, 26.2 cm); however, the bulk of the precipitation came in July for Boston and in August for Montreal.

To determine the possible effect of soil composition on the vitamin K_1 content of vegetables, soils from both the Boston and Montreal plots were analyzed, and the results are presented in Table II. Overall, the Montreal soil appeared to be richer than the Boston soil as reflected by higher cation-exchange capacity and higher levels of phosphorus (10×), potassium (3×), calcium (3×), and magnesium (2.5×). Values for pH, nitrogen, and micronutrient levels were in the normal range and comparable for both cities.

Table II. Report of Soil Analysis

parameter	Boston	Montreal
soil wt, g/5 cm^3	4.1	4.1
pH	6.3	7.3
cation-exchange capacity, mequiv/100 g	14.9	38.5
nitrogen as NO_3, ppm	30.0	30.0
nitrogen as NH_4, ppm	6.0	12.0
base saturation, %		
K	1.7	2.0
Mg	6.8	6.5
Ca	76.5	91.6
P, ppm	9.0	93.0
K, ppm	79.0	238.0
Ca, ppm	1854.0	5795.0
Mg, ppm	100.0	251.0
Al, ppm	45.0	5.0
Pb	low	low
micronutrients	normal	normal

Table III. Vitamin K_1 Content (Micrograms per Gram) for the Vegetables Grown in Boston and Montreal at the End of Their Maturation (100%)[a]

vegetable	Boston	Montreal
broccoli	not available	1.78 ± 0.02
cabbage inner leaves	0.72 ± 0.01*b	2.28 ± 0.02*a,b
cabbage outer leaves	4.49 ± 0.02	7.19 ± 0.02*a
Swiss chard	7.43 ± 2.23	9.17 ± 1.37
leaf lettuce	5.19 ± 0.49	11.80 ± 2.47*a
spinach	10.01 ± 1.65	14.39 ± 3.00*a
kale	6.21 ± 0.67	16.57 ± 0.62*a

[a] Each value was derived from triplicate determinations of three different plants. (mean ± SEM). * a $p < 0.05$ when compared to corresponding Boston value. * b $p < 0.05$ when compared to cabbage outer leaves.

The vitamin K_1 contents of the six green vegetables that were simultaneously grown in Boston and Montreal are presented in Table III. The results reported in this table represent the vitamin K_1 content at the end (100%) of maturation. In agreement with results reported by other investigators (Siefert, 1979; Shearer et al., 1980; Olson, 1988) the plants with the highest vitamin K_1 contents were the dark green and leafy vegetables (kale, spinach, Swiss chard, and leaf lettuce) with values ranging between 5 and 16 μg/g depending on the growth location. Regardless of the growth location, cabbage was found to contain 3–6 times more vitamin K_1 in the outer compared to the inner leaves ($p < 0.05$). It is of interest that similar trends were reported for vitamin E. Cabbage was found to contain almost nondetectable levels of vitamin E in the colorless heart but higher levels in the outer leaves (Bauernfeind, 1980).

For every vegetable analyzed, the vitamin K_1 contents were higher for samples grown in Montreal than for those obtained from Boston ($p < 0.05$). The geographical differences were particularly marked for cabbage (inner leaves), kale, and leaf lettuce with Montreal values being, respectively, 3.2, 2.6, and 2.3 times higher than the Boston values. The vitamin K_1 contents of the vegetables presented in Table III are much higher than previous estimates obtained by the chick bioassay (Richardson et

al., 1961; Olson, 1988). Generally, the vitamin K_1 contents of the vegetables grown in Boston compare favorably with the results obtained by Shearer et al. (1980), who, using HPLC, reported vitamin K_1 values of 7.24 μg/g for kale, 4.15 μg/g for spinach, 1.47 μg/g for broccoli, 1.37 μg/g for cabbage outer leaves, and 0.83 μg/g for cabbage inner leaves. However, the situation is somewhat different for the vegetables grown in Montreal, which were found to contain between 1.5 and 3.3 times more vitamin K_1 than those vegetables raised in Boston. In fact, the vitamin K_1 contents of the vegetables grown in Montreal represent the highest published values so far for these vegetables. This discrepancy of values between the two geographical locations is surprising and is difficult to explain. Factors such as seeds and harvest plant size are unlikely to account for these differences since common seeds were sown simultaneously in both locations and harvest plant sizes were standardized and established for each vegetable prior to the start of the experiment. In fact, for all of the vegetables studied, the individual and final plant sizes were comparable for both cities. Discrepancies due to sample handling are also improbable since all of the samples (Boston and Montreal) were processed and analyzed under identical conditions in the Vitamin K Laboratory at the USDA Human Nutrition Research Center on Aging at Tufts University in Boston.

Slight differences were observed in the climatological data recorded at both locations during the interval of the experiment. Although these differences appear to be minor, they could have contributed to the variations observed between the two locations. The average temperature and amount of daylight were less in Montreal than in Boston. In addition, the precipitation patterns through the growing season were different. One possibility for the higher values obtained for the vegetables grown in Montreal is that the lower amount of daylight may have had a protective effect against the known photodecomposition of vitamin K. On the other hand, the differences in the amount of daylight may have influenced the concentration of chlorophyll and resulted in changes in vitamin K_1 synthesis in the chloroplasts where vitamin K is known to function in photoinduced electron transfer. Differences in soil composition between the two locations may also have influenced the vitamin K_1 content of the plants. Table II demonstrates that the Montreal soil was much richer than the Boston soil in regard to the higher cation-exchange capacity and higher levels of minerals. Although it is not known how soil composition can affect vitamin K_1 synthesis in plants, the data suggest, given seed and gardening homogeneity, comparable climatological conditions, and sample handling techniques, that soil composition differences could be a major factor influencing the vitamin K_1 content of green vegetables.

Figures 1 and 2 demonstrate the effects of maturation stage on the vitamin K_1 content of the vegetables. Except for the inner leaves of cabbage, the vitamin K_1 content of most vegetables tended to increase during development. This means that on a wet weight basis vitamin K_1 is more concentrated in mature vegetables. The effect of maturation was independent of the geographical location; however, the magnitude of the changes occurring during maturation varied among the vegetables. The greatest increases were observed for Montreal kale ($p < 0.05$) followed by Montreal Swiss chard, Boston spinach, and Boston Swiss chard. It is noteworthy that similar maturation-related changes have also been observed in cabbage for vitamin E, with tocopherols being more concentrated in the larger more mature leaves than in smaller immature

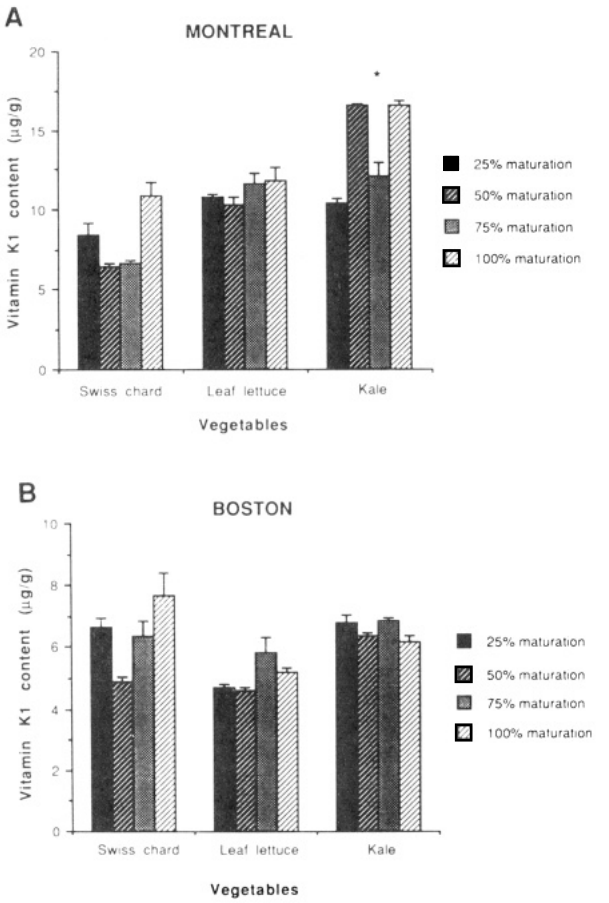

Figure 1. Effect of maturation on the vitamin K_1 content of Swiss chard, leaf lettuce, and kale grown in Montreal (A) and Boston (B). Values for each maturation stage were derived from triplicate determinations of three different plants (mean ± SEM). *$p < 0.05$ at 50 and 100% maturation when compared to 25% maturation.

leaves (Bauernfeind, 1980). Finally, although the vitamin K_1 content of cabbage outer leaves varied little with maturation, the inner leaves decreased by approximately 50% during the growth period.

The current recommended dietary allowance (RDA) for vitamin K has been established at 1.0 μg/kg of body weight (National Research Council, 1989). The adequacy of the typical American diet to furnish the RDA for vitamin K has not been determined and needs to be evaluated. The data presented in this paper demonstrate that green vegetables represent good sources of vitamin K_1, with dark green, leafy vegetables being the richest sources. The green and leafy vegetables (Swiss chard, leaf lettuce, spinach, and kale) provide anywhere from 5 (leaf lettuce, Boston) to 15 μg/g (kale, Montreal), with an average value of 10 μg/g vitamin K_1. Therefore, the current RDA for vitamin K can be supplied by approximately a 10-g serving of a green and leafy vegetable. Unfortunately, virtually nothing is known about the bioavailability of vitamin K_1 from these vegetables and the influences of cooking on their vitamin K_1 content and subsequent bioavailability. To assess the contribution that these vegetables make to the maintenance of adequate vitamin K nutriture, additional research into the bioavailability of vitamin K_1 from different food sources and the effects of cooking and processing must be performed.

In summary, it can be said that green vegetables represent excellent sources of vitamin K_1, with leafy vegetables being the richest. For most of the greens studied, the vitamin K_1 content increased during matu-

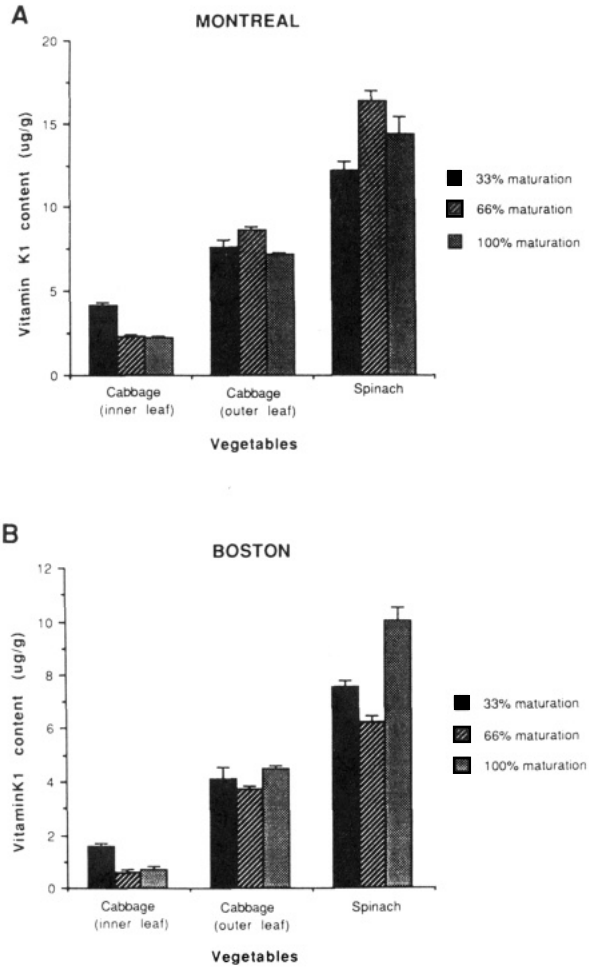

Figure 2. Effect of maturation on vitamin K_1 content of cabbage (inner and outer leaves) and spinach grown in Montreal (A) and Boston (B). Values for each maturation stage were derived from triplicate determinations of three different plants (mean ± SEM).

ration, though the extent of the changes varied with the vegetable being studied. Although it is not known how climate and soil composition influence the synthesis of vitamin K_1 in plants, the results of this study suggest that, given seed homogeneity, climate and soil could be factors influencing the vitamin K_1 content of green vegetables. It is apparent from our data that geographical differences may contribute to the significant variations observed in the determination of the vitamin K content of foods, further suggesting that minor changes in climate and soil conditions may result in significant seasonal changes in the vitamin K content of vegetables grown at the same location. From a nutritional perspective, these findings have their significance when dietary vitamin K_1 intakes in humans are assessed.

LITERATURE CITED

Bauernfeind, J. Tocopherols in food. In *Vitamin E: a comprehensive treatise*; Machlin, L. J., Ed.; Dekker: New York, 1980; pp 99–167.

Ferland, G.; MacDonald, D. L.; Sadowski, J. A. Development of a diet low in vitamin K_1 (phylloquinone) content. *J. Am. Diet. Assoc.* **1992**, *92*, 593–597.

Haroon, Y.; Bacon, D. S.; Sadowski, J. A. Liquid chromatographic determination of vitamin K_1 in plasma with fluorometric detection. *Clin. Chem.* **1986**, *32*, 1925–1929.

Haroon, Y.; Bacon, D. S.; Sadowski, J. A. Reduction of quinones with zinc metal in the presence of zinc ions: Application of post-column reactor for the fluorometric detection of vitamin K compounds. *Biomed. Chromatogr.* **1987a**, *2*, 4–8.

Haroon, Y.; Bacon, D. S.; Sadowski, J. A. Chemical reduction system for the detection of phylloquinone (vitamin K_1) and menaquinones (vitamin K_2). *J. Chromatogr.* **1987b**, *384*, 383–389.

National Research Council. *Recommended dietary allowances*, 10th ed.; National Academy Press: Washington, DC, 1989.

Olson, R. E. Vitamin K. In *Modern nutrition in health and disease*, 7th ed.; Shils, M. E., Young, V. R., Eds.; Lea & Febiger: Philadelphia, 1988; pp 328–339.

Richardson, L. R.; Wilkes, S.; Ritchey, S. J. Comparative vitamin K activity of frozen, irradiated and heat-processed foods. *J. Nutr.* **1961**, *73*, 369–373.

Shearer, M. J.; Allan, V.; Haroon, Y.; Barkhan, P. Nutritional aspects of vitamin K in the human. In *Vitamin K metabolism and vitamin K-dependent proteins*; Suttie, J. W., Ed.; University Park Press: Baltimore, 1980; pp 317–327.

Siefert, R. M. Analysis of vitamin K_1 in some green leafy vegetables by gas chromatography. *J. Agric. Food Chem.* **1979**, *27*, 1301–1304.

Statistical Analysis System Institute. *SAS user's guide: statistics*, 5th ed.; SAS Institute: Cary, NC, 1985.

Vermeer, C. γ-Carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. *Biochem. J.* **1990**, *266*, 625–636.

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