Evaluation of an HPLC Method for the Determination of Phylloquinone (Vitamin K_1) in Various Food Matrices

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The amount of phylloquinone (vitamin K_1) in five different foods was determined by applying a highly sensitive HPLC method that incorporates postcolumn chemical reduction of the quinone followed by fluorescence detection of the hydroquinone form of the vitamin. After initial extraction in 2-propanol and hexane, the food extracts were purified by solid-phase extraction (SPE) on silica gel and analyzed by HPLC or, depending on the matrix, further purified using liquid-phase reductive extraction or reversed-phase SPE on C_{18}. Intra- and interday precision of the assay ranged from 6.6 to 13.6%. The between sample coefficient of variation in the phylloquinone content of 10 random bulk samples ranged from 7.8 to 44.6%, depending on the food item analyzed. In addition, endogenous dihydrophylloquinone was identified in foods containing hydrogenated oils, although its biological activity is unknown.

INTRODUCTION

Advances in understanding the metabolism and function of vitamin K have clearly established its role as a cofactor for the posttranslational synthesis of γ-carboxyglutamic acid (gla) in vitamin K-dependent proteins (Suttie, 1985). Vitamin K is predominantly known for its role in the synthesis of coagulation proteins (factors II, VII, IX, and X) (Suttie, 1988). Recent findings, however, suggest a much wider role for gla-containing proteins and vitamin K outside the hemostatic system (Shearer, 1992). This has led to a renewed interest in methods for the assessment of vitamin K nutritional status and requirements. To date, little systematic analysis of vitamin K_1 (phylloquinone) in foods and beverages has been reported (Sadowski et al., 1991). Therefore, in the absence of reliable food composition data, it has not been possible to estimate dietary intake and/or adequacy of phylloquinone in a given population. This situation has also created difficulties in providing reliable dietary guidelines for individuals with thromboembolic diseases managed through the use of warfarin, a vitamin K antagonist (Howard and Hannaman, 1985; Kempin, 1983; Pedersen et al., 1991). It is now clear that the management of warfarin therapy is sensitive to dietary intake of phylloquinone. Dietary intake of phylloquinone may be a critical factor influencing the efficacy of low-dose or minidose warfarin in the future (Sadowski et al., 1991).

Out of concern for the validity of the earlier methods used for the determination of phylloquinone in foods, a provisional table was recently compiled exclusively using high-performance liquid chromatography (HPLC) data (Booth et al., 1993). The application of HPLC to the determination of phylloquinone at physiological concentrations in plasma has facilitated the development of more sensitive and precise methods for routine analysis in food (Rizzolo and Polesello, 1992). Unfortunately, most of the recently published data using HPLC are limited to human and cow milk, infant formulas, and leafy green vegetables (Canfield et al., 1990; Fournier et al., 1987; Haroon et al., 1982; Shearer et al., 1980). Previous food composition tables that included data from outdated methods such as the chick bioassays, in addition to more current procedures (Parrish, 1980; Pennington, 1989; Souci et al., 1986), had extremely large variations in the phylloquinone data presented.

In the absence of information on sampling techniques (sample size, portion of food sample analyzed, etc.), it is not known if different phylloquinone values for a given food item reflect sampling or analytical sources of variation. Estimates of precision and experimental variability in phylloquinone content, both within and among samples analyzed, are few in number (Canfield et al., 1990; Shearer et al., 1980).

Our laboratory has previously described a highly sensitive HPLC method for the determination of phylloquinone in plasma using fluorometric detection following postcolumn solid-phase chemical reduction of the quinone form of the vitamin to its fluorescent hydroquinone (Haroon et al., 1986). In this paper, we describe HPLC sample preparation procedures for the determination of phylloquinone in various food matrices that were selected to represent foods commonly consumed in the United States. The precision of the assay is presented for the different foods. Estimates of the heterogeneity of the phylloquinone content among different samples of a single food item are also presented.

MATERIALS AND METHODS

Sampling. Five food items were selected for analysis representing a range of food matrices and phylloquinone concentrations: vegetable juice (V8); whole milk (3.5% fat content); raw spinach leaves; plain bagel; and raw ground beef (75% lean). Food items were purchased from various retail stores within the greater Boston area, and the location, date of purchase, and lot number (where applicable) of each food item were noted.

Intraday variance of the assay was determined by analyzing each food sample 10 successive times on the same day. To determine interday variance, food items were analyzed in triplicate on three subsequent days. The same vegetable juice, bagel, and ground beef samples were used for intraday and interday studies. Due to spoilage of the milk and spinach, interday variability in these two food items was determined using a second set of samples.

To estimate the heterogeneity of the phylloquinone content among different samples of a given food item, 10 bulk samples of vegetable juice, milk, and spinach each were randomly selected. Each bulk sample was homogenized and analyzed in triplicate. Although different brands of each food item were analyzed to

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maximize sampling variability, all food items were purchased in the greater Boston area between July 1992 and January 1993. Food items were stored at 4 °C and wrapped in aluminum foil to protect them from light until analysis. All analyses were performed within 5 days of purchase.

Reagents and Standards. The solvents used in sample extraction and chromatography were of HPLC grade (Fisher Scientific Inc., Springfield, NJ). Vitamin $K_{1(20)}$ or phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) was purchased from Sigma Chemical Co. (St. Louis, MO). The internal standard 2,3-dihydrophylloquinone that was used for vegetable juice, milk, and spinach samples was synthesized by hydrogenation of phylloquinone using a modified procedure originally described by Langenberg and Tjaden (1984). The internal standard $K_{1(25)}$ [2-methyl-3-(3,7,11,15,19-pentamethyl-2-eicosenyl)-1,4-naphthalenedione] that was used exclusively for bread and beef samples was a gift from Hoffman-La Roche and Co. (Basle, Switzerland). Standard solutions were diluted to known concentrations in 100% methanol and characterized spectrophotometrically and chromatographically. Working solutions were stored at 4 °C and shielded from light. All procedures involving sample processing and preparation were performed under yellow lighting since vitamin K compounds are sensitive to photooxidation. Glassware and utensils were washed in a chromic–sulfuric acid solution (Fisher Scientific) to minimize contamination of samples from fluorescent material or carry-over of analyte.

Extraction of Phylloquinone from Vegetable Juice and Cow Milk. Juice and milk samples were individually mixed before analysis to ensure homogeneity. Aliquots (0.25–0.50 g) from each sample were weighed directly into 50-mL polypropylene centrifuge tubes (Corning Co., Corning, NY). Twenty microliters of the internal standard dihydrophylloquinone (1.014 ng) was added directly to the samples. Fifteen milliliters of 2-propanol/hexane (3:2 v/v) was added, followed by 4 mL of H_2O. The mixtures were dispersed by sonication (continuous output at 40% duty cycle for 30 s) using a Branson Model 350 sonifer–cell disruptor with a $^1/_8$-in. tapered microtip (Branson Ultrasonics Corp., Danbury, CT), vortexed (10 min), and centrifuged at 1000*g* (10 min). After phase separation, the upper hexane layer was aspirated into clean 16 × 100 culture tubes and evaporated to dryness in a centrifugal evaporator (Savant Instruments Inc., Farmingdale, NY). The residue was redissolved in 2 mL of hexane. Whole milk samples required an additional partitioning into hexane when the residue from the initial extraction did not completely evaporate to dryness. This was performed by the addition of 4 mL of hexane and 2 mL of H_2O to the first residue, followed by vortexing (1 min) and centrifugation (5 min).

Solid-Phase Extraction on Silica Gel. The hexane extracts were further processed by solid-phase extraction (SPE) using silica gel columns (3 mL) (J. T. Baker Inc., Phillipsburg, NJ) that were preconditioned by a wash of 8 mL of hexane/diethyl ether (93:3 v/v), followed by 8 mL of 100% hexane. The 2-mL extract was applied directly onto the preconditioned column packing, followed by a wash with 8 mL of 100% hexane. The phylloquinone-containing fraction was eluted from the SPE columns with an 8-mL wash with hexane/diethyl ether (93:3 v/v). The eluants were collected into clean 16 × 100 screw-cap culture tubes and evaporated to dryness. The vegetable juice residue was reconstituted in 20 μL of 100% methylene chloride with swirling to completely dissolve the residue. Immediately after dissolving, 180 μL of methanol (containing 10 mM $ZnCl_2$, 5 mM acetic acid, and 5 mM sodium acetate) was added. A sample volume of 100 μL was injected into the HPLC.

Liquid-Phase Reductive Extraction. Given the high fat content of whole milk, a reductive extraction step was added. The milk residue remaining from SPE column eluate was redissolved in 2 mL of hexane, followed by the addition of 4 mL of a reductive extraction mixture containing 10 g/L $ZnCl_2$, 30 mL/L acetic acid, and 970 mL/L acetonitrile. Approximately 50 mg of zinc metal (−200 mesh; Alpha Products, Ward Hill, MA) was then added, and the tubes were capped and vortexed vigorously for 5 min to reduce phylloquinone and dihydrophylloquinone to their acetonitrile-soluble hydroquinones. The tubes were centrifuged for 2 min at 1000*g* and the upper hexane layer which contained the contaminating lipids was removed and discarded. Two milliliters of H_2O and 4 mL of hexane were added to the remaining tube contents to oxidize the hydroquinones to their hexane-soluble quinones. The tubes were mixed (1 min) and centrifuged at 1000*g* for 5 min. After centrifugation, the hexane (top) layer containing the phylloquinone and dihydrophylloquinone was removed and evaporated. The final residue was reconstituted in the same manner as the juice.

Preparation of Spinach Samples. Spinach leaves were ground to a uniform consistency in a commercial food processor (Waring Products Div., New Hartford, CT), and 0.5 g was weighed out for analysis. The weighed samples were further ground to a fine powder in 5.0 g of anhydrous sodium sulfate with a mortar and pestle. The ground spinach powder was quantitatively transferred to a 50-mL centrifuge tube. Twenty microliters of an internal standard containing 202.7 ng of dihydrophylloquinone was added to the sample. Fifteen milliliters of 2-propanol/hexane (3:2 v/v) and 32 mL of H_2O were added, followed by sonication (30 s), vortexing (10 min), and centrifugation (5 min). The upper layer containing the phylloquinone and dihydrophylloquinone was aspirated into a clean 16 × 100 culture tube and evaporated to dryness in a centrifugational evaporator. Ten milliliters of hexane was added to redissolve the residue. A 50-μL aliquot was placed in a clean 16 × 100 culture tube and further processed by SPE on silica gel columns and reconstituted for injection as described for the juice samples. A 100-μL sample was injected into the HPLC.

Preparation of Bread Samples. Bread samples were ground to a powder using a mortar and pestle. Aliquots of 0.25–0.30 g were removed from the powder and weighed directly into 50-mL centrifuge tube. Fifty microliters of the internal standard $K_{1(25)}$ in methanol (1.151 ng) was added directly to the sample. Sample preparation consisted of the initial liquid-phase extraction in 2-propanol and hexane that was described for juice and milk samples. The dried lipid residue from the hexane extract was dissolved in 4 mL of hexane and applied to a SPE silica gel column (6 mL) (J. T. Baker) following the procedure outlined for vegetable juice and milk. The final residue was reconstituted initially in 30 μL of 100% methylene chloride, followed by 270 μL of methanol containing 10 mM zinc chloride, 5 mM acetic acid, and 5 mM sodium acetate. A 150-μL sample was injected into the HPLC.

Preparation of Beef Samples. Ground beef was ground to a uniform "paste-like" consistency in a Waring commercial food processor, and 0.25–0.5 g was weighed out for analysis. The samples were further processed to a fine powder by grinding with 10 times their weight in anhydrous sodium sulfate, followed by quantitative transfer to a 50-mL centrifuge tube. Fifty microliters of the internal standard $K_{1(25)}$ in methanol (1.151 ng) was then added directly to the sample. Fifteen milliliters of 2-propanol/hexane (2:2 v/v) and 32 mL of H_2O were added to the sample, followed by sonication (30 s), vortexing (10 min), and centrifugation (5 min). Sample preparation followed that described for bread samples where the initial liquid-phase extraction in 2-propanol and hexane was followed by extraction on SPE silica gel columns (6 mL). To further purify the beef sample for injection on the HPLC, a reversed-phase C_{18} SPE extraction was used to separate phylloquinone from the large quantity of saturated fats present. After evaporation, the residue from the eluate of the silica gel SPE columns was dissolved in 200 μL of 2-propanol while heating (45 °C) for 10 min. The reversed-phase C_{18} columns (6 mL) (J. T. Baker) were preconditioned by successive washes with 6 mL of methanol/methylene chloride (80:20 v/v), followed by 6 mL of 100% methanol and 6 mL of 100% H_2O. The samples were applied directly to the preconditioned packing. The column was washed with 6 mL of methanol/H_2O (95:5 v/v) followed by 6 mL of 100% acetonitrile and the sample eluted from the column with 10 mL of methanol/methylene chloride (80:20 v/v). The C_{18} SPE column eluant was evaporated and redissolved in the same manner outlined for reconstituting the bread extracts.

Separation of Phylloquinone by HPLC. The chromatographic system consisted of a Model 510 pump and a Model 712B (WISP) injector (Waters Associates, Milford, MA). Pump control, integration, and quantitation were achieved using a Model 860 VAX based data station with Expert Ease software (version 3.0) (Waters). Fluorescence was monitored using a Model 980 fluorescence detector (ABI Analytical, Ramsey, NJ) with exci-

tation and emission wavelengths of 244 and 418 nm, respectively (high voltage setting, 1250 V; photomultiplier tube setting, 1.0 μA).

The analytical column (150 × 4.6 mm) was packed with 3 μm of Hypersil ODS (Keystone Scientific, Bellefonte, PA). Fluorescent derivatives of the injected quinones were produced online using a postcolumn chemical reactor (2.0 × 50 mm) packed with zinc metal (−200 mesh; Alpha Products). The mobile phase consisted of methanol/methylene chloride (90:10 v/v), to each liter of which we added 5 mL of a solution containing 2 M zinc chloride, 1 M acetic acid, and 1 M sodium acetate, and was pumped at a constant flow rate of 1.0 mL/min. Quantitation was achieved by direct comparison of peak area ratios of the food samples to authentic standards of phylloquinone and the corresponding internal standards using a Waters 860 chromatography data system (Waters Chromatography, Milford, MA).

Peak Confirmation and Purity Tests. In the bread extracts a peak corresponding to the retention time of authentic dihydrophylloquinone was found, suggesting a reduction of endogenous phylloquinone to dihydrophylloquinone during the commercial hydrogenation of oils. To confirm the identity of this peak, we extracted partially hydrogenated soybean oil (Crisco shortening) and a food source rich in partially hydrogenated oil (commercially prepared chocolate cake) using the procedure outlined for bread samples. Removal of the postcolumn reactor used in our analytical system for reducing quinones to their fluorescent derivatives eliminated the peaks corresponding to phylloquinone and dihydrophylloquinone in the shortening, cake, and standard solutions, providing preliminary evidence about the chemical nature of the compounds in question. To assess the peak purity of the dihydrophylloquinone found in these foods, the fluorescence ratio of the height of the peak corresponding to dihydrophylloquinone was compared at three specific excitation wavelengths (244, 272, and 330 nm) while keeping the emission wavelength constant at 418 nm. The ratios of the peak heights for the shortening and cake samples were compared with the ratios obtained at the same wavelengths for a standard solution of pure dihydrophylloquinone.

Statistical Analysis. Phylloquinone concentrations were expressed as the mean with corresponding standard deviations (SD). Precision of the assay was defined as the coefficient of variation (% CV) for intra- and interday variations. For the 10 random bulk samples, the within sample variations were averaged to obtain a single within sample coefficient of variation per food item. The coefficients of variation of the individual sample means were used to estimate the between sample coefficient of variation for the corresponding food item. The between sample coefficients of variation were slightly overestimated in this approach because the individual sample means were influenced by analytical error.

RESULTS AND DISCUSSION

Assay of Phylloquinone in Foods. Our laboratory has previously described an HPLC methodology for the routine determination of phylloquinone in plasma based on fluorescence detection (Haroon et al., 1986). This analytical method has been applied to the analysis of a variety of biological (Huber et al., 1992) and nonbiological samples (Lennon et al., 1993), including selected food matrices (Ferland and Sadowski, 1992a,b; Ferland et al., 1992). Presented here are procedures for the determination of phylloquinone in food samples representative of a range of food matrices commonly encountered in food analysis. A flow chart summarizing the basic analytical procedures is presented in Figure 1.

Representative chromatograms for extracts of vegetable juice, whole milk, raw spinach, bread (bagel), and ground beef are shown in Figure 2. Phylloquinone and the internal standards dihydrophylloquinone and $K_{1(25)}$ were readily separated from the coextractable or coeluting compounds found in these foods. Under the conditions outlined, phylloquinone eluted by approximately 7.5 min, while elution of dihydrophylloquinone (Figure 2A–C) occurred at approximately 8.5 min. The retention time of the internal standard $K_{1(25)}$, used in the preparation of the bread and beef samples, was approximately 11.5 min (Figure 2D,E). Since these foods were analyzed on different days, slight day to day variation in retention times for the three peaks probably results from small

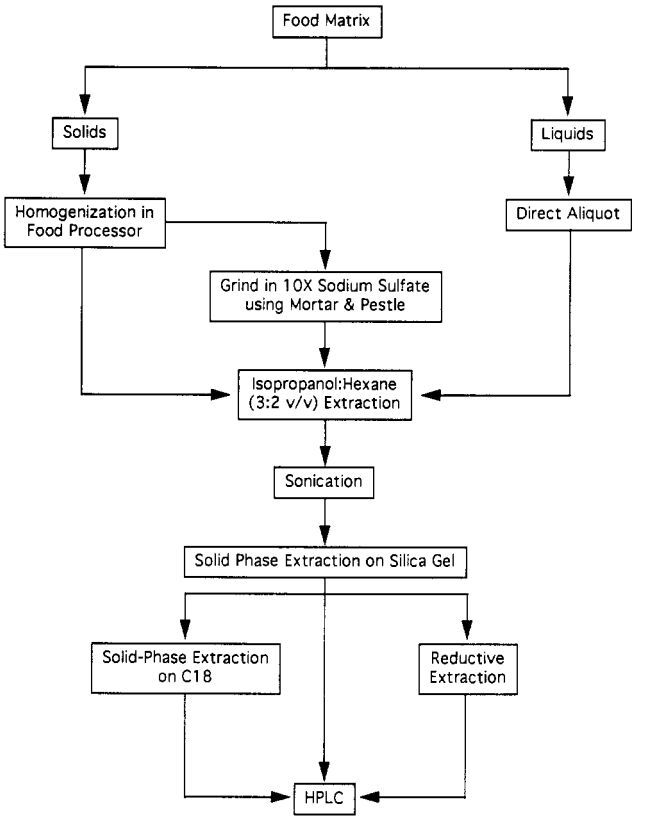

Figure 1. Flow chart for the analytical scheme of phylloquinone determination in different food matrices.

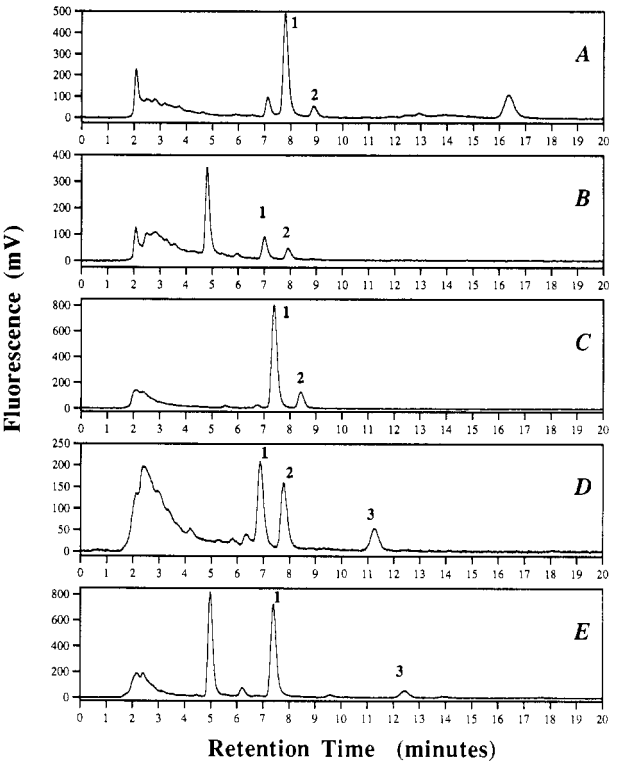

Figure 2. Representative chromatograms for extracts of (A) vegetable juice, (B) milk, (C) spinach, (D) bread, and (E) beef. Peaks correspond to (1) phylloquinone (2) dihydrophylloquinone, and (3) $K_{1(25)}$.

Table 1. Precision of the Assay As Indicated by the Intraday (n = 10) Coefficient of Variation in the Phylloquinone Content of Five Food Items

food	phylloquinone (μg/100 g)	CV (%)
vegetable juice	5.29	6.6
milk[a]	0.21	12.1
spinach[a]	482.9	6.3
bread	0.36	10.9
ground beef	2.98	9.5

[a] Intraday variations in phylloquinone contents of milk and spinach samples were determined from samples different from those used to determine interday variations

Table 2. Precision of the Assay As Indicated by the Interday (n = 3) Coefficient of Variation in the Phylloquinone Contents of Five Food Items

food	phylloquinone (μg/100 g)	CV (%)	internal standard
vegetable juice	5.74	12.3	dihydro-K_1
milk	0.32	13.8	dihydro-K_1
spinach	202.0	7.4	dihydro-K_1
bread	0.39	10.8	$K_{1(25)}$
ground beef	2.57	12.6	$K_{1(25)}$

changes in chromatographic conditions (temperature, flow, mobile phase). However within-run variation in the retention times for these peaks of interest was insignificant. A calibration standard containing phylloquinone and the corresponding internal standard was injected after every five samples to correct for changes in conditions.

Analytical Variation. The precision of the assay as defined by the coefficients of variation among 10 successive determinations on a sample on the same day (intraday) is presented in Table 1 and on the same food sample on three different days (interday) in Table 2. In spite of potential error in sample preparation, including homogenization, and the complexity of the matrices from which phylloquinone is extracted from, the analytical variation for the assay (CV = 6.6–13.8%) is respectable.

Internal Standards. Dihydrophylloquinone was found to be a suitable internal standard for the analysis of vegetable juice, whole milk, and spinach and would be appropriate for the analysis of most commonly consumed foods. Recoveries of the internal standard averaged 71.9 ± 8.4%, 61.6 ± 8.2%, and 81.0 ± 7.1% for vegetable juice, milk, and spinach, respectively. These recovery rates correspond to those previously reported from our laboratory (Ferland and Sadowski, 1992a,b). Likewise, Lambert et al. (1992) reported 62.5% recovery of their internal standards in their phylloquinone analysis in milk.

In our initial attempts to analyze bread samples we found recoveries of the internal standard dihydrophylloquinone in excess of 100%. When these samples were analyzed in the absence of the internal standard, a peak corresponding to the retention time of dihydrophylloquinone was found. For preliminary confirmation of the suspected dihydrophylloquinone, we removed our postcolumn reactor, which eliminated the suspected peak from the chromatogram. Peak purity was established using the calculated fluorescence ratios for the peak heights of the shortening and commercial cake at three different excitation wavelengths as divided by the corresponding peak heights of the standard solution of dihydrophylloquinone. As shown in Table 3, there was good agreement in the fluorescence ratios between the two food items and the standard dihydrophylloquinone solution. It would appear that endogenous dihydrophylloquinone, often found in bread and other bakery products, is related to the content of hydrogenated oils. Since several oils are relatively good sources of phylloquinone (Ferland and Sadowski, 1992b),

Table 3. Peak Purity Test for Dihydrophylloquinone

	calculated fluorescence ratio		
excitation wavelength (nm)	dihydrophylloquinone standard	Crisco	chocolate cake
272/330	0.43	0.38	0.41
272/244	0.05	0.05	0.05

Table 4. Determination of the Within and Between Sample Variation in the Phylloquinone Content of 10 Random Bulk Samples of 3 Food Items, Each Analyzed in Triplicate

food	N	phylloquinone[a] (μg/100 g)	range (μg/100 g)	CV (%) between[b]	CV (%) within[c]
vegetable juice	10	4.7 ± 2.1	2.1–9.1	44.6	6.0
milk	10	0.3 ± 0.03	0.2–0.3	7.8	9.9
spinach	10	299.5 ± 96.0	175.6–505.6	32.6	5.0

[a] Mean ± SD. [b] Between sample coefficient of variation. [c] Within sample coefficient of variation.

saturation of the 2,3 double bond of the side chain of the vitamin would normally occur during the hydrogenation process. However, it is not known if dietary dihydrophylloquinone can contribute to overall vitamin K nutriture in humans.

Since the dihydro form of the vitamin is not a suitable internal standard for those foods containing hydrogenated or partially hydrogenated oils, we substituted $K_{1(25)}$ (a synthetic analog of phylloquinone produced by the substitution of a 25-carbon side chain to menadione). However, this precluded the use of the liquid-phase reductive extraction used in the preparation of the milk samples since the chemistry of the reductive extraction, due to the polarity of the solvents, is such that recovery diminishes as side-chain length increases beyond 20 carbons. To remove the remaining lipids which would otherwise be removed with the reductive extraction, a reversed-phase, SPE using 6-mL silica columns was employed. The reductive extraction did not adequately separate phylloquinone from the large quantity of saturated fats present in the beef samples, so an additional solid-phase extraction was added using 6-mL C_{18} columns. Recovery of the internal standard from the bread and beef samples averaged 67.8 ± 8.9% and 51.8 ± 7.7%, respectively. Lower recoveries in the beef samples are attributable to the use of the reversed-phase extraction on C_{18}.

While under these conditions we chose to use $K_{1(25)}$ as the internal standard, other vitamin K analogs can be used such as menaquinone 4 (MK_4). However MK_4, like dihydrophylloquinone, is naturally occurring in a variety of foods (Sakano et al., 1988; Hirauchi et al., 1989). We have not found any evidence to support the existence of $K_{1(25)}$ in foods or a peak with the same elution characteristics of $K_{1(25)}$, and so we feel confident about its suitability for food analyses.

Sampling Variation. The total sample variation for the vegetable juice, milk, and raw spinach leaf samples (n = 10), which was defined as the between sample coefficient of variation in the mean phylloquinone content for the corresponding food item, varied from 7.8 to 44.6% (Table 4). The within sample coefficient of variation in milk samples, which is an indication of analytical error, was slightly greater than the variation attributable to actual sampling (between sample coefficient of variation). As milk contains very low concentrations of phylloquinone, the within sample variation may reflect the lower limits of the assay. In contrast, both vegetable juice and spinach samples had greater sampling variation (7.4 and 6.5 times

greater, respectively) than that attributable to the assay. The sampling variation observed in spinach would reflect the natural sources of variation inherent within a plant species such as the amount of rainfall, light exposure, and soil conditions. Since vegetable juice is a composite of several plants that are relatively good sources of phylloquinone, sampling variation would reflect differences among product lots and different companies coupled with natural sources of variation associated with each raw ingredient. Therefore, it is likely that composite or processed foods will have greater variation attributable to sampling error than that of individual raw or unprocessed foods. Since samples were only obtained from the greater Boston area during a single season, these between sample coefficients of variation are most likely underestimates. For example, Ferland and Sadowski (1992a) reported significant differences in the phylloquinone content of green leafy vegetables grown in two different geographical locations. Likewise, Fournier et al. (1987) reported significant increases in the phylloquinone concentration of cow milk between the months of January and July.

The ranges of the phylloquinone content in the 10 samples of each of the foods presented in Table 4 (vegetable juice, milk, and spinach leaves) are approximately 3 times the standard deviation of the corresponding food item. These data support observations by Elkins and Dudek (1985) that a sample size of 10–20 is sufficiently large when the distribution is normal. There is, therefore, a 95% probability that the mean phylloquinone content of 10 samples lies within a range of the hypothetical true mean (±2 SE). Although the precision of the reported phylloquinone content of a food item could be improved with a larger sample size, the other sources of variation associated with sampling, such as geographic and seasonal differences, would not be addressed. Instead, composite samples collected from different regions during different seasons would be more cost and time effective (Roberts, 1974).

Phylloquinone Concentration in Foods. Of the five food items analyzed, three have been previously reported in the literature. The average phylloquinone content of spinach leaves reported by different investigators ranges from 240 to 1220 μg/100 g of fresh leaf (Ferland and Sadowski, 1992; Kodaka et al., 1986; Langenberg et al., 1986; Sakano et al., 1988; Shearer et al., 1980). In our analyses of 10 random bulk samples purchased from local stores, we found a mean phylloquinone content of 299.5 μg/100 g of fresh spinach leaf (Table 4). For the phylloquinone content of whole milk, Hirauchi et al. (1989) reported 0.3 μg of phylloquinone/100 g for milk (3.5% fat content), which is identical to the mean value of 0.3 μg/100 g reported in this study. Shearer (personal communication) reported a range of 0.30–1.78 μg of phylloquinone/100 g of different types of pasteurized milk. Both Ferland et al. (1992) and Hirauchi et al. (1989) reported lower phylloquinone values for ground beef (0.60 and 0.30 μg/100 g, respectively) than the mean value of 2.7 μg/100 g presented here. In contrast, Shearer (personal communication) found that minced beef contained a mean phylloquinone content of 2.2 μg/100 g. However, since only one sample of ground beef was used for analyses and was not included in the determination of sampling variation, our value cannot be considered representative.

No accurate description of the vitamin K content of the typical American diet has been determined. However, estimates place the value between 300 and 500 μg/day (Olson, 1988). We are currently using the procedures described here to improve both the quality and quantity of the data for the phylloquinone content of foods so that an accurate and reliable estimation can be made. This determination will be facilitated by the analysis of samples from the Food and Drug Administration's United States Total Diet Studies (Pennington, 1992). Over 250 commonly consumed foods in this study are collected four times per year from different regions and consist of a representative sample of foods consumed in the United States. An analysis of this type will determine precisely how much phylloquinone is in the typical American diet in relation to the recommended daily allowance of 0.5–1.0 μg/kg of body weight (NRC, 1989).

In addition, dietary tools for the evaluation of nutrient intake such as food records and frequencies could be developed for determining vitamin K intakes if a better database was available. The validated nutrient intake tools for vitamin K will be helpful for epidemiological studies designed to look at the relationship of vitamin K nutritional status with different disease states such as osteoporosis and vascular disease.

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