



Original Article

Phylloquinone and dihydrophyloquinone contents of mixed dishes, processed meats, soups and cheeses [☆]

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Abstract

Assessment of dietary intakes of phylloquinone (VK-1) and dihydrophyloquinone (dK) has been limited by an overall deficit of food composition data, especially for mixed dishes and processed foods. Ninety-eight geographically representative food samples, obtained as part of the National Food and Nutrient Analysis Program (NFNAP), were analyzed for VK-1 and dK using reversed phase HPLC with fluorescent detection. The VK-1 concentrations of the mixed dishes, processed meats, soups, and cheeses ranged from zero (non-detectable, ND) to 11.1 µg/100 g; the dK concentrations ranged from zero (ND) to 22.4 µg/100 g. No dK was detected in the cheese samples. Minimal variation in VK-1 content was observed between the cooked and uncooked samples. Mixed dishes, processed meats, soups, and cheeses contain relatively small amounts of phylloquinone and dK when compared with vegetables and certain plant oils. However, since these foods may frequently be consumed in large amounts, they may be important dietary contributors of vitamin K. © 2003 Elsevier Ltd. All rights reserved.

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1. Introduction

Vitamin K is a fat-soluble vitamin that occurs in nature as a series of molecular forms (Shearer, Bach, & Kohlmeier, 1996). Phylloquinone, or vitamin K-1, is predominately found in photosynthetic plants and is the major source of dietary vitamin K in most food systems (Booth,

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Webb, & Peters, 1999a). The hydrogenation of phylloquinone-rich oils results in the conversion of phylloquinone to dihydrophyloquinone (dK) (Booth, Pennington, & Sadowski, 1996a; Davidson, Booth, Dolnikowski, & Sadowski, 1996).

Interest in vitamin K as it relates to risk of chronic disease, such as osteoporosis (Feskanich, et al., 1999; Booth et al., 2000), has led to an increased demand for vitamin K food composition data. In addition, case reports from patients taking warfarin, a vitamin K antagonist, suggest a potential sensitivity to fluctuating dietary vitamin K intake (Booth, 1999b). In order for health care workers to appropriately counsel patients on diets that have a constant vitamin K content, accurate food composition data are required. Early food composition data used chick bioassays that were more qualitative than quantitative. The development of reliable chromatographic procedures has resulted in greater sensitivity and accuracy of vitamin K measurement in foods (Booth, Davidson, & Sadowski, 1994; Booth & Sadowski, 1997; Jakob & Elmadfa, 2000; Koivu-Tikkanen, Ollilainen, & Piironen, 2000; Schurgers & Vermeer, 2000).

Phylloquinone is assumed to be present in most foods, whereas dK is exclusively found in foods that use hydrogenated oils (Davidson et al., 1996; Booth et al., 1996a). Although inclusion of phylloquinone measures in food composition databases has increased (Booth, Sadowski, Weihrauch, & Ferland, 1993; USDA Agricultural Research Service, 1994; Booth, Sadowski, & Pennington, 1995; Shearer et al., 1996; Deharveng, Charrondiere, Slimani, Southgate, & Riboli, 1999; Schurgers et al., 1999; Bolton-Smith, Price, Fenton, Harrington, & Shearer, 2000; Piironen & Koivu, 2000), minimal data are available on phylloquinone or dK concentrations in processed foods. Furthermore, common foods in the US food supply, including mixed dishes, such as soups, pizza, and macaroni and cheese, and processed meats such as chicken nuggets, hot dogs and cheeses, have not been adequately represented in previous food analysis studies.

In 1997, the National Food and Nutrient Analysis Program (NFNAP) initiated an integrated system for identifying foods and nutrients, food sampling, food preparation, sample preparation, and chemical analysis (Pehrsson, Haytowitz, Holden, Perry, & Beckler, 2000). Approximately 1000 foods and ingredients were identified as important contributors of various nutrients in the US food supply. As part of the NFNAP, foods were prioritized for analysis. The Key Foods approach (Haytowitz et al., 1996) was used to select the foods to be analyzed. This approach combines food composition and food intake data to determine important food contributors of nutrients of public health significance to the diet. Pizza was the highest ranked mixed dish using the Key Foods approach and was therefore among the first foods to be sampled. Other mixed dishes, processed meats, soups, and cheeses were also highly ranked. These were selected for sampling and subsequently analyzed for phylloquinone and dihydrophyloquinone, as reported here.

2. Materials and methods

2.1. Sampling

Nationally representative food samples used in this analysis were obtained from the USDA Nutrient Data Laboratories (NDL) as part of the Food and Nutrient Analysis Program (NFNAP), which has been described in detail elsewhere (Pehrsson et al., 2000, 2002). The goal of this program is to obtain national level estimates of the nutritional components for common

foods consumed in the United States. A three-stage sampling plan was employed. The first stage is to identify the counties where sampling was to occur. Within each region of roughly equal population, three Consolidated Metropolitan Statistical Areas were selected and sorted in descending order by population. A probability proportional to size systematic sample of size three was selected and sorted in descending order by their urbanicity (Goodall, Kafadar, & Tukey, 1998). A systematic sample of size two was selected for a total of 24 locations. Sorting counties by urbanicity ensured that the sample contained both more urban and lesser urban areas.

The second stage was to select a sample of grocery store outlets from within each county selected in the first stage. To accomplish our desire for a self-weighting sample, outlets were selected proportional to their value of annual sales; larger outlets, in terms of annual sales, had higher probabilities of selection. Only stores with annual sales of \$2 000 000 were included as it was felt they would have the diversity of products needed for this study.

The goal of the third sampling stage was to select specific food products (brands and package sizes) for nutrient analysis from food types (e.g., cheese pizza, chili with beans) identified by NDL. This information was obtained from Nielsen Market Research SCANTRACK data, which is obtained from checkout price scanners and, consequently, exclude products sold in stores without such equipment. To maximize the likelihood of having selected products available in all outlets, we restricted the product sampling universe to products with at least 1% market share. 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. After purchase by agents at the selected retail outlets, food samples are shipped overnight to the Food Analysis Laboratory Control Center at Virginia Polytechnic Institute and State University in Blacksburg, Virginia in appropriate containers. Samples from 12 to 24 locations are then composited and homogenized to form national composites for 6–12 brands. In general, one or two samples were selected from each food product for vitamin K analysis and shipped in a frozen state to the Vitamin K Laboratory at Tufts University, Boston, Massachusetts. Samples were then stored at -80°F until time of analysis.

2.2. Reagents and standards

All solvents used in sample extraction and chromatography were of HPLC grade (Fisher Scientific, 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); purified 2',3'-dihydrophylloquinone was a gift from J. Pyrek, University of Kentucky Mass Spectrometry Facility; and the internal standard, $\text{K}_{1(25)}$, was a gift from Hoffman La Roche (Basel, Switzerland). Primary and secondary stock solutions were diluted to known concentrations in methanol and characterized spectrophotometrically and chromatographically. To reduce potential contamination from fluorescent residue the glassware and utensils were washed with acetone. Yellow light was used during extraction and analysis because vitamin K is sensitive to photo-oxidation.

2.3. Extraction of phylloquinone and dihydrophylloquinone

The phylloquinone and dK contents of the food samples were determined using a modification of the method described by Booth and Sadowski (1997) and Peterson et al. (2002). All samples

were analyzed in duplicate. Samples were repeated if the CV of duplicates was greater than 15% (for samples with phylloquinone concentrations $> 5 \mu\text{g}/100 \text{ g}$), and/or a control sample of peach baby food run with each batch of foods was outside the SD of the established mean.

2.4. Quantitation of phylloquinone by HPLC

A reversed phase C_{18} column and an HPLC instrument were used to determine concentrations of phylloquinone and dihydrophyloquinone in foods. 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 analytical column ($150 \times 3 \text{ mm}$) was packed with $5 \mu\text{m}$ BDS Hypersil C_{18} (Keystone Scientific, Bellefonte, PA). Fluorescent derivatives of the injected quinones were produced on-line using a post-column reactor ($2 \times 50 \text{ mm}$) dry packed with zinc (-200 mesh). The excitation wavelength was 244 nm and the emission wavelength was 430 nm . The mobile phase consisted of two solvents. Solvent A is methanol with 10 mM ZnCl_2 , $5 \text{ mM HC}_2\text{O}_2\text{H}_3$, and $5 \text{ mM NaC}_2\text{O}_2\text{H}_3$. Solvent B is methylene chloride. The 2690 was programmed to do the following gradient elution procedure: (a) pump a 90:10 (A:B) mixture at 0.60 ml/min from injection for the first 11.50 min ; (b) at 11.50 min , change the flow rate to 0.80 ml/min and the composition to 70:30 (A:B); (c) at 19.50 min , change the composition to 90:10 (A:B); (d) at 23.50 min , change the flow rate to 0.60 ml/min ; and (e) at 24.0 min , end the cycle.

Standard curves were prepared from each calibrator injection. The fluorescence responses for phylloquinone, dihydrophyloquinone and for $\text{K}_{1(25)}$ were linear with the slope of the lines bisecting zero. Therefore a single-point calibration was routinely performed, forcing the slope of the line through zero. Quantitation was achieved by direct comparison of peak area ratios (VK-1 or dK to $\text{K}_{1(25)}$) generated from the calibration standard to those generated by the sample. Peak integration and sample concentration calculations were performed using Waters Millennium³² software, version 3.05.01.

3. Results and discussion

Phylloquinone and dihydrophyloquinone (dK) concentrations of 98 food composites categorized as mixed dishes, processed meats, soups, and cheeses are presented in Table 1.

Consistent with earlier reports (Booth et al., 1993, 1995; USDA, 1994), mixed dishes, processed meats, soups, and cheeses analyzed in the current study contain relatively low amounts of phylloquinone when compared with green vegetables (Bolton-Smith et al., 2000; Booth et al., 1993). In the samples analyzed in the current study, the phylloquinone concentrations ranged from zero (ND) to $11.1 \mu\text{g}/100 \text{ g}$, with 94% of the samples containing $< 10 \mu\text{g}/100 \text{ g}$. The dK concentrations ranged from zero (ND) to $22.4 \mu\text{g}/100 \text{ g}$, with 90% of the samples containing $< 10 \mu\text{g}/100 \text{ g}$ of dK. Pepperoni pizza, chicken nuggets, and chicken tenders contained more than $10 \mu\text{g}/100 \text{ g}$ of both phylloquinone and dK. Based on their frequent consumption in large quantities, these three foods may be important contributors of phylloquinone and dK in the diet. In contrast to the processed foods described here, the unprocessed counterparts such as raw or cooked chicken and beef were reported to contain $1\text{--}3 \mu\text{g}/100 \text{ g}$ of phylloquinone (USDA, 1994).

Table 1
Phylloquinone and dihydrophyloquinone content of mixed dishes, meats, soups and cheeses

Category	Food	n	Amount (μg) in 100 g edible portion	
			Phylloquinone Mean \pm SD ^a	Dihydrophyloquinone Mean \pm SD
Mixed dishes	Frozen bean and cheese burrito (uncooked)	2	6.2 \pm 0.4	5.0 \pm 0.4
	Frozen beef and bean burrito (cooked)	2	7.5 \pm 0.1	2.4 \pm 0.5
	Frozen beef and bean burrito (uncooked)	2	6.8 \pm 0.0	2.0 \pm 0.0
	Canned chili w/ meat & beans (uncooked)	1	2.7 ^b	2.5 ^b
	Canned chili w/ meat, no beans (uncooked)	2	2.1 \pm 0.3	2.5 \pm 0.4
	Beef stew (uncooked)	2	1.3 \pm 0.1	2.9 \pm 0.4
	Canned spaghetti, no meat (cooked)	1	0.8 ^b	4.2 ^b
	Canned spaghetti, no meat (uncooked)	1	0.7 ^b	2.7 ^b
	Canned spaghetti w/ meat (uncooked)	1	1.1 ^b	1.8 ^b
	Frozen lasagna w/ meat, regular (uncooked)	2	3.3 \pm 1.3	1.3 \pm 0.4
	Frozen lasagna w/ meat, lower fat (uncooked)	2	5.5 \pm 4.7	0.9 \pm 0.7
	Frozen cheese lasagna, no meat or veg (cooked)	1	9.1 ^b	ND ^c
	Frozen cheese lasagna, no meat or veg (uncooked)	1	5.2 ^b	ND
	Frozen cheese pizza, regular thin crust (cooked)	4	7.1 \pm 1.6	5.1 \pm 2.0
	Frozen cheese pizza, rising crust (uncooked)	2	2.7 \pm 1.7	1.8 \pm 1.7
	Pepperoni pizza (cooked)	4	11.0 \pm 5.2	14.3 \pm 7.4
	Sausage & pepperoni pizza (cooked)	4	9.2 \pm 4.8	13.0 \pm 4.6
	Frozen meat and vegetable pizza (cooked)	3	7.0 \pm 2.2	5.1 \pm 5.6
	Canned macaroni & cheese (cooked)	1	0.4 ^b	1.9 ^b
	Boxed mac & cheese w/ prepared sauce (cooked)	1	0.5 ^b	1.1 ^b
Boxed mac & cheese w/ dry powder (cooked)	2	6.4 \pm 1.3	5.8 \pm 0.1	
Chicken pot pie (cooked)	3	6.4 \pm 8.1	4.0 \pm 3.6	
Turkey pot pie (cooked)	2	1.6 \pm 0.2	1.2 \pm 1.1	
Processed meats	Frozen chicken nuggets (cooked)	2	11.0 \pm 0.9	22.4 \pm 3.8
	Frozen chicken nuggets (uncooked)	1	9.9 ^b	18.7 ^b
	Frozen chicken tenders (cooked)	3	10.9 \pm 11.0	10.2 \pm 17.6
	Frozen chicken tenders (uncooked)	1	5.9 ^b	ND
	Canned chunk light tuna in water	3	2.3 \pm 3.7	ND

Table 1 (continued)

Category	Food	n	Amount (μg) in 100 g edible portion	
			Phylloquinone Mean \pm SD ^a	Dihydrophyloquinone Mean \pm SD
	All beef hot dogs, regular fat (cooked)	2	4.7 \pm 5.2	ND
	All beef hot dogs, regular fat (uncooked)	2	3.9 \pm 0.5	ND
	All chicken hot dogs, regular fat (cooked)	1	0.2 ^b	ND
	All chicken hot dogs, regular fat (uncooked)	2	0.1 \pm 0.0	ND
	Meat franks, regular fat (cooked)	1	0.5 ^b	ND
	Meat franks, regular fat (uncooked)	3	3.1 \pm 4.3	ND
Soups	Onion soup, dry	2	8.5 \pm 6.6	2.6 \pm 1.3
	Chicken noodle cup of soup, dry	2	11.1 \pm 0.2	ND
	Ramen noodle chicken flavor, dry	2	2.6 \pm 0.1	1.0 \pm 0.6
	Ramen noodle beef Flavor, dry	2	2.6 \pm 0.5	0.7 \pm 0.4
	Condensed tomato soup	2	3.1 \pm 0.1	ND
	Condensed chicken noodle soup	2	0.7 \pm 0.9	ND
	Condensed cream of mushroom soup	2	6.4 \pm 5.9	ND
	Condensed cream of chicken soup	2	2.3 \pm 1.9	ND
Cheeses	Cheddar cheese	2	3.1 \pm 0.2	ND
	Swiss cheese	3	2.5 \pm 0.1	ND
	Low moisture part-skim shredded mozzarella cheese	2	1.3 \pm 0.4	ND
	American cheese	2	1.8 \pm 2.2	ND
	Grated parmesan cheese	2	1.9 \pm 2.3	ND
	Whole milk mozzarella cheese	2	2.3 \pm 0.0	ND

All samples analyzed in duplicate.

^aSD: standard deviation.

^bNot applicable—only one sample.

^cNot detectable.

It is assumed that oils and other ingredients added during processing of the samples analyzed in the current study increased the phylloquinone and dK content.

In comparing the cooked and uncooked sample concentrations of similar foods (Table 1), cooking had little or no effect on phylloquinone or dK concentrations. A few exceptions did exist, such as frozen cheese lasagna and meat franks (regular fat). However, the differences were small and probably attributable to analytic and sampling variations because different foods were used for the determination of uncooked and cooked sample concentrations.

Compared to data obtained from the US-FDA/Total Diet study (Booth et al., 1995), the phylloquinone concentrations measured in the current study were higher for chicken nuggets

(1.5 ± 0.2 and 10.7 ± 0.9 , respectively) and tomato soup (1.5 ± 0.7 and 3.1 ± 0.1 , respectively), but lower for beef stew (4.8 ± 0.1 and 1.3 ± 0.1 , respectively) and canned chili with beans (4.7 ± 0.9 and 2.7 , respectively). Similar results between the two studies were found for macaroni and cheese, lasagna with meat, spaghetti with tomato sauce, and canned condensed chicken noodle soup. Variation in phylloquinone concentrations could be derived from analytical variation or from differences in sampling methods, sample variation, industrial formulation of recipes, geographical variation, or type and amount of oil used in processing and/or cooking (Ferland & Sadowski, 1992a, b). DK concentrations were not reported for most of these foods in the previous study (Booth et al., 1995), and therefore could not be compared with the current data. Likewise, vitamin K (phylloquinone or DK) data were not available for similar mixed dishes from other regions for comparison (Bolton-Smith et al., 2000).

The recommended adequate (AI) intake of vitamin K varies by age and sex. The recommended intake is 30 and 55 $\mu\text{g}/\text{days}$ for girls and boys, aged 1–8 years, respectively, 60 and 75 $\mu\text{g}/\text{days}$ for girls and boys aged 9–18 years, respectively, and 90 and 120 $\mu\text{g}/\text{days}$ for women and men, aged ≥ 19 years, respectively (National Academy of Science, 2001). Published dietary intake data indicate that the mean reported vitamin K intake for men and women aged 13 and over is $\sim 80 \mu\text{g}/\text{days}$ and increases with age to $\sim 150 \mu\text{g}/\text{days}$ for people over age 64 (Booth et al., 1999a). The mean reported dK intake for the same group is $\sim 20 \mu\text{g}/\text{d}$ (Booth et al., 1999a). Children have the highest reported intake of dK per body weight (30% of total vitamin K intake) of all age groups (Booth, Pennington, & Sadowski, 1996b). The reported data suggested that a majority of the population does not meet the suggested adequate intake set for vitamin K (National Academy of Science, 2001).

Menaquinones, a form of vitamin K that is limited to animal products, were not measured in this study, but may be present in some of the foods, as suggested by Koivu-Tikkanen et al. (2000). As more food composition data become available for vitamin K, particularly with the addition of dK to databases, the vitamin K intake data may be challenged. Data from the current study indicate that a typical serving of two slices of pepperoni pizza would contribute 22.0 μg of phylloquinone and 24.6 μg of dK, which combined, is approximately 50% of the total recommended intake of an adolescent (National Academy of Science, 2001). However, little is known about the relative bioavailability of different forms of vitamin K from different foods. In a recent metabolic study, dK was less absorbed than phylloquinone and had no measurable effect on vitamin K dependent proteins involved in bone formation (Booth et al., 2001). Therefore, while the absolute intakes of vitamin K may be underestimated using existing databases, differences in the biological activity among forms of vitamin K may complicate the interpretation of vitamin K intakes required for adequate vitamin K status.

4. Conclusions

The phylloquinone and dK concentrations of mixed dishes, processed meats, soups, and cheeses vary considerably depending on the ingredients. The foods analyzed here contain relatively low amounts of phylloquinone and dK as compared to previously published data on vegetables and plant oils, but are often eaten more frequently and in greater amounts. Therefore, mixed dishes may be an important contributor of dietary vitamin K in the diet. In order to properly monitor

vitamin K intake, ongoing analysis of foods for vitamin K is required to develop an accurate food composition database which includes the variety of foods in the US diet.

Disclaimers

Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors, and do not necessarily reflect the view of the US Department of Agriculture.

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