Conversion of Vitamin K_1 to 2′,3′-Dihydrovitamin K_1 during the Hydrogenation of Vegetable Oils

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When vitamin K_1 (phylloquinone) was measured by HPLC in foods containing partially hydrogenated vegetable oils, a peak corresponding to the retention time of 2′,3′-dihydrovitamin K_1 was observed. Dihydrovitamin K_1 has a molecular mass 2 Da greater than that of vitamin K_1 due to saturation of the 2′,3′ double bond of the side chain. For confirmation of this putative compound, samples of a commercial shortening, soybean oil, and soybean oil subjected to light and heavy hydrogenation were purified by preparative HPLC and analyzed by GC/MS. Authentic dihydrovitamin K_1 and the oil samples produced molecular ions at *m*/*z* 452 and fragment ions at *m*/*z* 186. Although absolute levels of both vitamin K_1 and dihydrovitamin K_1 decreased with an increase in hydrogenation, there was an overall increase in the ratio of dihydrovitamin K_1 to vitamin K_1. The biological activity of dihydrovitamin K_1 remains to be determined; however, if biologically active, it could have a significant role in vitamin K nutriture.

Keywords: *Vitamin K; phylloquinone; dihydrovitamin K_1; vegetable oils; hydrogenation*

INTRODUCTION

There has been a gradual increase in the consumption of plant oils in the American diet in response to health concerns associated with the intake of saturated fats and cholesterol from animal sources (Enig et al., 1990; Hunter and Applewhite, 1986). Plant oils, such as soybean, cottonseed, and corn, rank high in their contribution to the overall dietary intake of fats and oils, most often in the form of processed foods, baked goods, margarines, and salad dressings (Block et al., 1985). Hydrogenation is a common process used by the food industry to increase the oxidative stability of polyunsaturated oils and to convert liquid oils into semisolid fats, thereby increasing their commercial applications (Torrey, 1983). One of the outcomes of hydrogenation of plant oils is the isomerization change of fatty acids from the *cis* to the *trans* form. It has been reported that between 20 and 40% of the total fatty acids in some margarines and shortenings are in the *trans* form (Nawar, 1983). The health implications of this reaction remain unresolved and have recently been reviewed by several authors (Dupont et al., 1991; Willet et al., 1994; Lichtenstein, 1994).

In addition to their fatty acid composition, plant oils are important dietary sources of the fat-soluble vitamins E and K (Murphy et al., 1990; Ferland and Sadowski, 1992; Booth et al., 1993). In an analysis of margarines and margarine-like products, some of which contained partially hydrogenated oils, Slover et al. (1985) reported variable concentrations of tocopherols. These authors postulated that the wide margin of variation in tocopherol content may have been a combination of natural variation and losses due to processing and storage. To date, the effect of hydrogenation on the vitamin K content of plant oils has not been studied.

Our laboratory has described a high-pressure liquid chromatography (HPLC) method for routine determination of vitamin K_1 in various food matrices (Booth et al., 1994). In our analysis of vitamin K_1 in several finished foods, we observed a peak corresponding to the retention time of 2′,3′-dihydrovitamin K_1. Dihydrovitamin K_1 (452 Da) has a molecular mass 2 Da greater than that of vitamin K_1 (Figure 1A) due to saturation of the 2′,3′ double bond of the phytyl side chain (Figure 1B). Dihydrovitamin K_1 is synthesized from vitamin K_1 and used for analytical purposes such as an internal standard. However, prior to our initial identification of endogenous dihydrovitamin K_1 (Booth et al., 1994) in finished foods, there have been no reports in the literature citing it as a constituent of food or hydrogenated oils or shortening. Since partially hydrogenated vegetable oils were used in the composition of one of the finished foods (commercial whole wheat bread) we analyzed, we theorized that dihydrovitamin K_1 might have been produced during the commercial hydrogenation of the oils. This was further supported by the presence of this putative compound in other foods containing partially hydrogenated oils.

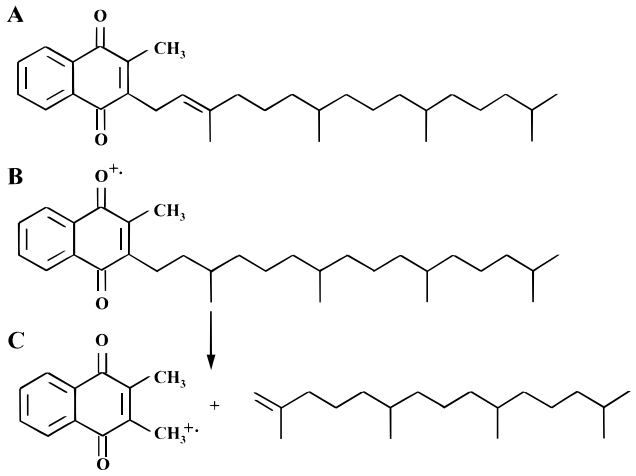

Figure 1. Structures of (A) vitamin K_1 (MW 450), (B) dihydrovitamin K_1 (MW 452), and (C) dihydrovitamin K_1 following fragmentation.

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In this paper we describe the identification of 2′,3′-dihydrovitamin K_1, a hydrogenated form of vitamin K_1, associated with the hydrogenation of vitamin K_1-rich vegetable oils. In addition, we present the vitamin K_1 and dihydrovitamin K_1 concentrations of a commercial shortening containing partially hydrogenated oils, soybean oil, and soybean oil subjected to light and heavy hydrogenation.

MATERIALS AND METHODS

Standards and Reagents. The extraction and chromatography solvents used were all of HPLC grade (Fisher Scientific Inc., Springfield, NJ). Vitamin $K_{1(20)}$ or vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone) was purchased from Sigma Chemical Co. (St. Louis, MO); 2′,3′-dihydrovitamin K_1 was synthesized by hydrogenation of vitamin K_1 using a modification of the procedure reported by Langenberg and Tjaden (1984). The internal standard vitamin $K_{1(25)}$ used for quantitative HPLC of the oil samples was a gift from Hoffman-La Roche and Co. (Basle, Switzerland). Standard solutions of vitamin K_1 and dihydrovitamin K_1 were prepared in 100% methanol for HPLC and in 100% hexane for gas chromatography/mass spectrometry (GC/MS) and were characterized spectrophotometrically before being used.

Oil Samples. A partially hydrogenated shortening (Crisco, Procter & Gamble, Cincinnati, OH) produced from soybean and cottonseed oils was purchased locally in the Boston area. Three soybean oil samples, taken from sequential states of the hydrogenation process, were donated by the Institute of Shortening and Edible Oils (ISEO) (Washington, DC). These included a refined, bleached, and deodorized soybean oil (RBD), a lightly hydrogenated soybean oil, and a heavily hydrogenated soybean oil. The three samples provided by ISEO were taken from the same lot, which allowed for direct comparisons among the different states of hydrogenation.

Sample Preparation for Quantitative and Preparative HPLC. Extracts of the oils were prepared for both quantitative and preparative HPLC using a modification of a procedure previously described by Ferland and Sadowski (1992) and Booth et al. (1994). Briefly, for quantitative HPLC, 0.1 g of each oil was weighed into an acid-washed glass culture tube. Four nanograms of the internal standard vitamin $K_{1(25)}$ and 10 mL of 100% hexane were then added, and the samples were vortexed for 20 min. A 1.0 mL aliquot was removed and brought to a total volume of 4.0 mL with 100% hexane. The extracts were then purified by two-stage, solid-phase extraction (SPE) before injection on the HPLC. The samples were applied to preconditioned 6 mL silica gel columns. The eluate in 3% ethyl ether in hexane was collected, evaporated to dryness, and reconstituted in 200 μL of 2-propanol with heat (45 °C) for 10 min. The samples were then applied to preconditioned C_{18} columns. The eluate in 20% methylene chloride in methanol was evaporated and redissolved initially in 30 μL of methylene chloride, followed by 270 μL of methanol (containing 10 mM zinc chloride, 5 mM sodium acetate, and 5 mM acetic acid). A 150 μL sample was injected into the HPLC. All oil samples were analyzed in triplicate.

For preparative HPLC and confirmation by mass spectrometry, 0.1 g of oil was dissolved in 4.0 mL of hexane (no internal standard was added) and the samples were vortexed for 20 min. Between 10 and 20 samples of each oil were extracted for fractionation. Oil extracts were applied directly to the SPE silica columns using the procedure followed for quantitative analyses. The preparative samples were reconstituted in a volume of 200 μL of mobile phase, and the total volume was injected on the column. Fractions were collected from successive injections of each sample corresponding to the retention times of dihydrovitamin K_1 and pooled. The pooled fractions were extracted from the mobile phase into 100% hexane. The extracts were evaporated and redissolved in 400 μL of mobile phase for reinjection on the chromatograph. This process was repeated two more times for a total of three fractionations for each oil sample. Pooled fractions containing the putative dihydrovitamin K_1 were stored in hexane at −70 °C and protected from light before injection on the mass spectrometer.

HPLC Instrumentation and Operating Conditions. The chromatography system used for quantitative and preparative HPLC consisted of a model 510 pump and a Model 712B (WISP) injector (Waters Chromatography, Milford, MA). The 150 mm × 4.6 mm column was packed with 3 μm Hypersil ODS (Keystone Scientific Inc., Bellefonte, PA). Fluorescent hydroquinone derivatives of the quinones were produced on-line, postseparation using a chemical reactor. Fluorescence was monitored at an excitation wavelength of 244 nm and emission at 418 nm using a Model 980 fluorescence detector (ABI Analytical, Ramsey, NJ). The mobile phase consisted of 10 mM zinc chloride, 5 mM sodium acetate, and 5 mM acetic acid in methanol/methylene chloride at 90/10 (v/v) or 95/5 (v/v) for quantitative or preparative HPLC, respectively. The flow rate was held constant at 1.0 mL/min. A Waters 860 VAX-based data station was used for pump control, integration, and quantitation.

GC/MS Instrumentation and Operating Conditions. The gas chromatograph (GC) was a Model 5890 with a 7673A autoinjector (Hewlett-Packard Co., Wilmington, DE). The GC was fitted with a 0.25 m × 0.53 mm uncoated deactivated fused silica retention gap connected to a 30 m × 0.25 mm fused-silica capillary column containing DB-5 stationary phase (J&W Scientific, Folsom, CA). The column oven and the on-column injector temperature were programmed to increase from 50 to 300 °C at 20 °C/min. The transfer line to the mass spectrometer was maintained at a constant 300 °C. The helium carrier gas pressure was increased throughout the run to maintain a constant flow rate of 1.3 mL/min. The spectrometer used was a Hewlett-Packard Model 5988A quadrupole mass spectrometer, operated in 70 eV electron ionization mode. The ion source was heated to 200 °C, and the quadrupole was scanned from *m/z* 10 to 500 during the run.

For injection, the pooled fractions were evaporated to dryness and brought to a concentration of approximately 20 ng/μL of the putative dihydrovitamin K_1 in 100% hexane. A 1.0 μL sample was injected onto the column. The data were collected by a Hewlett-Packard RTE data system and further processed using GRAMS/386 software (version 3.01) (Galactic Industries Corp., Salem, NH).

RESULTS AND DISCUSSION

Identification of Dihydrovitamin K_1 by GC/MS. Retention times for authentic dihydrovitamin K_1 were identical to those peaks observed by GC/MS in extracts of the commercial shortening and the light and heavily hydrogenated soybean oils. The dihydrovitamin K_1 standard produced a molecular ion at *m/z* 452 and a fragment ion at *m/z* 186 (a structure is proposed in Figure 1C). The extracts of the commercial shortening and the light and heavily hydrogenated soybean oils produced mass spectra consistent with spectra of the authentic dihydrovitamin K_1 (Figure 2). Dihydrovitamin K_1 was not detected in the RBD soybean oil, thus supporting our theory that dihydrovitamin K_1 is a product of the commercial hydrogenation process.

Dihydrovitamin K_1 Content of Hydrogenated Oils. HPLC chromatograms of the oil sample extracts are shown in Figure 3. Dihydrovitamin K_1 is slightly less polar than vitamin K_1 and elutes immediately after the latter under the chromatographic conditions outlined. Dihydrovitamin K_1 could not be detected in the RBD soybean oil (Figure 3A). The vitamin K_1 and dihydrovitamin K_1 contents of the commercial shortening and oils are presented in Table 1. Soybean oil is a rich dietary source of vitamin K_1, and these data are consistent with other HPLC analyses of vitamin K_1 in soybean oils (Ferland and Sadowski, 1992; Zonta and Stancher, 1985). Since the RBD oil analyzed in this study was not obtained "off the shelf" from a grocer or supermarket, but prior to the commercial hydrogenation process, there would have been minimal losses due to light and storage conditions that have been reported

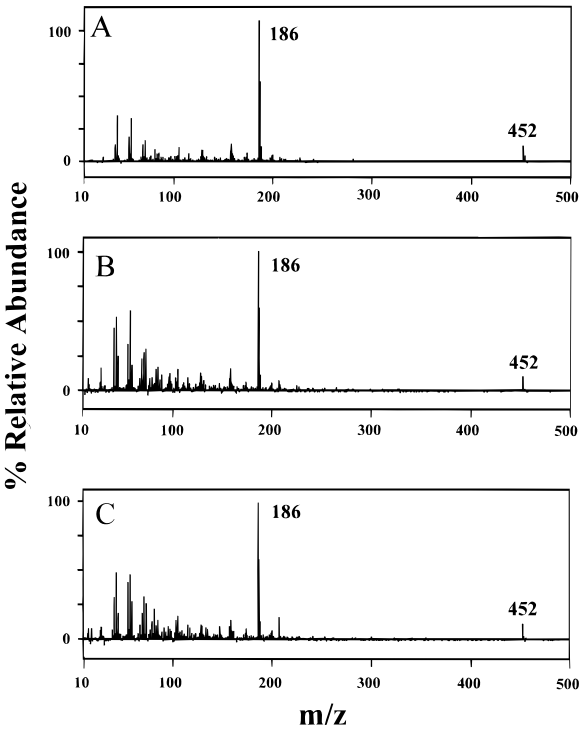

Figure 2. Mass spectra of (A) dihydrovitamin K_1 standard, (B) lightly hydrogenated soybean oil, and (C) heavily hydrogenated soybean oil. Mass spectra are background subtracted.

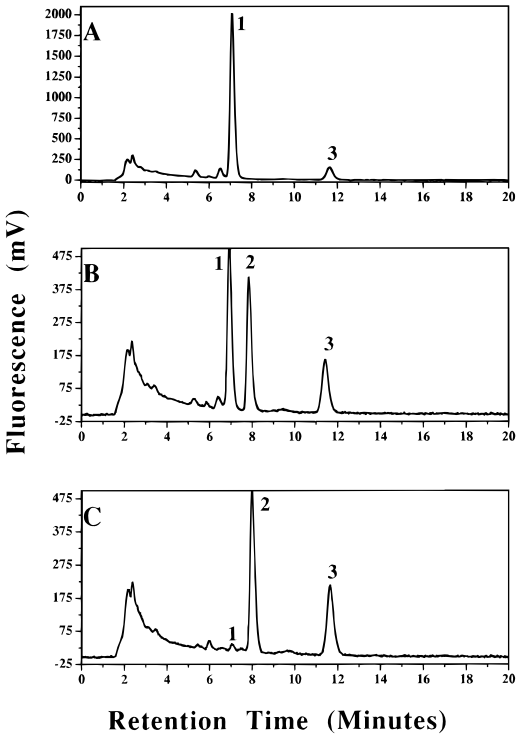

Figure 3. HPLC chromatogram of (A) RBD soybean oil, (B) lightly hydrogenated soybean oil, and (C) heavily hydrogenated soybean oil. Peaks correspond to (1) vitamin K_1, (2) dihydrovitamin K_1, and (3) $K_{1(25)}$.

elsewhere (Ferland and Sadowski, 1992). The mean vitamin K_1 concentration obtained for the RBD oil (314 ± 37 μg/100 g) is indeed higher than what has been previously reported for soybean oil (193 ± 28 μg/100 g) sampled from grocers and supermarkets (Ferland and Sadowski, 1992).

The commercial shortening, which is comprised of both "partially" or "lightly" hydrogenated soybean and cottonseed oils, had a higher concentration of vitamin K_1 and dihydrovitamin K_1 than the lightly hydrogenated pure soybean oil (Table 1), even though cottonseed oil (40−80 μg/100 g) is notably lower in vitamin K_1 than soybean oil. This would suggest a higher initial concentration of vitamin K_1 in the mixture of shortening oils, prior to hydrogenation, than in the RBD oil. However, the concentration of constituent oils used in commercial products can vary, as can the conditions of the actual hydrogenation process itself. In addition, the single sample sizes of each that were used in this study were insufficient for adequate comparison given the sources of possible variation associated with vitamin K_1 concentration (Booth et al., 1993).

Table 1. Mean (± SD)[a] Vitamin K_1 and Dihydrovitamin K_1 Contents of Shortening and Soybean Oil at Different Stages of Hydrogenation

	(μg/100 g of oil)	
description of oil	vitamin K_1	dihydrovitamin K_1
Crisco commercial shortening[b]	80.0 (± 9.0)	133.0 (± 15.0)
RBD soybean oil[c]	314.0 (± 37.0)	nd[d]
lightly hydrogenated soybean oil[c]	71.0 (± 7.0)	85.0 (± 10.0)
heavily hydrogenated soybean oil[c]	1.0 (± 0.1)	52.0 (± 2.0)

[a] The mean and standard deviations of each oil sample analyzed in triplicate. The mean recoveries of the internal standard, $K_{1(25)}$, ranged from 62.0 to 66.0%. [b] This was a single sample of shortening obtained commercially. [c] These were single samples of soybean oil obtained from the same lot prior to hydrogenation and were donated by the Institute of Shortening and Edible Oils. [d] nd not detectable.

The lightly hydrogenated soybean oil had approximately equivalent amounts of vitamin K_1 and dihydrovitamin K_1 (Figure 3B). However, only 50% of the initial vitamin K_1 concentration in the RBD oil could be accounted for as either vitamin K_1 or dihydrovitamin K_1 in the lightly hydrogenated oil (Table 1). Moreover, in the heavily hydrogenated oil, vitamin K_1 was greatly reduced (Figure 3C). Only 17% of the initial vitamin K_1 content of the RBD oil could be accounted for as vitamin K_1 or dihydrovitamin K_1 in the heavily hydrogenated oil, of which 98% (52.0 μg/100 g) was dihydrovitamin K_1. Since the commercial hydrogenation of oils involves the use of hydrogen gas in the presence of catalyst under pressures of at least 200−300 psig at temperatures well in excess of 150 °C (Torrey, 1983), it would seem plausible that degradation of vitamin K_1 and/or dihydrovitamin K_1 might occur. Comparatively, dihydrovitamin K_1 was more stable under the conditions of hydrogenation than was vitamin K_1.

Ferland and Sadowski (1992) demonstrated that oils exposed to a temperature of 185−190 °C for up to 40 min had measured vitamin K_1 losses of up to 15%. As the samples in this study were hydrogenated using a continuous process, it is not known how long they were exposed to the elevated temperature required for hydrogenation prior to sampling. Since the oils were protected from light during the hydrogenation process, it is likely that overall degradation of the vitamin was associated with either elevated temperature, pressure, or the hydrogenation reaction.

The addition of fats and oils to mixed dishes and desserts has a significant impact on the quantity of vitamin K_1 consumed in the American diet (Booth et al., 1996). Hydrogenated vitamin K_1-rich vegetable oils are widely used by the food industry in food preparation because of their physical characteristics and oxidative

stability. We have shown that there is an overall loss in vitamin K_1 associated with the hydrogenation of oils such as soybean and cottonseed with a subsequent partial conversion of vitamin K_1 to 2′,3′-dihydrovitamin K_1. In partially or lightly hydrogenated products the dihydrovitamin K_1 concentration was approximately equal to or greater than that of vitamin K_1. Given the prevalence of foods containing hydrogenated oils in the American diet, dihydrovitamin K_1, if shown to be biologically active, may be an important factor in vitamin K nutriture.

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