FOOD COMPOSITION AND ADDITIVES

Determination of Vitamin K_1 Isomers in Foods by Liquid Chromatography with C_{30} Bonded-Phase Column

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Vitamin K_1 was determined in a variety of foods by using reversed-phase liquid chromatography with a C_{30} column followed by post-column reduction to the fluorescent hydroquinone derivatives. Lipids were removed by lipase digestion, followed by single extraction into hydrocarbon, and the protocol was extended to selected natural and processed foods. Biologically active *trans*- and inactive *cis*-vitamin K_1 isomers were measured individually to evaluate the true nutritional status of the products. Method performance parameters confirmed the validity of the technique. The use of the triacontyl-bonded C_{30} phase for selective phylloquinone isomer measurement extends previously validated AOAC Method 999.15 for vitamin K_1 in milk and infant formula to a wider range of foods important in the human diet. The *cis*-vitamin K_1 isomer contributes up to about 15% of total phylloquinone in certain foods.

Vitamin K_1 (phylloquinone) is an obligatory cofactor in the post-translational γ-carboxylase modification of glutamate residues within a small group of specialized vitamin K-dependent calcium (Ca)-binding proteins. Tissue carboxylase distribution studies suggest 3 physiological processes in which these γ-carboxyglutamic acid (Gla)-proteins function: blood coagulation, bone metabolism, and signal transduction (1–5). Vitamin K_1 is ubiquitous within the chloroplast of green plants, which constitute the major dietary source of this vitamin. The higher prenylated and diverse menaquinones are of bacterial origin; MK4 is atypical of this group in that there is increasing evidence of a nonbacterial, tissue-specific in vivo conversion from phylloquinone (6–8). The utilization and physiological role of the menaquinones in vitamin K metabolism are currently unresolved (9–11).

Liquid chromatography (LC) has superceded other techniques for the estimation of K vitamins in both clinical tissues and foods. In view of its potential impact on bone integrity, there has been an increasing demand from regulatory and nutritional organizations for a comprehensive and credible dietary foods database for vitamin K. Several reports have described various sample preparation and LC protocols for the estimation of K_1 (12–24), MK4 (16, 21, 24), higher menaquinones (13, 16, 24), and dihydrophylloquinone (21, 25, 26) in many diverse foods present in the human diet, and these analytical techniques have been recently reviewed (27, 28). The clinical and nutritional significance of food-derived dihydro-K_1 and MK4 relative to K_1 is under intense study, but currently remains uncertain (8, 14, 16, 21, 25, 26, 29).

The K vitamins are subject to side-chain structural isomerization, and naturally occurring K_1 is found exclusively as the biologically active 2′-*trans*-isomer. However, foods may contain appreciable quantities of the essentially nonactive *cis*-isomer as a consequence of either its presence in synthetic K_1 used during food supplementation, or photoisomerization of the *trans*-isomer during exposure of the food to light (30, 31). The potential contribution of dietary *cis*-K_1 is therefore a further complication in establishing a credible food database and will require attention.

We previously developed and validated an LC method for the routine estimation of vitamins K_1, menaquinones, and dihydro-K_1 in milk and infant formulas, at either supplemental or endogenous levels (21). The analytical scheme incorporates enzymatic digestion, solvent extraction, and direct analysis by reversed-phase LC with post-column reduction and fluorescence detection, and has recently been subjected to interlaboratory collaborative study (32) and adopted as AOAC Official Method **999.15**.

Normal-phase LC on unmodified silica has previously been successful in separating the *cis*- and *trans*-isomers of vitamin K_1 (30, 31). However, the recognized operational disadvantages of this chromatographic mode have compromised

Received July 24, 2001. Accepted by JL December 3, 2001.
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its application to routine analysis of foods. The application of polymeric triacontyl C_{30} column to the separation of phylloquinone isomers in infant formula and margarine has recently been reported (32, 33). This study reports the extension of the enzymatic digestion and C_{30} LC-fluorescence protocol, as applied to survey both vitamin K content and phylloquinone isomer distribution in a range of foods representative of the human diet.

Experimental

Apparatus

(**a**) *Liquid chromatograph*.—Automated Summit LC system (Dionex Corp., Sunnyvale, CA) configured with ASI-100 autosampler, P580A-HPG pump, RF-2000 fluorescence detector (λ_{ex} 243 nm, λ_{em} 430 nm), and Chromeleon v6.01 software. The detector exhibited a Raman spectra signal-to-noise ratio of >200:1. The LC system contained an in-line vacuum degasser and column heater at 25°C. Alternative LC systems (Waters, Milford, MA, and Shimadzu, Tokyo, Japan) were used in the authors' laboratories and were configured similarly.

(**b**) *Columns*.—Two low-ligand density C_{30} analytical column types were used during the study: (*1*) 5 and 3 µm polymeric YMC 250 × 4.6 mm (Wilmington, NC); and (*2*) polar end-capped, 5 µm monomeric Develosil 250 × 4.6 mm (Phenomenex, Torrance, CA).

(**c**) *Reductor*.—The post-column reductor was a 20 × 4 mm stainless steel post-column assembly configured between the analytical column and the fluorescence detector.

(**d**) *Spectrophotometer*.—UV-1201 with digital readout to 3 dp (Shimadzu).

(**e**) *Water bath*.—Y28 (Grant, Cambridge, UK) set at 37 ± 1°C.

(**f**) *Wrist-action shaker*.—SF1 8-place (Stuart Scientific, UK).

(**g**) *pH meter*.—42A (Orion Research, Inc., Boston MA) with combination electrode and calibration buffers.

(**h**) *Rotary evaporator*.—Buchi, Switzerland; at 40 ± 1°C with 100 mL pear-shaped flasks.

(**i**) *Centrifuge*.—GS200 operable at 2000 rpm (H.I. Clements Ltd., Sydney, Australia).

(**j**) *Homogenizer*.—High-speed (ASE Ltd., UK).

(**k**) *Filtration apparatus*.—With 0.45 µm nylon membranes for samples and mobile phase clarification (Alltech, Deerfield, IL).

(**l**) *Freeze drier*.—Used when required; Modulyo (Edwards High Vacuum Systems, Crawley, UK).

(**m**) *Glassware*.—Pipets (or adjustable pipettors, 1 and 5 mL), Schott bottles (100 mL and 1 L) with leak-proof caps, low-actinic volumetric flasks (10, 20, 50, and 100 mL), measuring cylinders (100 mL and 1 L), centrifuge tubes with ground-glass stoppers, and autosampler vials (2 mL) with Teflon sealed caps.

Reagents

(**a**) *Lipase*.—L-1754, Type VII from *Candida rugosa*, ca 1000 U/mg (Sigma, St. Louis, MO).

(**b**) *Papain*.—390303G, from *Carica papaya*, >30 000 USP U/mg (BDH, Poole, UK).

(**c**) *Zinc chloride, sodium acetate (anhydrous), glacial acetic acid, potassium carbonate (anhydrous), and zinc powder (<60 µm)*.—BDH.

(**d**) *Solvents*.—Isopropanol, methanol, ethanol, hexane, and dichloromethane, LC grade (Mallinckrodt, Phillipsburg, PA). Water was purified to >18 MΩ resistivity.

(**e**) *Phosphate buffer (0.2M)*.—Dissolve 27.2 g potassium dihydrogen phosphate in ca 800 mL water. Adjust to pH 7.9–8.0 with potassium hydroxide solution (40%, m/v) and dilute to 1 L.

(**f**) *Vitamin K standards*.—Vitamin K_1 (phylloquinone) was obtained from United States Pharmacopeia (USP; Rockville, MD); MK4 from Sigma; 2′,3′-dihydrophylloquinone, MK5, MK6, MK7, MK8, and MK9 were donated by Hoffmann-LaRoche (Basel, Switzerland); and K_1-2,3-epoxide was gifted by H. Thijssen (University of Limburg, The Netherlands).

(**g**) *Vitamin K_1 standard solutions*.—(*1*) *Stock (ca 2.0 mg/mL)*.—Dissolve ca 100 mg phylloquinone with gentle warming (30°C) in isopropanol (50.0 mL). (*2*) *Intermediate I (ca 20 µg/mL)*.—Dilute 1.0 mL stock solution (*1*) to 100 mL with ethanol. Measure absorbance at 248 nm against ethanol and calculate accurate concentration by using $E^{1\%}$ of 408. Both standards (*1*) and (*2*) are stable for 6 months at –10°C. (*3*) *Intermediate II (ca 200 ng/mL)*.—Dilute 1.0 mL intermediate I to 100 mL with methanol. (*4*) *Working standards (ca 2–40 ng/mL)*.—Separately dilute 1.0 mL intermediate II with methanol to 5, 10, 25, 50, and 100 mL. Calculate accurate concentrations from calibrated intermediate I standard. Individual contributions of the *cis*- and *trans*-isomers are estimated after LC area ratio measurement.

Sample Preparation

Samples were extracted exclusively under subdued incandescent lighting in the absence of direct sunlight. Foods were homogenized by the most appropriate means (in general, with standard homogenization equipment) to obtain representative test portions. For certain nonprocessed foods, e.g., raw vegetables, test portions were either freeze-dried before extraction or lipid-extracted; a portion of the extract was then subjected to assay. For intractable foods, the proteolytic enzyme papain was useful for dissolution of the matrix. All assays were performed in replicate to establish acceptable food homogeneity.

Milk powders, infant formula powders, and hard cheeses (1 g), retorted baby foods (5–10 g, depending on estimated content), yogurt (2.5 g), liquid milks, soymilk, health beverages (10 mL), vegetable oils and other high-fat foods (0.25 g), and meats and raw vegetables (minced, 5–10 g) were accurately weighed into 100 mL Schott bottles, and 1.0–1.5 g lipase was added. A 20 mL volume of 0.2M phosphate buffer was dispensed, and the solutions were incubated at 37°C for 2 h with frequent shaking. For each new food type, the pH of the buffered solution was verified to ensure that it remained within the optimal digestion range 7.6–8.2; additional buffer was added if required. The digestion was extended (up to 4 h)

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for samples exhibiting evidence of incomplete lipid hydrolysis, although the analytical LC column is tolerant to moderate levels of residual triglycerides. An alternative source of lipase (from porcine pancreas) was used for some hard cheeses. Addition of ca 200 mg papain enzyme, concurrent with lipase, aided in breaking down cellular structure and was beneficial during sample preparation of many natural foods.

The digests were cooled to ambient temperature, and 10 mL ethanol plus 1 g solid potassium carbonate was added. After thorough mixing, 30.0 mL hexane was added and the flasks were shaken vigorously for 30 min on a wrist-action shaker. Phase separation was accomplished either by overnight standing at 4°C, centrifugation (2000 rpm), or addition of 2 mL ethanol.

For certain processed foods with dispersion or solubility problems (e.g., baked beans), samples were homogenized with a high-speed food processor and a 5–10 g representative test portion was taken. For some samples, this process was repeated after addition of buffer. For hygroscopic foods (e.g., dried soups, dried vegetables, and cereal products), it was beneficial to initially hydrate the test sample with 20–40 mL phosphate buffer. Additional buffer was also used for foods vulnerable to gelation (e.g., jams and confections), with other conditions remaining unchanged. For certain low-fat foods of very low vitamin K content, the extraction scheme was scaled up for a 20 g test portion.

National Institute of Standards and Technology (NIST) SRM 1846 infant formula, SRM 2383 baby food certified reference materials, in-house reference, and a blank were systematically included with each batch of samples to monitor within-laboratory method performance and to establish between-laboratory precision estimates.

Extraction

An aliquot of the upper organic phase was transferred and hexane was removed by evaporation under nitrogen. The dried extracts were stable at –18°C for up to 4 weeks, until ready for LC analysis. Residues were redissolved in methanol depending on the expected vitamin K content. Certain high-fat samples containing residual lipid were reconstituted in methanol–isopropanol (1 + 1); occasional extracts containing particulates were filtered through a 0.45 μm PTFE filter. Volumes typically used during extraction are shown in Table 1 and provided optimal fluorescence sensitivity based on detector characteristics.

Table 1. Parameters used in extractions

Sample	Hexane extract (V_1), mL	Methanol (V_2), mL	Dilution factor ($30 \times V_2/V_1$)
Fortified foods (10–100 μg/100 g)	5	5	30
Natural foods (1–10 μg/100 g)	15	5	10
Natural foods (<1 μg/100 g)	20	2	3

Chromatography

The mobile phase generally used for the YMC column was prepared by dissolving 0.41 g anhydrous sodium acetate, 1.37 g zinc chloride, and 0.30 g glacial acetic acid in 920 mL methanol. An 80 mL volume of dichloromethane was added, and the solution was filtered through a 0.45 μm nylon membrane. Other proportions of dichloromethane (4–8%, v/v) were required for optimal separation of certain food extracts; the Develosil column was typically operated with 5% (v/v) dichloromethane.

The post-column reductor (30–200 mm) was dry-packed with zinc powder by sequentially adding small amounts with frequent gentle tapping, and the assembly was sealed securely. Before connection of post-column zinc reductor assembly, the LC system was equilibrated with mobile phase (1.0–1.5 mL/min for 30 min) to ensure that it was free of moisture. The zinc reductor assembly was then installed between the analytical column and fluorescence detector (λ_{ex} 243 nm; λ_{em} 430 nm), and flow continued for another 15 min to establish a stable baseline.

The working calibration standards were injected, followed by sample extracts (20–50 μL). Control extracts and calibrants were interpolated regularly through the analytical sequence to ensure system stability. The *trans*- and *cis*-vitamin K_1 isomers eluted at ca 11 and 12.5 min, respectively, for the YMC column, depending on specific mobile phase composition and flow rate. Reagent blanks were included to ensure the absence of chromatographic interferences; removal of the post-column reductor facilitated identification of nonvitamin K fluorescence coextractives in certain foods.

Multilevel calibration was performed by linear least-squares regression, with quantitation of vitamin K in unknowns obtained by the external standard technique after interpolation and computation of appropriate dilution factors. Individual *cis*- and *trans*-phylloquinone contents were calculated against the respective isomeric vitamin K_1 external standard.

Results

Initial work established the verification and extension of AOAC Method **999.15** (32) to foods, using both the monomeric Develosil and polymeric YMC C_{30} analytical columns. Both columns were of comparable selectivity with respect to the *cis*-K_1, *trans*-K_1, MK4, K_1-2,3-epoxide, and 2′,3′-dihydro-K_1 analytes targeted in selected foods. However, these columns exhibited dissimilar efficiencies, with the 5 μm YMC column providing enhanced resolution of the *cis*- and *trans*-K_1 isomers compared with the 5 μm Develosil column. Although the 5 μm YMC column was used routinely, the 3 μm form adds further efficiency and may be preferred, despite increased system pressure.

Figure 1 illustrates the chromatograms obtained with a YMC C_{30} column and with a conventional C_{18} column. The effect of temperature on resolution of the K_1 isomers was in-

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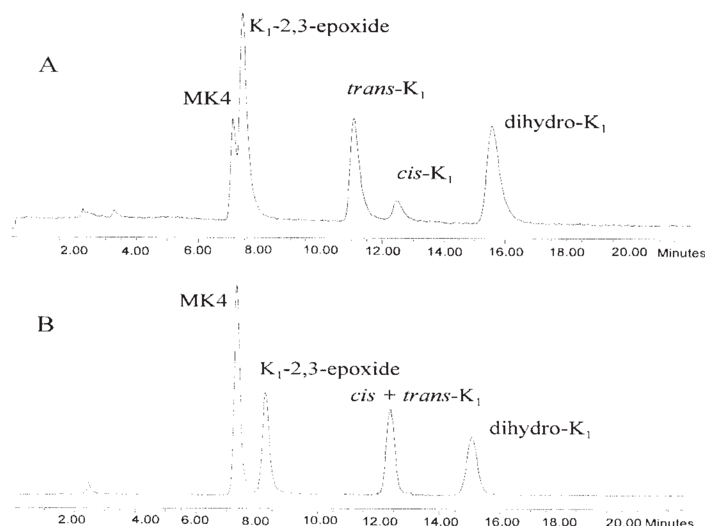

Figure 1. Liquid chromatograms of vitamin K standards on a C_{30}, 3 µm YMC column (A) and a C_{18} column (B).

vestigated for both column types between 4 and 60°C. Predictably, overall retention decreased and isomer resolution degraded with increasing temperature, resulting in coelution at 60°C. Resolution of the K_1-isomers was improved for both phases at subambient temperature (4°C) compared with ambient conditions, although fluorescence yield was significantly diminished under such conditions.

Under the isocratic conditions used, authentic menaquinones eluted with a linear relationship between log k' and number of side-chain isoprenoids ($r^2 = 0.9997$). Gradient conditions, however, were used when higher menaquinones (>MK6) were present in certain foods as illustrated for cheese in Figure 2. Peak identities in sample extracts were established by coelution with authentic standards and elimination of fluorescence response subsequent to removal of the reduction column.

Detector linearity was evaluated for *cis*-K_1, *trans*-K_1, and MK4 with multilevel standards (50–2500 pg on-column) and typical least-square regressions were $y = 856x + 1347$, $y = 859x + 5013$, and $y = 898x + 14\,393$, respectively, with $r^2 > 0.999$. Fluorescence response of K vitamers, and hence sensitivities, were comparable, as indicated by slope values.

The detection (3σ) limit was typically estimated at 10 pg on-column throughout the study. This parameter was predominantly influenced by both zinc reductor condition and detector performance, such that under optimum conditions, 2 pg on-column was detectable. Estimated quantitation limits (10σ) in milk powders were 41, 9, and 9 ng/100 g for MK4, *cis*-, and *trans*-K_1, respectively. To confirm complete conversion to the hydroquinone with a 20 mm zinc reductor, further reductor columns were added in series with no improvement in signal.

Further verification studies involved confirming method recovery after independent standard additions of *cis*-K_1, *trans*-K_1, and MK4 to unfortified whole milk, whole milk powder, skim milk powder, and other foods, with mean recoveries of 103 ± 4% ($n = 9$), 99 ± 3% ($n = 9$), and 102 ± 1% ($n = 2$), respectively.

Intermediate precision was estimated in a single laboratory by between-day replicate analysis of a range of food samples (infant formulas, milk, yogurt, cheddar cheese), with RSD_R of 3–5, 4–7, and 5–8% for MK4, *trans*-, and *cis*-K_1, respectively.

The certified NIST SRM 1846 milk-based infant formula and SRM 2383 composite infant food were both used to verify the overall vitamin K method under the conditions described (Figure 3). The infant formula SRM contains supplementary synthetic phylloquinone, which is responsible for the presence of the *cis*-isomer; the presence of 2′,3′-dihydro-K_1 confirms the incorporation of hydrogenated vegetable oil.

Three laboratories performed independent replicate analyses of SRM 1846, yielding aggregate *cis*+*trans*-vitamin K_1 levels of 95.4 (RSD_R 4.10%, $n = 10$), 96.7 (RSD_R 1.50%, $n = 26$), and 94.9 µg/100 g (RSD_R 7.47%, $n = 15$), which compare with the assigned reference value of 94.4 ± 4.1 µg/100 g. *cis*-K_1 represented 10.8, 10.9, and 12.4% of total phylloquinone, with *trans*:*cis*-K_1 ratios of 8.28, 8.31, and 8.89, respectively.

Although there is no certification value for vitamin K in SRM 2383, it is reported to contain about 15 µg/100 g total phylloquinone with use of a conventional C_{18} analytical column (personal communication, Katherine Sharpless, NIST, Gaithersburg, MD). Both *cis*- and *trans*-isomers of vitamin K_1 are evident (Figure 3). Spinach constitutes a major component in this material, which accounts for most of the vitamin; however, because supplementary vitamin K is also present within the infant formula component of this SRM, *cis*-phylloquinone is also detected. Three laboratories performed independent replicate analyses using a C_{30} stationary phase, yielding aggregate *cis*+*trans*-vitamin K_1 levels of 13.1 (RSD_R 8.61%, $n = 30$), 14.9 (RSD_R 5.34%, $n = 17$), and 13.9 µg/100 g (RSD_R 10.78%, $n = 18$). These data compare with a mean value of

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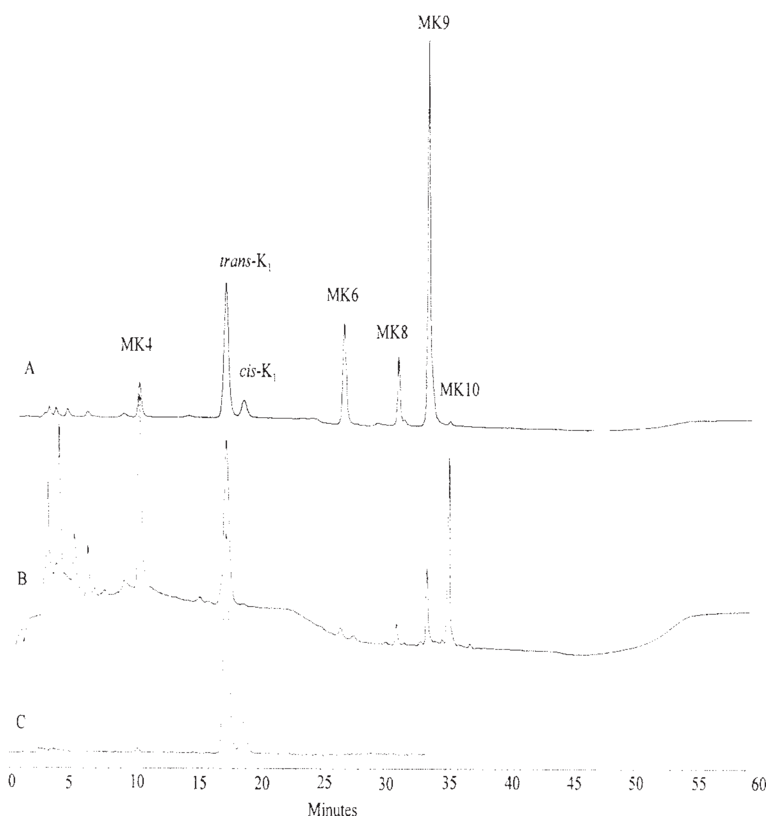

Figure 2. Liquid chromatograms of menaquinones in a standard mixture (A), mature cheddar cheese (B), and fortified milk powder (C). Column: C_{30}, 3 μm YMC; mobile phase: isocratic (0–18 min) 100% methanol–dichloromethane (95 + 5, v/v) followed by linear gradient (18–30 min) to 80% methanol–dichloromethane (50 + 50, v/v) used for the higher menaquinones.

13.9 μg/100 g estimated with use of a conventional C_{18} column. *cis*-K_1 represented 8.2, 7.8, and 8.3% of total phylloquinone, with *trans-*:*cis*-K_1 ratios of 11.4, 12.0, and 11.3, respectively.

Determination of vitamin K was compromised in certain foods by the presence of an unidentified peak eluting between the phylloquinone isomers on both C_{30} phases. This artifact was particularly significant in canola and soy oils and foods containing these oils, although it has occasionally been detected in certain unprocessed foods. A typical canola oil extract using both C_{30} and C_{18} chromatographic chemistries confirmed the different column selectivities for this unknown (Figure 4).

The polymeric YMC column achieved superior resolution of this chromatographic interference compared with the monomeric Develosil, which was enhanced further through inherent efficiency attributes of the 3 μm material and a modified eluent (0.5%, v/v, dichloromethane). Several antioxidant additives (BHA, TBHQ, tocopherol) and coenzyme Q_{10} were eliminated as potential candidates for the unknown artifact; in the absence of the zinc reductor it remained undetected, suggesting a prenylquinone structure related to vitamin K. This possibility was also indicated by its equivalent fluorescence spectral characteristics compared with phylloquinone, under conditions of varied excitation (230–260 nm) and emission (390–450 nm) wavelengths. Further evidence was indicated on the basis of its equivalent first order degradation kinetics relative to vitamin K_1 when exposed to 350 nm UV irradiation ($k = 1.4 \times 10^{-3}$/s). However, neither vitamin K_1-2,3-epoxide, nor any authentic menaquinone standards, coeluted with this unknown.

The vitamin K_1 content in infant diets, oils, and other foods, both natural and processed, is presented in Table 2, illustrating the contribution of the nonbioactive *cis*-isomer. Of the menaquinones, only MK4 was quantitated in selected dairy foods (milk, yogurt, and cheese) with levels typically comparable with *trans*-vitamin K_1; higher homologs were viewed qualitatively under gradient conditions. Similarly, 2′,3′-dihydrophylloquinone was detected in certain foods, but was not quantitated.

Unprocessed foods (e.g., meat, fruit, and vegetables) were generally more difficult to assay because of the intractability of native cellular material. However, freeze-drying or fat-extraction techniques before analysis facilitated quantitative recovery, although for certain such foods, extended sample preparation and direct lipase digestion yielded equivalent vitamin K levels. An effective alternative was to add papain enzyme to the digest. Although papain has a slightly different pH and temperature optimum to lipase, it proved successful in digesting protein in meat and animal products. These products

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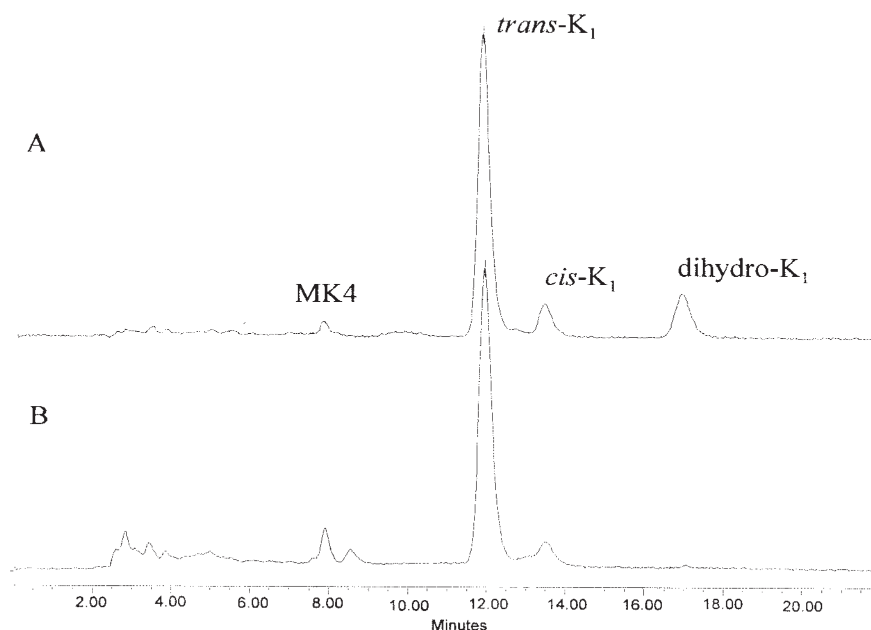

Figure 3. Liquid chromatograms of (A) NIST 1846 (fortified infant formula) and (B) NIST 2383 (infant food composite). Column: C_{30}, 3 μm YMC.

contained exclusively *trans*-phylloquinone, with no evidence of the *cis*-isomer.

Discussion

Conventional silica-based C_8 and C_{18} bonded-phase columns have been used extensively in the analysis of the fat-soluble vitamins and carotenoids. A wide variety of such phases have been exploited, including monomeric, polymeric, and a range of end-capped configurations, each with unique attributes depending on the specific application. Polymeric phases have generally demonstrated enhanced shape selectivity for carotenoid geometric and positional isomers when compared with monomeric equivalents, despite lower capacity and reduced efficiency (34, 35).

The development of the polymeric triacontyl silica C_{30} column was initiated to improve the separation of nonpolar carotenoids and their geometric isomers (36) and was further applied to the more polar xanthophylls (37). This material has consistently enhanced selectivity for natural carotenoid isomers relative to C_{18} phases traditionally used (38–41), and has been similarly applied to capillary electrochromatography, with greater efficiency than LC (42).

The polymeric C_{30} phase has also been applied to analysis of tocopherol stereoisomers (43–45), tocotrienols (46, 47), and retinoids (43, 48–51), and its application to analysis of foods has been recently reviewed (52). On-line coupled C_{30} LC/MS and LC/NMR studies have overcome inherent limitations of UV detection, as applied to spectroscopically equivalent vitamin isomers (41, 44, 45, 47–49, 53, 54). The selectivity attributes and bonded-phase parameters of the polymeric C_{30} column have been optimized for enhanced molecular shape recognition, high retention, and silanol activity (36). Further chromatographic, spectroscopic, and molecular modeling studies have reported the significance of extended surface dimensions, higher order of the C_{30} ligand (39), significance of polar-dipole or hydrogen-bonding interactions and specific hydrophobicity (46), the influence of temperature on ligand morphology and stereochemistry (40, 43, 48), and increased sample capacity and recovery, despite reduced column efficiency relative to conventional C_{18} phases (43).

To date, applications of the C_{30} phase to vitamin K analysis have been restricted to infant formulas and margarines (32, 33), and rat liver tissue (55), where resolution of the *cis*- and *trans*-phylloquinone isomers has been reported. The present comparative study confirmed the enhanced selectivity of the polymeric C_{30} phase for *cis*- and *trans*-K_1 as compared with a monomeric form of this surface modification, consistent with similarly shape-constrained fat-soluble vitamins previously reviewed (52). The ability to distinguish the K_1-isomers will likely become analytically significant in view of their dissimilar biological activities.

More speculatively, there is some evidence for the presence of minor contributions of putative *cis*-menaquinones in the synthetic standards examined, because each menaquinone resolves under the described conditions into 2 peaks with similar selectivity as for K_1. The potential existence of *cis*-MK isomers has not yet been reported, and it will be of interest to evaluate the potential for the C_{30} material to establish the possible contribution of such MK isomers in foods. Similarly, the potential contribution of *cis*-tocotrienol phytyl-isomers in

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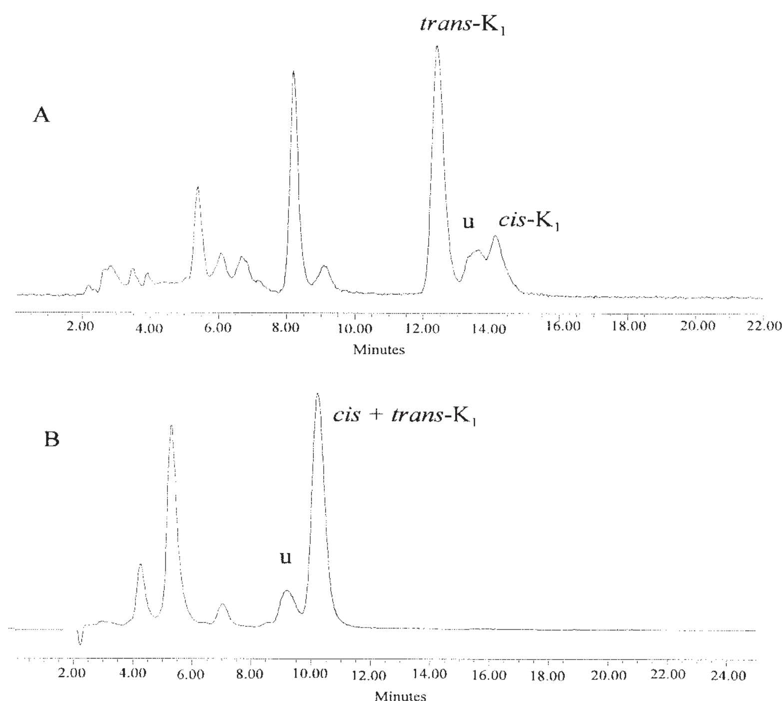

Figure 4. Liquid chromatograms of canola oil sample on C_{30}, 3 μm YMC column (A) and C_{18} column (B). Unidentified component is labeled as 'u'.

foods has been recently investigated through use of a β-cyclodextrin chiral phase (56).

Previous studies have also reported the presence of an unidentified component during analysis of canola oil, soybean oil, and margarines (26, 33, 57), where the component eluted before vitamin K_1 on conventional C_{18} columns. We confirmed that this unknown component elutes between *cis*- and *trans*-phylloquinone on the C_{30} phase used in the present study. Apart from its presence at moderate levels in vitamin K-rich oils and margarines, it has also been detected at low levels in certain processed foods (e.g., baby foods, canned soups, baked beans). Although the evidence suggests a prenylnaphthoquinone structure related to vitamin K, its identity has not yet been confirmed. It may be relevant that vitamin K_1-chromenol, a novel phylloquinone metabolite, has been identified in plasma through MS analysis, suggesting that such naphthotocopherol derivatives may be distributed in other tissues (58).

Consistent with recent food database studies (12, 13, 15, 16, 18, 24), total phylloquinone (aggregate *cis*- and *trans*-K_1 isomers) values reported presently confirm its association with photosynthetic tissues, with the highest values found in green leafy vegetables and significant amounts in fruits, oils, dairy products, eggs, meat products, and certain processed foods. It should be noted, however, that the bioavailablity of dietary vitamin K (K_1 and menaquinones) is reported to be dependent not only on content, but also the form in which it is ingested (59).

Apart from animal liver, the only other significant sources of dietary menaquinones in the Western diet are fermented foods, and the present study has confirmed the presence of MK8 and MK9 in both cheese and yogurt. However, although menaquinones are barely detectable in cow's milk, MK4 has been verified to be present at levels comparable with phylloquinone. The presence of 2′,3′-dihydrophylloquinone has been confirmed in foods containing hydrogenated vitamin K-rich oils. This vitamer has recently been demonstrated to be biologically active for the hepatic vitamin K-dependent proteins, although it is absorbed less efficiently relative to *trans*-K_1 (29).

Supplementation of foods with vitamin K is uncommon, except for infant formula feeds to which synthetic preparations of phylloquinone are invariably added. Depending on the method of synthesis, the contribution of the *cis*-K_1 isomer in these diets will vary, and the present survey confirms about 10% of total phylloquinone to be present as the inactive *cis*-form. The presence of variable levels of *cis*-phylloquinone in other processed foods may be attributed to incorporation of refined vegetable oils, or exposure to UV light under storage or display conditions (30, 31, 33); in other cases its origins remain undetermined. The influence of UV light, either within the living plant or under display, may rationalize the presence of this isomer in a number of natural foods, although nonplant-based foods (mussels, liver) also contain appreciable *cis*-phylloquinone content, possibly because of the slower hepatic turnover of *cis*-vitamin K_1 relative to the *trans*-isomer (55). The distribution of vitamin K_1 isomers in the human diet has a potential impact on both nutritional label claims and clinical intervention studies. At present, there is no legal requirement to specifically declare the *trans*-phylloquinone

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Table 2. Vitamin K_1 content of foods (μg/100 g)

Food	*trans*-K_1	*cis*-K_1	K_1 (total)	*cis*-K_1 (%)
Tallow	46.6	0.02	46.7	0.1
Ghee	11.9	0.03	11.9	0.3
Cod liver oil	1.1	0	1.1	0
Palm olein oil	5.9	1.5	7.4	20.8
Palm oil	5.0	1.0	6.0	16.1
Coconut oil	1.5	0.2	1.7	9.4
Sunflower oil	14.9	1.6	16.5	9.8
Soybean oil	236.2	33.6	269.8	12.5
Canola oil	90.1	21.4	111.5	19.2
Safflower oil	5.7	0.8	6.5	12.9
Olive oil	80.9	12.8	93.6	13.6
Almond oil	8.7	0.2	8.9	2.5
Peanut oil	1.6	0.1	1.6	4.9
Avocado oil	79.2	0	79.2	0
Broccoli	239.8	2.1	241.9	0.9
Parsley	663.0	4.6	667.6	0.7
Spinach	489.4	3.9	493.3	0.8
Lettuce	86.7	0.2	86.9	0.2
Carrots	7.4	0	7.4	0
Pumpkin	4.0	0	4.0	0
Potatoes	4.0	0	4.0	0
Cabbage	93.8	0.4	94.2	0.4
Asparagus	103.9	1.0	104.9	1.0
Sweet corn	0.5	0	0.5	0
Peas	18.7	0	18.7	0
Mushroom	1.0	0	1.0	0
Eggs	0.3	0	0.3	0
Mussels	0.4	0.02	0.4	5
Tuna fish	0.1	0	0.1	0
Apples	7.7	0	7.7	0
Tomato	9.8	0.1	9.9	0.7
Avocado	24.0	1.1	25.0	4.2
Bovine liver	14.5	2.1	16.6	12.8
Beef	1.1	0	1.1	0
Chicken	0.3	0	0.3	0
Baby rice	3.8	0.4	4.1	8.5
Baby food	1.2	0	1.2	0
Baby food	0.6	0	0.6	0
Baby food, lamb and bacon	0.6	0	0.6	0
Baby food, chicken	0.9	0	0.9	0
Baby food, vegetables and lamb	1.8	0	1.8	0
Baby food, vegetables and beef	2.1	0.04	2.1	1.9
Baked beans, canned	0.3	0	0.3	0
Desiccated liver	2.0	0	2.0	0
Crackers	1.2	0.04	1.2	3.3
Milk, homogenized, 3.4% fat	0.3	0	0.3	0
Milk powder, whole, 26% fat	3.1	0	3.1	0
Yogurt, unsweetened	0.4	0	0.4	0
Cheddar cheese, mature, 35% fat	3.2	0	3.2	0
Porridge	1.4	0	1.4	0
Yogurt, plum	0.6	0	0.6	0
Tea leaves	857.8	4.0	861.8	0.5
Coffee	0.1	0	0.1	0
Corn flakes	0.0	0	0.0	0
Ground oats	2.7	0	2.7	0
Dried soup, chicken and vegetables	8.3	0	8.3	0
Dried soup, bacon and ham	7.7	0	7.7	0
Dried soup, tomato	47.7	5.6	53.4	10.6
Canned soup, mushroom	0.1	0	0.1	0
Canned soup, oxtail	3.6	0.1	3.6	1.9
Tomato sauce	2.0	0.04	2.0	2.0
Canned tomato soup	13.8	2.0	15.8	12.6
Biscuit	0.2	0	0.2	0
Short bread	0.8	0	0.8	0
White bread	0.2	0.03	0.3	11.5
Frankfurters	0.5	0	0.5	0
Minced meat	0.3	0	0.3	0
Sausages, pork	0.7	0.01	0.7	1.4
Sausages, beef	0.2	0	0.2	0
Pate, pork	0.4	0	0.4	0
Canned cat food (fish-based)	3.8	0.4	4.2	8.9
Canned cat food (meat-based)	2.2	0.2	2.4	8.4
Canned dog food (meat-based)	7.9	0.5	8.4	6.0

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content of fortified or natural foods, mainly because of limitations of current analytical methods based on conventional C_{18} chemistries which are restricted to the nonselective measurement of total vitamin K_1.

The need for preliminary cleanup techniques has generally been dependent on food type, analyte level, and detection mode, and most reported schemes have variously incorporated lipid extraction, enzymatic digestion, solid-phase fractionation, and semipreparative LC schemes before analytical LC (27, 28). However, the present study has confirmed that lipase digestion and hexane extraction, followed by direct analytical LC quantitation, are expedient for the many diverse foods selected for analysis.

The validity of the method at endogenous and supplemental vitamin K_1 levels in both milk and infant formula was established by AOAC collaborative study, Method **999.15** (32). The current work therefore represents an extension of the analytical scheme to other foods, by using the enhanced selectivity of the C_{30} column for selective measurement of the phylloquinone isomers. Several recent studies have compiled comprehensive surveys of total vitamin K_1 content of foods (12–29), and the present study has not attempted to duplicate such efforts. Rather, the primary focus in this study has been to quantitate the contribution of the vitamin K_1 isomers in selected foods, in view of their disparate biological activities and potential significance in human nutrition.

Acknowledgments

We acknowledge the contribution of Mark Reynolds (New Zealand Dairy Research Institute) for his technical expertise, and the New Zealand Dairy Research Institute for partial funding of this study.

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