Determination of Phylloquinone in Vegetables, Fruits, and Berries by High-Performance Liquid Chromatography with Electrochemical Detection

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The amount of phylloquinone (vitamin K_1) in the most important vegetables, fruits, and berries available in Finland was analyzed by a reversed-phase high-performance liquid chromatographic (HPLC) method. In this method phylloquinone was quantified with a dual-electrode electrochemical (EC) detector using menaquinone 4 (MK-4) as an internal standard. The seasonal variation of phylloquinone in some vegetables was also investigated. The highest phylloquinone content was analyzed in parsley (mean content = 360 μg/100 g), while other green vegetables were also found to be good sources of phylloquinone (general mean content > 100 μg/100 g). In contrast, red and yellow vegetables and fruits contained considerably lower amounts of phylloquinone (mean content < 20 μg/100 g). The mean phylloquinone content of berries ranged from 5.5 μg/100 g (strawberry) to 30 μg/100 g (black currant). Variation in the phylloquinone content of vegetables was considerable, although the main reason for this could not be determined. The contribution of vegetables, fruits, and berries to the average daily dietary intake of phylloquinone in Finland was estimated to be approximately 40 μg.

Keywords: *Phylloquinone; vitamin K_1; vegetables; HPLC*

INTRODUCTION

Vitamin K is a cofactor in the posttranslational synthesis of γ-carboxyglutamic acid in several proteins. It was earlier believed that vitamin K affects only blood coagulation; discoveries made in the past two decades have, however, revealed that a diverse group of proteins with no function in blood coagulation are dependent on vitamin K. These proteins include osteocalsin, which is important in bone metabolism (Shearer, 1995).

These new findings on the role of vitamin K in bone metabolism, in addition to evidence suggesting that menaquinones synthesized by the intestinal microflora are less important than previously believed (Vermeer et al., 1995), raise doubts about the adequacy of the current Recommended Dietary Allowance (RDA; NRC, 1989). In addition, research performed in the United States showed that 25−30-year-old adults consumed less vitamin K than suggested in the current RDA (Booth et al., 1996). Poor vitamin K status may, therefore, be more prevalent than previously believed (Vermeer et al., 1995). On the other hand, dietary vitamin K has an effect on the efficiency of anticoagulant drugs. For further understanding of the nutritional role of vitamin K, more information is needed on the concentration of vitamin K-active compounds, including phylloquinone (vitamin K_1) and menaquinones (vitamin K_2), in food (Booth et al., 1993).

Green vegetables are in general recognized as the richest source of dietary phylloquinone; the dark green vegetables (spinach, collards, lettuce, and broccoli) were shown to be the top four contributors of dietary phylloquinone in the United States (Booth et al., 1996). More data on phylloquinone contents in vegetables are needed, however, since they have been investigated with modern, reliable analytical methods in only a few studies (Ferland and Sadowski, 1992; Langenberg et al., 1986). Moreover, in some studies the validity of the extraction methods used was insufficiently confirmed or documentation of the sampling techniques was often inadequate. Furthermore, significant variations in phylloquinone contents may occur due to various growing conditions such as climate and soil (Ferland and Sadowski, 1992). To our knowledge no studies have been conducted on the presence of phylloquinone in vegetables, fruits, and berries grown in the Nordic countries.

Here we describe a high-performance liquid chromatographic (HPLC) method for determining phylloquinone in vegetables, fruits, and berries. The aim of the present study was to develop an extraction method that disrupts cell walls for efficient extraction of vitamin K. The method was applied for determining phylloquinone in the most important vegetables, fruits, and berries available in Finland. We also investigated seasonal variation in the phylloquinone content of vegetables.

MATERIALS AND METHODS

Sampling. The most important vegetables, fruits, and berries available in Finland were selected (Table 1) for analysis. All samples, except berries and domestic apples, were purchased from 10 retail stores representing the 4 major food chains in the Helsinki area during the summer and fall of 1996 and the winter of 1997. Domestic berries (red and black currants, strawberry, raspberry, blueberry, and lingonberry) as well as apples (red and yellow varieties) were purchased from four market places in Helsinki during the summer of 1996. Most vegetables were domestic and were purchased at the peak of their season; all fruits, expect apple, and also some vegetables were imported. Eight to ten subsamples weighing 0.2−1.0 kg were obtained of each food item.

Seasonal variations in the phylloquinone contents of some vegetables were also studied. White cabbage, carrot, and pot-

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Table 1. Phylloquinone Content of Vegetables, Fruits, and Berries

food item	phylloquinone[a] (μg/100 g)	CV (%)
berries		
black currant	30 ± 2	6.1
blueberry	12 ± 2.0	16.8
lingonberry	9 ± 0.5	5.6
raspberry	10.2 ± 0.06	0.6
red currant	11 ± 0.8	7.1
strawberry	5.5 ± 0.24	4.4
fruits		
apple		
domestic, unpeeled	5 ± 0.4	8.5
imported, unpeeled	5 ± 0.4	9.6
avocado	20 ± 0.7	3.6
banana	nd[e]	
grapes, green	19 ± 0.3	1.8
kiwi fruit	34.3 ± 0.32	0.9
orange	nd	
plum, red	8 ± 1.0	11.8
vegetables		
broccoli[b]	110 ± 13	11.0
Brussels sprouts	220 ± 6	2.5
carrot,[b] unpeeled	19 ± 0.9	4.5
cauliflower	20 ± 1	7.2
Chinese cabbage[b]	80 ± 11	13.6
cucumber,[b] unpeeled	15 ± 0.7	4.8
dill	280 ± 3	1.0
leek	54 ± 2.7	5.1
lettuce		
Iceberg lettuce[b]	40 ± 3	8.8
leaf	160 ± 27	17.6
lettuce in pot[c]	100 ± 10	7.7
mixed carrot, swede, leek, and celery, frozen	6 ± 0.8	13.3
mixed peas, corn, and pepper, frozen	10 ± 0.9	9.1
onion	0.7 ± 0.05	6.8
parsley	360 ± 24	6.6
pea, frozen[d]	28 ± 1.7	3.2
pepper		
green	9.4 ± 0.15	1.6
red	4.6 ± 0.19	4.1
yellow	2.36 ± 0.034	1.4
potato, without skin	1.04 ± 0.030	2.8
red beets, canned	nd	
spinach, frozen	270 ± 3	1.0
swede	2 ± 0.2	11.0
tomato[b]	5 ± 0.4	8.0
white cabbage[c]	60 ± 5	8.3

[a] Mean ±SD. [b] Two sampling times. [c] Four sampling times. [d] $n = 36$. [e] nd, not detected at a detection limit of 0.3 μg/100 g. Only edible parts were analyzed. All samples were raw.

grown lettuce (*Grand grapit*) are commonly consumed throughout the year in Finland. White cabbage and pot-grown lettuce were purchased four times (in May, August, and October 1996 and January 1997), and carrot was purchased twice (in August 1996 and January 1997); these samples were all domestic, except white cabbage purchased in May 1996. Some other regularly consumed vegetables were purchased twice to investigate the effects of season and country of origin. Domestic cucumber, tomato, and apple were purchased during the summer of 1996 and the same vegetables as imported during the winter of 1997. Iceberg lettuce, Chinese cabbage, and broccoli were purchased in May 1996 (imported) and in August 1996 (domestic). Ten subsamples of these vegetables were obtained at every sampling time from 10 different retail stores. In addition, the variation in phylloquinone content among carrot, white cabbage, and pot-grown lettuce was studied by analyzing six individual subsamples of these items at one sampling time.

In general, one pooled sample was prepared representing each item and the samples were determined as for consumption, i.e. only edible parts were analyzed. Domestic apple and cucumber were analyzed as unpeeled, but imported varieties were determined as both peeled and unpeeled. The phylloquinone content of their peels was also investigated. Each subsample was diced, and identical amounts (usually 100 g) of each were added to the pool. The pooled samples were mixed and vacuum-packed as 50−100-g portions in plastic bags and stored at −70 °C in the dark until analyzed (generally 1−3 weeks).

Phylloquinone Determination. All work was performed under subdued light. The phylloquinone content of each sample was determined in triplicate (each analytical sample was weighed from its own separate plastic bag). Reference samples and individual subsamples used in analyzing variation were, however, determined only in duplicate. Phylloquinone and the internal standard menaquinone 4 (MK-4) were purchased from Sigma Chemical Co. (St. Louis, MO), and standard stock and working solutions were used as previously described (Piironen et al., 1997).

The phylloquinone content of the samples was determined with reversed-phase HPLC (Hart et al., 1985; Piironen et al., 1997). MK-4 was selected for an internal standard as the only commercially available alternative. For each item a blank sample without the internal standard (MK-4) was analyzed first to confirm validity of its use. Samples containing other compounds eluting at the retention time of MK-4 in analytical HPLC were purified with semipreparative straight-phase HPLC (Piironen et al., 1997) prior to quantification. These samples included carrot, tomato, apple, onion, red and yellow pepper, plum, banana, orange, kiwi fruit, avocado, and all berries.

For the extraction of phylloquinone from vegetables two methods were compared: extraction with 2-propanol/hexane and with chloroform/methanol. The efficiencies of these extraction methods were investigated using two highly different matrices: carrot and white cabbage. In the development of the extraction method the influences of certain modifications in the procedures were also tested. The efficiency of each method was estimated by monitoring phylloquinone contents, recovery of MK-4, and repeatability of the results. The reliability of the methods was further confirmed by comparing the ratios of phylloquinone with MK-4 in the first and second extractions. Differences between methods were determined using the paired *t* test.

When extraction was performed with 2-propanol/hexane [a modification of the method of Langenberg et al. (1986)] a homogenized 2−3-g sample was weighed into a centrifuge tube. An appropriate amount (10−3100 ng) of MK-4 and 10 mL of 2-propanol were added prior to digestion in a boiling water bath; various digestion times were tested (range 0−20 min). After cooling, 10 mL of 2-propanol was added and the sample was homogenized with an Ultra-Turrax T25 mixer (Janke & Kunkel, Seelze, Germany) for 2 min. *n*-Hexane (10 mL) was added to the mixture twice and homogenized for 2 min after each addition. Finally, 10 mL of water was added, followed by rapid shaking. The extract was centrifuged at 1500g for 5 min (Sorvall RC2-B, Ivan Sorval Inc., Norwalk, CT), after which time an appropriate amount (0.5−10 mL) of *n*-hexane phase was evaporated to dryness. The residue of samples, which required purification with semipreparative HPLC, was redissolved in *n*-hexane (0.5−5 mL) and filtered through a membrane filter (Puradisc 25 TF 0.45 μm, Whatman, Ann Arbor, MI). The residues of the other samples were dissolved directly in the mobile phase of the analytical HPLC and filtered.

When the efficiency of the extraction method was evaluated, the second extraction with hexane (20 mL) was performed for the residue of the first extraction. The mixture was homogenized for 1 min and centrifuged for 5 min, and evaporation, dissolving, and filtering were performed as in the first extraction. To further confirm the efficiency of the method, extraction of the sample material after 15 min in boiling water was also monitored. After cooling, the water was removed and the change in weight of the vegetables registered. Extraction was performed as described above using 2-propanol/hexane as the solvent.

In the second method chloroform/methanol was used as the extraction solvent; its efficiency was monitored using white cabbage only. The MK-4 and 10 mL of 2-propanol were first added to a homogeneous, weighed (2−3 g) sample. The tube

was immersed in boiling water for 10 min; after cooling, 10 mL of 2-propanol was added and the sample homogenized with the Ultra-Turrax for 2 min. The mixture was centrifuged at 2000*g* for 5 min; after phase separation, the upper layer was collected in a flask. Twenty milliliters of chloroform/methanol (1:1) was added to the residue and the mixture homogenized for 2 min and filtered into the flask. The combined extract was filtered and evaporated to dryness using a rotavapor. Ethanol (10 mL) was added and the evaporation repeated. The residue was dissolved in *n*-hexane and handled as described for the extraction with 2-propanol/hexane; in this case, the second extraction was performed with 40 mL of chloroform/methanol. The mixture was shaken with a magnetic stirrer for 1 h, after which time the sample was filtered, evaporated, and redissolved in the same manner as in the first extraction.

For routine determinations, extraction with 2-propanol/hexane was chosen. The digestion time in the boiling water bath was 5 min for vegetables, fruits, and berries; for carrot and other root crops a longer digestion time (10 min) was used. Extraction and homogenization were performed as described above.

When necessary, purification of the extract was performed using the semipreparative straight-phase HPLC system described by Piironen et al. (1997). The vitamers (*cis* and *trans* isomers of phylloquinone and MK-4) were separated with a μPorasil column (5 μm, 300 × 3.9 mm; Millipore Corp., Milford, MA), in which the mobile phase was *n*-hexane containing 1% diethyl ether with a flow rate of 1.5 mL/min and an injection volume of 300 μL. The collection time was begun 2 min prior to elution of the *cis*-phylloquinone and ended 1.5 min after the elution of MK-4. The collected fraction was evaporated and redissolved in 0.5 mL of the mobile phase used in analytical HPLC.

The phylloquinone contents were quantified with reversed-phase HPLC with a dual-electrode electrochemical (EC) detector as described by Piironen et al. (1997); the method used was based on that of Hart et al. (1985). The analytical column was a Vydac 201 TP54 column (5 μm, 250 × 4.6 mm; The Separation Group, Hesperia, CA) with a mobile phase consisting of 96% methanol/0.05 mol of sodium acetate buffer (pH 3) flowing at a rate of 1 mL/min; the injection volume was 30 μL. The detector was operated in the redox mode, in which the upstream electrode (−1.1 V) reduced the vitamin K compounds and the downstream electrode (0 V) reoxidized them. The phylloquinone content of the samples was quantified according to the internal standard method based on the peak areas, in which the response factor was determined monthly at three concentration levels (0.2−0.9 ng per injection) and daily at one concentration level (0.7 ng per injection).

Method Validation. The accuracy of the method chosen was tested by determining the recovery of phylloquinone added in various materials (white cabbage, carrot, spinach, and pea) calculated by the internal standard method. The recoveries of phylloquinone and MK-4 calculated by the external standard method were also compared. Repeatability of the determinations was investigated by analyzing a reference sample (frozen peas) in duplicate in every second sample series. The daily variations in detector response and the retention times of the analytical HPLC were monitored with standard injections after every third sample. In preparative HPLC standards were injected to confirm the retention times for the collection. To avoid the carry-through effect, the mobile phase was injected after every standard injection in both HPLC systems.

RESULTS AND DISCUSSION

Analytical Method. We have previously described an HPLC method for the determination of phylloquinone in oils and margarines (Piironen et al., 1997). In the present study the method was modified for determining the presence of phylloquinone in vegetables, fruits, and berries. Special attention was focused on the efficiency of the extraction method.

Statistically significant differences were not observed in the extraction efficiency between 2-propanol/hexane and chloroform/methanol; the phylloquinone contents of white cabbage were similar with both methods (Table 2). The recovery of MK-4 was, however, significantly better ($p < 0.05$) in 2-propanol/hexane extraction. On the other hand, the ratios of phylloquinone to MK-4 were similar in the first and second extractions in both methods, indicating that the extractability of endogenous phylloquinone was similar to that of the added standard. In addition, extraction with 2-propanol/hexane was shown to be reproducible and easy to perform. After consideration of all the reasons mentioned above, extraction with 2-propanol/hexane was chosen for routine determinations.

Table 2. Extraction of Phylloquinone from Cabbage by 2-Propanol/Hexane (A) and by Chloroform/Methanol (B)

method	*N*	phylloquinone[a] (μg/100 g)	recovery of MK-4[a] (%)
A[b]	13	64 ± 14.3	80 ± 8.5
B[b]	9	60 ± 8.8	70 ± 8.7

[a] Mean ± SD. [b] Digestion time 10 min.

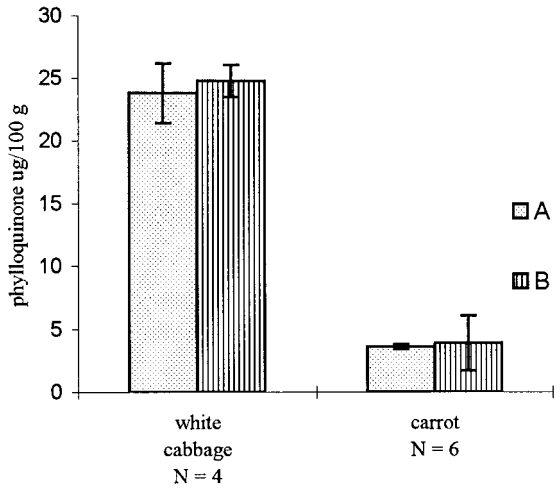

Figure 1. Phylloquinone contents of white cabbage and carrot extracted (A) after the samples were cooked in boiling water and (B) without cooking.

When extraction was performed with 2-propanol/hexane, digestion of the samples in 2-propanol prior to extraction was shown to be useful. It was shown that prolonging digestion time (from 5 min for cabbage and from 10 min for carrot) did not increase the phylloquinone content of samples. Furthermore, no significant differences in recovery of MK-4 between various digestion times (5−20 min) were observed, which indicated that the digestion procedure did not destroy vitamin K. The ratios of phylloquinone to MK-4 were similar in the first and second extractions after every tested digestion time. The selected times (5 min for white cabbage and 10 min for carrot) gave the most reproducible results.

Higher fat-soluble vitamin contents have been reported for cooked than for corresponding raw vegetables in several studies. The reason for this may be better extractability of fat-soluble vitamins from cooked materials. Due to this finding, the efficiency of 2-propanol/hexane extraction was confirmed by testing the influence of cooking in boiling water before extraction. As illustrated in Figure 1, extraction performed after the sample was cooked in boiling water led to slightly lower amounts of phylloquinone both for white cabbage and for carrot; the differences between procedures were not statistically significant nor was cooking in boiling water prior to extraction observed to influence recovery of MK-

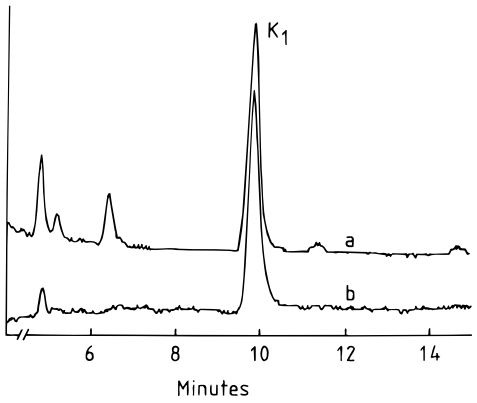

Figure 2. Analytical HPLC chromatograms of a kiwi fruit sample without (a) and after (b) purification with semipreparative HPLC.

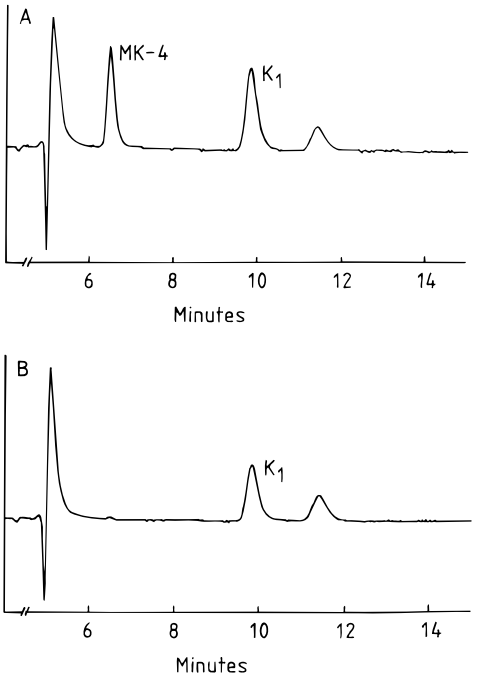

Figure 3. Analytical HPLC chromatograms of a white cabbage sample with (A) and without (B) the internal standard (MK-4).

4. When extracted after the sample was cooked, the ratio of phylloquinone to MK-4 did not change from the first extraction to the second extraction.

Semipreparative HPLC was used to purify samples with interfering peaks eluting at the retention time of MK-4 in analytical HPLC. The purification step removed also other impurities from the samples. The analytical chromatograms of kiwi fruit (Figure 2) illustrate the efficiency of the purification. Good separation of phylloquinone and MK-4 from the other components of many green vegetables was obtained in analytical HPLC without the purification step (Figure 3). Under the conditions described, phylloquinone eluted in approximately 9.9 min and MK-4 in 6.5 min. Minor day-to-day variation appeared in retention times of the two peaks, but the within-run variation was insignificant.

The variation in EC detector response in analytical HPLC was tested daily. The coefficient of within-day variation (CV) for the peak areas of phylloquinone was 3.3% and for the internal standard (MK-4) 3.5% ($n > 3$ per day, 72 days). Respectively, the day-to-day CV values were 6.6% and 6.3% ($n = 72$). The response factor for phylloquinone, using MK-4 as the internal standard, was 1.02 ± 0.019 (day-to-day CV = 1.8%, $n = 52$). When the response factor was determined at three different concentration levels, slightly more variation was observed. The day-to-day CV between determinations was 6.3% (six determination times), and the within-day CV between different concentration levels was 1.7%.

Table 3. Recoveries of Phylloquinone and the Internal Standard (MK-4) in the Different Food Items

		recovery[a] (%)		
food item	*N*	phylloquinone[b]	phylloquinone[c]	MK-4[c]
carrot	6	96 ± 12.4 (2.0)	107 ± 5.0 (3.4)	100 ± 8.7 (7.6)
pea, frozen	6	97 ± 3.8 (1.7)	92 ± 7.4 (2.0)	87 ± 4.8 (5.8)
spinach, frozen	4	98 ± 10.3 (6.7)	104 ± 15.0 (9.1)	99 ± 4.3 (1.9)
white cabbage	7	99 ± 12.1 (1.8)	82 ± 12.3 (3.6)	82 ± 2.2 (1.6)

[a] Mean ±SD (CV%). [b] Calculated by the internal standard method. [c] Calculated by the external standard method.

Recoveries of the phylloquinone and MK-4 in various sample matrices are summarized in Table 3. The good recovery of phylloquinone calculated according to the internal standard method for all tested samples (96−99%) indicated the accuracy of the method used. The similar recoveries of phylloquinone and MK-4 calculated by using the external standard method proved the similarity of their behavior during extraction and analysis. In routine determinations the recovery of MK-4 was usually 75−100%.

The repeatability of the analysis was further confirmed by the small day-to-day variation observed in the reference sample results. The CV in phylloquinone contents of pea (reference sample) was 6.1% (number of days = 36). When vegetable, fruit, or berry samples were analyzed in triplicate, the CV between determinations was normally below 10%. For some sample items, a greater CV (10−20%) was acceptable, because the sample matrix was very heterogeneous or the phylloquinone content low (<2 μg/100 g).

Phylloquinone in Vegetables, Fruits, and Berries. The main purpose here was to determine the phylloquinone contents in vegetables, fruits, and berries available in Finland. The phylloquinone contents of the analyzed samples are summarized in Table 1; as expected, the highest contents were found in dark green vegetables. The best sources of phylloquinone were parsley, dill, spinach, and Brussels sprouts, the phylloquinone contents of which were >200 μg/100 g. The amounts of phylloquinone were also moderately high in leaf lettuce (mean content = 160 μg/100 g), broccoli (110 μg/100 g), and in pot-grown lettuce (100 μg/100 g). These results are generally within the ranges previously reported for these important sources of phylloquinone (Careri et al., 1996; Booth et al., 1995; Ferland and Sadowski, 1992; Langenberg et al., 1986), although some of the results reported for broccoli (113−247 μg/100 g), spinach (202−1439 μg/100 g), and leaf lettuce (123−1180 μg/100 g) are slightly higher than our results.

The phylloquinone levels were considerably lower both in yellow and red vegetables and in root crops, which provide only moderate levels of phylloquinone. The phylloquinone contents reported here for these vegetables are also generally in accordance with previous data on phylloquinone (Careri et al., 1996; Booth et al., 1995; Ferland and Sadowski, 1992; Langenberg et al., 1986). Our results for carrot (19 μg/100 g), pea (28 μg/100 g), and tomato (5 μg/100 g) are within previously reported ranges (Careri et al., 1996; Jakob

Table 4. Seasonal Variation in the Phylloquinone Content of Vegetables

food	N^a	phylloquinone[b] (μg/100 g)	range (μg/100 g)	CV[c] (%) between
broccoli	2	110 ± 13	91.1−135.8	11.1
carrot	2	19 ± 0.9	16.0−22.6	17.6
Chinese cabbage	2	80 ± 11	72.4−99.8	5.3
cucumber	2	15 ± 0.7	14.6−16.9	2.3
Iceberg lettuce	2	39 ± 3.3	30.2−45.6	13.5
tomato	2	5 ± 0.4	4.4−5.7	7.9
lettuce in pot	4	100 ± 10	82.6−129.9	14.1
white cabbage	4	60 ± 5	53.4−73.4	6.3

[a] Sampling times. [b] Mean ±SD. [c] Between sampling time coefficient of variation.

et al., 1996; Booth et al., 1995; Ferland and Sadowski, 1992; Langenberg et al., 1986). In these studies the phylloquinone content of carrot was reported to be 3−25 μg/100 g and those of pea and tomato 24−33 and 3−9.5 μg/100 g, respectively; however, for several vegetables analyzed in the present study, e.g. Chinese cabbage and red and yellow peppers, no previous data were available.

Among the fruits analyzed, green fruits were moderate sources of phylloquinone. The results for avocado (20 μg/100 g), grapes (19 μg/100 g), and kiwi fruit (34.3 μg/100 g) are slightly higher than previously reported: 14, 8.3, and 25 μg/100 g, respectively (Booth et al., 1995; Weihrauch and Chatra, 1993). Other fruits are, however, insignificant sources of phylloquinone; their contents were at most 8 μg/100 g.

Most Finnish berries contained phylloquinone in amounts varying from 5.5 to 11 μg/100 g; black currant was, however, a significantly better source of phylloquinone (30 μg/100 g) than the other berries. Previous data were available only for strawberry; Booth et al. (1995) reported that strawberry contains 1.5 μg/100 g of phylloquinone, which is considerably less than found here (5.5 μg/100 g).

We investigated the degree to which peel affects the phylloquinone content of apple and cucumber. Peeling decreased the amount of phylloquinone by 60% in both items. Our results for the fleshy portions of cucumber (6.1 μg/100 g) and apple (2 μg/100 g) are, however, higher than those reported by Weihrauch and Chatra (1993; 2 and 0.4 μg/100 g, respectively). In our study, the phylloquinone contents of cucumber peel (92 μg/100 g) and various apple peels (red, 16.6 μg/100 g; and green, 20.3 μg/100 g) are, much lower than those reported earlier [360 μg/100 g for cucumber peel and 20 and 60 μg/100 g for apple peels (Weihrauch and Chatra, 1993)]. Yellow apple peels contained the lowest amount of phylloquinone (9.5 μg/100 g). The reason for the differences between our study and the results of others is probably the wide individual variation in the phylloquinone content of plants.

To monitor the seasonal variation in phylloquinone content, some important vegetables were analyzed twice (broccoli, carrot, Chinese cabbage, cucumber, Iceberg lettuce, and tomato) or four times (lettuce in pot and white cabbage) (Table 4). Statistically significant differences between sampling times were observed only in pot-grown lettuce ($p < 0.01$), Iceberg lettuce ($p < 0.02$), and carrot ($p < 0.01$). Variation in other vegetables analyzed two or four times was quite small. Usually phylloquinone content was higher in summer (in samples representing the new crop of that year) than in winter. The phylloquinone content of pot-grown lettuce, which is grown throughout the year in Finland, ranged between 84 and 120 μg/100 g. The highest phylloquinone level was found in May and the lowest in October.

Individual subsamples of white cabbage, carrot, and pot-grown lettuce were analyzed to monitor the variation further. The phylloquinone contents of six individual pot-grown lettuces (purchased in October) ranged from 54 to 130 μg/100 g (mean = 100 ± 30 μg/100 g, CV 31%), while the amounts in white cabbage (in January) were 28−72 μg/100 g (mean = 60 ± 23 μg/100 g, CV 42%). On the other hand, variation among individual carrot subsamples (in January) was lower; phylloquinone content ranged between 9 and 14 μg/100 g (mean = 11 ± 1.5 μg/100 g, CV 14%). These findings are in good agreement with previous studies on variation in green vegetables (Booth et al., 1994; Ferland and Sadowski, 1992).

To our knowledge no previous studies have been published in which seasonal variation has been directly investigated. Many confounders made monitoring of variation difficult also in this study; however, the results confirm that considerable variation may occur in the phylloquinone content of vegetables. Previously, Ferland and Sadowski (1992) showed that climate and growth locations affect the phylloquinone contents of vegetables. In this study other factors such as harvesting time, storage, or genetic factors may also play a role in variation.

The use of fresh vegetables, fruits, and berries has been fairly low in Finland. The average consumptions of vegetables, fruits, and berries have been 169, 119, and 29 g/day, respectively (Ministry of Agriculture and Forestry, 1996; Tikkanen, 1993). The various cabbages, cucumber, tomato, and carrot were the most commonly used vegetables. On the basis of the above consumption figures, the average intake of vitamin K from these foods was estimated to be 37 μg/day, when the proportion of vegetables accounts for ~90%. Previously we estimated (Piironen et al., 1997) that oils and margarines give about the same amount of phylloquinone per day; thus, these two food groups together will almost satisfy the daily RDA (NRC, 1989). In the vegetable group, the greatest amount of phylloquinone was estimated to be supplied by various cabbages (12.4 μg/day) and lettuces (5.6 μg/day). A significant amount of phylloquinone can also be supplied by carrot (3.9 μg/day) and cucumber (2.6 μg/day); however, a fairly high variation in the consumption of vegetables among individuals is to be expected. In consequence, the daily dietary intake of phylloquinone may vary considerably. In conclusion, green vegetables are very good sources of phylloquinone; for example, eating 50 g of pot-grown lettuce per day will fulfill >50% of the daily RDA (NRC, 1989).

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LITERATURE CITED

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