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Determination of phylloquinone in oils, margarines and butter by high-performance liquid chromatography with electrochemical detection

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A high-performance liquid chromatographic (HPLC) method for the determination of phylloquinone in oils and margarines following purification of hexane solutions of oils or hexane extracts of margarines by straight-phase semi-preparative HPLC is described. Phylloquinone was quantified by reverse-phase HPLC with a dual-electrode electrochemical detector operating in the redox mode. Menaquinone-4 (MK-4) was used as an internal standard. By this method the phylloquinone present in the main oils and margarines available on the Finnish market was determined.The same method, but without the internal standard, was applied to butter. The detection limit of phylloquinone was 50 pg per injection, and its recovery when added to oil and margarine samples and quantified by the internal standard method was 98% and 102%, respectively. The mean phylloquinone content of oils ranged from 1·5 μg (refined rapeseed oil) to 0·10 μg (sunflower oil). In the soft margarines with 80% fat, the phylloquinone levels were 0·89–1·1 μg g^{-1}. The phylloquinone content of margarines with 40% and 60% fat correlated with their fat content. Blended and hard margarines contained less phylloquinone than the soft margarines with corresponding fat contents. The contribution of oils and margarines to the average daily dietary intake of phylloquinone in Finland was estimated to be approximate 40 μg.

INTRODUCTION

In addition to its role in blood coagulation, it has been suggested that vitamin K performs other roles, such as in bone metabolism (Shearer, 1995; Vermeer *et al.*, 1995). These roles are based on the function of vitamin K as a cofactor in the post-transational synthesis of γ-carboxyglutamic acid in vitamin-K-dependent proteins. New, sensitive tests based on detecting under-carboxylation of these proteins have indicated that a poor vitamin K status may be far more prevalent than was previously assumed (Vermeer *et al.*, 1995). Furthermore, most recent evidence suggests that menaquinones synthesized by intestinal microflora are less important in vitamin K nutrition than previously thought (Suttie, 1995), emphasizing the significance of the dietary intake. On the other hand, certain population groups, such as those undergoing anticoagulant therapy, are advised to avoid foods rich in vitamin K. These findings have led to a need for more reliable data on the occurrence of the vitamin-K-active compounds phylloquinone and menaquinones in foods.

Due to the complexity of the matrices and problems in detection, the quantification of vitamin K compounds in foods has remained an analytical challenge (Lambert & De Leenheer, 1992). Quantification by reverse-phase high-performance liquid chromatography (HPLC) after several purification procedures has recently been the method of choice. Electrochemical detection (EC) or fluorometric detection after post-column reduction is needed to give sensitivity and specificity to the HPLC analysis. Although much progress has been made in analytical methods, data on vitamin K compounds in foods based on reliable methods and well-documented sampling procedures are still very limited (Booth *et al.*, 1993).

Green vegetables are generally regarded as the best dietary sources of phylloquinone. Certain oils, especially rapeseed and soybean oils, have also been shown to contain high amounts of phylloquinone (Zonta & Stancher, 1985; Ferland & Sadowski, 1992*a*; Gao & Ackman, 1995). Oils and oil-based products may, therefore, contribute significantly to the daily dietary vitamin K

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intake. Their role as dietary vitamin K sources may further be emphasized by the new research indicating poor availability of phylloquinone in green vegetables. The importance of membrane-bound vitamin K compounds in general may be overestimated (Vermeer *et al.*, 1995). Some research has recently been done on phylloquinone in oils (Ferland & Sadowski, 1992*a*; Moussa *et al.*, 1994; Zonta & Stancher, 1985; Gao & Ackman, 1995) and a very limited amount on phylloquinone in margarines (Booth *et al.*, 1993, 1995). The phylloquinone contents of plant materials have been shown to be affected by their growing conditions (Ferland & Sadowski, 1992*b*). On the other hand, the stability of phylloquinone in oils and margarines may be dependent on storage and processing conditions. Phylloquinone in oil stored in clear bottles was rapidly destroyed by daylight and fluorescent light (Ferland & Sadowski, 1992*a*). Therefore, considerable variation in phylloquinone contents of oils and margarines is possible, depending on the origin of the oil plants and on oil and margarine processing and storage practices.

In the present study an HPLC method for determining phylloquinone in oils and margarines is presented. Semipreparative HPLC was used as the only purification step before quantification by reverse-phase HPLC with EC detection. The method was applied for determining phylloquinone in the main oils available in Finland, as well as in different margarines and butter. The stability of phylloquinone in oil and margarine processing was also evaluated.

MATERIALS AND METHODS

Chemicals

HPLC-grade methanol, n-hexane and isopropanol were purchased from Rathburn (Walkerburn, UK) and diethyl ether was from Merck (Darmstadt, Germany). Sodium acetate, acetic acid (Merck), petroleum ether (Riedel-de Haen, Seelze, Germany) and ammonia (J. T. Baker, Deventer, The Netherlands) were of pro analysis grade.

The standard stock solutions were prepared by dissolving approximately 20 mg of phylloquinone (contains both *cis* and *trans* isomers; Sigma, St Louis, USA) and menaquinone-4 (MK-4; Sigma) in 25 ml of n-hexane; they were stored at −18°C in the dark for up to 4 weeks. Standard working solutions (approx. 70 ng ml^{-1}) in the mobile phase were prepared weekly. The concentrations of the standard solutions were confirmed by measuring their absorbance at a wavelength of 249 nm and by applying a molar absorptivity of 18 900 for both phylloquinone and MK-4 (Gao & Ackman, 1995).

Samples

Samples of the main oils available in Finland, refined rapeseed, soybean and sunflower oils, and of ten popular brands of margarine with 40–80% fat content (soft and hard margarines), three margarine blends, one oil blend for baking and butter were each purchased on two occasions (in December 1995 to January 1996, and in March 1996) from ten retail stores representing the four major food chains in the Helsinki area (Table 1). The number of subsamples of the oils of minor importance in Finland, i.e. cold-pressed rapeseed oil and olive oils, was smaller, averaging 6–8. The margarine samples represented both of the two Finnish manufacturers (Van den Berg Foods and Raisio Group), whose margarines account for approximately 90% of the margarine sold in Finland. The butter samples were produced by Valio, which makes 70% of the butter in Finland. The refined rapeseed oil, soybean oil and sunflower oil were all produced in Finland by the two margarine manufacturers referred to above who produce approximately

Table 1. Margarine samples (composition, as stated by the manufacturer)

Sample	Code	Oil and fat composition
Soft margarines		
Fat content 80%	A	Rapeseed and sunflower oil 87%, vegetable fat 13%
	B	Rapeseed oil 60%, vegetable fat 40%
	C	Vegetable oil 65%, vegetable fat 35%
Fat content 70%	D	Rapeseed oil 66%, vegetable fat 34%
Fat content 60%	E	Rapeseed oil 70%, vegetable fat 30%
	F	Vegetable oil 65%, vegetable fat 35%
Fat content 40%	G, H	Vegetable oil 60–63%, vegetable fat 37–40%
Blended margarines		
Fat content 80%	I	Vegetable fats, rapeseed oil 40%, milk fat 10·5%
	J	Rapeseed oil 25%, milk fat 75%
Fat content 40%	K	Rapeseed oil 40%, milk fat 60%
Hard margarines		
Fat content 80%	L, M	Animal and vegetable fat, vegetable oil 30–40%
Oil blend for baking	N	Rapeseed oil 98%, hardened rapeseed oil

70% of the oils sold in Finland. The rapeseed oil was mainly Finnish turnip rapeseed oil, high in oleic acid (*Brassica rapa* subsp. *oleifera* DS). The raw materials of the soybean and sunflower oils were imported. The olive oils were imported from Italy. The cold-pressed rapeseed oil was produced by a small Finnish manufacturer.

Generally, one pooled sample representing each oil type was prepared at each of the two sampling times. The margarine samples bought at the same sampling time were pooled according to their brand. Identical 100 g amounts of each margarine subsample and 60–100 ml of each oil subsample were added to the pool and the pooled samples mixed, vacuum-packed as 50 g portions in plastic bags (margarines, butter) or as 30 ml portions in plastic cans (oils), and stored at −18°C in the dark until analysis. The variation in the phylloquinone content of the most popular margarine brand and of refined rapeseed oil was studied by analysing separately, in addition to the pooled sample, six individual subsamples of the first sampling.

The effect of oil processing on phylloquinone content was studied by determining the phylloquinone in two batches of crude rapeseed oil and in the corresponding refined oil. In addition, the phylloquinone content in two batches of crude rapeseed oil and in the corresponding margarine compositions (approximately 70% of refined rapeseed oil and 30% of hardened vegetable fats), and in margarines with 70% fat content, was also determined. The process samples were obtained from the two Finnish manufacturers mentioned above.

Sample preparation

All work was carried out under subdued light conditions. The samples were generally analysed in triplicate. Reference samples and individual subsamples for studying variation were, however, analysed in duplicate.

Oil samples

A sample of oil (0·5–1·0 g) was weighed into a 10 ml volumetric flask. After adding 170–700 ng of MK-4 as an internal standard, the sample was diluted to volume with n-hexane. The solution was filtered through a membrane filter (Millex-HV, 0·45 μm; Millipore Corp., France) before purification by semipreparative HPLC.

Margarine and butter samples

For extraction of phylloquinone from margarines with 80% and 40% fat content, three methods were tested: extraction by hexane, by isopropanol–hexane and by diethyl ether and petroleum ether after ammonia treatment. For the routine determinations, extraction by hexane was chosen. With this method, a sample of 0·5–1·0 g was weighed into a 10 ml volumetric flask and the internal standard (300–700 ng) added. The vitamin K compounds were extracted by shaking in approximately 5 ml of hexane for 1 min, after which the samples were diluted to volume with n-hexane and left to stand for 30 min. A 2 ml aliquot was evaporated and the residue dissolved again in hexane (2 ml). Before purification by HPLC the extract was filtered. The same method without the internal standard was used for butter samples.

Extraction by the second method, using isopropanol-hexane, involved first weighing an aliquot of 1 g into a centrifuge tube. After adding the internal standard, 15 ml of isopropanol–hexane (1:1) were then added and the sample was homogenized for 1 min (Ultra-Thurrax T25 mixer; Janke & Kunkel, Germany). Water (5 ml) was then added and the mixture was shaken. An aliquot of the hexane extract was handled as described for the first method.

In the third method (IDF, 1983; modification), 2 ml of water and 2 ml of 25% ammonia solution were added to the sample (1 g) in a Mojonnier flask. After shaking, 5 ml of 95% ethanol were added and the sample was mixed again. Diethyl ether (25 ml) was then added, and the mixture shaken for 1 min, after which petroleum ether (25 ml) was added and the mixture shaken again. The diethyl ether–petroleum ether extract was separated and the extraction was repeated twice (the second extraction with 15 ml and the third with 5 ml of the solvents). The combined extract was washed with water in a separatory funnel until a neutral pH was achieved. The extract was then evaporated using a rotavapor. Ethanol (10 ml) and n-hexane (10 ml) were added and the evaporation repeated. The residue was then dissolved in hexane (10 ml) and handled as described for the first method.

Purification by semipreparative straight-phase HPLC

Purification of the hexane extracts by semipreparative HPLC was performed using an HPLC apparatus consisting of a Waters Model 510 HPLC pump (Waters Associates, Milford, MA, USA), a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA), a Merck-Hitachi L-4200 UV-VIS detector (Hitachi, Tokyo, Japan) set at 248 nm and an LKB 2220 recording integrator (LKB, Bromma, Sweden). A Waters Guard-Pak holder with a silica insert (Millipore, Milford, MA, USA) was installed before a μPorasil column (5 μm, 300 mm×3·9 mm; Millipore).

The flow rate of the mobile phase containing 1% diethyl ether in n-hexane was 1·5 ml min^{-1}. An aliquot of 300 μl of the hexane extract (sunflower oil and butter, 500 μl) was injected. The *cis* and *trans* isomers of the phylloquinone standard were separated from each other and from MK-4 in the chromatographic system. The collection time began 2 min before elution of the *cis*-phylloquinone and ended 1·5 min after the elution of MK-4. The collected fraction (phylloquinone + MK-4) was evaporated and the residue redissolved in 0·5 ml of the mobile phase used in the analytical HPLC. The

column was washed after every second sample with 50% diethyl ether in hexane flowing at the rate of 1·9 ml min^{-1}. To prevent a carry-through effect of the standards injected to confirm the retention times, it was necessary to inject 0·5 ml of hexane before the first sample injection.

Analytical HPLC

Quantification of the phylloquinone contents was performed by reverse-phase HPLC with a dual-electrode EC detector. The method was based on the method of Hart *et al.* (1985). The apparatus consisted of a Merck-Hitachi L-2000 pump (Hitachi), a Merck T-6300 column thermostat (Merck, Darmstadt, Germany), a Waters 717 autosampler, an ESA Coulochem II EC detector equipped with a guard cell (Model 5020) and a dual-electrode analytical cell (Model 5011) containing two porous graphite electrodes in series (ESA, Chelmsford, MA, USA) and Millenium 2010 chromatography manager (Waters). The separation was performed on a Vydac 201 TP54 column (The Separation Group, Hesperia, CA, USA) (5 μm, 250 mm×4·6 mm). A Waters Guard-Pak holder with a Nova-Pak C18 insert was used as a guard column. The mobile phase was 95% methanol/0·05 M sodium acetate buffer (pH 3) (Hart *et al.*, 1985), the flow rate 1·0 ml min^{-1} and the injection volume 40 μl.

The detector was operated in the redox mode. Vitamin K compounds were reduced at the upstream electrode (electrode potential −1·1 V). The quantification was based on reoxidizing the compounds at the downstream electrode set at 0 V. To optimise the applied electrode potentials, voltammograms were run for both electrodes. For the upstream electrode the voltammogram was obtained by keeping the downstream electrode at 0·2 V and varying the potential of the upstream electrode between −0·5 V and −1·2 V in 100 mV steps. For the downstream electrode, the voltammogram was obtained by keeping the upstream electrode at −1·1 V and varying the potential of the downstream electrode between 0·05 V and 0·45 V. The same batch of mobile phase was recycled for one sample series which was run overnight. To avoid contaminating the electrodes, the mobile phase was recycled for the next day so that the electrode potentials were set at 0·2 V and −0·2 V (Hart *et al.*, 1985). To avoid the carry-through effect after the standard injections, 40 μl of the mobile phase were injected after the standard injection. The chromatographic system was washed with water and methanol once a week.

Phylloquinone was quantified by the internal standard method based on the peak areas. Phylloquinone in butter was, however, quantified by the external standard method with recovery corrections. For every sample, a blank without the internal standard was analysed. The response factor was determined once a month at three concentration levels (0·7–2·8 ng per injection).

Method validation

The linearity ranges of the standard curves for phylloquinone and MK-4, as well as the detection limits of phylloquinone and MK-4, were determined. To establish the peak purity, the response ratios of phylloquinone and MK-4 were compared in the standard solution and in an oil and a margarine sample at the downstream electrode potentials 0·4 V and 0 V. The recovery of phylloquinone quantified by the internal standard method and the recoveries of both phylloquinone and MK-4 quantified by the external standard method were tested for oils as well as for margarines with different fat content. The recovery of phylloquinone added to butter was also determined. The day-to-day repeatability of the determinations was investigated by analysing a reference sample in every second sample series. Two rapeseed oil samples (pooled samples of the two sampling times) were used as reference samples when analysing oils, and two samples of margarine with 80% fat content (pooled samples of one margarine brand) when analysing margarines. The within-day variations of the detector response and the retention times were monitored by standard injections after every third sample.

RESULTS AND DISCUSSION

Analytical method

In the method presented above, phylloquinone was quantified by reverse-phase HPLC with EC detection after purification of the hexane solutions of the oils or hexane extracts of margarines and butter by straight-phase semipreparative HPLC. No other purification procedures were necessary.

When testing the three different extraction methods for margarines, extraction by shaking in hexane was shown to be effective and reproducible. The extraction by isopropanol–hexane led to the greatest variation in the results. The other two methods gave quite similar phylloquinone contents and recoveries for both a margarine of 80% fat content and one of 40% fat content. Due to its simplicity, the hexane extraction method was chosen.

At the analytical HPLC step it was necessary for the samples to be soluble in the mobile phase, which consisted of methanol and acetate buffer. Therefore, nonpolar components such as triacylglycerols had to be removed from the hexane extracts. That was most effectively and easily done by the semipreparative HPLC system. Solid-phase extraction (Ferland & Sadowski, 1992*a*) and lipase hydrolysis together with solid-phase extraction (Gao & Ackman, 1995) were the methods previously used for purification of oil extracts when fluorometric detection allowing use of non-aqueous mobile phases was applied. Without further development these methods were not effective enough for oils and

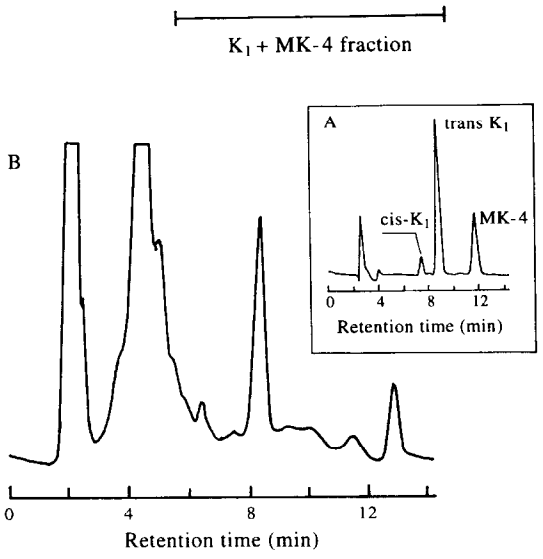

Fig. 1. Semipreparative HPLC chromatograms of a standard solution (A) and (B) a margarine sample (collected fraction is marked on the chromatogram).

margarines when the residue had to be dissolved in a solvent suitable for the EC detector.

The HPLC purification also adequately removed other possible interfering components when the separation capacity of the column was maintained by washing it regularly. The *cis* and *trans* isomers of phylloquinone and MK-4 were eluted from the semipreparative column in approximately 6·8, 8·1 and 10·7 min, respectively (Fig. 1). Particular care was necessary to avoid a carry-through effect of the standards used to verify the collection time of the vitamin K fraction in the sample. Fairly large quantities of the standards had to be injected due to the insensitivity of the UV detection. The carry-through effect was effectively avoided by injecting a large volume of hexane before the next sample. As a purification method, one major benefit of HPLC is that it is possible to easily modify the purification according to the samples by changing the chromatographic conditions.

In the analytical HPLC, good separation of phylloquinone and MK-4 from the other components of the sample was obtained. The chromatograms of a rapeseed oil and a margarine sample are shown as an example (Figs 2 and 3). Phylloquinone eluted in approximately 10·5 (*cis* and *trans* isomers eluted together) and MK-4 in 6·8 min. The within-day variations in the retention times were small: 0·22% (phylloquinone) and 0·96% (MK-4). The day-to-day variations were 0·17% and 0·09%, respectively. No interfering peaks eluted at the retention time of MK-4 in the samples containing no milk fat.

The EC detector gave the highest response for phylloquinone when the electrode potentials were set at 1·1 V (upstream electrode) and 0·4 V (downstream electrode). Reducing the potential of the downstream electrode to 0 V eliminated extra peaks from the

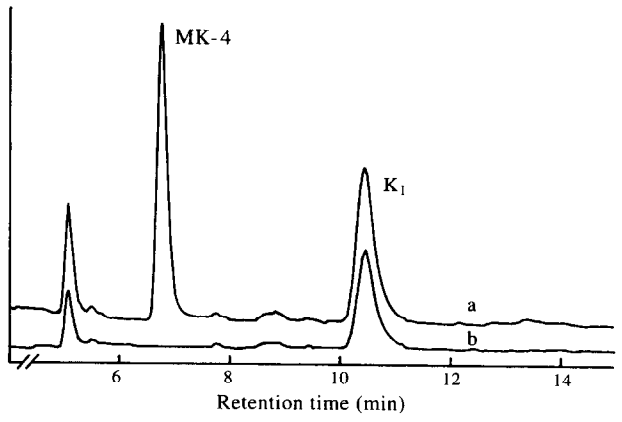

Fig. 2. Analytical HPLC chromatograms of a rapeseed oil sample with (a) and without (b) the internal standard (MK-4).

chromatograms and stabilized the detection. This potential was therefore selected for the routine determinations, although it caused a 25% drop in the peak heights. The detection limits for phylloquinone and MK-4, defined as a signal twice the height of the noise level, were 50 pg and 20 pg, respectively. Previously, a limit of 50 pg was obtained for phylloquinone with the same detector (Hart *et al.*, 1985). The phylloquinone contents of the analysed samples were well above this limit. The detector response was linear in the tested range of 0·1–50 ng per injection (coefficient of correlation 0·9992). When testing the within-day variation of the detector response, the coefficients of variation for the peak areas of phylloquinone and MK-4 were 3·4% and 3·0% ($n > 3$ per day, 38 days). The day-to-day variations were 6·8% and 5·8% ($n = 38$). The response factor for phylloquinone using MK-4 as the internal standard was $1{\cdot}055 \pm 0{\cdot}0139$ (day-to-day variation 1·3%, $n = 5$). The response ratios of phylloquinone and MK-4 in the standard solutions and in the samples when two reoxidizing potentials were compared were equal, which is an indication of the peak purity.

The main purpose of the present study was to determine phylloquinone in oils and margarines made from

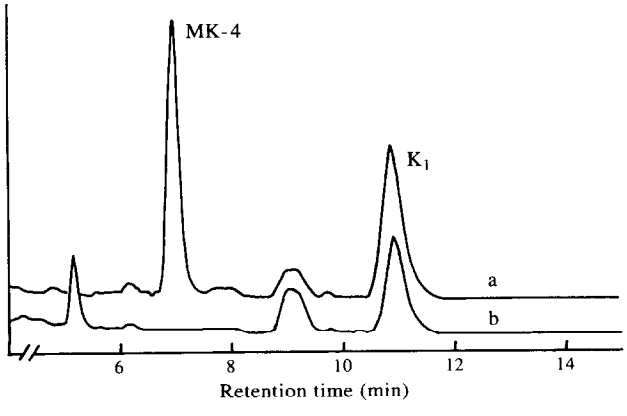

Fig. 3. Analytical HPLC chromatograms of a margarine sample with (a) and without (b) the internal standard (MK-4).

vegetable oils and fats. Thus, MK-4, whose benefit is its commercial availability, was selected for the internal standard. MK-4 proved to be a good internal standard for phylloquinone in oils and margarines. The similarity of its behaviour in the analysis to phylloquinone was confirmed by the recovery tests. The overall recoveries (calculated using the external standard method) of phylloquinone and MK-4 added to rapeseed or sunflower oil samples ($n = 8$) were 98 ± 4·8% (CV% 4·9) and 97 ± 7·6% (CV% 7·8), and 84 ± 21% (CV% 25) and 87 ± 10% (CV% 12) when added to margarine (40–80% fat) samples ($n = 12$). Care was necessary in analysing samples which may contain endogenous MK-4. To confirm reliable quantification, blank tests were carried out for every sample in this study. When quantifying phylloquinone in blended margarines, minor amounts of MK-4 were detected. In these cases the internal standard method could be applied by taking into account the proportion of endogenous MK-4. Phylloquinone in butter which contained endogenously more MK-4 than phylloquinone had to be quantified by the external standard method with recovery correction. The recovery of phylloquinone added into butter samples was 78 ± 8·6% (CV% 11, $n = 6$).

The good recoveries for phylloquinone obtained when calculating the results using the internal standard method were an indication of the accuracy of the method. For oil samples the recovery was 98 ± 6·1% (CV 6·2%, $n = 20$), and for different margarines 102 ± 7·2% (CV% 7·1, $n = 12$). The reliability of the results was further confirmed by the good day-to-day repeatability of the method. The CV in the phylloquinone contents of two rapeseed oil reference samples were 3·3% (number of days 7) and 10% ($n = 5$) and those of two margarine samples 6·3% ($n = 10$) and 6·9% ($n = 4$). When analysing triplicate oil and margarine samples, the CV was normally below 4% (range 0·1–10·7%).

Phylloquinone in oils and margarines

Among the refined oils analysed, the best sources of phylloquinone were rapeseed oil (mean content 1·5 μg g^{-1}) and soybean oil (mean content 1·30 μg g^{-1}) (Table 2). These contents are within the ranges previously reported: 1·7–2·78 μg g^{-1} (Ferland & Sadowski, 1992*a*; Gao & Ackman, 1995) and 1·08–3·33 μg g^{-1} (Zonta & Stancher, 1985; Ferland & Sadowski, 1992*a*),

Table 2. Phylloquinone contents (μg g^{-1}) of oils, margarines and butter

Food item	First sampling		Second sampling		Mean
	$x \pm SD$	CV%	$x \pm SD$	CV%	
Oils					
Olive oil, refined	0·34 ± 0·023	6·7	0·251 ± 0·0034	1·4	0·30
Olive oil, extra virgin	0·44 ± 0·03	6·9	0·5 ± 0·04	8·3	0·5
Rapeseed oil, refined[a]	1·6 ± 0·07	4·6	1·4 ± 0·14	9·7	1·5
Rapeseed oil, cold pressed	1·43 ± 0·013	0·9	1·17 ± 0·011	0·9	1·30
Soybean oil, refined	1·32 ± 0·013	1·0	1·585 ± 0·0013	0·1	1·45
Sunflower oil, refined	0·092 ± 0·002	1·8	0·10 ± 0·061	6·0	0·10
Soft margarines					
A (80% fat)	1·06 ± 0·011	1·0			
B (80% fat)	0·96 ± 0·013	1·4	0·82 ± 0·016	1·9	0·89
C (80% fat)[b]	1·1 ± 0·07	7·1	1·01 ± 0·086	8·4	1·1
D (70% fat)	1·04 ± 0·015	1·5	0·83 ± 0·009	1·1	0·94
E (60% fat)	0·74 ± 0·021	2·9	0·64 ± 0·021	3·2	0·69
F (60% fat)	0·77 ± 0·015	2·0	0·66 ± 0·016	2·4	0·72
G (40% fat)	0·45 ± 0·048	10·7	0·65 ± 0·048	7·4	0·55
H (40% fat)	0·53 ± 0·019	3·6	0·6 ± 0·06	9·8	0·6
Blended margarines					
I (80% fat)	0·9 ± 0·01	1·6	0·66 ± 0·021	3·2	0·8
J (80% fat)	0·5 ± 0·02	3·2	0·5 ± 0·05	10·0	0·5
K (40% fat)	0·42 ± 0·013	3·0	0·3 ± 0·01	3·8	0·4
Hard margarines					
L (80% fat)	0·46 ± 0·010	2·2	0·40 ± 0·009	2·3	0·43
M (80% fat)	0·70 ± 0·022	3·1	0·51 ± 0·010	1·9	0·61
Oil blend (100% fat)	1·3 ± 0·02	1·6			
Butter	0·08 ± 0·012	14·3	0·06 ± 0·007	10·8	0·07

$x \pm SD$, mean ± standard deviation.
[a] $n = 16$ (first sampling) or $n = 10$ (second sampling).
[b] $n = 22$ (first sampling) or $n = 11$ (second sampling).

respectively. Accordingly, sunflower oil was the poorest source of phylloquinone among the oils analysed (mean content 0·10 μg g^{-1}). Non-systematic results were obtained when the phylloquinone contents of the refined and cold-pressed unrefined oils were compared. The phylloquinone content of the cold-pressed olive oil was higher than that of the refined oil. On the other hand, the reverse was true in the case of rapeseed oil. It must, however, be noted that the oils were of different origin. Therefore, in addition to the processing conditions (e.g. recovery of phylloquinone into the crude oil and losses during refining), the origin of the oil plants and the different stability of phylloquinone in these oils may have an impact. Based on the analysis of the crude rapeseed oils and the corresponding refined oils in this study, refining caused a 20% loss in phylloquinone. To our knowledge, there are no previous studies directly comparing the crude and the corresponding refined oil.

To investigate further the variation to be expected in the phylloquinone levels of refined rapeseed oil, six individual bottles were analysed. The phylloquinone contents were in the range 1·4–1·87 μg g^{-1} (1·6 ± 0·17 μg g^{-1}, CV% 11). No correlation was found between the phylloquinone content and the storage time, but the results indicated that the variation may have been caused mainly by other factors, such as differences in the raw materials or storage conditions. No difference between manufacturers was observed. For different oils, the results of the two sampling times differed 0–27%. The fairly high variation found in the phylloquinone levels of the crude rapeseed oil samples of the processing experiments in this study (1·4, 1·8, 2·0 and 2·1 μg g^{-1}, CV% 14) indicate that occasionally significant variation may also occur in retail oils.

Due to the extensive use of rapeseed oil for margarines in Finland, the margarines were also shown to be good sources of phylloquinone. In the soft margarines with 80% fat content, the mean phylloquinone values ranged from 0·89 to 1·1 μg g^{-1}. The phylloquinone content of margarines with 40% and 60% fat correlated with their fat content. The phylloquinone contents could, therefore, be estimated on the basis of their fat content, provided the phylloquinone levels of the margarines with 80% fat are known. The hard margarines contained lower amounts of phylloquinone, reflecting the lower proportion of vegetable oils in their composition. As expected, the blended margarines also contained less phylloquinone than the soft margarines with similar fat contents. The phylloquinone content of butter, 0·07 μg g^{-1}, was comparable with the value 0·056 μg g^{-1} reported in Japan (Hirauchi *et al.*, 1989) and 0·07 μg g^{-1} reported in the USA (Booth *et al.*, 1995).

A variation of 0·96–1·17 μg g^{-1} was found within one margarine brand when six individual packages were analysed (1·1 ± 0·07 μg g^{-1}, CV% 6·6). The difference in the results of the two sampling times was fairly remarkable, 0–36%. It was probably caused mainly by differences in raw materials. On the other hand, the margarine samples obtained from the manufacturers right after production (process samples) contained 13% more phylloquinone than the retail samples of the same brand. This difference indicates that the storage may also have some influence.

No phylloquinone losses were found in the investigation of the effect of processes used in margarine production by comparing the phylloquinone contents of the margarine compositions (oil-based raw materials) and the corresponding margarines.

The phylloquinone contents of the margarines could only be compared with values from two previous studies. In the USA, the stick margarine was reported to contain 0·33 μg g^{-1} of phylloquinone (Booth *et al.*, 1995), which is less than found in the present study for hard margarines, and 0·51 μg g^{-1} for regular hard stick margarine (Weihrauch & Chatra, 1993). The latter figure is comparable with the results of the present study for hard margarines. Significant variation in the phylloquinone contents of margarines produced in different countries can be expected due to differences in their oil compositions.

Use of oils and margarines has been fairly low in Finland. In 1995, the average consumption of butter, margarines and vegetable oils was 14, 30, and 8 g per day, respectively (Information Centre of Finnish Margarine Industry, 1996). Rapeseed oil, shown to be a good source of phylloquinone, comprises, however, 80% of total sales of oils in retail stores. The proportion of rapeseed oil in the oils used in margarine production is approximately 70% (40% of all oil and fat raw materials). Therefore, oils and margarines may contribute significantly to the average daily dietary vitamin K intake in Finland. Based on the above consumption figures, their contribution was estimated to be 40 μg, which is approximately 50–60% of the recommended daily allowance (National Research Council, Food and Nutrition Board, 1989). On the hand, consumption of 60–70 g soft margarine with 80% fat content would fulfil the recommendation. The new data on bioavailability of phylloquinone from different food sources may further emphasize the significance of oils and margarines as dietary vitamin K sources.

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