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Dihydrovitamin K_1 in oils and margarines

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Abstract

In the present study the vitamin K_1 and 2′,3′-dihydrovitamin K_1 contents of several crude vegetable oils, partially hydrogenated oils used as raw materials in the margarine industry and margarines were analysed with a high-performance liquid chromatographic (HPLC) method. Prior to quantification by reverse-phase HPLC with an electrochemical (EC) detector, hexane extracts of samples were purified by straight-phase semipreparative HPLC. Menaquinone-4 (MK-4) was used as an internal standard. All hydrogenated oils (rapeseed, soybean and mixture of rapeseed and palm) contained considerable amounts of 2′,3′-dihydrovitamin K_1, which accounted for approximately 60% of the sum of 2′,3′-dihydrovitamin K_1 and vitamin K_1; however, among the margarines analysed 2′,3′-dihydrovitamin K_1 was found only in hard margarines meant for the baking industry. The proportion of 2′,3′-dihydrovitamin K_1 in these products was 25% of the total. The appearance of 2′,3′-dihydrovitamin K_1 among the samples analysed correlated with that of *trans* fatty acids. During recent years the Finnish margarine industry has focused on reducing the amount of *trans* fatty acids in soft margarines. It was concluded that because of these changes in the manufacturing process, 2′,3′-dihydrovitamin K_1 plays no significant role in vitamin K nutrition in Finland.

1. Introduction

It is currently known that vitamin K functions as an essential cofactor both in blood coagulation and in bone metabolism (Shearer, 1995). It has also been suggested that menaquinones synthesized by the intestinal microflora are less important for these processes than previously believed (Vermeer et al., 1995). The significance of dietary vitamin K is therefore increased.

Several studies have shown that certain vegetable oils (especially soybean and rapeseed oils) are important dietary sources of vitamin K_1 (Zonta and Stancher, 1985; Ferland and Sadowski, 1992; Booth et al., 1993, 1995; Moussa et al., 1994; Gao and Ackman, 1995; Jakob and Elmadfa, 1996; Piironen et al., 1997), while high vitamin K_1 contents were also found in Finnish soft margarines (Piironen et al., 1997). On the other hand, hydrogenation of vegetable oils was recently shown to convert part of the vitamin K_1 to 2′,3′-dihydrovitamin K_1, the biological activity of which is not known (Davidson et al., 1996). In addition to hydrogenated margarines, other substances, e.g. fast-food items and infant formulas containing hydrogenated oils, were also found to contain appreciable amounts of dihydrovitamin K_1 (Booth et al., 1996; Indyk and Woollard, 1997).

In our previous study, we determined the vitamin K_1 contents of several oils and margarines (Piironen et al., 1997). The aim of the present study was to determine the 2′,3′-dihydrovitamin K_1 contents of some hydrogenated oils and margarines, and to estimate the significance of 2′,3′-dihydrovitamin K_1 as a source of vitamin K in Finland.

2. Materials and methods

2.1. Samples

The effect of hydrogenation on the vitamin K_1 and 2′,3′-dihydrovitamin K_1 contents of oils was studied by determining their concentrations in partially hydrogenated rapeseed and soybean oils as well as in the corresponding crude oils. In addition, the contents were analysed in a hydrogenated mixture of rapeseed (80%) and palm (20%) oils. To monitor variation in the contents two batches of hydrogenated soybean oil and the

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mixture of rapeseed and palm oils were analysed. Oil samples were obtained from two leading manufacturers (Van den Berg Foods and the Raisio Group) in Finland during the winter of 1997.

Vitamin K_1 and 2′,3′-dihydrovitamin K_1 contents were also analysed in two popular soft margarines and one hard margarine, representing both the manufacturers mentioned above. Samples were purchased from 10 retail stores in the Helsinki area, and one pooled sample representing each brand was prepared as described in our previous study (Piironen et al., 1997). In addition, four margarine samples, filling cream (cream preparation used for filling in bakery products), puff pastry margarine and hard margarines meant for the baking industry and for household use, were obtained from one manufacturer. All margarine samples were analysed during the winter of 1997.

2.2. Vitamin K_1 determination

All work was performed under subdued light conditions. Vitamin K_1 and the internal standard menaquinone-4 (MK-4) were purchased from Sigma Chemical Co. (St Louis, MO, USA). 2′,3′-Dihydrovitamin K_1 standard was a gift from Hoffman-La Roche and Co. (Basel, Switzerland). All reagents used were the same as in our previous study (Piironen et al., 1997). The samples were analysed in duplicate or triplicate (Table 1). In addition, a blank without the MK-4 was analysed for every sample.

Sample preparation is described in our previous paper (Piironen et al., 1997). Briefly, the extraction procedure of oils included diluting the sample and the MK-4 to 10 ml with *n*-hexane. Margarine samples were shaken for 1 min in approximately 5 ml *n*-hexane before diluting to volume. After standing for 30 min a 2-ml aliquot of sample was evaporated and the residue dissolved in *n*-hexane. The hexane extracts of both sample types were filtered through a membrane filter (Puradisc 25 TF 0.45 μm; Whatman, Ann Arbor, MI, USA).

The high-performance liquid chromatographic (HPLC) methods and apparatus used are described elsewhere (Piironen et al., 1997). Semipreparative straight-phase HPLC was used for purification of the hexane extracts; the mobile phase used was *n*-hexane containing 1% diethyl ether with a flow rate of 1.5 ml min^{-1}. The vitamers (2′,3′-dihydrovitamin K_1, *cis* and *trans* isomers of vitamin K_1 and MK-4) were separated with a μPorasil column (5 μm, 300×3.9 mm; Millipore Corp., Milford, MA, USA). The collection time began 2 min before elution of 2′,3′-dihydrovitamin K_1 and ended 1.5 min after elution of MK-4. The collected fraction was evaporated and redissolved in 0.5 ml of the mobile phase used in analytical HPLC.

Quantification of the vitamin K_1 and 2′,3′-dihydrovitamin K_1 was performed by reverse-phase HPLC with a dual-electrode electrochemical (EC) detector (Piironen et al., 1997). The analytical column was a Vydac 201 TP54 column (5 μm, 250×4.6 mm; The Separation Group, Hesperia, CA, USA) with a mobile

Table 1
2′,3′-dihydrovitamin K_1 and vitamin K_1 contents **(g g^{-1}) of oils and margarines**

Food item	Vitamin K_1		2′,3′-Dihydrovitamin K_1		*N*
	$x \pm SD$	CV (%)	$x \pm SD$	CV (%)	
Oils					
Crude rapeseed oil	1.9		ND[b]		2
Crude soybean oil	1.9		ND		2
Hydrogenated rapeseed oil	0.6 ± 0.04	5.8	1.55 ± 0.030	2	5
Hydrogenated rapeseed-palm oil 1	1.23 ± 0.021	1.7	1.0 ± 0.05	4.8	3
Hydrogenated rapeseed-palm oil 2	1.05 ± 0.031	2.9	1 ± 0.14	13.3	3
Hydrogenated soybean oil 1	0.9 ± 0.08	8.7	1.2 ± 0.17	14.2	4
Hydrogenated soybean oil 2	0.57 ± 0.016	2.7	1.25 ± 0.006	0.4	3
Margarines for household					
Soft margarine A (fat 80%)	0.9		ND		2[c]
Soft margarine B (fat 80%)	0.8		ND		2[c]
Hard margarine (fat 80%)	0.6 ± 0.006	1.0	ND		4[c]
Hard margarine for household[a]	0.67		ND		2[c]
Margarines for industry					
Hard margarine	0.9 ± 0.04	4.8	0.3 ± 0.03	9.2	4
Puff pastry margarine	0.3 ± 0.03	10.4	0.06 ± 0.005	7.5	4
Filling cream	0.6		0.28		2

$x \pm SD$ = mean ± standard deviation.
[a] Obtained from manufacturer.
[b] ND = not detected.
[c] Determined by the external standard method.

phase consisting of 95% methanol–0.05 M Na acetate buffer (pH 3) flowing at a rate of 1 ml min^{-1}; 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 concentrations were quantified by the internal standard method based on the peak areas; however, the vitamin K_1 contents of margarines that did not contain 2′,3′-dihydrovitamin K_1 were analysed without added MK-4. The response factors for both forms (vitamin K_1 and 2′,3′-dihydrovitamin K_1) were determined daily at one concentration level (0.7 ng per injection for vitamin K_1 and 0.6 ng for 2′,3′-dihydrovitamin K_1).

Trans fatty acid concentrations of samples were determined as described by Hyvönen et al. (1993). Fatty acids were determined as their methyl esters using capillary gas chromatography with a CP Sil 88 column (50 m×0.25 mm, 0.2 μm film thickness; Chrompack, The Netherlands). Apparatus and other analysis conditions were similar to those in the study by Hyvönen et al. (1993). Quantification of *trans* fatty acids was based on an internal standard method using C19:0 methyl ester (Sigma) as the internal standard.

2.3. Method validation

Daily variations in EC detector response and the retention times in analytical HPLC were monitored with standard injections after every third sample. In semi-preparative HPLC, standards were injected to confirm the retention times for collection. To avoid the carry-through effect, the mobile phase was injected after every standard injection in both HPLC systems. Recovery of the internal standard (MK-4) was determined by the external standard method in every sample.

3. Results and discussion

3.1. Analytical method

The method used here was previously described for determination of vitamin K_1 in oils and margarines (Piironen et al., 1997). Here the 2′,3′-dihydrovitamin K_1 contents of some oils and margarines were determined by the same method; only the collection time in semi-preparative HPLC was modified.

Semipreparative HPLC was used for purification of the hexane extracts of samples. 2′,3′-Dihydrovitamin K_1, *cis* and *trans* isomers of vitamin K_1 and MK-4 were eluted from the semipreparative column in approximately 5.8, 6.6, 7.7 and 10.3 min, respectively. In the analytical HPLC good separation of vitamin K_1, 2′,3′-dihydrovitamin K_1 and MK-4 from the other components was obtained; the chromatogram of hard margarine meant for the food industry is shown as an example (Fig. 1). The retention times of the vitamers were approximately 10.8, 13.0 and 6.7 min, respectively. The day-to-day variations in retention times were small: the coefficients of variation (CV) were 1.7% (2′,3′-dihydrovitamin K_1), 1.1% (vitamin K_1) and 0.7% (MK-4); the within-day variation was insignificant.

The variation in EC detector response in analytical HPLC was tested with a standard daily. The within-day CV for the peak areas of 2′,3′-dihydrovitamin K_1 was 2.8%, for vitamin K_1 2.4% and for MK-4 2.6% ($n>3$ per day, 17 days). Respectively, the day-to-day CV values were 4.1%, 5.3% and 7.0% ($n=17$). The response factor for 2′,3′-dihydrovitamin K_1, using MK-4 as the internal standard, was 1.20 ± 0.072 (day-to-day CV 3.8%, $n=12$). Respectively, the response factor for vitamin K_1 was 1.02 ± 0.046 (day-to-day CV 2.1%, $n=17$). The repeatability of analyses was further confirmed by the small variation between replicated samples (generally below 10%); the recovery of the internal standard was usually 80–100%.

3.2. 2′,3′-dihydrovitamin K_1 and vitamin K_1 in samples

The present results are summarized in Table 1. The vitamin K_1 contents analysed here for crude rapeseed and soybean oils are consistent with previous studies of oils (Zonta and Stancher, 1985; Ferland and Sadowski, 1992; Moussa et al., 1994; Gao and Ackman, 1995; Jakob and Elmadfa, 1996; Davidson et al., 1996; Piironen et al., 1997). In these samples 2′,3′-dihydrovitamin K_1 could not be detected; their *trans* fatty acid contents were also lower than the detection limit. However, all partially hydrogenated oils (*trans* fatty acid content 26–44 g/100 g) contained considerable amounts of 2′,3′-dihydrovitamin K_1, thus supporting the earlier observation of formation of 2′,3′-dihydrovitamin K_1 during the hydrogenation process (Davidson et al., 1996).

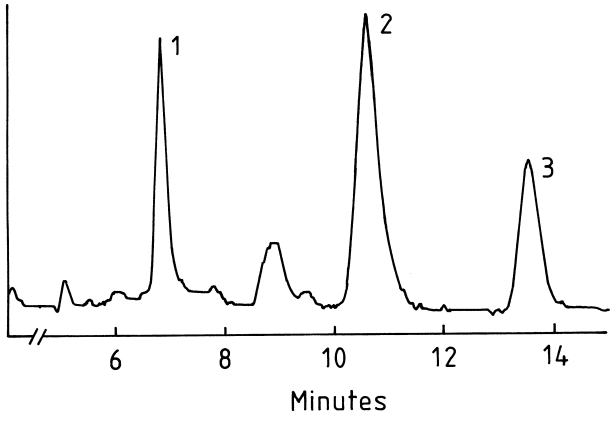

Fig. 1. HPLC chromatogram of hard margarine meant for the food industry. Peaks correspond to (1) menaquinone-4, (2) vitamin K_1 and (3) 2′,3′-dihydrovitamin K_1.

2′,3′-Dihydrovitamin K_1 accounted for 47–72% of the sum of dihydrovitamin K_1 and vitamin K_1, which is within the range previously reported for hydrogenated soybean oils, i.e. 54–98% (Davidson et al., 1996). Only slight variation was found in proportions of 2′,3′-dihydrovitamin K_1 between different batches of hydrogenated oils: 4.3% for the mixture of rapeseed and palm oils and 9.2% for soybean oil. Variation in their *trans* fatty acid content was insignificant. The sums of 2′,3′-dihydrovitamin K_1 and vitamin K_1 in these hydrogenated oils were similar to those of the vitamin K_1 contents in the corresponding crude oils. The observation of Davidson et al. (1996) of vitamin K_1 degradation during hydrogenation was not confirmed here.

In soft and hard margarines meant for household use 2′,3′-dihydrovitamin K_1 was not detected (Table 1) nor were *trans* fatty acids found in these products. Their vitamin K_1 contents were within the ranges previously reported (Piironen et al., 1997). Low amounts of 2′,3′-dihydrovitamin K_1 were found in those margarines meant for the baking industry: hard margarine, puff pastry margarine and filling cream. The proportion of 2′,3′-dihydrovitamin K_1 in these products was approximately 25% of the total. The appearance of 2′,3′-dihydrovitamin K_1 in these margarines was confirmed by their *trans* fatty acid contents (5–13 g/100 g); however, Davidson et al. (1996) found a considerably higher proportion of 2′,3′-dihydrovitamin K_1 in a commercial shortening (62%). We believe that the main reasons for this difference may lie in the different raw materials and industrial procedures used in these products. Exact comparison of the results was difficult because the *trans* fatty acid content of the commercial shortening was not known.

During recent years the Finnish margarine industry has focused on reducing the amount of *trans* fatty acids in soft margarines. Because of these changes in the manufacturing process, 2′,3′-dihydrovitamin K_1 was not found in these products. The significance of 2′,3′-dihydrovitamin K_1 is also reduced by its small or even non-existent proportion in margarines and other products meant for the food industry. In addition, fast-food items are not very popular in Finland and infant formulas used in Finland do not contain hydrogenated oils. As a result of all these factors 2′,3′-dihydrovitamin K_1 probably plays no significant role in vitamin K nutrition in Finland.

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