ELSEVIER

Food Chemistry 68 (2000) 223–226

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Chemical Methods Section

Quality of vitamin K analysis and food composition data in Finland

V. Piironen*, T. Koivu

Department of Applied Chemistry and Microbiology, University of Helsinki, PO Box 27, FIN-00014, Helsinki, Finland

Received 29 July 1998; accepted 29 July 1998

Abstract

The phylloquinone content of plant-based foods, e.g. oils and margarines, vegetables, fruits, berries, and cereal products consumed in Finland were recently published. Based on this data the quality of vitamin K analysis and food composition data are discussed, emphasising method validation and the effects of the sampling system. The details of the procedures are carefully documented in the original papers to facilitate evaluation of the data by the users. The results showed great variation in the phylloquinone content of individual subsamples of different items, as well as in pooled samples of vegetables and margarines. The number of subsamples should thus always be considerable. Furthermore, repeating the sampling of items regarded as important is recommended.

Keywords: Vitamin K; Phylloquinone; Food composition; Quality

1. Introduction

Research on vitamin K at the University of Helsinki investigates the occurrence of phylloquinone and menaquinones in various types of food. So far, the phylloquinone content of oils and margarines (Piironen, Koivu, Tammisalo & Mattila, 1997), vegetables, fruits and berries (Koivu, Piironen, Henttonen & Mattila, 1997) and cereal products (Koivu, Piironen & Mattila 1999) has been analysed. The significance of dihydrophylloquinone, a compound formed from phylloquinone when vegetable oils are hydrogenated, was also studied (Koivu, Piironen & Mattila, 1998). At the moment the focus is on vitamin K compounds in animal products.

This work has enabled discussion of the quality of vitamin K analysis and food composition data in Finland, particularly method validation and the effect of sampling, e.g. analysing one pooled sample versus repeating the sampling and pooling versus analysing individual subsamples. To provide some background to the procedures chosen, the criteria proposed by Holden, Beecher, Doherty, Davis and Finglas (1997) for food composition studies are also briefly summarised.

2. Analytical method

Holden et al. (1997) offered these criteria for evaluating food composition data, which an analytical method has to fulfil to achieve the best score: published documentation with validation for the foods analysed; use of reference material with results within an acceptable range or 95–105% recovery from similar food; use of other method or laboratory on the same sample with excellent agreement; acceptable repeatability. Some other criteria were also included. Evaluating vitamin K data, Booth, Sadowski, Weihrauch and Ferland (1993), on the other hand, gave a best score of 3 where HPLC with fluorometric detection or other method documented by a complete write-up with validation studies for the foods analysed was used.

In this study, phylloquinone in vegetables, fruits, berries and cereal products was extracted with isopropanol-hexane. Oils were analysed after dissolution in hexane and margarines after extraction with hexane. These hexane solutions were purified using semipreparative normal-phase HPLC. Extracts of the other samples were analysed either directly or after the purification step. Reverse-phase HPLC equipped with a dual-electrode electrochemical detector was used for quantification. Menaquinone-4 was used as an internal standard (Fig. 1). The main procedures in confirming the reliability of the method in the extraction, identification and

* Corresponding author.

0308-8146/99/$ - see front matter
PII: S0308-8146(99)00159-4

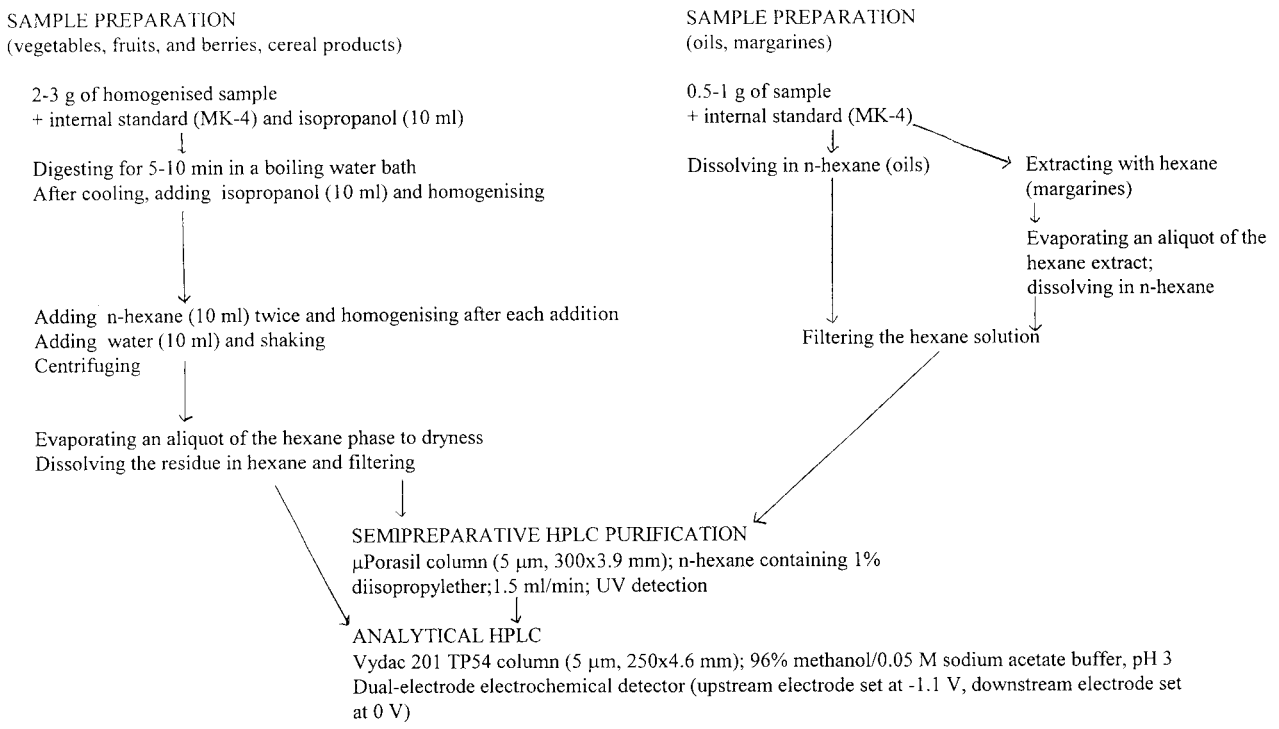

Fig. 1. Scheme of procedure for determining phylloquinone content of plant-based foods.

quantification step, and covering the entire analytical method will be discussed in this paper.

2.1. Extraction

Extraction efficiencies of at least two extraction methods for each type of food were compared in order to find as efficient a method as possible. The selection was based on the results, variation in them, and recoveries. In addition, simplicity of the procedure was considered. Higher fat-soluble vitamin content is frequently found in cooked than in the corresponding raw vegetables. This is probably caused by variable extractability. In the case of vegetables, results obtained for raw and the corresponding cooked material were thus compared. A comparison study with the Food Administration in Sweden was also carried out. Because of the lack of certified reference material for vitamin K in foods, this validation mean could not be used. The main difference in the selected extraction method, compared with previous methods, is that a digestion step, heating the sample in a small amount of isopropanol for 5 or 10 min to soften the structure and to inactivate enzymes, was used before extraction.

2.2. Purification

Purification using semipreparative HPLC was needed to remove lipids from the hexane solutions of oils and margarines before quantification with reverse-phase HPLC. The need for this step with other samples was checked with blank tests without adding the internal standard. The purification step was carried out if there were interfering peaks. In that step the most important way to confirm reliable operation was to check collection times for the vitamin K compounds regularly.

2.3. Quantification

The parameters determined to confirm reliable quantification included linearity of the detector response and detection limits. Linearity was shown in the tested range of 0.1–50 ng per injection. The detection limit for phylloquinone was 50 pg and for menaquinone-4 20 pg. Reliability was further confirmed by determining the response factors regularly and by showing that variation in the retention times and detector responses was small. Recovery tests using external standard quantification confirmed similar extractability of phylloquinone and the internal standard. To confirm peak purity, the response ratios of phylloquinone and menaquinone-4 in the standard solution and in the sample at two different electrode potentials were compared. Participation in the Quality of Vitamin K Analysis Scheme coordinated by St. Thomas Hospital, UK, offered an additional opportunity to test the quantification step. In that project standards and plasma samples are circulated twice a year. To monitor the analytical level, standards sent to our laboratory were quantified against our own standards.

2.4. Entire analytical procedure

The reliability of the entire analytical procedure was confirmed by recovery tests. When the internal standard quantification method was used, the mean values for different matrices were as follows: 98% for oils ($n=20$), 102% for margarines ($n=12$), 96–99% for vegetables, fruits and berries ($n=23$), 96% for rye meal ($n=5$) and 92% for rye bread ($n=3$). Repeatability was monitored by using in-house reference materials. The day-to-day repeatabilities for different matrices ranged from 3% (oil) to 7% (margarine). Variation in the results of triplicated samples was usually below 4% for oils and margarines and less than 10% for other materials. These figures include variation caused by the pooling and homogenisation practices because each replicate was weighed from a separate package of the pooled sample.

3. Sampling

The criteria for sampling in food composition studies established by Holden et al. (1977) deal with number of samples, sample handling and sampling plans. For the best score the number of samples has to be extensive. The various steps in sample handling have to be documented. Samples should be taken using multiple geographical sampling and confirming that they are representative of brands/varieties consumed or commercially used. Our sampling system was based on ranking the samples in various categories. The more important the sample type was thought to be, the more effort was put into the sampling procedures.

One pooled sample of 10 subsamples was analysed for those samples regarded as less important. These items included cereal products, berries, fruits and some vegetables. These subsamples as well as samples of the other categories were bought from carefully selected retail stores or market places in the cities of Helsinki, Vantaa and Espoo in the Helsinki area. Knowing the food production and distribution system in Finland, sampling from different parts of the country was not regarded as necessary in this first study. In the case of oils, margarines and flours, products of the same few manufactures are sold all over the country. Similarly, imported vegetables and fruits are distributed through the same wholesalers to various parts of Finland. In the case of domestic vegetables, fruits and berries, which are consumed more locally, our procedure was to document their origin as carefully as possible and to see that the subsamples came from different producers.

Sampling of all oils and margarines and some vegetables was repeated, the total number of subsamples thus being 20. Some important vegetables were investigated in more detail. Their sampling was repeated four times, yielding 40 subsamples. Furthermore, some items within each food group were selected for more detailed study, variation in individual subsamples being investigated. In addition, our intention was to select those food items which need to be investigated further from this first study. The variation found in the results permits evaluation of the reliability of the vitamin K data after different sampling approaches.

Variation between the two sampling times of oils ranged from 0 to 27%. The difference for rapeseed oil, which is the most important oil in Finland, was about 13%. When this most important oil was investigated further by analysing six individual oil bottles, phylloquinone content varied from 140 to 187 μg/g (coefficient of variation, CV, 11%). No difference between manufactures or correlation with storage time was found in the phylloquinone content, but the results indicated that the variation was caused by other factors, such as differences in the raw materials or storage conditions.

The differences in the results of the two pooled samples of margarine brands were considerable, 0–36%, and were probably caused by differences in raw materials. On the other hand, a batch of samples taken from the manufacturer immediately after production contained 13% more phylloquinone than the retail samples of the same brand, indicating that storage may also have had some influence. The variation in the most

Table 1
Variation in the phylloquinone content of vegetables (Koivu et al., 1997)

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	2.4–5.7	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.

popular margarine brand in Finland was monitored further. The phylloquinone content ranged from 96–117 μg/g when six individual packages were analysed (CV 6.6%).

Variation between the pooled samples of vegetables is given in Table 1. It was considerable given that each pooled sample was composed of 10 subsamples, and was statistically significant for pot-grown lettuce, Iceberg lettuce and carrot. When domestic samples were compared, results were usually higher in summer, when the samples represented the new crop of that year. The phylloquinone content of individual subsamples of white cabbage, pot-grown lettuce and carrots differed remarkably; they ranged 54–130, 28–72 and 9–14 μg/100 g for lettuce, white cabbage and carrot, respectively.

Significant variation in the phylloquinone contents of the pooled samples means that sampling of those items regarded as important should be repeated if possible. As shown by variation in the subsamples, attention should always be paid to the number of subsamples.

4. Conclusions

The procedure used in this study for validating the analytical method fulfils the criteria proposed for food composition studies. The sampling system was a compromise seeking reliable vitamin K data at reasonable costs. This was achieved by focussing the effort on items which were regarded as important in vitamin K nutrition. Careful documentation of the procedures in the original papers enables the users to evaluate the data. Furthermore, aspects shown to require more detailed investigation can be identified.

References

Booth, S. L., Sadowski, J. A., Weihrauch, L. L., & Ferland, G. (1993). Vitamin K1 (Phylloquinone) content of foods: a provisional table. *J. Food Composition and Analysis, 6*, 109–120.

Holden, J., Beecher, G., Doherty, R., Davis, C., & Finglas, P. (1997). Evaluation system for the assessment of the quality of folate composition data of foods. Presented as a poster SR647 at the 16th International Congress of Nutrition, 31 July 1997, Montreal, Canada.

Koivu, T., Piironen, V., Henttonen, S., & Mattila, P. (1997). Determination of phylloquinone in vegetables, fruits, and berries by high-performance liquid chromatography with electrochemical detection. *Journal of Agricultural and Food Chemistry, 45*, 4644–4649.

Koivu, T., Piironen, V., & Mattila, P. (1998). Phylloquinone in cereal products. *Cereal Chemistry, 75*, 113–116.

Koivu, T., Piironen, V., & Mattila, P. (1999). Dihydrovitamin K1 in oils and margarines available in Finland. *Food Chemistry*. *64*, 411–414

Piironen, V., Koivu, T., Tammisalo, O., & Mattila, P. (1997). Determination of phylloquinone in oils, margarines and butter by high performance liquid chromatography with electrochemical detection. *Food Chemistry, 59*, 473–480.