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Phylloquinone (vitamin K_1) content of commercially-available baby food products

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Abstract

The contents of phylloquinone (vitamin K_1) were investigated in two batches of 62 commercially available baby food products, by HPLC. They comprised vegetable products for babies from 5, 8 and 12 months and fruit and cereal products of three different brands. Vitamin K_1 was detected in all analyzed products. In vegetable products for babies from 5 months, the mean content of phylloquinone ranged from 30 to 100 μg/100 g, except for 'Samples with spinach', which provided 700 μg vitamin K_1/100 g on average. Vegetable products for babies from 8 months and from 12 months showed similar values. In the group of fruit and cereal products, the contents of phylloquinone were lower and varied between 10 and 20 μg/100 g only, except for 'Various cereals' (46 μg/100 g). The analyzed baby food products are a very good source of phylloquinone and are therefore excellent as a supplement to breast milk.

Keywords: Phylloquinone; Baby food; HPLC method

1. Introduction

1.1. Sources of vitamin K for infants

Because of the risk of haemorrhagic disease of the newborn as a result of a deficit of vitamin K, it is generally agreed that newborns should receive vitamin K. However, there is no consensus concerning the route of administration, dose, number of doses, or dose frequency (Jonville-Bera, & Autret, 1997; Shearer, 1995). The potential source of vitamin K for newborns and infants is the transplacental passage of phylloquinone (vitamin K_1) from mother to infant, which is very limited (Greer, Marshall, Foley, & Suttie, 1997; Hogenbirk, Peters, Bouman, Sturk, & Büller, 1993; Shearer, Barkhan, Rahim, & Stimmler, 1982). The use of menaquinones (vitamin K_2) synthesized by bacteria in the human gastrointestinal tract is impossible due to the fact that the intestinal bacteria predominating in breast-fed infants (lactobacillus) do not synthesize menaquinones (Greer et al., 1997; Shearer, 1995). Considering these facts, the newborn vitamin K stores are precariously low. Therefore, the infant depends on a dietary source. The primary source of vitamin K for unsupplemented infants would seem to be the small amounts of phylloquinone present in human milk (Canfield, Hopkinson, Lima, Silva, & Garza, 1991). The vitamin K concentrations in human milk reported by different authors range from 1 to 3 μg/l (Bolisetty, Gupta, Graham, Salonikas & Naidoo, 1998; Canfield et al., 1990; 1991; Hogenbirk et al.; Pietschnig et al., 1993).

1.2. Requirements and recommendation for vitamin K for infants

Intake recommendations for vitamin K are estimated at 5 μg/day for the first 6 months and 10 μg/day for the second 6 months (National Research Council, 1989; D-A-CH, 2000). Mature milk provides about 50% of the recommended amount at 1 month but less than 15% at 6 months (Canfield et al., 1991). It is possible to increase the vitamin K content of the breast milk by daily oral supplementation of vitamin K to mothers (Bolisetty et al., 1998). It would also be possible, especially with

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older infants, to make sure that the baby is supplied with an optimal amount of vitamin K within the diet. From the fifth month at the earliest and the seventh month at the latest, an additional feeding should be started, since it is at this age that the infant can no longer be sufficiently supplied with energy and nutrients by breast milk.

1.3. Phylloquinone in foods

The vitamin K concentration is very low in most foods (<10 μg/100 g), and the majority of the vitamin is obtained from a few green and leafy vegetables (e.g. spinach, broccoli) and four plant oils (soybean, cotton seed, canola and olive) that also contain high amounts of phylloquinone (Booth & Suttie, 1998). Because of the importance of vitamin K for infants and considering the fact that, only a few studies have dealt with and analyzed vitamin K contents of vegetables and fruit (Booth, Sadowski & Pennington, 1995; Ferland & Sadowski, 1992a; Jakob & Elmadfa, 1996; Koivu, Piironen, Henttonen, & Mattila, 1997; Langenberg, Tjaden, De Vogel, & Langerak, 1986), there is not enough present data concerning the concentrations of vitamin K_1 in baby food products containing vegetables and fruit. Therefore it was of interest to determine the phylloquinone (vitamin K_1) contents in vegetable, fruit and cereal paps of the baby food assortment commonly available in Austria.

2. Materials and methods

2.1. Chemicals

Phylloquinone (vitamin K_1) was obtained from Merck (Darmstadt, Germany). 2′, 3′- dihydrophylloquinone, to serve as an internal standard, was kindly donated by Hoffman-La Roche (Basel, Switzerland). All other chemicals were obtained from Merck (Darmstadt, Germany) or Riedel-de Haen (Seelze, Germany).

2.2. Sampling

The vitamin K_1 contents of 62 commercially-available baby food products, of two different batches each, (one jar per batch, two jars per brand, 124 samples altogether) were determined. Analyzed samples included vegetable products for babies from 5, 8 and 12 months, and fruit and cereal products of three different brands (B1, B2, B3). The products were purchased in two lots, from retail markets, between August and December 1998. The time interval between sampling of the two batches was 2 months for each brand.

The contents of one unit (jar = 190, 220 or 250 g) were homogenized and then, immediately weighed and extracted.

2.3. Sample extraction

Sample extraction and the HPLC assay for the determination of phylloquinone was carried out according to the slightly modified (modification: filtration after extraction and addition of methanol; only dichloromethane/methanol was taken as an extraction mixture; for details see the text below) HPLC method of Jakob and Elmadfa (1996).

One to two grams of homogenized samples were taken for extraction with a mixture of dichloromethane/methanol (2:1 v/v, sample/solvent 1:30). The extract was filtered through dehydrated sodium sulfate and made up, with methanol, to 100 ml. 1 ml of this was taken, to which 1 ng of the internal standard (2′, 3′- dihydrophylloquinone dissolved in ethanol) was added, and then evaporated. The residue was redissolved in hexane, which was followed by purification, as described below. Four ml of the mixture of methanol/water (9:1 v/v) were added to the hexane extracts, vigorously mixed for 2 min, and centrifuged for 5 min at 3000 rev/min. The upper hexan layer was removed and evaporated to dryness (15 min, 40°C, vacuum). After dissolving the residue in 150 μl of the eluent, it was injected into the HPLC.

2.4. HPLC system

The HPLC system consisted of a high precision pump Model 300B, an analytical column Gynkotek ODS Hypersil (250×4.6 mm i.d., 5 μm), a guard-column Gynkotek ODS Hypersil (20×4.6 mm i.d., 5 μm) — all from Gynkotek, Germering, Germany, a reduction-column dry-filled with zinc powder (20×4.0 mm i.d.; Bischoff, Leonberg, Germany), injection device (Rheodyne, Cotati, USA), column-heating, cosytherm (Labortechnik Barkey, Bielefeld, Germany) and U3 (Julabo, Seelbach, Germany), Fluoroscence Spectrophotometer F-1050 and Chromato-Integrator D-2500 (both from Merck, Darmstadt, Germany).

The mobile phase contained 100 ml of dichloromethane and 900 ml of methanol. This solvent was then combined with 5 ml of a methanolic solution, containing 1.37 g of zinc chloride, 0.41 g of sodium acetate and 0.30 g of acetic acid. The flow rate was 1.0 ml/min, and the column was heated at 40°C. The injection loop volume was 100 μl. Detection was carried out with an excitation wavelength of 243 nm and an emission wavelength of 430 nm. The concentrations were calculated by peak height ratios, using a linear regression curve from standard solutions containing 0.13, 0.5, 2.0 and 10 ng phylloquinone per injection (100 μl) and each containing 1.0 ng 2′, 3′- dihydrophylloquinone as an internal standard. The intra-assay variation was 5.6% and the inter-assay variation was 7.1%. The detection limit, defined as the lowest quantitatively measurable concentration, was 0.04 ng/ml. The rate of recovery was found to be in the range of 92–104%.

2.5. Statistical methods

Results for the three brands (B1, B2, B3) are expressed as the mean of two different batches as μg/100 g of fresh weight. The Mann–Whitney U-test was employed to determine differences between the phylloquinone contents of two specific groups.

3. Results and discussion

3.1. Vegetable products for babies from 5 months

In the group of vegetable products for babies from 5 months (Table 1) 'Samples with Spinach' showed the highest concentrations of phylloquinone, 700 μg/100 g on average, which can be explained by the contents of phylloquinone in spinach (270–400 μg/100 g) (Booth et al., 1995; Booth, Sadowski, Weihrauch, & Ferland, 1993; Booth & Suttie, 1998; Koivu et al., 1997; Shearer, Bach, & Kohlmeier, 1996). The differences between the brands mainly resulted from different recipes. The same was true for the product groups 'All Sorts of Vegetables' and 'Tender garden vegetables'. Although these products basically consisted of the same ingredients (carrots, potatoes, peas), the jars 'All sorts of vegetables' of the brand B3 provided twice as much phylloquinone as the jars of the other two producers, since they additionally contained spinach. Compared with other samples, also 'Tender garden vegetables' of the brand B2 showed higher values of phylloquinone, which is caused by the portion of broccoli included. Broccoli contains high amounts of phylloquinone (110–270 μg/100 g) (Booth et al., 1993, 1995; Booth & Suttie, 1998; Ferland & Sadowski, 1992a; Jakob & Elmadfa, 1996; Koivu et al., 1997; Langenberg et al., 1986; Shearer, Bach & Kohlmeier, 1996).

3.2. Effect of plant maturation, seasonal variation and types of plant oil

High contents of vitamin K_1 were also found in products purely consisting of carrots, although carrots are not particularly rich in phylloquinone (5–19 μg/100 g) (Booth et al., 1993, 1995; Booth & Suttie, 1998; Jakob & Elmadfa, 1996; Koivu et al., 1997; Langenberg et al., 1986; Shearer et al., 1996). We can conclude, from the homogeneity of results within one group of products, that the differences in the amounts between such similar products as 'Early Carrots' and 'Carrots' are on the one hand caused by different recipes and on the other by a seasonal variation or a different maturation stage. Corn and sunflower oil, the oils that are most frequently used in baby food preparation, contain only small amounts of phylloquinone (3 and 6–10 μg/100 g, respectively) but soya bean oil contains 145–190 μg/100 g (Booth & Suttie, 1998; Ferland & Sadowski, 1992b; Piironen, Koivu, Tammisalo, & Mattila, 1997; Shearer et al., 1996). Since there is no declaration of the types of oil, it is not possible to discuss results in detail. Koivu et al., 1997

Table 1
Vitamin K_1 content of vegetable products (μg/100 g)[a]

Product group (*n*)	Brand			Mean±SD
	B1	B2	B3	
Vegetable products for babies from 5 months				
Early carrots (4)	51.7	50.7		51.2±0.76
Carrots (6)	93.1	86.2	88.7	89.4±3.48
Carrots and apple (6)	29.0	48.4	69.7	49.0±20.4
All sorts of vegetables(6)	81.73	64.1	152	99.4±46.7
Tender garden vegetables (6)	71.64	94.3	58.8	74.9±18.0
Samples with spinach (6)		1152	468.35[b]	696±395
Vegetables and turkey(4)	89.5	5.72		47.6±59.23[c]
Vegetables and chicken (4)	62.3	40.7		51.5±15.3
Vegetables and veal (6)	74.7	34.6	52.0	53.8±20.11
Vegetables and beef (6)	111	72.6	83.5	89.1±19.9
Carrots and sweetcorn (2)		35.3		35.3±0.52
Tomatoes and zucchini (2)		32.2		32.2±0.53
Vegetable products for babies from 8 months and from 12 months				
Vegetables + rice + turkey (4)	61.2	68.2		64.7±4.96
Vegetables + noodles (6)	45.8	42.1	66.8	51.6±13.3
Broccoli + turkey (2)	148			148±15.8
Cream-broccoli (2)			39.77	39.8±2.54
Vegetables + barley (2)			55.73	55.7±3.81
Vegetables + beef (4)	62.6	95.3		78.9±23.1

[a] Each value of brands B1, B2 and B3 was a mean of two different batches; *n*, amounts of the jars in each product group.
[b] Mean of four samples, two different products with spinach of brand B3.
[c] Because of the different recipes.

reported that the phylloquinone contents in some important vegetables (e.g. broccoli, carrot, Iceberg lettuce, tomato) were higher in summer than in winter, although the seasonal variation in the phylloquinone contents of carrots was not statistically significant. The values ranged between 16 and 23 μg/100 g. Ferland and Sadowski (1992a) reported that the phylloquinone contents of most vegetables tend to increase during development. Although carrots are relatively low in their phylloquinone content, they are frequently consumed in large enough quantities to be ranked among the top 25 contributors to total dietary intake of phylloquinone (Booth & Suttie, 1998). Since beef itself is not particulary rich in phylloquinone (0.5–0.8 μg/100 g) (Booth et al., 1993; Shearer et al., 1996) and the other vegetable ingredients in the product group 'Vegetables and beef' are not very good sources of this vitamin either (potatoes ~ 1μg/100 g, tomatoes 5–6 μg/100 g) (Booth & Suttie, 1998; Booth et al., 1993; Koivu et al., 1997; Shearer et al., 1996), the high result in this product group can be explained by the use of plant oil that, according to the specification, belongs to the ingredients. We might assume that peas (peas 23–28 μg/100 g) (Booth & Suttie, 1998; Booth et al., 1993; Koivu et al., 1997), which were also used during the preparation, could have contributed to these results, but since products of the brand B1 showed the highest contents, although they did not contain any peas at all, this suggestion can be excluded.

3.3. *Role of different recipes*

All the other samples among vegetable products for babies from 5 months showed average values between 30 and 50 μg phylloquinone/100 g. Since chicken (0.1 μg/100 g) and turkey (0.02 μg/100 g) contain low amounts of phylloquinone (Booth et al., 1993; Shearer et al., 1996) and there are no data available concerning veal, we can assume that the differences between the products are due to different types of vegetables. 'Vegetables and Turkey' were a very interesting product group, since in this we could find the biggest differences. This can most probably be explained by different recipes. The jars of both producers contained turkey and plant oil (no declaration concerning the types of oil), the one (B1) having carrots and butter (7 μg/100 g) (Booth & Suttie, 1998; Piironen et al., 1997; Shearer et al., 1996), the other (B2) having sweetcorn (0.5 μg/100 g) (Booth et al., 1993) as the only vegetable.

3.4. *Vegetable products for babies from 8 months and 12 months*

The highest amounts of phylloquinone among vegetable products for babies from 8 months and from 12 months (Table 1) were found in the products 'Broccoli + Turkey'. This was mainly caused by broccoli and other vegetable ingredients, such as spinach, since turkey only shows a minimal content of phylloquinone (see above). The lowest contents, on the other hand, were shown by the products 'Cream-Broccoli'. They did contain broccoli, but not as the main ingredient. The rice (0.1–1 μg/100 g) (Booth et al., 1993; Shearer et al., 1996) or other types of vegetable, e.g. potatoes (see above) or onions (0.7–2 μg/100 g) (Booth et al., 1993; Koivu et al., 1997) could not enrich the amounts of phylloquinone in this product. The differences in the other products can be explained by different vegetable ingredients. The jars with leek ('Vegetables + beef' of B2 or 'Vegetables + noodles' of B3) or with parsley ('Vegetables + rice + turkey') showed higher values of vitamin K_1. Koivu et al., 1997 reported that leek contains 54 μg phylloquinone/100 g and parsley 360 μg/100 g. In the studies of Shearer et al. (1996) and Booth et al. (1993) the phylloquinone content of leek was reported to be only 10–14 μg/100 g but that of parsley ~500 μg/100 g.

3.5. *Fruit and cereal products*

The phylloquinone values in fruit and cereal products were evidently lower than in vegetable products (Table 2). This is explained by the fact that some types of fruit contain only small amounts of vitamin K_1. Booth et al. (1993), Koivu et al. (1997) and Shearer et al. (1996) reported, for banana, 0.1–0.5 μg phylloquinone/100 g, for peach, 3–4 μg/100 g, for apricots, 5 μg/100 g, for apples and pears 6 μg/100 g and for blueberries 6–12 μg/100 g. The sample 'Various cereals' was an exception. This product was actually a milk pap with different grains. The result can either be explained by high amounts of oatmeal (3–10 μg phylloquinone/100 g) (Booth et al., 1993; Jakob & Elmadfa, 1996; Shearer et al., 1996), which was contained in this product or by the addition of plant oil, since the milk itself is not a good source of vitamin K_1 (0.3–0.6 μg/100 g) (Booth et al.,

Table 2
Vitamin K_1 content of fruit and cereal products (μg/100 g)[a]

Product group (*n*)	Brand B1	Brand B2	Brand B3	Mean ± S.D.
Peach and apple (6)	19.9	11.9	19.6	17.1 ± 4,53
Samples with apricots (6)	15.0	23.7	23.6	20.8 ± 5.01
Fruit pap and rice (6)	11.9	9.59	12.8	11.4 ± 1.66
Blueberry and Apple (4)	15.7	18.3		17.0 ± 1.85
Mandarin + banana + apple (2)	10.5			10.5 ± 0.52
Banana + mandarin + pear (2)	11.5			11.5 ± 0.48
Wholegrain fruit pap (6)	18.2	10.3	12.6	13.7 ± 4.05
Various cereals (2)	45.8			45.8 ± 0.85
Apple-banana-muesli (6)	27.2	10.6	13.7	17.1 ± 8.82
Wholegrain pap and pear (2)			12.9	12.9 ± 0.35

[a] Each value of brands B1, B2 and B3 was a mean of two different batches; n, amounts of the jars in each product group.

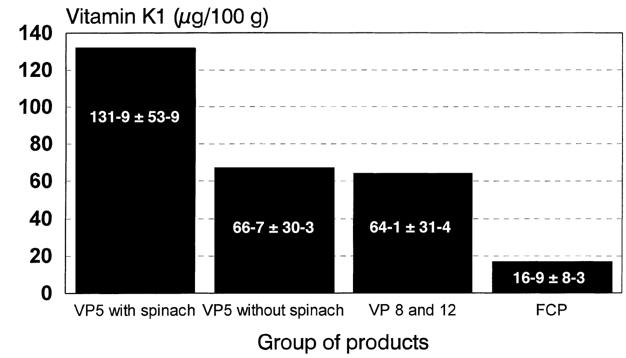

Fig. 1. Comparison of vitamin K_1 contents (mean±S.D.) between the three groups of baby food products. VP5 with spinach, vegetable products for babies from 5 months with 'Samples with spinach' VP5 without spinach, vegetable products for babies from 5 months without 'Samples with spinach' VP 8 and 12, vegetable products for babies from 8 months and from 12 months; FCP, Fruit and cereal products.

1993; Jakob & Elmadfa; Shearer et al., 1996). The enormous differences in the product group 'Apple-banana-muesli' were probably also caused by the addition of plant oil. The jars of the brand B1 had a three times higher content of fat than the other products in this group.

Generally, our phylloquinone contents in baby food products were considerably higher than the results for American infant and junior foods reported by Booth et al. (1995). In this study the phylloquinone content, e. g. of creamed spinach, was 292 µg/100 g, of mixed vegetables, 7.4 µg/100 g, of vegetables and beef, 4.1 µg/100 g, of vegetables and chicken, 3.5 µg/100 g, of pears and peaches, 4 and 5 µg/100 g, respectively. The reason for these discrepancies is probably the different recipes of the products, the wide individual variation in the phylloquinone content of plants (Koivu et al., 1997) and geographical variation or maturation stage, as reported by Ferland and Sadowski (1992a). Alternatively, the lower values could be indicative of phylloquinone losses associated with enzymatic destruction during preparation (grinding to fine powder with anhydrous sodium sulfate) and storage.

3.6. Comparison of vitamin K_1 contents between the three groups of products

The amounts of phylloquinone in vegetable products from 5 months were similar to the amounts contained in vegetable products from 8 months and from 12 months (Fig. 1). Significant differences ($P<0.01$) between these two vegetable groups of products were only for 'Samples with spinach'. This shows that spinach products should in any case be used for feeding the baby. As to recommendations (D-A-CH, 2000; National Research Council 1989) concerning infants between 4 and 12 months (10 µg vitamin K/day), all vegetable products, showing a mean content of 65 µg phylloquinone/100 g, can make a good contribution to the vitamin K supply (1 jar = 190, 220, 250 g). For 'Samples with spinach', which showed 700 µg phylloquinone/ 100 g on average, this contribution could be essentially increased. Compared with vegetable products, fruit and cereal products showed the significantly ($P<0.001$) lower mean value 20 µg phylloquinone/100 g. They can be regarded as an additional source of vitamin K.

4. Conclusion

It has been estimated that healthy adults absorb approx. 80% of an oral dose of phylloquinone given in free form and efficiency of absorption of phylloquinone from naturally-occuring foods depends on the food source (Shearer, McBurney, & Barkhan, 1974). Garber, Binkley, Krueger and Suttie (1999) reported that the absorption of phylloquinone was about six times greater after the consumption of a 500 µg phylloquinone tablet than after the ingestion of 495 µg phylloquinone as 150 g of raw spinach. A similar relatively poor absorption of phylloquinone in spinach was observed earlier by Gijsbers, Jie, and Vermeer (1996). The bioavailability, in human subjects, of 1 mg phylloquinone in spinach was only 4% that of pure phylloquinone. Adding butter to the spinach increased this to 13%. On the one hand, the analyzed baby food products are a very good source of vitamin K and in this respect an excellent complementary food to breast milk. On the other hand, the low availability of phylloquinone from food sources, compared with that of the pure vitamin and its high excretion (Shearer et al., 1974), will have to be considered carefully to see if baby food products, showing high amounts of phylloquinone, can sufficiently supply infants between 4 and 12 months with vitamin K.

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