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Application of an HPLC assay for the determination of folate derivatives in some vegetables, fruits and berries consumed in Finland

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The folate content of 30 commodities of vegetables, fruits and berries, including a few processed products, was determined by high-performance liquid chromatography (HPLC). HPLC was used with a combination with fluorescence and ultraviolet detectors to analyze folate monoglutamate derivatives and their distribution after extraction at pH 6.0 and deconjugation with hog kidney deconjugase at pH 4.9. 5-Methyltetrahydrofolate was the main derivative in all foods studied, but tetrahydrofolate, 5-formyltetrahydrofolate and 10-formylfolic acid were also detected. The sum of the monitored folate derivatives (as μg folic acid) in vegetables ranged from 9 to 114 μg per 100 g and that of berries and fruits from 3 to 36 μg per 100 g. The variation in folate content, which was studied by analyzing raw potato, carrot and cabbage bought from retail shops three times a year, was small. Some of the studied processed vegetable foods were also reasonably good sources of folate. The results obtained with HPLC are rather similar to the previously reported values for vegetables determined by a microbiological method. Several measures for method improvement and quality control of the analysis allowed reliable determination of the main folate forms, particularly 5-methyltetrahydrofolate, in a wide range of plant-derived foods. © 1997 Elsevier Science Ltd

INTRODUCTION

In recent years the consumption of vegetables, fruits and berries in Finland has increased. For example, the consumption of fresh vegetables increased from 43 kg in 1993 to 54 kg in 1994 per person (Ministry of Agriculture and Forestry, 1996). These foods are important sources of folates in Finnish diets, particularly because fortification of grain or cereals with folic acid is not practised in Finland. However, reliable data are lacking on the folate content of many foods, including most plant and plant-derived foods. No systematic studies have been reported on the folate content of vegetables and berries produced in Finland, nor is there much reliable data available on the distribution of folate derivatives.

Most of the existing data on the folate content of foods have resulted from microbiological methods, which provide one figure for the sum of all folates present and do not, therefore, give any information on the different folate forms involved. Other limitations of existing folate bioassays (e.g. growth response dependence on pH and folate form, interference from standard impurities in quantitative calibration, and stimulation or inhibition of bacterial growth by non-folate components) should also be considered when quantifying food folates, as discussed by Lucock *et al.* (1995) and Martin (1995). The development of improved methods for folate analysis such as high-performance liquid chromatography (HPLC) makes it possible to obtain data on various chemical forms of folates and possibly also allows better prediction of their stability and bioavailability; this gives a more precise indication of the value of the food or food product as a folate source.

Variation in the folate values for vegetables, fruits and berries is caused, in addition to extensive variation resulting from differences in analytical methodology, by several other factors. Folate metabolism is highly compartmented in eukaryotes. For example, photosynthetic

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tissues, mitochondria and cytoplasm have distinct folate pools for separate physiological purposes (Cossins, 1980; Carl *et al.*, 1995; Wagner, 1995). In plants, folate derivatives and their distribution are affected by light. Sponk and Cossins (1972) compared radish cotyledons harvested during different phases of the light–dark period and observed that formylfolates declined and unsubstituted derivatives increased during the light phase. Plants contain mainly polyglutamylfolates, but storage of vegetables may lead to hydrolysis of these forms by endogenous deconjugases (Leichter *et al.*, 1979; Mullin *et al.*, 1982; Müller, 1993). However, Mullin *et al.* (1982) noted that intervarietal differences in folate content of several vegetable crops were not evident or were difficult to measure.

This study describes an HPLC method for folate analysis in plant foods and reports folate data on vegetables, fruits and berries commonly consumed in Finland. A comparison of different extraction methods and suitability of 3′,5′-dichlorofolic acid for use as an internal standard are also presented. Advantages and disadvantages are discussed concerning the use of HPLC in folate analysis.

MATERIALS AND METHODS

Calibrants

Tetrahydrofolic acid trihydrochloride (H_4folate), 5-methyltetrahydrofolic acid (5-CH_3-H_4folate, calcium salt), 5-formyltetrahydrofolic acid (5-HCO-H_4folate, calcium salt), folic acid, pteroyltri-γ-L-glutamic acid ($PteGlu_3$), 10-formylfolic acid (10-HCO-folic acid) 5,10-methylenetetrahydrofolic acid, magnesium salt and 5,10-methenyltetrahydrofolic acid hydrochloride were obtained from Dr Schirck's Laboratories (Jona, Switzerland). 3′,5′-Dichlorofolic acid was obtained from Sigma Chemical Co. (St Louis, MO). Standards were dissolved as described by van den Berg *et al.* (1994), and purities were calculated using molar extinction coefficients at pH 7 (Blakley, 1969). Standard solutions were stored in 0.01 M acetate buffer (pH 4.9) with 1% ascorbate at −18°C.

Sampling

Samples of vegetables, fruit and berries were purchased from retail stores, supermarkets and market stalls (ten in total) in the cities of Helsinki, Espoo and Vantaa between May 1995 and May 1996. Four main retail chains were represented according to their market share. Samples were purchased in units in which they are sold, representing the food as normally obtained by the consumer. These foods were selected primarily according to food consumption patterns (Statistics Finland, 1993; Ministry of Agriculture and Forestry, 1996), but foods suspected to be good folate sources were also given attention.

Fresh samples were prepared within 2 days after purchase and storage at +4°C; canned and frozen foods were prepared within 4 days after storage in appropriate conditions. A representative retail sample was prepared by pooling equal portions (100–150 g) of each sample obtained. Peeling or other handling of samples was carried out using normal household practices. Large samples were cut into quarters, and diagonally opposite portions were then pooled. Composite samples (1–1.5 kg) were cut into cubes of 1–1.5 cm or sliced; liquid and semiliquid samples were mixed as such. After mixing the cubes, a 100 g subsample was quickly cut or chopped into cubes of 100–200 mg pieces. Sample preparations were carried out under reduced light, keeping the temperature low by storing the sample containers over ice.

Sample extraction and incubation

Duplicate 5–7 g samples of the chopped sample (10 g of juices) were weighed into glass tubes and covered with two volumes (10–12 ml) of cold extraction buffer and flushed with nitrogen gas before storage at −20°C. Samples were stored for a maximum of a few weeks before analysis.

Sample extraction and incubation were performed as described by Vahteristo *et al.* (1996*a*). Hog kidney (HK) deconjugase (γ-carboxypeptidase), which was prepared according to Gregory *et al.* (1984), was used for deconjugation of folate polyglutamate forms. Samples were incubated at pH 4.9 in a water bath at 37°C for 3 h (5 h for orange). The extracts were then kept 5 min in a boiling water bath, to inactivate enzymes, and cooled in ice.

Samples rich in starch (raw and boiled potato, french fries, swede, banana, frozen peas and pea soup concentrate) were treated with α-amylase prior to deconjugation. α-Amylase (Sigma A0273, 200 µl, 50 mg ml^{-1} water) was added to the extract (3 ml) omitting prior centrifugation of the extract, and incubated for 3 h before bringing the pH to 4.9 with hydrochloric acid before HK deconjugase treatment.

Enzyme blanks were used and $PteGlu_3$ (17 µg per 3 ml sample extract) was added to one of each sample extract to check its complete deconjugation to folic acid during incubation. All the analyses were carried out in duplicate from the pooled sample, except the pooled orange and banana samples which were analyzed in triplicate.

The extraction method of Wilson and Horne (1984) at pH 7.85 was compared to the extraction at pH 6 using five commodities. Also the method of Gregory *et al.* (1984) was evaluated for providing data on the presence of formylfolates, because that method should convert 10-formyl-H_4folate (10-HCO-H_4folate) completely into 5-HCO-H_4folate and therefore allow indirect determination of 10-HCO-H_4folate. Folates in all extracts were quantitated after deconjugation with HK deconjugase.

Purification

The incubated sample extracts were applied on 3 ml strong anion exchange (SAX) solid phase extraction (SPE) cartridges (quaternary amine; J. T. Baker Inc., Phillipsburg, NJ) for purification as described in detail by Vahteristo *et al.* (1996*a*). Prior to application, the extracts were usually filtered with syringe filters of 0.8 μm pore size (Nylon Acrodisc; GelmanSciences) to avoid blockage of the cartridges with denatured sample and enzyme material.

SPE purification in ion-pair mode was also tested for improving purification according to Rebello (1987). Standard recoveries of SAX purification from pH 7.85 extraction buffer were tested with H_4folate (75 ng), 5-CH_3-H_4folate (195 ng), 5-HCO-H_4folate (65 ng) and folic acid (1.34 μg) in triplicate (amount of calibrant applied on SAX column in parentheses).

Chromatography

A Varian Vista 5500 liquid chromatograph equipped with cooled Waters 700 Satellite WISP autosampler and Waters 470 fluorescence detector together with Varian UV-200 detector were used. A data acquisition system (Millennium 2010 Chromatography Manager) was used to collect chromatographic data, and to evaluate peak height. Folate vitamers were separated with a Spherisorb ODS column (Phase Separations, Clwyd, UK; 5 μm, 250 mm×4.6 mm) in front of a Shandon (Cheshire, UK) Hypersil ODS column (3 μm, 150 mm× 4.6 mm i.d.) protected with a guard column (Novapak C_{18} Guard-Pak; Waters, Milford, MA). Column temperature was kept at 30°C. The excitation and emission wavelengths were set at 290 and 356 nm for H_4folate, 5-CH_3-H_4folate and 5-HCO-H_4folate, and at 360 and 460 nm for 5,10-methenyl-H_4folate and 10-HCO-folic acid. Detector sensitivity was increased at 24 min to allow small amounts of 5-HCO-H_4folate to be detected. Folic acid and $PteGlu_3$ were detected with the ultraviolet detector at the wavelength of 290 nm. Gradient elution with acetonitrile (from 9% to 25% within 14 min) and 30 mM potassium phosphate buffer pH 2.2 was used to separate the vitamers and impurities. The run time was 30 min and the time from injection to injection was 45 min. Flow rate was 0.5 ml min^{-1}.

Ion-pair chromatographic separation of folate monoglutamates according to the method of Witthöft & Bitsch (1993) was used for verifying peak purity and peak identity for certain samples.

Quantitation and evaluation of method

External calibration plots were prepared every day for quantitation with folate levels similar to those of samples. Quantitation against calibrants taken through sample purification did not affect the results, and was not considered necessary. Results are presented as micrograms of free vitamers, and the sum of the vitamers is calculated as μg folic acid per 100 g fresh weight, taking into account the small differences in molecular weights of different folate monoglutamates. Recovery of calibrants from the analysis were performed according to AOAC (1990). Results were not corrected for recoveries as the recoveries with monoglutamate forms did not always produce repeatable results, unlike the food samples in which mainly polyglutamated folate forms are present.

Because of its absence in the chromatograms 5,10-methenyl-H_4folate was not taken through full method validation. However, the possible presence of that vitamer was checked in samples with unknown peaks appearing with a relative retention close to that of this calibrant. Conversion of 5,10-methenyl-H_4folate to 10-HCO-H_4folate with the method used by Scott (1980) seemed to succeed based on spectral properties of the product, but 10-HCO-H_4folate can not be determined in such acidic LC conditions as ours (pH 2.2). Also ion-pair chromatography at pH 5.1 was also not suited for its analysis, or conversion to 10-HCO-H_4folate had not been adequately acquired. Due to its high lability, this folate form could not be determined in this study.

Retention times and spiking were not the only ways of peak identification, but 5-CH_3-H_4folate could often be quantitated with ultraviolet detection as well. Comparison of ratio of sample peaks from ultraviolet and fluorescence detectors to ratio of standard peaks, particularly for 5-HCO-H_4folate, was used for verifying peaks before quantitation.

A quality control sample (fortified milk powder) was used in each set of analyses. A control chart was prepared for 5-CH_3-H_4folate and total folates for this sample. An average content of 101 ± 11 μg folate was obtained during these analysis. This material has been referenced to contain 124 ± 14.4 μg folate per 100 g in an intercomparison study using microbiological method as carried out by laboratories experienced in using the microbiological method (*Lactobacillus rhamnosus*, pH 6.2, HK deconjugase) (Seale & Finglas, 1995).

The possibility to use 3′,5′-dichlorofolic acid as an internal standard was tested in SPE purification by applying 3 and 6 μg of 3′,5′-dichlorofolic acid on the cartridge in 3 ml of extraction buffer. The recoveries from the deconjugation procedure were determined in an incubation experiment where 30 μg of dichlorofolic acid was incubated in 3 ml of buffer, onion or chicken meat extract for 2 h with HK deconjugase. The recovery of dichlorofolic acid from incubation buffer was determined without SPE purification and from sample extract after the purification step.

The data of the two different extractions for five samples were analyzed using a two-tailed *t*-test for paired samples. A two-tailed *t*-test for comparison of two means was used for analyzing differences between two different extractions of broccoli and peas (Minkkinen, 1992).

Table 1. Minimum detectable amounts of folate vitamers

Vitamer	Limit of detection (ng per injection)
H_4folate[a]	0.003
5-CH_3-H_4folate[a]	0.002
5-HCO-H_4folate[a]	0.05
10-HCO-folic acid[b]	0.04
Folic acid[c]	1.4

[a]Fluorescence at excitation 290 nm, emission 356 nm.
[b]Fluorescence at excitation 360 nm, emission 460 nm.
[c]Ultraviolet detection at 290 nm.

Dry matter

To determine the dry matter, minced samples were oven-dried in dublicate at 102 ± 2°C overnight.

RESULTS AND DISCUSSION

Results from method evaluation

Detection limits (defined as signal three times the height of the noise level, except for 5-HCO-H_4folate which was signal two times the height of the noise level) for all the analytes were very low (Table 1). Standard recoveries were good from SPE purification for all calibrants (see below). The recovery of 5-CH_3-H_4folate from lyophilized vegetable mixture was 79% ($n = 2$), from non-deconjugated sample it was 92% (level of addition prior to extraction was 2.5 μg per 2 g sample). Recoveries for H_4folate and 5-HCO-H_4folate were 60% and 50–80%, respectively. The recovery of 10-HCO-folic acid in buffer from the whole sample treatment was 60–70% (1–10 μg).

Duplicate analysis of each commodity produced very comparable results. Coefficient of variation of the analysis was below 10% for total folate in orange and banana.

Calibration curves, illustrated in Fig. 1, show a linear response of peak height versus concentration of folate vitamers. The range of each calibration curve depended somewhat on the folate amount detected in the sample.

3′,5′-Dichlorofolic acid was quantitatively recovered from anion exchange cartridges (97–100%), producing similar results as reduced folate forms H_4folate, 5-CH_3-H_4folate, 5-HCO-H_4folate, and folic acid (102 ± 8.7%) at levels of 0.1–2 μg in 3 ml of extraction buffer, and 10-HCO-folic acid and 5,10-methenyl-H_4folate (about 85% and 95%) at levels of 20–40 μg. Incubation in buffer caused detectable losses (32 ± 4% loss, $n = 3$) and incubation in sample extracts markedly reduced dichlorofolic acid recoveries. Therefore, 3′,5′-dichlorofolic acid was not tested further. Our results are, however, somewhat conflicting with the data obtained with liver samples by Hahn *et al.* (1991).

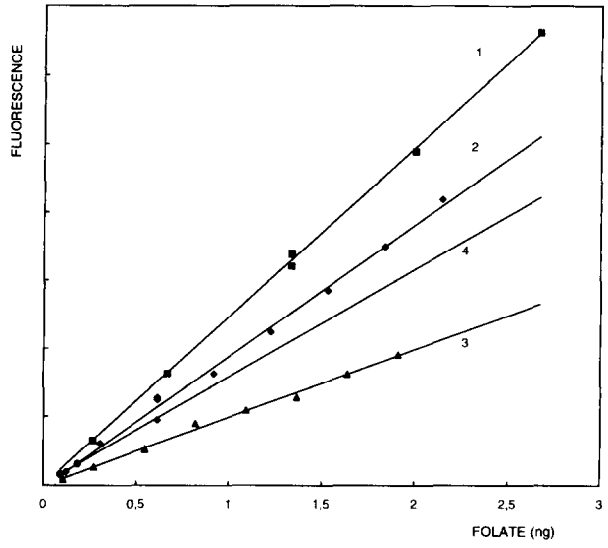

Fig. 1. Linearity of peak height with folate vitamer concentration. Calibrants were chromatographed on two C_{18} columns as described under Materials and Methods. The calibrants were 1) 5-CH_3-H_4folate, 2) H_4folate, 3) 5-HCO-H_4folate and 4) 10-HCO-folic acid.

Extraction at pH 7.85 produced similar amounts of 5-CH_3-H_4folate in white cabbage, chinese cabbage, tomato, carrot, pea soup concentrate and lyophilized pig liver as extraction at pH 6.0. Standard recovery of the purification step from pH 7.85 extraction buffer was 83 ± 2.8% for H_4folate, 5-CH_3-H_4folate, 5-HCO-H_4folate and folic acid. Statistical analysis supported the finding that the differences between the extraction methods were not significant for these samples.

Table 2. Comparison of folate content for broccoli and frozen green peas after two different extraction methods at pH 4.9 and pH 6.0

	Folate vitamers (μg per 100 g, mean ± SD)				
	Broccoli			Green peas	
	H_4folate	5-CH_3-H_4folate	10-HCO-folic acid ($n = 1$)	H_4folate	5-CH_3-H_4folate
pH 6.0 ($n = 3$)	18 ± 1.9	98 ± 10.1	1.2	10 ± 1.0	51 ± 0.9
pH 4.9 ($n = 3$)	11 ± 0.6	94 ± 3.5	3.3	7 ± 1.0	41 ± 3.8
P	0.005	NS		0.05	0.02

NS, not significant.

Extraction for 1 h at pH 4.9 produced for broccoli and frozen green peas slightly lower folate values than extraction for 10 min at pH 6.0, with no detectable increase for 5-formylfolates (Table 2). A possible slight increase in 10-HCO-folic acid content in broccoli was, however, observed after extraction at pH 4.9. Previous comparison between extraction methods presented by Gregory *et al.* (1990) produced higher folate content in frozen green peas and calf liver at pH 7.85 than at pH 4.9 or pH 7.0. Microbiological assay was used and therefore the effect of extraction method on the vitamers remains unanswered. Possibly the folate forms not determined in the present study account for the detected increase in folate content.

Ion-pair chromatography at pH 5.1 produced results for 5-CH_3-H_4folate in samples extracted at pH 7.85 similar to the results obtained with the method used in this study, providing more evidence for peak identity and purity. An attempt to quantitate 10-HCO-H_4folate with ion-pair chromatography at pH 5.1 did not succeed. However, that vitamer might be of importance in vegetables as well. Seyoum and Selhub (1993) reported that 10-HCO-H_4folates constitute about 9% of folates in lima beans. In the early work of Sponk and Cossins (1972) they estimated spinach leaves to contain 13–16% 10-HCO-H_4folates.

Low recoveries of 5-HCO-H_4folate are partly due to its low fluorescence intensity at the levels studied, which causes difficulties for quantitation, and partly due to disturbing substances with chromatographically similar properties. Almost no fluorescent peaks with similar retention times in these conditions were identified as 5-HCO-H_4folate based on ratios of fluorescence and ultraviolet responses. These fluorescent compounds could not be adequately removed from the chromatogram using additional purification steps. Although good

Table 3. Folate composition of vegetables (μg per 100 g fresh weight)

Vegetable	5-CH_3-H_4 folate	H_4-folate	5-HCO-H_4 folate	10-HCO-folic acid	Sum (as μg folic acid)	Dry matter (%)	Description of composite made from ten primary samples
Potato, raw (*Solanum tuberosum* L.)	21	3	—	tr	23	22.0	Mean of three composite samples (see Table 6)
Carrot, raw (*Daucus carota* L. subsp. *sativus*)	16	1	msk	—	16	9.9	Mean of three composite samples (see Table 6)
White cabbage, raw (*Brassica oleracea* L. var. *capitata*)	27	4	—	—	30	8.4	Mean of three composite samples (see Table 6)
Broccoli (*Brassica oleracea* L. var. *italica* Plank)	98	18	—	1	114	10.7	Exported, composite of 12 samples
Brussel sprouts (*Brassica oleracea* L. var. *gemmifera* DC)	88	9	msk	msk	94	15.2	Exported, composite of 12 samples
Cauliflower (*Brassica oleracea* L. var. *botrytis* L.)	80	9	msk	—	85	8.2	Domestic, composite of 12 samples
Chinese cabbage (*Brassica pekinensis* (Lour.) Rupr.)	50	4	—	—	52	5.5	Domestic, size of head 0.5–2.1 kg, average 1.1 kg
Swede (*Brassica napus* L. subsp. *napus*)	50	2	—	—	49	11.4	Average size 660 g each
Cucumber (*Cucumis sativus* L.)	9	1	—	—	9	3.4	Exported from Spain, average size 446 g each
Tomato (*Lycopersicon esculentum* Miller)	11	1	—	tr	11	5.4	Domestic, size 60–120 g, average 96 g
Tomato	11	1	—	—	11	5.6	Exported, size 53–133 g each, average 102 g
Onion, yellow (*Allium cepa* L. var. *cepa*)	13	1	—	—	13	15.8	Domestic, average size 72 g (43–120 g each)
Sweet pepper, red (*Capsicum annuum* L.)	50	5	3	—	55	9.4	Exported from Spain, average size 203 g (136–278 g each)
Lettuce (leaf) (*Lactuca sativa* L. var. *capitata* L.)	44	9	—	—	51	4.6	Domestic, loose leaf head lettuce

Each value is the mean of duplicate determinations for a composite sample.
msk, masked by impurities; —, not detected; tr, traces.

recoveries with ion-pair SPE purification were obtained, the chromatogram impurities remained. When SPE in ion-pair mode and strong anion exchange SPE purifications were performed consecutively (off-line), chromatogram purity did not improve either. Combination of two purification steps also reduced standard recoveries from matrix, although the results for incubated samples were almost the same as those obtained using only SAX.

A slight increase in folate content was observed after α-amylase treatment with potato extract, and the enzymatic hydrolysis was carried out without further testing

Table 4. Folate composition of fruits, berries and fruit products (μg per 100 g fresh weight)

Product	5-CH_3H_4 folate	H_4-folate	5-HCO-H_4 folate	Sum (as folic acid)	Dry matter (%)	Description of composite
Apple, peeled and cored (*Malus domestica*)	3	—	msk	3	12.9	Exported, mixture of red and yellow varieties
Banana (*Musa*× *paradisiaca* L.)	12	1	—	13	24.2	Average size unpeeled 190 g
Orange (*Citrus sinensis* (L.))	27	< 1	msk	27	13.2	Average size unpeeled 230 g
Strawberry (*Fragraria* L.×*ananassa*)	36	1	—	36	11.8	Domestic, composite of six samples
Bilberry, wild (*Vaccinium myrtillus* L.)	12	—	—	11	14.7	Domestic, composite of 7 samples
Black currant (*Ribes nigrum* L.)	8	—	—	8	18.8	Composite of nine samples
Orange juice	16	< 1	—	15	9.8	Non-sweetened
Juice concentrate, mixed, red	—	—	—	—	ND	Sweetened, 50–60% juice (e.g. grape, apple, pear, black currant, raspberry), dilution 1 + 3 for use
Pineapple, canned	9	< 1	—	9	14.7	In its own juice, drained for 15 s

Results are means of duplicate determinations.
For abbreviations, see Table 3. ND, not determined.

Table 5. Folate composition of vegetable products (μg per 100 g fresh weight)

Product	5-CH_3-H_4 folate	H_4 folate	5-HCO-H_4 folate	10-HCO-folic acid	Sum (as folic acid)	Dry matter (%)	Description of composite
Potato, boiled with skin	11	tr	msk	0.5	11	22.0	Obtained from four university cafeterias, 200 g subsamples, peeled in laboratory, average size 78 g each (50–120 g)
Carrot, boiled	10	1	3	—	13	7.5	Boiled in slices, 300 g in 500 ml boiling water for 15 min, water discarded
French fries, frozen	15	1	—	—	15	39.1	Six brands, ten primary samples
Spinach, frozen	50	—	msk	msk	48	7.5	Four brands, 12 primary samples
Crushed tomatoes, canned	12	3	—	—	15	6.0	Four brands, nine primary samples
Red beets, pickled	37	—	tr	—	36	15.4	In glass containers, drained for 20 s, 25–40 g average size
Peas, green, frozen	51	10	—	—	59	22.7	Three brands, 11 primary samples
Pea soup, canned concentrate	1	< 1	5 (msk)	—	7	25.6	Canned concentrate made with dried peas and meat, dilute for use 1 + 0.5 litre with water. Two brands, nine primary samples

Results are means of duplicate determinations.
For abbreviations, see Table 3.

Table 6. Content of folate (μg per 100 g) in raw potato, carrot and white cabbage sampled at three different times of the year (pooled sample)

Pooled item	Sampling time	5-CH_3-H_4folate	H_4folate	5-HCO-H_4folate	Dry matter (%)
Potato, raw	May	26[a]	4	—	22.2
	August	20	1	—	21.5
	January	16	2	—	22.3
	Mean ± SD	21 ± 5.3	3 ± 1.6	—	22.0 ± 0.44
Carrot, raw	May	14	1	msk	9.3
	August	16	1	6	9.8
	January	17	2	5	10.5
	Mean ± SD	16 ± 1.3	1 ± 0.2	6	9.9 ± 0.60
White cabbage, raw	May	27	2	—	9.0
	August	27	4	—	6.8
	January	27	5	—	9.3
	Mean ± SD	27 ± 0.2	4 ± 1.8	—	8.4 ± 1.36

[a]Mean of duplicate determination for a composite of ten primary samples, each 120–150 g.

for other samples high in starch. HK deconjugase was well suited for liberating folate monoglutamtes from their respective polyglutamates, as was also concluded by Engelhardt and Gregory (1990). Only the extract from oranges showed detectable inhibition of the enzyme after 3 h. In a previous study with this method, the use of different HK preparations and human plasma produced similar folate results (Vahteristo *et al.*, 1996*b*).

Folates in vegetables, fruits, berries and processed plant-derived foods

The folate content of vegetables, fruits, berries and vegetable products are presented in Tables 3–5. Folic acid, 5,10-methenyl- and 5,10-methylene-H_4folate were not detected in any of these samples, and 10-HCO-folic acid was not detected in fruits and berries; these folates are therefore not included in the tables. The chromatogram in Fig. 2 illustrates the separation of folate forms for potato and carrot samples.

The folate contents found in this study are in good accordance with the results previously reported in the USDA Nutrient Database (US Department of Agriculture, 1993), although those results are produced with a microbiological method and with samples of very different origin compared to this study. Compared to the USDA data, our results were considerably higher for potato, broccoli, brussel sprouts, cauliflower and sweet pepper, but lower for white cabbage. In a Danish study, the microbiological method used for folate analysis in vegetables produced somewhat higher results for most vegetables when compared to the results of this study (Okholm-Hansen & Brogren, 1991). For example, folate contents of tomatos and onions were more than twice that determined in the present study.

5-CH_3-H_4folate was the predominating folate form detected in the vegetables studied, as well as in fruits, berries and vegetable products (Tables 3–5). Müller (1993) estimated about 70% of folates in vegetables to be 5-CH_3-H_4folate based on his analyses.

Broccoli, brussel sprouts and cauliflower were high in folate content; also lettuce, chinese cabbage, red peppers and raw swedes contained more than 50 μg folate per 100 g product (Table 3). Strawberries and oranges contained relatively high amounts of folates (Table 4). Orange juice contained about half of the folate amount detected in fresh oranges, but juice concentrate prepared from a selection of berries and fruit had a folate content below our detection limits.

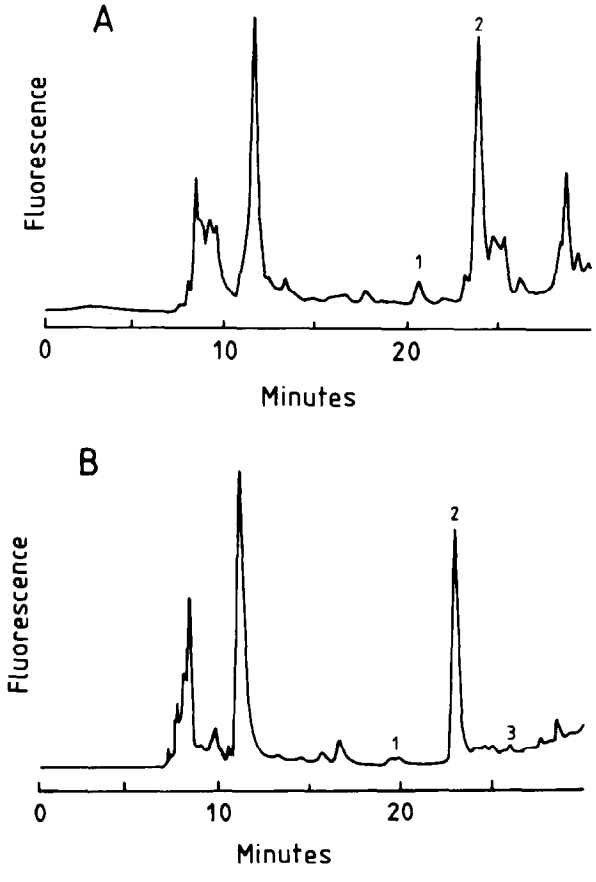

Fig. 2. Representative HPLC chromatograms of potato (A) and carrot (B). 1 = H_4folate, 2 = 5-CH_3-H_4folate, 3) 5-HCO-H_4folate.

Frozen spinach and frozen green peas were high in folate content (Table 5). DeSouza and Eitenmiller (1986) used a microbiological method for folate determination and reported results for frozen, water-blanched spinach (31 μg per 100 g) and for raw broccoli (about 100 μg per 100 g) that were similar to those detected in this study. However, a folate content as high as 126 μg in frozen spinach has recently been reported by Martin *et al.* (1990). Canned pea soup concentrate, which is produced from dried and soaked peas with a small amount of meat, was a poor folate source (Table 5). The study on folate retention in dried legumes conducted by Hoppner and Lampi (1993) indicated also low retention on cooking, possibly mainly due to leaching. Frozen french fries and boiled potatoes contained about half of the folates that were detected in raw potatoes (on dry weight basis). Sliced carrot lost about 40% of its folate content when boiled (the same pooled sample), but leaching losses into the cooking water were not studied. Processed vegetables, with few exceptions, were still reasonably good sources of folate in a diet according to this study.

Variation in folate content

To assess the seasonal variation in folate content, a few staple vegetables (raw potato, carrot and white cabbage) were analyzed three times a year (Table 6). Those samples collected in August all represent the new crop of that year. Also domestic and exported tomatoes were analyzed as separate pools (Table 3). In all of these composite samples the variation was small, being the highest for potatoes. On a dry weight basis, white cabbage exhibited the highest folate content of these samples.

Vegetables, fruits and berries as sources of folates

Among the food groups studied, the best sources of folate in the Finnish diet were potatoes, fruit juices, oranges and strawberries based on average consumption of the year 1990 (Statistics Finland, 1993). Since then the household budget surveys have been carried out only on an economical basis excluding the amounts consumed, and they cannot be used for estimating actual consumption of foods. The actual increase in vegetable consumption in the recent years (Ministry of Agriculture and Forestry, 1996) may, however, have extended and changed the list of most frequently consumed vegetables and fruits and the order of the best folate sources of the Finnish population among vegetables, fruits and berries.

CONCLUSIONS

The results for folate content in vegetables, fruits and berries studied are well in line with the previously reported values obtained with microbiological methods. Dual detection was successfully used for estimating peak purity, allowing discrimination between fluorescent non-folate peaks and folate peaks. Despite some analytical difficulties and lack of suitable calibrants for a few other folate forms, HPLC allowed sensitive and reliable determination of the main folate forms, particularly 5-CH_3-H_4folate, in a wide range of vegetables, fruits and berries. The significance of formylfolates contributing to folate activity remained unclear in few samples.

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REFERENCES

AOAC (1990). *Official Methods of Analysis*, 15th edn. Association of Official Analytical Chemists, Washington, D.C.

Blakley, R. L. (1969). *The Biochemistry of Folic Acid and Related Pteridines*. North Holland Publishing Company, Amsterdam.

Carl, G. F., Hudson, F. Z. & McGuire, B. S. (1995). Rat liver subcellular folate distribution shows association of formyltetrahydropteroylpentaglutamates with mitochondria and methylterahydropteroylhexaglutamates with cytoplasm. *J. Nutr.*, **125**, 2096–2103.

Cossins, E. A. (1980). One-carbon metabolism. In *The Biochemistry of Plants*, Vol. 2, ed. D. D. Davies. Academic Press, New York, pp. 365–418.

DeSouza, S. C. & Eitenmiller, R. E. (1986). Effects of processing and storage on the folate content of spinach and broccoli. *J. Food Sci.*, **51**, 626–628.

Engelhardt, R. & Gregory, J. F. (1990). Adequacy of enzymatic deconjugation in quantification of folate in foods. *J. Agric. Food Chem.*, **38**, 154–158.

Gregory, J. F., Sartain, D. B. & Day, B. P.F. (1984). Fluorometric determination of folacin in biological materials using high performance liquid chromatography. *J. Nutr.*, **114**, 341–353.

Gregory, J. F., Engelhardt, R., Bhandari, S. D., Sartain, D. B. & Gustafson, S. K. (1990). Adequacy of extraction techniques for determination of folate in foods and other biological materials. *J. Food Comp. Anal.*, **3**, 134–144.

Hahn, A., Flaig, K. H. & Rehner, G. (1991). Optimized high-performance liquid chromatographic procedure for the separation and determination of the main folacins and some derivatives. *J. Chromatogr.*, **545**, 91–100.

Hoppner, K. & Lampi, B. (1993). Folate retention in dried legumes after different methods of meal preparation. *Food Res. Int.*, **26**, 45–48.

Leichter, J., Landymore, A. F. & Krumdieck, C. L. (1979). Folate conjugase activity in fresh vegetables and its effect on the determination of free folate content. *Am. J. Clin. Nutr.*, **32**, 92–95.

Lucock, M. D., Green, M., Priestnall, M., Daskalakis, I., Levene, M. I. & Hartley, R. (1995). Optimisation of chromatographic conditions for the determination of folates in foods and biological tissues for nutritional and clinical work. *Food Chem.*, **53**, 329–338.

Martin, C. (1995). Folate analysis in foods. *BNF Nutr. Bull.*, **20**, 8–15.

Martin, J. I., Landen, W. O.Jr & Soliman, A.-G. M. (1990). Application of a tri-enzyme extraction for total folate determination in foods. *J. Assoc. Off. Anal. Chem.*, **73**, 805–808.

Ministry of Agriculture and Forestry (1996). Balance sheet for food commodities in 1993 and 1994 (preliminary). Information Centre of the Ministry of Agriculture and Forestry, Helsinki, Finland.

Minkkinen, P. (1992). *Rutiinianalyysien laadun valvonta.* Lappeenrannan Teknillinen Korkeakoulu, Lappeenranta, Finland.

Mullin, W. J., Wood, D. F. & Howsam, S. G. (1982). Some factors affecting folacin content of spinach, swiss chard, broccoli and brussel sprouts. *Nutr. Rep. Int.*, **26**, 7–16.

Müller, H. (1993). Bestimmung der Folsäure—Gehalt von Gemüse und Obst mit Hilfe der Hochleistungsflüssigchromatographie (HPLC). *Z. Lebensm. Unters. Forsch.*, **196**, 137–141.

Okholm-Hansen, B. & Brogren, C.-H. (1991). Content of folacin in foods. Publication No. 206, Levnedsmiddelstyrelsen, Soborg, Denmark.

Rebello, T. (1987). Trace enrichment of biological folates on solid-phase adsorption cartridges and analysis by high-pressure liquid chromatography. *Anal. Biochem.*, **166**, 55–64.

Scott, J. M. (1980). Thin-layer chromatography of pteroylmonoglutamates and related compounds. *Methods Enzymol.*, **66**, 437–443.

Seale, R. & Finglas, P. M. (1995). Final report of 4th EU MAT folate study. CT. No. MAT1-CT93-0022. Institute of Food Research, Norwich, UK.

Seyoum, E. & Selhub, J. (1993). Combined affinity and ion pair column chromatographies for the analysis of food folate. *J. Nutr. Biochem.*, **4**, 488–494.

Sponk, A. M. & Cossins, E. A. (1972). Folate derivatives of photosynthetic tissues. *Phytochemistry*, **11**, 3157–3165.

Statistics Finland (1993). Kotitaloustiedustelu 1990. Elintarvikkeiden kulutusmäärät ja ateriat. [Household budget survey. Income and consumption.] Statistics Finland, Helsinki.

US Department of Agriculture (1993). USDA Nutrient Database for Standard Reference, Release 10. National Technical Information Service, Springfield, VA. Computer tape, PB93-502771.

Vahteristo, L., Ollilainen, V., Koivistoinen, P. & Varo, P. (1996*a*). Improvements in the determination of reduced folate monoglutamates and folic acid in food using high-performance liquid chromatography. *J. Agric. Food Chem.*, **44**, 477–482.

Vahteristo, L., Finglas, P. M., Witthöft, C., Wigertz, K., Seale, R. & de Froidmont-Görtz, I. (1996*b*). Third EU MAT intercomparison study on food folate analysis using HPLC procedures. *Food Chem.*, **57**, 109–111.

van den Berg, H., Finglas, P. M. & Bates, C. (1994). FLAIR intercomparisons on serum and red cell folate. *Int. J. Vitam. Nutr. Res.*, **64**, 288–293.

Wagner, C. (1995). Biochemical role of folate in cellular metabolism. In *Folate in Health and Disease*, ed. L. Bailey. Marcel Dekker, New York, pp. 23–42.

Wilson, S. D. & Horne, D. W. (1984). High-performance liquid chromatographic determination of the distribution of naturally occurring folic acid derivatives in rat liver. *Anal. Biochem.*, **142**, 529–535.

Witthöft, C. & Bitsch, I. (1993). HPLC methods to analyse folate pattern in food and in human plasma as a precondition to evaluate availability of food folates by biokinetic methods. *Proceedings of Bioavailability '93.* Part 2. Bundesforschungsanstalt für Ernährung, Karlsruhe, Germany, pp. 436–439.