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Folates – food sources, analyses, retention and bioavailability

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ABSTRACT

Health benefits of folates regarding their prevention of neural tube defects in babies and occlusive vascular diseases caused by elevated plasma homocysteine, their link to mental fitness and possibly certain forms of cancer have already been recognised. However, analyses of food folates is still tedious because of a lack of validated methods for characterisation and quantitation of the great number of native folate forms, but also due to a lack of adequate methods for sample pretreatment. Therefore, the assessment of folate losses through industrial and household food processing is still incomplete, as well as knowledge on folate bioavailability in humans. This paper reviews the state of art for the occurrence and analysis of folates in foods. Furthermore, results are summarised from studies of folate retention during food processing and the assessment of folate bioavailability.

Key words: Analysis, bioavailability, folates, food table data, retention/losses

Introduction

Folates represent an important group among the B-vitamins, participating in one-carbon transfer reactions required within the cell, especially for purine and pyrimidine biosynthesis (DNA and RNA) and amino acid interconversions. Optimal folate nutrition and status are linked to diminished risk for neural tube defects, occlusive vascular diseases (1-4) and possibly some forms of cancer (5). Recently, a relationship to cognitive and mental function has also been discussed (6).

In the latest edition of the Nordic Nutritional Recommendations (1996), the daily intake for adults was increased from 200 µg to 300 µg folate and for pregnant women a daily intake of 400 µg was recommended (7). When publishing the dietary reference intakes (DRI) in 1998, the US Food and Nutrition Board included the concept of possible health-protective effects of folate by increasing recommendations for adults to 400 µg/d from the previous 200 µg/day (8). Moreover, the US Food and Nutrition Board recommends women who plan a pregnancy to consume an additional 400 µg synthetic folic acid from fortified foods or supplements. Such dramatic increases of recommended intakes for folate combined with the fact that the average daily intake of folate among Western populations is generally lower than recently set recommendations, emphasise the need for a critical evaluation of the dietary sources of folates. Most of the food folate data derive from microbiological analysis with often insufficient methodological control. There is still today little reliable information about which folate forms and concentrations are present in food and what impact food processing techniques have on folate retention. Moreover, knowledge about human folate bioavailability from native food sources or after fortification is still incomplete (9). Possible risks and benefits from food fortification with folic acid are currently a subject of controversy.

This review aims to give brief information on folate contents and analysis in food, folate losses during food processing and the assessment of folate bioavailability (for more detailed information see 10-13).

Folate chemistry and stability

According to recommendations of the IUNS Committee on Nomenclature (1986), "folate" should be used as the generic term for the class of compounds having similar chemical and nutritional properties to pteroyl-L-glutamic acid (folic acid) (14). While the pteridine ring of folic acid exists in oxidised form, native folates have either two or four additional hydrogens in their pteridine ring forming dihydro- or tetrahydrofolates. Thus, dietary folates exist primarily as reduced, one-carbon-substituted forms of pteroylglutamates, with up to seven glutamyl residues attached to the p-aminobenzoic group by γ-peptide linkage. Five different one-carbon units are known to be linked at N_5- and/or N_{10}-position of the pteroyl group: methyl (5-CH_3), formyl (5- or 10-HCO), formimino (5-CHNH), methylene (5,10-CH_2) and methenyl (5,10-CH) (Figure 1). Altogether the theoretical number of all native folate vitamers reaches several hundred (11).

All folates are in danger of oxidative degradation enhanced by oxygen, light and heat, resulting in a splitting of the molecule into biologically inactive forms, of which p-aminobenzoylglutamate is one major form. There are considerable differences in stability between various reduced folate forms; the order of stability is:
5-HCO-H_4folate > 5-CH_3-H_4folate > 10-HCO-H_4folate > H_4folate.
Moreover, the stability is pH-dependent. Folic acid exhibits substantially greater stability than the reduced folate forms. The chemistry of folates makes the vitamin one of the most vulnerable to losses during food processing. If present in adequate amounts, antioxidants, e.g. ascorbic acid and thiols, protect folates. The rate of reaction for folate breakdown in the presence of oxygen depends on the type of folate derivative and the nature of the food matrix, in particular with respect to pH, buffer composition, catalytic trace elements and antioxidants (11-13).

List of abbreviations

5-HCO-H_4folate	5-formyltetrahydrofolate
5-CH3-H_4folate	5-methyl-tetrahydrofolate
10-HCO-PGA	10-formyl-folic acid
FBP	folate-binding protein
HPLC	high performance liquid chromatography
PBA	(competitive) protein-binding assay
PteGlu	pteroylglutamic acid
RIA	radioimmuno assay
H_4folate	tetrahydrofolate

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Folate content in foods

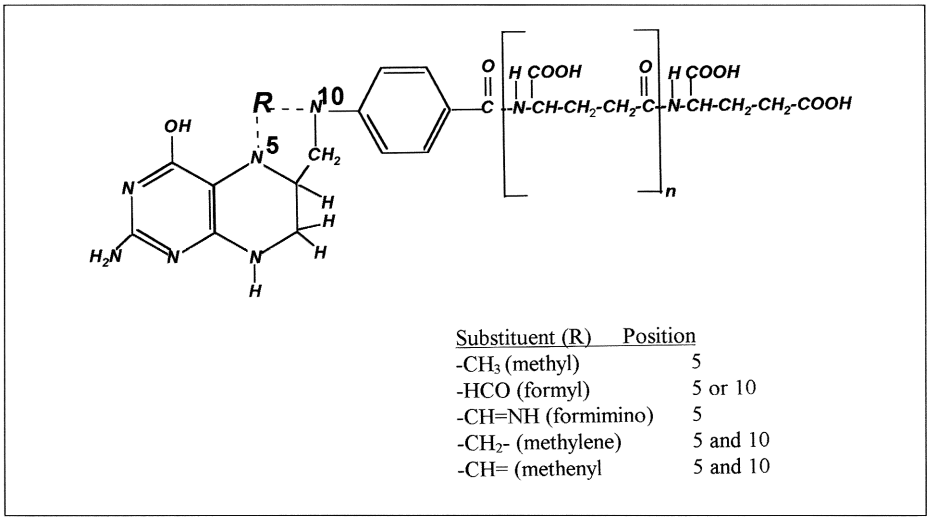
A brief look into various food tables from different countries (Table 1) shows that many vegetables and pulses are rich sources of folate, with folate concentrations up to 600 μg/100 g in some beans and chick peas and around 200 μg/100 g in leafy vegetables. A sort of general rule is that the lower the water content in the vegetable the higher the folate concentration, and moreover that leafy vegetables are good folate sources (folium means leaf). Folate concentrations in fruits and berries are usually one-tenth those of vegetables, ranging from a few μg to approx. 50 μg/100 g. The highest concentrations are to be found in frozen concentrated orange and grapefruit juice, strawberries and several nuts with folate concentrations of about 50–100 μg/100 g or more.

Meat and meat products, except liver which is the storage organ, contain little folate, while chicken and fish are moderate sources (15). Folate concentrations in milk are only around 5 μg/100 ml, but milk is however of interest due to its content of a specific high-affinity folate-binding protein. This may play an important role in the regulation of absorption and bioavailability of dietary folates from the gastrointestinal tract (16). An increase of the folate content usually results from fermentation of milk and whey products. In hard cheeses, up to 40 μg/100 g and more were quantified. Soft cheeses like Camembert and Brie contain 60–100 μg/100g (17). High in folate is egg yolk, with up to 90 μg/100 g (15).

Cereals are also an important dietary source for folate. Some cereal fractions like bran and germs contain a few hundred μg folate per 100 g, while bread from wholemeal flour contains 50–100 μg/100 g (Table 1). Baker's yeast with its extremely high folate concentration (approx. 1000–4000 μg/100 g) contributes to the folate content in soft bread. Commonly, foods are ordered into groups being "rich", "good" and "moderate" sources, with folate concentrations of >100 μg, 50–100 μg and 15-50 μg per serving, respectively (18).

Folate analysis by HPLC can provide information on individual folate forms present in food, but currently only few data are available; some examples from vegetables, fruits and dairy products are given in Table 2. *The sum of concentrations* from individual folate forms analysed by HPLC cannot be directly compared with *total folate concentrations* assessed by microbiological methods, as the latter response to nearly all tetrahydro-, dihydro- and fully oxidised folate forms in the food sample. It is still unclear, however, which concentration best reflect the amount of folate bioavailable for humans.

Figure 1. Structure of tetrahydropteroylpoly-γ-glutamate.



Substituent (R)	Position
$-CH_3$ (methyl)	5
-HCO (formyl)	5 or 10
-CH=NH (formimino)	5
$-CH_2-$ (methylene)	5 and 10
-CH= (methenyl	5 and 10

Both tables show notable discrepancies between folate data for some foods, which cannot only be explained by differences with folate content but rather by methodological differences in respect to folate quantification and sample pretreatment, emphasising the need for re-assessment using better controlled methods.

Folate analysis

Traditionally, microbiological assay procedures with *Lactobacillus casei* (ATCC 7469), which responds to most folate derivatives with up to three polyglutamate residues, are used for folate quantification (19). Most folate values published in food tables today were established by microbiological methods.

The use of (radio-) protein binding procedures is common for clinical diagnostics in plasma, serum or whole blood mainly containing 5-CH_3-H_4folate (20). These tests are based on non-specific competitive binding of folates from the test sample and radiolabelled folic acid to a folate-binding protein. Accurate control of the assay pH is required, and formylated folates are only bound to a small percentage (21,22). Protein-binding procedures have not often been used successfully for food folate analyses and their application might be restricted to food matrixes which contain mainly 5-CH_3-H_4folate (23-30). Most foods, however, contain a variety of folate forms (31-36).

In recent years, HPLC methods for the simultaneous determination of several individual folate monoglutamates were established (31,37-42). Often fluorescence detection is used, and consequently determination is restricted to reduced folate forms H_4folate, 5-HCO-H_4folate and 5-CH_3-H_4folate, which show native fluorescence (43).

Folate stabilisation before and during analysis is necessary as folates are sensitive to oxygen, heat, light, pro-oxidants and extreme shifts of pH-value (10-13). Optimisation of stabilisation procedures is hampered, because individual folates forms possess different pH-optima for maximum stability. With the use of antioxidants throughout sample preparation, folates are successfully protected from interconversion (44) and from oxidative degradation (45-48). Additional exclusion of oxygen by overlay with nitrogen (49-51), as well as use of low temperatures and shelter from light, should already be applied when homogenising the samples.

Commonly, folates are extracted in slightly acidic to slightly alkaline conditions by heat, using autoclaving with microbiological folate assay (19,25,52) or a boiling water bath with HPLC procedures (40,41).

Prior to quantification with both microbiological and HPLC procedures, deconjugation of folate polyglutamates to monoglutamates is required (9,10). As deconjugase preparations are not commercially available, they have to be prepared by the investigator. Partially purified suspensions of γ-glutamyl-hydrolases (EC 3.4.22.12) from hog kidney (37), chicken pancreas (53) and plasma from humans or other species (25,54,55) are used; to a lesser extent enzyme preparations from intestines (56-58). For HPLC determination, the use of human plasma or hog kidney deconjugation is advisable, because these exopeptidases produce folate monoglutamates (37,59). Procedures for folate deconjugation have to be optimised in respect to time, temperature, incubation milieu, folate stabilisation and substrate-enzyme ratio depending on the characteristics of the sample

Table 1. Total folate contents in foods compiled from different national food tables.

Food item	Folate concentration in µg/100g			
	S[a]	UK[b]	D[c]	DK[d]
Vegetables				
aubergine	18	5	31	27
asparagus	119	175	108[e]	150
beet red	86	150	83e	91
beans, green	36	80	70[e]	64
beans, white, dried	488	+[f]	187	219
broccoli	175	90	111[e]	187
Brussels sprouts	61	135	182[e]	130
butterhead lettuce	73	57	75	86
cabbage, white	57	75	31e	48
cabbage, fermented, sauerkraut	40	31	31	nr[g]
cabbage, Chinese	150	77	79[e]	66
cabbage, red	21	39	35	46
carrot	14	12	55[e]	27
cauliflower	47	66	125[e]	140
chick peas, dried	557	180	340	180
cucumber	14	9	27[e]	12
garlic	3	5	nr	103
kale	30	120	187[e]	60
leek	91	56	103	82
lentils, dried	433	110	168	35
onion	20	17	7	37
parsley	183	170	149	116
parsnip	67	87	59	67
peas, green	65	11	159[e]	25
peppers, sweet, green	14	19	60[e]	31
potato	19	35	20[e]	22
radishes	27	38	24	28
spinach	194	150	145[e]	220
squash	22	52	nr	46
tomato	21	17	44.5[e]	26
Fruit, berries, nuts				
almonds	56	48	45	96
apricot	9	5	3.6	9
avocado	62	8	30	91
banana	19	14	17[e]	28
black currants	23	+	16e	8.2
blueberry	6	6	6	6
cherries	3	5	75[e]	nr
grapes	4	2	43[e]	5
hazelnuts	72	72	71	72
lemon	11	+	6.3	32
mango	36	+	36	71
orange juice	44	20	24	16
peach	4	3	2.7	4.2
peanuts	101	110	169	210
rosehip, dried	2	nr	nr	210
strawberries	99	20	65[e]	63
Yeast, cereals, cereal products				
baker's yeast, compressed	1000	nr	716[e]	1000
corn flour	10	nr	10	20
rye flour, wholemeal	56	78	15	72
wheat bran	260	260	195[e]	140
wheat flour	21	22	10	18
wheat germs	330	331	520	190
wheat flour, plain	21	22	nr	18
barley, rolled	20	20	nr	nr
bread, white	36	29	nr	43
oats, rolled	56	60	87[e]	46
spaghetti, raw	12	34	11	30
rice, parboiled, raw	31	nr	29	31
Milk and dairy products				
Brie, 28 % fat	65	58	65	90
Camembert, 23 % fat	62	102	44[e]	62
Cheddar, 33 % fat	nr	33	19	20
cheese, blue, 30 % fat	36	50	40	36
cottage cheese	12	27	nr	nr
milk, whole	6	6	7	9
yoghurt, plain, 3 % fat	15	18	13	17
whipping cream	4	7	4	6

[a] (119); [b] (120); [c] (121); [d] (122); [e] = analysed by HPLC; [f] + = folate present in food sample, but no reliable value available; [g] nr = no value reported

Table 2. Folate contents and derivates in foods assessed by HPLC.

Food item	Folate concentration in µg/100g			
	H_4 folate	5-HCO-H_4 folate	5-CH_3-H_4 folate	Ref
Vegetables				
asparagus	17.0	20.1	75.7	b
butterhead lettuce	9	-[g]	44	a
	2.2	17.0	59.3	b
	18	-	44	d
broccoli	18	-	98	a
	14.8	17.9	83.2	b
	15	-	76	d
Brussels sprouts	9	msk[h]	88	a
	14.8	17.9	83.2	b
	6	-	56	d
cabbage, white	4	-	27	a
	5.2	9.2	18.2	b
	3.1	-	5.3	c
	14	-	16	d
cauliflower	9	msk	80	a
	24.7	4.2	100.7	b
carrot	1	msk	16	a
	3.6	13.0	41.3	b
Chinese cabbage	4	-	50	a
	0.7	44.9	33.8	b
peas, green, frozen	10	-	51	a
	5	-	48	d
peppers, sweet	5	3	50	a
	2.2	6.5	53.9	b
potato	tr[i]	msk	11	a
	2.4	2.4	15.7	b
	4	-	19	d
potato, cooked	tr	msk	11	a
	-	-	17	d
turnip	2	-	50	a
turnip, cooked	3	-	11	d
tomato	1	-	11	a
	1.2	35.7	16.4	b
	18	-	2	d
tomato, conserve	3	-	12	a
	-	-	25	d
Fruits and berries				
apple	-	msk	3	a
	1.0	3.3	1.8	b
banana	1	-	12	a
	1.9	2.3	13	b
	-	-	13	4[d]
black currants	-	-	8	a
	5.7	4.0	7.1	b
orange	<1	msk	27	a
	0.9	7.4	36.1	b
	-	-	30	d
orange juice	<1	-	16	a
	-	-	17	c
strawberries	1	-	36	a
	5.0	2.7	59.3	b
	1	-	19	d
Milk and dairy products				
milk, 1.5 % fat	<1	-	4	a
	-	-	7	f
whey cheese	-	-	51	f
yoghurt, plain	2	3	1	a
yoghurt, plain, 3% fat	-	-	5	f
yoghurt, plain, 3.5 % fat	<1	13	1	e
Camembert	14	15	17	e
hard cheese (Edam type)	1	4/msk	2	a
hard cheese (Herrgård)	-	-	12	f
hard cheese (Emmentaler)	<1	6	<1	e

[a] Vahteristo et al.,1997 (36), [b]Müller, 1993 (38),[c]Gregory et al, 1984 (87), [d]Lawrance, 1996 (31); [e]Müller, 1993 (123); [f] Wigertz, 1997 (17); [g] - = not detected; [h]msk = masked; [i]tr = traces; foods are raw if not mentioned otherwise; H_4folate = tetrahydrofolate, 5-HCO-H_4folate = 5-formyltetrahydrofolate, 5-CH_3-H_4folate = 5-methyl-tetrahydrofolate

Table 3a. Folate losses in foods subjected to thermal processing.

Thermal processing	Conditions	Food sample	Folate losses (%)	Effecting factors	Reference
water blanching, steam blanching	3-6 min	spinach	83-42	leakage, oxidation	DeSouza and Eitenmiller 1986 (24)
		broccoli	70-91		
steaming (pressure)	20-40 min	broccoli	24-41	leakage, oxidation	Petersen 1993 (124)
sous-vide processing in vacuum bags	40 min	broccoli	11	oxidation	Petersen 1993 (124)
tinning		spinach, broccoli	50	leakage, oxidation	DeSouza and Eitenmiller 1986 (24)
tinning		Brussels sprouts	30	leakage, oxidation	Malin et al 1977 (74)
boiling or pressure cooking	10-30/5 min	Brussels sprouts	0	leakage, oxidation	Malin et al 1977 (74)
infra-red or convection heating		cooked vegetables	4-24	oxidation	Williams et al 1995 (125)
warm holding	1 h (72°C)	cooked vegetables	14	oxidation	Williams et al 1995 (125)
autoclaving	20 min	folate standards in food	0[a]	oxidation	Ristow et al 1982 (85)
		model	75[b]		
oven baking	25-35 min (200°C)	rainbow trout	30[b]	oxidation	Vahteristo et al 1998 (126)
		pollack	46[b]		
		chicken breast fillets	34[b]		
pasteurisation	15 s (74°C)	milk	8[b]	oxidation	Wigertz et al 1997 (127)
UHT	5 s (140°C)	milk	19[b]		

[a] losses of folic acid; [b] losses of 5-methyltetrahydrofolate

Table 3b.Folate losses from combined food processing.

Combined processing	Conditions	Food sample	Folate losses (%)	Effecting factors	Reference
blanching + tinning		spinach	84	leakage, oxidation	DeSouza and Eitenmiller 1986 (24)
quick soak + cooking	1 h + 20-150 min	peas + lentils	55-79	leakage, oxidation	Hoppner and Lampi 1993 (128)
		beans	55-81		
		various pulses	34-69		
overnight soak + cooking	16 h + 20-150 min	peas + lentils	40-71	leakage, oxidation	Hoppner and Lampi 1993 (128)
		beans	40-31		
		various pulses	34-69		
blanching + freezing		spinach	87	leakage, oxidation	DeSouza and Eitenmiller 1986 (24)
blanching + blast-freezing + storage	14-180 days	Brussels sprouts	0	leakage, oxidation	Malin et al 1977 (74)
freeze drying + rehydration		space shuttle food	36-71	enzymes, oxidation	Lane et al 1995 (129)
ionised radiation	10 kGy	various foods	5-30	oxidation	Müller and Diehl 1996 (130)
ionised radiation	2.5, 5,10 kGy	spinach	10-30	radiation	Müller and Diehl 1996 (130)
		white cabbage		10-60	
		Brussels sprouts		10-40	
cook/chill + reheating	3 days (3°C)	various vegetables	26	leakage, oxidation	Williams et al 1995 (125)
cook/hot-hold	30 min (72°C)	various vegetables	19	leakage, oxidation	Williams et al 1995 (125)
	2h (72°C)		32		

Table 3c. Folate losses from storage of food

Storage	Time	Food sample	Folate losses (%)	Effecting factors	Reference
frozen	8 month	blanched spinach	17	oxidation, enzymes	DeSouza and Eitenmiller 1986 (24)
frozen	188 days	fresh Brussels sprouts	42	oxidation, enzymes	Malin et al 1977 (74)
frozen	7, 6 month	beef liver, strawberries	0	oxidation, enzymes	Vahteristo et al 1998 (129)
chilled (3°C)	1 day	cooked vegetables	5	oxidation	Williams et al 1995 (125)
storage at room temperature	8 weeks	UHT-milk	0[a]	oxidation, enzymes	Wigertz et al 1997 (127)
chilled	2 weeks	filmjölk, yoghurt	0[a]		

[a] losses of 5-methyltetrahydrofolate

matrix and enzyme source.

Recently, increased total folate concentrations were reported after additional use of proteolytic and amylolytic enzymes in cereal-based foods, some vegetable and dairy products or composite food samples (33,52,60-62). Procedures for this so called tri-enzyme extraction are yet not widely established. Further investigations are needed for accurate food folate quantification in order to revise current folate data in food tables (62).

Purification of food extracts is necessary when using HPLC determination. Several solid phase extraction procedures with commercial disposable cartridges have been reported (37,40,41,51,63,64). Less common is the use of affinity chromatography with folate-binding protein attached to agarose gel (33,65-67). The affinity columns have to be prepared by the investigator, they are comparatively expensive, show low affinity to 5-HCO-H_4folate (65) and possess a short lifetime (31).

The need for validated methods for folate determination becomes obvious. Attention should not only be focused on the method of determination, but also on techniques for extraction, stabilisation and deconjugation of folates including quality control of methods by e.g. recovery studies, use of certified reference material and adequate calibration procedures. These tasks were recently tackled in the studies of the "EU SMT project on

improvements in the determination of folates in food" (25,68-70), which have resulted in the preparation of four certified reference materials (CRM) from milk powder, wholemeal wheat flour, lyophilised pig's liver and mixed vegetables.

Folate losses or retention during storage and processing

Most foods in modern nutrition are consumed after being processed by household or industrial procedures. This aims for microbiological safety, convenience regarding storage and distribution and optimal nutritional value and organoleptic appeal (13). Several methods of industrial and household processing were investigated regarding their impact on folate retention, as summarised in Table 3. Most studies reflect negative effects from processing, causing increasing losses with increasing severity of processing conditions in terms of heating temperature and time. Although quite a few studies could be found focusing on the influence of processing on folate concentration in vegetables, the picture is still fragmentary. For fruits and berries there is practically no information at all on processing and storage stability of folates.

Leakage and oxidative degradation are the major reasons for folate losses during processing and storage. After thermal processing of vegetables, as displayed in Table 3a, both leakage and oxidative degradation can cause folate losses up to 70–80%. In high-temperature short-time processing such as pasteurisation or UHT-treatment of milk, no leakage but oxidative degradation of folate occurs resulting in rather modest losses between 0 and 20% (71). Oxidation is also the major cause of folate losses during steam-flaking, spray-drying and extrusion cooking of cereals when producing pre-cooked cereal products for gruel, porridge, biscuits etc. (72).

Oxidation of tetrahydrofolates, e.g. 5-CH_3-H_4folate to 5-CH_3-dihydrofolate, can occur, which as a very unstable compound is prone to further degradation. However, *Lucock* et al. (73) showed that 5-CH_3-dihydrofolate can be reduced to 5-CH_3-H_4folate in the presence of ascorbic acid and can subsequently be salvaged under physiological conditions. Many food sources contain endogenous ascorbic acid which can prevent folate oxidation (74). From 10-HCO-H_4folate the formation of oxidation products like 10-HCO-dihydrofolate or 10-HCO-folic acid is possible.

Heat treatment can also cause *interconversion* of several folate forms. *Gregory* et al. (37) studied interconversion of the very labile 10-HCO-H_4folate to the more energetically stable 5-HCO-H_4folate. Heat-induced conversion is rapidly completed, so that formulated folates can only be analysed as one 5-HCO-H_4folate fraction by HPLC.

It is still questionable whether certain forms of processing, which include rise of temperature, may have an impact on folate pattern and concentrations due to *heat destruction of endogenous food enzymes*. An experiment by *Ristow* et al. (75) indicated lower folate bioavailability from cooked compared to raw cabbage in a bioassay using chicken. When endogenous folate deconjugase was not inactivated by heat treatment, folate polyglutamates from raw cabbage were presumably deconjugated to a greater extent prior to feeding. These "endogenously" deconjugated folate monoglutamates were according to the authors more bioavailable.

On the other hand, folate monoglutamates might be more susceptible to oxidative or enzymatic degradation after endogenous deconjugation. This idea could be supported by the findings of *Malin* et al. (74), who determined folate losses of 84% after 188 days of storage at –21°C in non-blanched Brussels sprouts samples. In blanched samples, no losses occurred during storage.

Food processing can lead to positive effect when applying *fermentation procedures*, as compiled and studied for milk products, e.g. yoghurt, buttermilk and cheeses by *Wigertz* (17), and as traditionally practised with some vegetables and during bread-making with yeast and sour dough.

Some food processing including *fractionation* results in fractions with either increased or decreased folate concentrations. A typical example of this is *milling*. When whole grains of cereals like wheat, rye and oats are milled, the grains become separated into bran, germs, wholemeal flours etc. resulting in markedly increased concentrations of folates in the bran parts and decreased folate content in the bran-free flour fractions (Table 1).

There is a need for intensified studies, especially with a view to developing new techniques enhancing folate content and bioavailability in products. Together with gentle techniques of processing, this can maximise folate retention which is desirable in the development of functional foods.

Human folate bioavailability

Bioavailability is commonly defined as the absorption and metabolic utilisation of a nutrient, involving processes of intestinal absorption, transport, metabolism and excretion (76,77). Conjugated dietary folates consumed by man require deconjugation to their monoglutamate forms prior to absorption (78). Human intestinal brush border deconjugase is a zinc-dependent exopeptidase with optimum activity at pH 6.5 catalysing stepwise hydrolysis of polyglutamyl folates (56,79). Once deconjugated, the folate monoglutamates are absorbed by an active energy-dependent carrier mediated process at physiological concentrations, and by passive diffusion at higher concentrations (80). Absorption takes place mainly in the jejunum and is markedly influenced by pH, with a maximum at pH 6.3. Oxidised folates in physiological amounts are reduced and methylated when passing through the intestinal mucosa or during passage of the liver, the storage organ of folates. All intracellular folates exist in polyglutamyl form, whereas transport in plasma occurs in the methylated monglutamyl form (80).

Folate bioavailability can be influenced by several intrinsic or extrinsic factors. Directly linked to the organism are folate status, health and gastro-intestinal function, age, sex and antagonistic effects from use of drugs and alcohol. Extrinsic factors are many depending on the ingested folate (e.g. oxidation status and substituent, length of polyglutamate chain), the food matrix (e.g. folate interaction with substances from the food matrix, substances with antifolate activity), or processing and storage.

Initially bioassays were performed to determine folate bioavailability using rats 81,82), pigs (83,84), chickens (85) or even monkeys (86). Bioassays make it possible to determine folate concentrations in several response tissues such as liver, kidneys, serum and whole blood and allow the assessment of growth or reproduction activity as parameters for folate bioavailability. Often bioassays are performed according to a so-called depletion-repletion protocol, whereby animals are fed a folate-free diet during the depletion period and then receive various doses of folate during repletion. Accumulation of folate until steady-state is determined in the response tissues of each group to create a dose response curve (87).

Clifford et al. (88,89), using a rat bioassay model, reported folate bioavailability between 70 and 120% from food items like lyophilised orange juice, cooked lima beans, cooked leafy vegetables, wheat germ, eggs and mushrooms. These figures are higher than usually found in human studies based on similar food items. One reason could be a more efficient folate deconjugation in rats, since pancreas juice of rats, in contrast to man, is a very rich source of deconjugases (90). Another factor is that the rat model is performed over a period of 1–2 weeks and folate intake might include a contribution

Table 4. Examples of studies on the absorption and bioavailability of folates in humans presented in chronological order

Tested effect	Marker	Summary of results	Reference
Inhibition of intestinal deconjugases by yeast	Plasma	Reduced plasma folate concentrations after consumption of $PteGlu_7$[a] and yeast	Rosenberg, Godwin, 1971 (130)
Absorption of pharmaceutical folate and native folate from 12 foods	Urine	Folate bioavailability for each food ranged from 0-137% compared to PteGlu response)	Tamura, Stockstad, 1973 (109
Absorption capacity from oral dose of 5-HCO-H_4folate[b]	Urine	No difference in plasma AUC[c] after oral and i.m. administration of 21 mg 5-HCO-H_4folate	Kirwan, Narebor, 1976 (103)
Inhibition of intestinal deconjugases by orange juice, organic acid	Urine	Reduced folate excretion (54/39%) into urine after consumption of $PteGlu_7$ with juice/ organic acids	Tamura et al 1976 (131)
Reduced folates absorption through age	Urine, brushborder biopsy samples	No difference in $PteGlu_7$ absorption in younger and older volunteers. Similar intestinal deconjugase activity in biopsy samples	Bailey et al 1984 (132)
Absorption and elimination kinetics from PteGlu	Plasma, urine	Pharmacokinetic parameters from plasma- and urine folate after i.m. application of 1.05 mg PteGlu	Loew et al 1987 (97)
Oral absorption from PteGlu linear	Plasma	Doses from 150-5000 µg PteGlu are absorbed completely independent from size of dose	Hages, Pietrzik, 1987 (96)
Effect of fibre-rich foods on $PteGlu_7$ absorption	Plasma	Reduced plasma folate AUC after consumption with wheat bran. No effects after consumption with spinach	Bailey et al 1988 (99)
Effect of food fibre on $PteGlu_7$ absorption	Plasma	No significant differences in plasma folate AUC after consumption with wheat bran and beans	Keagy et al 1988 (102)
Impact of polyglutamate chain on absorption	Urine	Urinary folate excretion of oral dose of $PteGlu_6$ in apple juice is 50% compared to equimolar dose of $PteGlu_1$	Gregory et al 1991 (133)
Bioavailability of various folate forms	Urine	Folate excretion into urine from equimolar PteGlu dose is higher than from reduced folate forms	Gregory et al 1992 (134)
Increase of dietary folate by natural foods vs. fortified foods vs. supplementation	Erythrocyte	Increased red-cell folate after additional consumption of 400 µg folate from fortified foods and folate supplements over 3 month. No increase after consumption of natural foods	Cuskelly et al 1996 (93)
Incorporation of milk into a mixed diet increases folate absorption	Ileostomal effluent	Reduced excretion of dietary 5-CH_3-H_4folated with stomal effluent consumption of of milk over three weeks. No significant effects comparing consumption of fermented or non-fermented milk	Wigertz 1997 (17)
Bioavailability of PteGlu from fortified cereal-grain foods	Urine	No significant differences in urinary folate excretion after consumption of equimolar doses of PteGlu in water, spaghetti, rice, bread	Pfeiffer et al 1997 (108)
Plasma folate pattern after oral ingestion of pharmaceutical 5-HCO-H_4folate and 5-CH_3-H_4folate	Plasma	Different plasma folate derivatives after ingestion of 15 mg 5-HCO-H_4folate and 5-CH_3-H_4folate during >10 h post dose	Witthöft 1998 (64)
Relative bioavailability of folates in spinach	Plasma	40% increase of plasma folate AUC after consumption of 600 compared to 300 g spinach	Prinz-Langenohl et al 1999 (98)

[a]$PteGlu_x$ = pteroylglutamic acid (suffix 1 - 7 for number of glutamate rests attached), no suffix = pteroylmonoglutamic acid (folic acid); [b]5-HCO-H_4folate = 5-formyltetrahydrofolate; [c]AUC = area under the plasma concentration curve; [d]5-CH_3-H_4folate = 5-methyl-tetrahydrofolate

from folates produced and absorbed in the colon or simply due to coprophagy. Data from human studies originate mainly from single dose protocols. There is a growing discussion about the appropriateness of bioassays in order to predict folate metabolism in humans, due to quantitative and qualitative physiological differences between species.

In vitro methods have been used to investigate properties of endogenous deconjugases from various species (12,91,92) or folate binding to other food components, e.g. dietary fibre (85). *Wigertz* (17) studied folate retention in milk products using an *in vitro* model simulating the human gastro-intestinal tract. However, *in vitro* methods can reflect the complexity of *in vivo* folate absorption, deconjugation and metabolism only to a limited extent.

Studies involving human volunteers provide the best means to assess folate bioavailability by the use of long-term or short-term protocols. Long-term folate kinetics usually investigate the impact of repeated folate supplementation on folate status, e.g. feeding fortified vs. natural foods vs. folic acid supplements (93,94). Subsequently, folate concentrations in whole blood or erythrocytes are quantified (93,95), which reflects the folate status of the past three months. These studies are intensive as regards costs and time.

Short-term folate absorption and elimination kinetics are often studied after application of a single oral folate dose in the form of a pharmaceutical preparation or a fortified or natural food sample. Plasma and urinary folate concentrations can be determined by protein-binding assay (96-98), microbiological (99-104) and HPLC methods (42,105-107). Comparison of the area under the plasma response curve or urinary folate excretion allow determination of relative bioavailability of different folate forms, from various doses or application techniques (98,108,109).

In recent years, the use of stable isotope protocols was introduced by *Gregory* et al. (95,110,111). The incorporation of isotopically labelled folates (from fortified food samples) into the diet provides a tool to estimate folate turnover by urinary and fecal excretion of the labelled compounds and their metabolites (108). Advantage of the use of stable isotopes is that isotopically labelled folates from the dose and endogenous folates from body stores can be differentiated. Due to lack of commercial availability of labelled compounds, intensive analytical procedures and the difficulty of mass spectrometric determination, stable isotope procedures are

not commonly applicable.

A drawback of some short-term protocols for the determination of folate kinetics is the fact that the application of a single folate dose of physiological magnitude only results in a small plasma and urine folate response. Therefore often pharmacological doses or loading of body stores are used (97,109), which do not reflect physiological conditions.

Table 4 summarises in chronological order sample results from human studies of folate bioavailability and absorption kinetics, even showing contradictory results. Whichever protocol for the determination of folate bioavailability is applied, the limitations of each approach have to be taken into consideration.

Summing up the state of the art concerning folate bioavailability in man, synthetic pteroylmonoglutamic acid (folic acid) is absorbed almost completely when ingested as a physiological dose without simultaneous consumption of food, while synthetic pteroylpolyglutamates seem to be less available (50–80%) even when given in the absence of food (112).

Figures describing absorption of native dietary folates, which exist as a mixture of reduced mono- and polyglutamate forms, have to be interpreted with care, but are generally in the magnitude 40–70%.

Matrix effects from foods, e.g. interaction with deconjugase inhibitors, binding or encapsulation to different food constituents, pH effects or losses during gastro-intestinal digestion, owing to oxidative degradation of native folates are some factors that might contribute to reduced bioavailability.

A food constituent of particular interest is folate-binding protein (FBP) from milk. In cow's milk most of the folate is bound to FBP. Folate bound to FBP is mainly absorbed in the ileum at a slower rate than the free folate which is absorbed in the jejunum. FBP has been suggested to play a role in the absorption of folate by preventing its uptake by intestinal bacteria, and it may also directly promote the transport of folate across the intestinal mucosa. Most of these studies investigating the role of FBP on folate bioavailability are performed using animals (16,113-116).

Studies performed on infants by *Ek* and *Magnus* (117,118) showed that breast-fed babies had a better folate status than bottle-fed babies. Breast-fed babies sustained their folate status on an intake of 55 μg folate per day while bottle-fed babies needed 78 μg folate per day.

It is suggested that these discrepancies are due to the presence of FBP in human milk which is destroyed in heat-processed milk formula.

Outlook – need for future investigations

The importance of an adequate folate intake to maintain health has already been recognised. Despite that, there is still uncertainty about folate requirements during e.g. infancy, growth, pregnancy or when suffering from chronically or inflammatory diseases. Quantitative aspects of human folate absorption, bioavailability and metabolism are still poorly understood.

Several methods for folate determination are established today, but the need for strict methodological validation has to be stressed, including international method calibration and harmonisation to revise current food data tables.

Another issue is the evaluation of current and development of new techniques of food processing with respect to beneficial or adverse effects on folate content, retention and bioavailability. Taken together, these aspects all point to a need to re-evaluate national health policies, and to participate in the discussion of (mandatory) food fortification and functional foods.

REFERENCES

1. Engbersen AM, Franken DG, Boers GHJ, Stevens EMB,Trijbels FJM, Blom HJ: Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hypercysteinemia. Am J Hum Genet 1995; 56:142-50.
2. Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PWF, Belanger AJ, O'Leary DH, Wolf PA, Rush D, Schaefer EJ, Rosenberg IH: Relationship between plasma homocysteine, vitamin status and extracranial carotid-artery stenosis in the Framingham study population. J Nutr 1996; 126:1258S-1265S.
3. Schorah CJ, Habibzadeh N, Wild J, Smithells RW, Seller MJ: Possible abnormalities of folate and vitamin B_{12} metabolism associated with neural tube defects. Maternal Nutr and Pregnancy Outcome 1993;678:81-91.
4. van der Put NMJ, Steegers-Theunissen RPM, Frosst P, Trijbels FJM, Eskes TKAB, van den Heuvel LP, Mariman ECM, den Heyer M, Rozen R, Blom HJ: Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet 1995;346:1070-1.
5. Giovannucci E, Stampfer MJ, Colditz GA, Rimm EB, Trichopoulos D, Rosner BA, Speizer FE, Willett W: Folate, methionine, and alcohol intake and risk of colorectal adenoma. J Nat Cancer Inst 1993;85:875-84.
6. Kihlberg R: Om homocystein och folsyra. Scand J Nutr 1998;42:89-90.
7. Sandström B, Aro A, Becker,W, Lyhne N, Pedersen, J, Torsdottir, I: Nordiska Näringsrekommendationer 1996. Nordiska Ministerrådet. Report. Nord 1996;28:129-33.
8. Yates AA, Schlicker SA, Suitor CW: Dietary reference intakes: the new basis for recommendations for calcium and related nutrients, B vitamins, and choline. J Am Diet Assoc 1998; 98: 699-702.
9. Gregory III JF: Bioavailability of folate. Eur J Clin Nutr 1997;51:S54-S59.
10. Ball GFM: Folate. In: Bioavailability and analysis of vitamins in foods. Chapman & Hall, London 1998;439-96.
11. Eitenmiller RR, Landen WO: Folate. In: Eitenmiller RR, Landen WO, eds. Vitamin Analysis for the Health and Food Sciences, CRC Press, Bocca Raton, London, N. Y., Washington 1999; 411-66.
12. Gregory III JF: Chemical and nutritional aspects of folate research: analytical procedures, methods of folate synthesis, stability, and bioavailability of dietary folates. Adv Food Nutr Res 1989;33:1-101.
13. Hawkes JG, Villota R: Folates in foods: reactivity, stability during processing, and nutritional implications. Food Sci Nutr 1989; 28:439-538.
14. Blakley L: IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN): Nomenclature and symbols for folic acid and related compounds, Recommendations 1986. J Biol Chem 1988;263:605-7.
15. Vahteristo L: Folates and their analysis: Determination of folate derivatives and their stability by high-performance liquid chromatography. Dissertation, University of Helsinki 1998.
16. Parodi PW: Cow's milk folate binding protein: Its role in folate nutrition. The Australian J Dairy Technol 1997;57:109-18.
17. Wigertz K: Milk folates. Characterisation and availability. Dissertation, Univ of Lund 1997.
18. Department of Health, Scottish Office Home and Health Department, Welsh Office, Department of Health and Social Services, Northern Ireland: Folic acid and the prevention of neural tube defects. Health Publications Unit, Heywood Lancashire, Great Britain 1992.
19. Tamura T: Microbiological assay of folates. In: Picciano MF, Stockstad ELR, Gregory III JF, eds. Folic Acid Metabolism in Health and Disease, Wiley-Liss, New York 1990;121-37.
20. Gutcho S: Source of error in determination of erythrocyte folate by competitive binding radioassay – comments. Clin Chem 1978;24:388-9.
21. Givas JK, Gutcho S: pH-dependence of the binding of folates to milk binder in radioassay of folates. Clin Chem 1975;21:427-8.
22. van den Berg H, Finglas PM, Bates C: FLAIR Intercomparisons on serum and red cell folate. Internat J Vit Nutr Res 1994;64:288-93.
23. Chen MF, Hill JW, McIntyre PA: The folacin contents of food as measured by a radiometric microbiologic method. J Nutr 1983;113:2192-6.
24. DeSouza S, Eitenmiller R: Effects of different enzyme treatments on extraction of total folate from various foods prior to microbiological assay and radioassay. J of Micronutrient Analysis 1990; 7:37-57.
25. Finglas PM, Faure U, Southgate DAT: First BCR-intercomparison on the determination of folates in food. Food Chem 1993;46:199-213.
26. Gregory III JF, Day BPF, Ristow KA: Comparison of high performance liquid chromatographic, radiometric and *Lactobacillus casei* methods for the determination of folacin in selected foods. J Food Sci 1982;47 (5):1568-71.
27. Klein BP, Kuo CHY: Comparison of microbiological and radiometric assay for determining total folacin in spinach. J Food Sci 1981;46:552-4.
28. Lumley I: A preliminary report on the second BCR intercomparison of methods for the determination of folates in food. Laboratory of the Government Chemist (internal report), Teddington, UK 1993.
29. Österdahl BG, Johansson E: Comparison of radiometric and microbiological assay for the determination of folate in fortified gruel and porridge. Internat J Vit Nutr Res 1989;59:147-50.
30. Shane B, Tamura T, Stokstad ELR: Folate assay: a comparison of radioassay and microbiological methods. Clin Chim Acta 1980; 100:13-9.
31. Lawrance P: Individual folates in foodstuffs. MAFF Project 2B033, Report no. AS20/96/88. Laboratory of the Government Chemist, UK 1996.
32. Müller H: Neubestimmung und Bewertung der Folsäuregehalte von ausgewählten Lebensmitteln pflanzlicher und tierischer Herkunft. Ern-Umschau 1995;42:170-4.
33. Pfeiffer CM, Rogers LM, Gregory III JF: Determination of folate in cereal-grain food products using trienzyme extraction and combined affinity and reversed-phase liquid chromatography. J Agric Food Chem 1997;45:407-13.
34. Vahteristo LT, Ollilainen V, Varo P: HPLC determination of folate in liver and liver products. J Food Sci 1996;61:524-6.
35. Vahteristo LT, Ollilainen V, Varo P: Liquid chromatographic determination of folate monoglutamates in fish, meat, egg, and dairy

products consumed in Finland. J AOAC Internat 1997; 80:373-8.
36. Vahteristo L, Lehikoinen K, Ollilainen V, Varo P: Application of an HPLC assay for the determination of folate derivatives in some vegetables, fruits and berries consumed in Finland. Food Chem 1997;59:589-97.
37. Gregory III JF: Determination of folacin in foods and other biological materials. J AOAC 1984;67:1015-9.
38. Müller H: Bestimmung der Folsäure-Gehalte von Gemüse und Obst mit Hilfe der Hochleistungsflüssigkeitschromatographie (HPLC). Z Lebensm Unters Forsch 1993;196:137-41.
39. White DR: Determination of 5-methyltetrahydrofolate in citrus juice by reversed phase high-performance liquid chromatography with electrochemical detection. J Agric Food Chem 1990; 38:1515-8.
40. Vahteristo L, Ollilainen V, Koivistoinen PE, Varo P: Improvements in the analysis of reduced folate monoglutamates and folic acid in food by high-performance liquid chromatography. J Agric Food Chem 1996;44:477-82.
41. Wigertz K, Jägerstad M: Comparison of a HPLC and radioprotein-binding assay for the determination of folates in milk and blood samples. Food Chem 1995;54:429-36.
42. Witthöft C, Bitsch I: HPLC methods to analyse folate pattern in food and in human plasma as a precondition to evaluate availability of food folates by biokinetic methods. In: Schlemmer U, ed. Proceedings of Bioavailability '93, Part 2, Bundesforschungsanstalt für Ernährung, Karlsruhe, Germany 1993;431-5.
43. Uyeda K, Rabinowitz JC: Flourescence properties of tetrahydrofolate and related compounds. Anal Biochem 1963;6:100-8.
44. Wilson SD, Horne DW: Evaluation of ascorbic acid in protecting labile folic acid derivatives. Proc Natl Acad Sci USA 1983;80:6500-4.
45. Bitsch I, Hammes B, Schulz A: Thermische Zersetzung der Tetrahydrofolsäure und ihre Stabilisierung durch Sauerstoffausschluß. Lebensmittelchem Gerichtl Chem 1988;42:56-7.
46. Chen T-S, Cooper RG: Thermal destruction of folacin: effects of ascorbic acid, oxygen and temperature. J Food Sci 1979;44:713-6.
47. Day BPF, Gregory III JF: Thermal stability of folic acid and 5-methyltetrahydrofolic acid in liquid model food systems. J Food Sci 1983;48:581-7,599.
48. O'Broin JD, Temperley IJ, Brown JP, Scott JM: Nutritional stability of various naturally occurring monoglutamate derivatives of folic acid. Am J Clin Nutr 1975;28:438-44.
49. Schulz A, Wiedemann K, Bitsch I: Stabilisation of 5-methyltetrahydrofolic acid and subsequent analysis by reversed phase high-performance liquid chromatography. J Chromatogr 1985; 328:417-21.
50. Lankelma J, van der Kleijn E, Jansen MJT: Determination of 5-methyltetrahydrofolic acid in plasma and spinal fluid by high-performance liquid chromatography, using on-column concentration and electrochemical detection. J Chromatogr 1980;182:35-45.
51. Gounelle J-C, Ladjimi H, Prognon P: A rapid and specific extraction procedure for folate determination in rat liver and analysis by high-performance liquid chromatography with fluorimetric detection. Anal Biochem 1989; 176:406-11.
52. Pedersen JC: Comparison of γ-glutamyl hydrolase (conjugase; EC 3.4.22.12) and amylase treatment procedures in the microbiological assay for food folates. Br J Nutr 1988;59:261-71.
53. Bell JG: Microbiological assay of vitamins of the B group in foodstuffs. Lab Pract 1974; 23:235-42,252.
54. Lakshmaiah N, Ramasastri BV: Folic acid conjugase from plasma, part II: studies on the source of enzyme in blood. Int J Vit Nutr Res 1975;45: 194-200.
55. Lakshmaiah N, Ramasastri BV: Folic acid conjugase from plasma, part III: use of the enzyme in the estimation of folate activity in foods. Int J Vit Nutr Res 1975;45:262-72.
56. Halsted CH: Jejunal brush-border folate hydrolase – a novel enzyme. West J Med 1991; 155:605-9.
57. Horne DW, Krumdieck CL, Wagner C: Properties of folic acid γ-glutamyl hydrolase (conjugase) in rat bile and plasma. J Nutr 1981;111:442-9.
58. Silink M, Reddel R, Bethel M, Rowe PB: γ-glutamyl hydrolase (conjugase); purification and properties of the bovine hepatic enzyme. J Biol Chem 1975;250:5982-94.
59. Engelhardt R, Gregory III JF: Adequacy of enzymatic deconjugation in quantification of folates in foods. J Agr Food Chem 1990;38:154-8.
60. Cerna J, Kas J: Folacin in cereals and cereal products. In: Holas J, Kratochvril J, eds. Progress in Cereal Chemistry and Technology. Proceedings of the VIIth World Cereal and Bread Congress 1882, Part A, Prague, Czechoslovakia. Elsevier, New York 1983:501-5.
61. Martin JI, Landen WO, Soliman A-GM, Eitenmiller RR: Application of a tri-enzyme extraction for total folate determination in foods. J Assoc Off Anal Chem 1990;73:805-8.
62. Tamura T, Mizuno Y, Johnston KE, Jacob RA: Food folate assay with protease, a-amylase, and folate conjugase treatments. J Agric. Food Chem 1997;45:135-9.
63. Rebello T: Trace enrichment of biological folates on solid phase absorption cartridges and analysis by HPLC. Anal Biochem 1987;166:55-64.
64. Witthöft, C: Entwicklung von HPLC-Methoden zur Folatbestimmung in biologischem Material. Validierung und Methodenvergleich im Rahmen der Europäischen Gemeinschaftsforschung. Dissertation, University of Giessen 1998.
65. Selhub J, Ahmad O, Rosenberg IH: Preparation and use of affinity columns with bovine milk folate-binding protein (FBP) covalently linked to sepharose 4B. In: McCormick DB, Wright LD, eds. Methods in Enzymology (Vol. 66), Academic Press, New York 1980;686-90.
66. Seyoum E, Selhub J: Combined affinity and ion pair chromatographies for the analysis of food folate. J Nutr Biochem 1993;4:488-94.
67. Konings EJM: A validated liquid chromatographic method for determining folates in vegetables, milk powder, liver and flour. JAOAC 1999;82: 119-27.
68. Finglas PM, van den Berg H, de Froidmont-Görtz I: Improvements in the determination of vitamins in foods: method intercomparison studies and preparation of certified reference materials (CRMs). Food Chem 1996;57:91-4.
69. Vahteristo L, Finglas PM, Witthöft C, Wigertz K, Seale R, de Froidmont-Görtz I. Third EU MAT intercomparison study on food folate analysis using HPLC procedures. Food Chem 1996;57: 109-11.
70. Finglas PM, Wigertz K, Vahteristo L, Witthöft CM, Southon S, de Froidmont-Görtz I: Standardisation of HPLC techniques for the determination of naturally-occuring folates in food. Food Chem 1998;64:245-55.
71. Wigertz K, Hansen SI, Høier-Madsen M, Holm J, Jägerstad M: Effect of milk processing on the concentration of folate-binding protein (FBP), the folate-binding capacity and the retention of 5-methyltetrahydrofolate. Int J Food Sci Nutr 1996; 47:315-22.
72. Håkansson B, Jägerstad M, Öste R, Åkesson B, Jonsson L: The effect of various thermal processes on protein quality, vitamins and selenium content in whole-grain wheat and white flour. J Cereal Sci 1987;6:269-82.
73. Lucock MD, Priestnall M, Daskalakis I, Schorah CJ, Wild J, Levene MI. Non-enzymatic degradation and salvage of dietary folate: physicochemical factors likely to influence bioavailability. Biochem Mol Med 1995;55:43-53.
74. Malin JD: Total folate activity in Brussels sprouts: the effects of storage, processing, cooking and ascorbic acid content. J Food Technol 1977;12: 623-32.
75. Ristow KA, Gregory III JF, Damron BL: Effects of dietary fiber on the bioavailability of folic acid monoglutamate. J Nutr 1982;112:750-8.
76. VLAG (Advanced Studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences): Bioavailability '97. Book of Abstracts, 25.-28. May 1997, Wageningen, The Netherlands, 1997.
77. Schlemmer U ed: Proceedings of Bioavailability '93. Part 1, 2, 9.-12. May, Ettlingen, Schriftenreihe der Bundesforschungsanstalt für Ernährung, Karlsruhe, Germany, 1993.
78. Halsted CH: Intestinal absorption of dietary folates. In: Picciano MF, Stockstad REL, Gregory III JF, eds. Folic Acid Metabolism in Health and Disease, Wiley-Liss, N. Y. 1990:23-45.
79. Reisenauer AM, Krumdieck CL, Halsted CH: Folate conjugase: two separate activities in human jejunum. Science 1977;198:196-7.
80. Selhub J, Powell GM, Rosenberg IH: Intestinal transport of 5-methyltetrahydrofolate. Am J Physiol 1984;246:G515-20.
81. Abad AR, Gregory III JF: Determination of folate bioavailability with a rat bioassay. J Nutr 1987; 117:866-73.
82. Keagy PM, Oace SM: Rat bioassay of wheat bran folate and effects of intestinal bacteria. J Nutr 1989;119:1932-9.
83. Anonymus: Interspecies differences in folate metabolism. Nutr Rev 1992;50:116-8.
84. Natsuhori M, Shimoda M, Kokue E-I, Hayama T, Takahashi Y: Tetrahydrofolic acid as the principal congener of plasma folates in pigs. Am J Physiol 1991;261:R82-6.
85. Ristow KA, Gregory III JF, Damron BL: Thermal processing effects on folacin bioavailability in liquid model systems, liver and cabbage. J Agric Food Chem 1982;30:801-6.
86. Editorial: Long-term effects of ethanol consumption on folate status of monkeys. Nutr Rev 1983; 41:227-8.
87. Keagy PM: Animal assays for folate bioavailability: a critical evaluation. In: Picciano MF, Stockstad ELR, Gregory III JF, eds. Folic Acid Metabolism in Health and Disease, Wiley-Liss, New York 1990;139-50.
88. Clifford AJ, Jones AD, Bills ND: Bioavailability of folates in selected foods incorporated into amino acid-based diets fed to rats. J Nutr 1990; 120:1640-7.
89. Clifford, AJ, Heid MK, Peerson JM, Bills ND: Bioavailability of food folates and evaluation of matrix effect with bioassay. J Nutr 1991; 121:445-53.
90. Jägerstad M, Lindstrand K, Westesson, A-K: Hydrolysis of conjugated folic acid by pancreatic conjugase. Scand J Gastroenterol 1972;7:593-7.
91. Bhandari SD, Gregory III JF: Inhibition by selected food components of human and porcine intestinal pteroylpolyglutamate hydrolase activity. Am J Clin Nutr 1990;51:87-94.
92. Chandler CJ, Wang TTY, Halsted CH: Pteroylpolyglutamate hydrolase from human jejunal brush borders. J Biol Chem 1986;261:928-33.
93. Cuskelly GJ, McNulty H, Scott JM: Effect of increasing dietary folate on red-cell folate: implications for prevention of neural tube defects. Lancet 1996;347:657-9.
94. Sauberlich HE, Kretsch MJ, Skala JH, Johnson HL, Taylor PC: Folate requirements and metabolism in nonpregnant women. Am J Clin Nutr 1987;46:1016-28.
95. von der Porten A, Gregory III JF, Toth JP, Cerda JJ, Curry SH, Bailey LB: *In vivo* folate kinetics during chronic supplementation of human subjects with deuterium-labeled folic acid. J Nutr 1992;122:1293-9.
96. Hages M, Pietrzik K: Untersuchung zur Bioverfügbarkeit der Folsäure aus unterschiedlichen Dosierungen. Ern-Umschau 1987;34: 298-302.
97. Loew D, Eberhardt A, Heseker H, Kübler W: Zur Plasmakinetik und Elimination der Folsäure. Klin Wochenschr 1987;65:520-4.
98. Prinz-Langenohl R, Brönstrup A, Thorand B, Hages M, Pietrzik K: Availability of food folates in humans. J Nutr 1999;129:913-6.
99. Bailey LB, Barton LE, Hiller SE, Cerda JJ: Bioavailability of mono- and polyglutamyl-folate in human subjects. Nutr Rep Internat 1988;38: 509-18.
100. Brown JP, Scott JM, Foster FG, Weir DG: Ingestion and absorption of naturally occurring pteroylmonoglutamates (folates) in man. Gastroenterol 1973;64:223-33.
101. Dencker H, Jägerstad M, Westesson A-K: Portal

and peripheral plasma folates after ingestion of folic acid. Acta Hepato-Gastroenterol 1976; 23:140-3.
102. Keagy PM, Shane B, Oace SM: Folate bioavailability in humans: effects of wheat bran and beans. Am J Clin Nutr 1988;47:80-8.
103. Kirwan JR, Narebor EM: Absorption of large oral doses of 5-formyltetrahydrofolic acid in man. Br J Cancer 1976;34:671-3.
104. Perry J, Chanarin I: Intestinal absorption of reduced folate compounds in man. Brit J Haematol 1970;18:329-39.
105. Etienne M-C, Speziale N, Milano G: HPLC of folinic acid diastereomers and 5-methyltetrahydrofolate in plasma. Clin Chem 1993;39:82-6.
106. Kelly P, McPartlin J, Goggins M, Weir DG, Scott JM: Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. Am J Clin Nutr 1997;65:1790-5.
107. Schilsky RL, Choi KE, Vokes EE, Guaspari A, Guarnieri C, Whaling S, Liebner MA: Clinical pharmacology of stereoisomers of leucovorin during repeated oral dosing. Cancer 1989;63: 1018-21.
108. Pfeiffer CM, Rogers LM, Bailey LB, Gregory III JF: Absorption of folate from fortified cereal-grain products and of supplemental folate consumed with or without food determined by using a dual-label stable-isotope protocol. Am J Clin Nutr 1997;66:1388-97.
109. Tamura T, Stockstad ELR: The availability of food folate in man. Br J Haematol 1973;25:513-32.
110. Gregory III JF, Toth JP: Stable-isotopic methods for in vivo investigation of folate absorption and metabolism. In: Picciano MF, Stockstad ELR, Gregory JF, eds. Folic Acid Metabolism in Health and Disease, Wiley-Liss, New York 1990;151-69.
111. Stites TE, Bailey LB, Scott KC, Toth JP, Fisher WP, Gregory III JF: Kinetic modeling of folate metabolism through use of chronic administration of deuterium-labeled folic acid in men. Am J Clin Nutr 1997;65:53-60.
112. Bates CJ, Heseker H: Human bioavailability of vitamins. Nutr Res Rev 1994;7:93-127.
113. Said HM, Horne D, Wagner D: Absorption of dietary (milk) folate. Gastroenterology 1986; Abstract no. 1612.
114. Tani M, Iwai K: Some nutritional effects of folate-binding protein in bovine milk on the bioavailability of folates to rats. J Nutr 1984; 114:778-85.
115. Tani M, Fushiki T, Iwai K: Influence of folate-binding protein from bovine milk on the absorption of folate in the gastro-intestinal tract of rats. Biochim Biophys Acta 1983;757:274-81.
116. Swiatlo N, O'Connor DL, Andrews J, Picciano MF: Relative folate bioavailability from diets containing human, bovine and goat milk. J Nutr 1990;120:172-7.
117. Ek J, Magnus EM: Plasma and red blood cell folate in breast fed infants. Acta Paediatr Scand 1979; 68:239-43.
118. Ek J, Magnus EM: Plasma and red blood cell folacin in cows' milk-fed infants and children during the first 2 years of life: The significance of boiling pasteurized cows' milk. Am J Clin Nutr 1980;33:1220-4.
119. Livsmedelsverket: Livsmedelstabell, Sverige 1996.
120. Holland B, Unwin ID, Buss DH: Vegetables, herbs and spices. The fifth supplement to McCane & Widdowson's The Composition of Foods (ISBN 0-85186-376-0) 1991.
121. Souci SW, Fachmann W, Kraut H: Die Zusammensetzung der Lebensmittel, Nährwert-Tabellen. Food Composition and Nutrition Tables (1989/90). Edited by Deutsche Forschungsgemeinschaft für Lebensmittelchemie, compiled by Scherz H, Senser F, (5th edition), Medpharm Scientific Publ., Stuttgart, 1994.
122. Levnedsmiddelstyrelsen: Levnedsmiddeltabeller, Danmark 1996.
123. Müller H: Die Bestimmung der Folsäure-Gehalte von Lebensmitteln tierischer Herkunft mit Hilfe der Hochleistungsflüssigkeitschromatographie (HPLC). Z Lebensm Unters Forsch 1993;196: 518-21.
124. Petersen MA: Influence of sous vide processing, steaming and boiling on vitamin retention and sensory quality in broccoli florets. Z Lebensm Unters Forsch 1993;197:375-80.
125. Williams PG, Ross H, Brand Miller JC: Ascorbic acid and 5-methyltetrahydrofolate losses in vegetables with cook/chill or cook/hot-hold food service systems. J Food Science 1995;60:541-6.
126. Vahteristo L, Lehikoinen K, Ollilainen V, Koivistoinen PE, Varo P: Ovenbaking and frozen storage affect folate vitamer retention. Lebensm-Wiss u -Technol 1998;31:329-33.
127. Wigertz K, Svensson UK, Jägerstad M: Folate and folate-binding protein content in dairy products. J Dairy Res 1997;64:239-52.
128. Hoppner K, Lampi B: Folate retention in dried legumes after different methods of meal preparation. Food Science Internat 1993;26:45-8.
129. Lane HW, Nillen JL, Kloeris VL: Folic acid content in thermostabilized and freeze-dried space shuttle foods. J Food Science 1995; 30:538-40.
130. Müller H, Diehl JF: Effect of ionizing radiation on folates in food. LWT 1995;29:187-90.
131. Rosenberg IH, Godwin HA: Inhibition of intestinal γ-glutamyl carboxypeptidase by yeast nucleic acid: an explanation of variability in utilisation of dietary polyglutamyl folate. J Clin Invest 1971;50:78a.
132. Tamura T, Shin YS, Buehring KU, Stokstad ELR: The availability of folates in man: effect of orange juice supplement on intestinal conjugase. Brit J Haematology 1976;32:123-33.
133. Bailey LB, Cerda JJ, Bloch BS, Busby MJ, Vargas L, Chandler CJ, Halsted CH: Effect of age on poly- and monoglutamyl folacin absorption in human subjects. J Nutr 1984;114:1770-6.
134. Gregory III JF, Bhandari SD, Bailey LB, Toth JP, Baumgartner TG, Cerda JJ: Relative bioavailability of deuterium-labeled monoglutamyl and hexaglutamyl folates in human subjects. Am J Clin Nutr 1991;53:736-40.
135. Gregory III JF, Bhandari SD, Bailey LB, Toth JP, Baumgartner TG, Cerda JJ: Relative bioavailability of deuterium-labeled monoglutamyl tetrahydrofolates and folic acid in human subjects. Am J Clin Nutr 1992;55:1147-53. □