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ScienceDirect
Food Chemistry 101 (2006) 1095–1107
Food Chemistry
www.elsevier.com/locate/foodchem

Retention of folates in cooked, stored and reheated peas, broccoli and potatoes for use in modern large-scale service systems

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Received 11 November 2005; received in revised form 20 February 2006; accepted 4 March 2006

Abstract

This study aimed to evaluate retention of folate in vegetables caused by different processes used in modern large-scale service systems and the food industry. The concentration of folates present in raw samples of peas, broccoli and potatoes was measured during different cooking methods, warm and cold holding and reheating. The main folate forms in vegetables, tetrahydrofolates and 5-methyltetrahydrofolates, were analysed using a validated high-performance liquid chromatography (HPLC) method.

This study shows the following decreasing order in folate retention, on DM basis, compared to raw potatoes during heat-processing: sous-vide (103%), boiling (72–59% (unpeeled and peeled)) and oven-baking (63%) and compared to raw green peas during heat processing: boiling (77%), microwaving (75%), steam boiling (73%) and blanching (71%). However, only blanching of peas, boiling of potatoes and oven-baking of unpeeled potatoes caused significant reduction. Storage at various temperatures and length of times followed by reheating caused no further significant losses of total folate.

Keywords: Folate; Vegetables; Food processing; Food analysis; High-performance liquid chromatography; Large-scale service systems

1. Introduction

Naturally occurring folate is present in a wide range of foods, especially leafy green vegetables, legumes, yeast and liver (Holland et al., 1991). The Europeans have a high consumption of bread and potatoes, which together with green vegetables, are the major contributors of folate from the diet (Becker, 2000; de Bree, van Dusseldorp, Brouwer, van het Hof, and Steegers-Theunissen, 1997).

According to the most recent edition of Nordic nutritional recommendations (2004) the daily intake of folate should be 300 μg for adults and 400 μg for fertile women. Pregnant and lactating women are advised to consume a daily intake of 500 μg folate (Becker et al., 2004). Research on folate intake in the Nordic countries shows that Swedish women and men have an overall folate intake of 217 and 232 μg/day respectively (Becker, 1999) whereas a Danish study shows a mean folate intake of 249 and 304 μg/day, respectively (Andersen et al., 1996).

Since folate is an unstable nutrient, the amount of folate available in processed and stored food could be considerably lower than that in raw food. Thus, it is important that the food industry consider how processing in different food service systems affects the content of folate in foods. In large-scale service systems, for example hospitals, knowledge of the content of folate in food can be critical because of special or increased requirements for many patients. This must be taken into consideration when calculating the real total folate intake from food.

Methods of processing used in small- and large-scale service systems often include steam-boiling, boiling, oven baking and microwave cooking. Loss of folate during heat treatments, such as boiling, blanching and steam boiling has been shown to be substantial (Hawkes and Villota,

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doi:10.1016/j.foodchem.2006.03.009

1989; Selman, 1994), whereas other forms of processing such as oven baking and microwave cooking have been found preferable in terms of folate retention (Augustin, Marousek, Tholen, and Bertelli, 1980). These earlier published studies show that losses during processing occur simply by leaching of folates into surrounding water used for washing, blanching or cooking. This is also confirmed by studies that measure both the folate content in the drained food samples and in the remaining water (juice) after processing (Dang, Arcot, and Shrestha, 2000).

Processing technologies used in the food industry, such as blanching and sous-vide have also been studied. Blanching of broccoli, spinach and beetroot has been reported to cause substantial losses of folate and the extent seems to depend on the time and amount of water used (DeSouza and Eitenmiller, 1986; Jägerstad, Jastrebova, and Svensson, 2004; Puupponen-Pimiä et al., 2003). In contrast, results from sous-vide processing of broccoli show less reduction of folate than in methods involving direct contact between the food and water (Petersen, 1993).

There is an increasing consumption of ready-made food, prepared both domestically and in large-scale service systems. These pre-cooked foods are often held warm and reheated when used in large-scale food service systems. Few studies have been published on losses of folate caused by warm holding and reheating. However, one study reported that reheating of seven different cooked vegetables resulted in less retention of folate after one day of cooled storage than those held warm after processing (Williams, Ross, and Miller, 1995). Losses during cooled storage followed by microwave reheating of potatoes have also been reported (Augustin et al., 1980). In contrast, storage of foods by freezing does not seem to affect the concentration of folate in spinach, potatoes and broccoli (Phillips et al., 2005; Puupponen-Pimiä et al., 2003).

This study aimed to provide information on how processing could influence the folate content in samples of broccoli, potatoes and industrial processed peas subjected to traditional cooking systems (boiling and oven baking) and minimal processing (microwaving, sous-vide and steam boiling) under conditions used in modern large-scale service systems. These vegetables were selected since they are amongst the major contributors of folate in the Nordic diet and commonly used in the food industry and small- and large-scale service systems. An important aspect was to study folate retention after warm holding, storage in refrigerator overnight and re-heating of cooked vegetables, since this is becoming more common and data on folate stability for these steps are scarce. Folate were analysed using a validated high-performance liquid chromatography (HPLC) method enabling quantification of different folate forms (Jastrebova, Witthöft, Grahn, Svensson, and Jägerstad, 2003). This is in contrast to most studies where data on folates in processed foods are based on microbiological assays (MA), which do not discriminate between different folate forms known to vary in stability.

2. Materials and methods

2.1. Vegetable samples

2.1.1. Processing of peas

Samples of green peas (*Pisum sativum*) were provided from Findus "green pea line" in Bjuv, Sweden. Green pea samples were collected in duplicates at six times: as raw, after transportation with pumping water, after sorting to medium sized peas, before and after blanching (98 °C/2 min), after brine grading, and after freezing (−18 °C). The cooking procedures (in duplicate) were performed in the laboratory at the Swedish University of Agricultural Sciences in Uppsala, Sweden. *Frozen, blanched and brine graded peas* (Fig. 1A) from Findus were *boiled* in a covered saucepan (120 g in 1 L tap water; 3 min) or*steam-boiled* (120 g in 0.3 L tap water; 4 min). The water in the saucepan and steamer was boiling prior to the addition of vegetables. Green peas were also *microwaved* (120 g in 0.07 L tap water; 4 min, 750 W). After 2 min, there was a short break to stir the sample. The peas were further drained and cooled on ice. After boiling, steam boiling and microwave cooking the green peas were stored in 4 °C for 24 h before *reheated* in a steam-boiler for 3 min. Pea samples were kept frozen (−20 °C) until folate analysis at the Swedish University of Agricultural Sciences, Uppsala, and dry matter (DM) determination at AnalyCen, Lidköping, Sweden.

2.1.2. Processing of broccoli

Samples of raw broccoli (*Brassica oleracea*, L. *botrytis asparagoides*, cultivar: Lord) were obtained from a local vegetable distributor (BAMA, Stavanger, Norway) on the evening prior to processing of the samples and stored overnight at 4 °C. For processing, inflorescence and some of the stem was used for broccoli. All processes, with duplicate samples, were performed in natural light while cold storage of samples occurred in rooms without lights.

Raw broccoli was *boiled* (Fig. 1B) in a covered saucepan (50 ± 2 g in 1 L tap water; 4 min) or *steam-boiled* (Fig. 1B) in a steam oven (50 ± 2 g at 100 °C; 5 min). The water in the saucepan was boiling prior to the addition of vegetables.

Samples of boiled and steam-boiled broccoli were *cooled* for 1 h at room temperature (20 °C) or *held warm* on a heating unit at 60 °C for 2 h (1 h covered to protect from light) respectively, before cooling for 24 h at 4 °C. All samples were further *reheated* in a steam oven for 5 min at 100 °C.

Raw broccoli was also *blanched* (Fig. 1B) in a saucepan (50 ± 2 g in 1 L tap water; 5 s) before cooling in ice water until temperature reaching 4 °C. The water was boiling in the saucepan prior to addition of vegetables. The samples of blanched broccoli were stored at −20 °C for 24 h before *reheating* in a steam oven for 5 min at 100 °C. Raw samples of blanched broccoli was *sous-vide processed* (50 ± 2 g at 90 °C, 99% vacuum; 7 min) (Fig. 1B) before cooling in ice water until temperature reaching 4 °C.

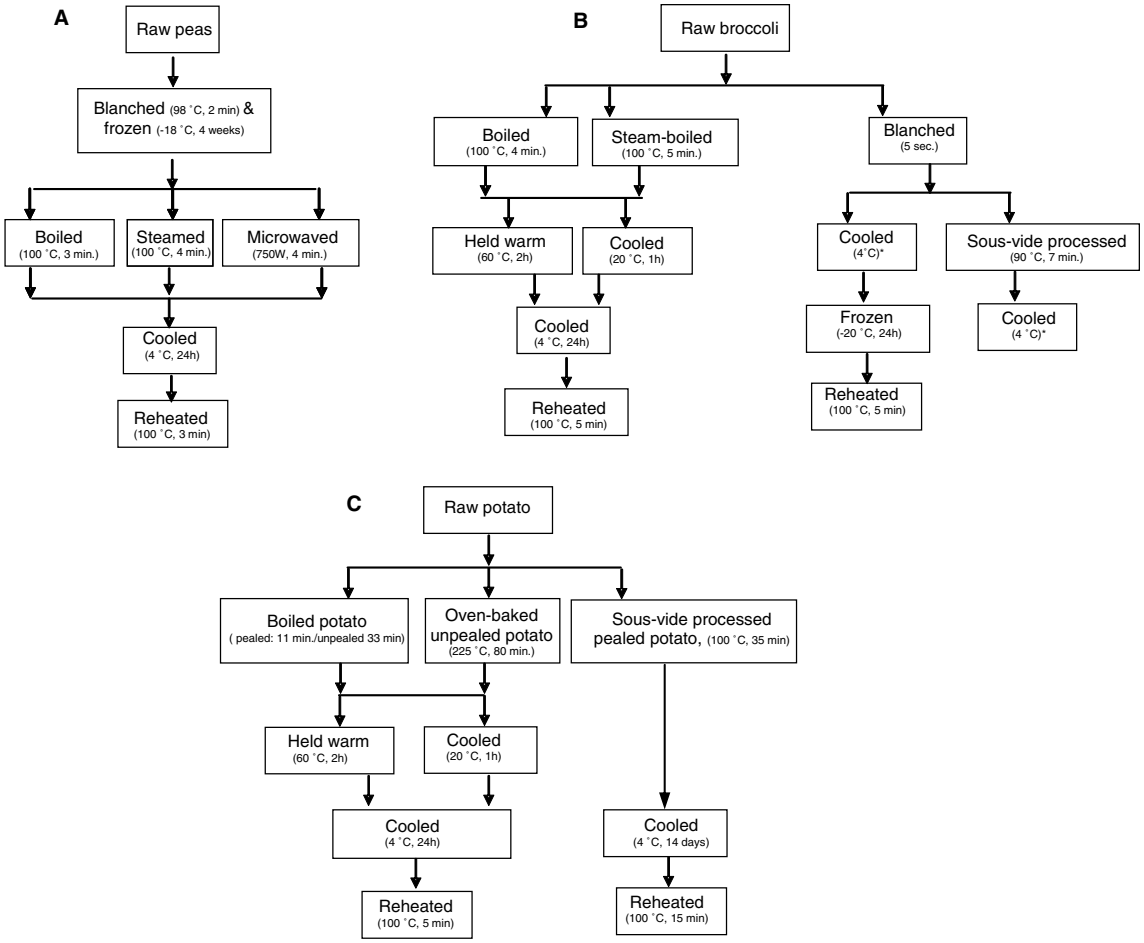

Fig. 1. Flow chart of the processes studied. (A) Blanched and frozen green peas, (B) raw broccoli and blanched broccoli and (C) unpeeled and pealed potatoes.

Samples of raw and processed (from every stage of processing) of broccoli were vacuum-packed and deep-frozen overnight at −40 °C and further stored at −20 °C until folate analysis and DM determination.

2.1.3. Processing of peeled potatoes and unpeeled potatoes

Potatoes (*Solanum tuberosum*, cultivar: Beate) were purchased from BAMA (Stavanger, Norway) and stored at room temperature during the night prior to processing at Norconserv AS in Stavanger, Norway. All processes were performed on duplicate samples and prepared in natural light while cold storage of samples occurred in rooms without lights.

Raw unpeeled potatoes (135 ± 10 g) and peeled potatoes (95 ± 10 g) were *boiled* (Fig. 1C) in covered saucepans containing 1 L tap water. Due to different size, peeled potatoes and unpeeled potatoes were boiled for 11 and 33 min respectively to obtain the same core temperature. The water was boiling in the saucepans prior to addition of the samples. The unpeeled potatoes (102 ± 7 g) were also *oven-baked* (Fig. 1C) at 225 °C for 80 min. Both boiled and oven-baked potatoes were *held warm* on a heating unit at 60 °C for 2 h (including 1 h hour covered to protect from light) or *cooled* in 1 h at room temperature (20 °C). Thereafter all samples were cooled for 24 h in 4 °C before *reheating* in a steam oven for 15 min.

Peeled potatoes (74 ± 2 g) were *sous-vide processed* (Fig. 1C) for 35 min (99% vacuum) at 100 °C. Samples of sous-vide processed potatoes were stored for 14 days at 4 °C before reheating in a steam oven for 15 min.

Samples of raw and processed (from every stage of processing) potatoes were vacuum-packed and deep-frozen overnight at −40 °C and further stored at −20 °C until folate analysis and DM determination.

2.1.4. Certified reference material CRM 485

A lyophilized mixed vegetable sample, CRM 485, with certified folate content, was obtained from The Institute for Reference Materials and Measurements (Geel, Belgium) and stored as vacuum-packed sub-samples (2 g) at −80 °C until analysis.

2.2. Dry matter

Dry matter of raw and processed peas, broccoli and potatoes were determined in duplicate at 102–105 °C for

16–18 h by the accredited laboratory, AnalyCen in Lidköping, Sweden, according to the NMKL method nr. 23 (NMKL, 1991).

2.3. Folate analysis

2.3.1. Preparation of enzymes, reagents and samples

All reagents were of analytical grade, except acetonitrile and methanol, which were of HPLC grade. Water was purified using Milli-Q system (Millipore, USA). All chemicals were purchased from Merck (Darmstadt, Germany) except 2,3-dimercapto-ethanol (BAL) which was obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.3.2. Folate standards

(6S)-5-CH_3-H_4folate, sodium salt, (6S)-H_4folate, sodium salt and (6S)-5-HCO-H_4folate, sodium salt were donated by Eprova AG, Schaffhausen, Switzerland. The purity of the folate standards was checked in phosphate buffer, pH 7.0 according to van den Berg, Finglas, and Bates (1994) and purities calculated using molar extinction coefficient reported by Eitenmiller and Landén (1999). The standard stock solutions, of 200 μg/ml (corrected for purity) were prepared under subdued light in 0.1 M phosphate buffer pH 6.1 containing 1% ascorbic acid (AA) and 0.1% 2-mercaptoethanol (MCE). Aliquots of standard stock solutions were placed in separate tubes, flushed with nitrogen and stored in −80 °C for maximum 90 days. The calibration solutions were prepared immediately before use by dilution of the stock solutions with elution buffer (0.1 M sodium acetate containing 10% (w/v) sodium chloride, 1% (w/v) ascorbic acid and 0.1% (v/v) BAL).

2.3.3. Enzyme preparation

Thermostable α-amylase solution (E-BLAAM, 3000 U/ml) was obtained from Megazyme International, Cork, Ireland and used for sample pre-treatment of potatoes without additional preparation. Rat serum (RS) (Scanbur Sollentuna, Sweden) was used as folate conjugase (γ-glutamyl hydrolase) source. RS was dialysed to remove endogenous folates at 4 °C during stirring in three steps (40 min each) using 800 ml of the 0.05 M phosphate buffer pH 6.1 containing 0.1% MCE in each step as earlier described (Patring, Jastrebova, Hjortmo, Andlid, and Jägerstad, 2005a). The dialysed RS was stored in small portions (1 ml) at −80 °C.

2.3.4. Preparation of food samples

All processes of broccoli, peas and potatoes were performed in duplicates, and these samples were all analysed for their folate content. Samples were protected against folate oxidation throughout the preparation process by nitrogen, subdued light and cooling on ice after heating. The florets were cut from each part of the broccoli head. The raw potato was cut in small pieces while the processed potatoes were mashed. The samples (3 g) were homogenized with an Ultra-Turrax T25 homogenizer (IKA, Germany) in freshly prepared extraction buffer: 15 ml of phosphate buffer, pH 6.1, containing 1% (w/v) AA and 0.1% (v/v) BAL. For CMR 485, 0.3 g was homogenized in 15 ml in extraction buffer. Homogenates were heated for 12 min in a boiling water bath and centrifuged (27,000*g*, 15 min at 4 °C) before dilution with 25 ml extraction buffer. For deconjugation of folate polyglutamates to monoglutamates, RS (100 μl for peas and broccoli samples and 150 μl for potato samples) was added to 3.2 ml supernatant and the sample was incubated in a shaking water bath at 37 °C for 3 h. However, preparation of potato samples also included treatment with 40 μl α-amylase prior to the deconjugation step (Johansson, Witthöft, Bruce, and Jägerstad, 2002) since the recovery of folates from potato samples was slightly higher after amylase treatment.

For enzyme inactivation, the samples were heated in boiling water for 5 min and subsequently centrifuged (12,000*g*, 8 min at 4 °C). The supernatant were frozen below −20 °C before sample clean-up.

2.3.5. Sample clean-up

Purification of sample extracts was carried out prior to HPLC analysis by solid-phase extraction (SPE) on strong anion exchange (SAX) Isolute cartridges (500 mg, International Sorbent Technology, UK) as described by Nilsson, Johansson, Yazynina, Strålsjö, and Jastrebova (2004). A Visiprep SPE vacuum Manifold (Supelco, USA) was used for elution under reduced pressure. The SAX cartridges were conditioned with 5 ml methanol and 5 ml water. Subsequently, 2.5 ml of the sample extracts were applied with a flow rate of 1 drop/s. To remove the matrix interfering components, the cartridges were rinsed with 5 ml water before the elution of retained folates was performed with freshly prepared 0.1 M sodium acetate containing 10% (w/v) sodium chloride, 1% (w/v) ascorbic acid and 0.1% (v/v) BAL. The first portion (0.7 ml) of eluate was discarded and the second portion (3.0 ml) was weighed and collected for HPLC analysis.

2.3.6. HPLC analysis

HPLC analysis was performed according to Jastrebova et al. (2003) using an AGILENT 1100 system. The folates were separated on a Zorbax SB C_8 column (5 μm, 150 × 4.6 mm) and a matching C_8 guard column (5 μm, 12.5 × 4.6 mm). Gradient elution was performed with acetonitrile and 30 mM potassium phosphate buffer, pH 2.3 at a flow rate of 0.4 ml/min.

The injection volume was 20 μl and the temperature was set to 23 °C and 8 °C in the column compartment and the autosampler respectively.

The gradient started at 6% (v/v) acetonitrile and was maintained isocratically for the first 5 min. before it was increased linearly to 25% within 20 min. The total run time was 33 min.

The excitation wavelength in the fluorescence detector was 290 nm whereas the emission wavelength was set to 360 nm. The diode-array detector (DAD) wavelength was

set to 290 nm. Peaks were identified by retention times. The identity of individual folate forms was confirmed by comparison of ratio sample peaks from fluorescence and DAD detectors to ratio of standard peaks as well as fluorescence and diode array spectra.

2.3.7. Quantification

Quantification was based on external standard method. The peak area was plotted against concentration and least-square regression analysis was used to fit lines to the data. A multilevel calibration curve was used ($n = 7$) and the amount of each folate form was calculated in its free acid form.

The enzyme preparations contained a small amount of endogenous 5-CH_3-H_4folate, and the sample was therefore corrected by subtraction of the 5-CH_3-H_4folate concentrations in blank sample.

2.3.8. Recovery tests

Recovery tests were performed by adding known amounts of H_4folate and 5-CH_3-H_4folate to CRM 485 and pooled pea samples before extraction. The recovery (R) was calculated as $R = (C_{found} - C_{sample})/C_{added}$, where C_{found} is the concentration in spiked sample, C_{sample} is the concentration in the sample prior to spiking and C_{added} is the concentration of the added standard.

2.4. Statistical analysis

The results were presented as mean values from duplicates, and the difference between two separate values for a duplicate was less than 10% for all samples from green peas and potatoes and less than 15% for all samples from broccoli. All results are based on both dry (DM) and fresh weight (FW). Statistical analysis were performed with Tukey's pairwise comparison ($\alpha = 0.05$) using software Minitab release 14 (Minitab Ltd., Coventry, UK) and significant variations were considered from $p < 0.05$.

3. Results

3.1. Folates in raw, heat-processed, stored and reheated samples of green peas

The concentrations of total and individual forms of folate in raw, blanched, heat-processed, stored and reheated green peas are presented on both dry matter (DM) and fresh weight (FW) in Table 1. Total folate is measured as the sum of H_4folate and 5-CH_3-H_4folate, expressed as folic acid. H_4folate constituted less than 7%. The DM content of green peas measured before and after blanching and freezing in industrial scale varied between 20% and 22%. After boiling, steam boiling and microwave heating, the DM content varied between 19% and 26%. Cooling and reheating resulted in DM contents of 21–26%, implying that the moisture content varied only between 74% and 81%.

Transporting of raw peas to the industrial plant (Findus) by pumping in water resulted in folate retention of 87% on DM basis (process 1). Blanching (98 °C/2 min) of medium size peas resulted in a significantly reduced ($p < 0.05$) retention (64%) compared with medium size peas before processing on both FW and DM basis.

Heat processing of green peas resulted in the following retention of total folate calculated from the frozen, blanched and brine graded green peas: boiling (87%) steam boiling (83%) and microwaving (84%, Fig. 2). None of these changes in retention were significant on DM basis; only the boiling procedure, on FW basis, resulted in a significant ($p < 0.05$) reduction in folate content.

Table 1
Mean content of individual and total folate in raw, commercially processed (process 1) cooked, stored and reheated (process 2) green peas[a]

Process	DM (g/100 g)	Folate content (μg/100 g DM)			Folate content (μg/100 g FW)
		H_4folate[b]	5-CH_3-H_4folate[c]	Total folate[d]	Total folate[d]
Commercially processed peas (1)					
Raw	20.8	12.8	226	230	47.9
Raw, after pumping in water	20.5	11.3	197	201	41.2
Medium size	20.6	16.4	248	254	52.5
Medium size, blanched	21.9	11.8	158	164	36.0
Blanched and brine graded	20.8	11.8	199	203	42.2
Blanched and brine graded peas processed with methods used in large-scale service systems (2)					
Frozen, after pumping in water	20.5	3.0	208	203	41.7
Boiled	18.9	8.5	175	177	33.4
Boiled, cooled and reheated	20.6	4.9	140	139	29.2
Steam-boiled	23.5	11.0	165	169	39.7
Steam-boiled, cooled and reheated	22.8	3.8	160	158	36.0
Microwaved	26.4	8.5	170	172	45.3
Microwaved, cooled and reheated	26.4	5.4	168	167	44.2

DM = dry matter; FW = fresh weight.
[a] All folate results are means of duplicates. The difference between two separate values for a duplicate was less than 10% for all samples.
[b] H_4folate = tetrahydrofolate.
[c] 5-CH_3-H_4folate = 5-methyltetrahydrofolate.
[d] Total folate = measured as the sum of H_4folate and 5-CH_3-H_4folate, expressed as folic acid.

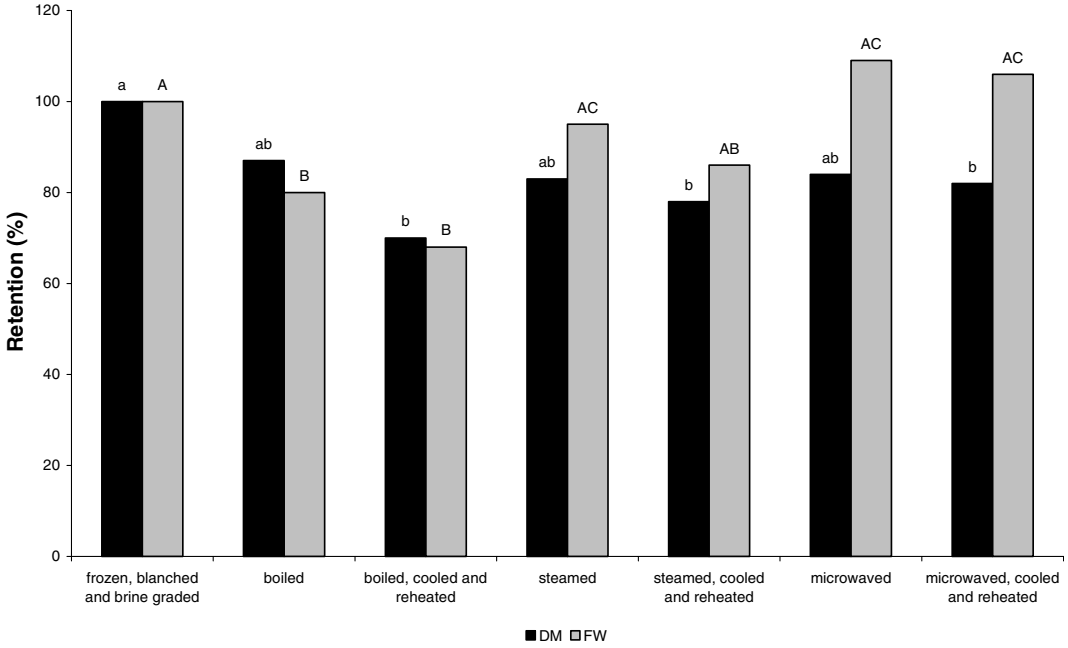

Fig. 2. Retention (%) of folate in green peas. Values are means of duplicates, and the difference between two separate values for a duplicate was less than 10% for all samples. Different letters show statistically significantly ($p < 0.05$) differences in folate content after different processes compared with frozen, blanched and brine graded peas (process 2, Table 1). DM: dry matter, FW: fresh weight.

Cooling and reheating of boiled, steam boiled or microwaved green peas gave the following retention of total folate: 79%, 93% and 97% on DM basis, respectively compared with cooked green peas. None of these results showed statistical significance.

Distribution of 5-CH_3-H_4folate seems to change individually under processing. Green peas from field to freeze-storage after blanching at the industry plant resulted mainly in reduced retention of 5-CH_3-H_4folate (process 1). However, this reduction was not statistical significant. Boiling, steam boiling and microwave cooking of peas resulted in a significant ($p < 0.05$) decreased retention of 5-CH_3-H_4folate compared to raw peas. However, only steam boiling caused a significant reduction of 5-CH_3-H_4folate compared to frozen, blanched and brine graded peas (Table 1).

3.2. Folates in raw, heat-processed, stored and reheated and reheated samples of broccoli

The folate contents (calculated on DM and FW) in raw and processed broccoli obtained with the reported method

Table 2
Mean content of individual and total folate in raw cooked, stored and reheated broccoli[a]

Process	DM (g/100 g)	Folate content (μg/100 g DM)			Folate content μg/100 g FW)
		H_4folate[b]	5-CH_3-H_4folate[c]	Total folate[d]	Total folate[d]
Raw	13.3	147	748	866	115
Blanched	10.2	148	1022	1130	115
Blanched, cooled and reheated	10.5	125	862	953	100
Blanched and sous-vide processed	9.6	116	920	1000	96.0
Steamed	12.9	123	1139	1219	157
Steamed and held warm	12.6	120	1077	1157	146
Steamed, held warm, cooled and reheated	13.6	103	982	1048	142
Steamed, cooled and reheated	12.9	112	1048	1118	144
Boiled	8.6	111	934	1009	86.8
Boiled and held warm	8.9	121	917	999	88.8
Boiled, held warm, cooled and reheated	9.3	110	895	968	89.8
Boiled, cooled and reheated	8.8	90	815	870	76.4

DM = dry matter; FW = fresh weight.
[a] All folate results are means of duplicates. The difference between two separate values for a duplicate was less than 15% for all samples.
[b] H_4folate = tetrahydrofolate.
[c] 5-CH_3-H_4folate = 5-methyltetrahydrofolate.
[d] Total folate = measured as the sum of H_4folate and 5-CH_3-H_4folate, expressed as folic acid.

are shown in Table 2. Total folate is expressed as folic acid and is the sum of H_4folate and 5-CH_3-H_4folate, where H_4folate constituted 10–20%. Moreover the DM content is shown in Table 2. For raw broccoli DM was approximately 13%. Blanching, steaming, boiling and sous-vide processing resulted in DM content between 8.5% and 13% with a little change after warm holding, cooling and reheating. Thus, as for green peas, the moisture content varied only moderately – between 87% and 91.5%.

Cooked samples of broccoli showed the following decreasing order of folate content on DM basis: steam boiling (1219 μg/100 g), blanching (1132 μg/100 g), boiling (1010 μg/100 g), and sous-vide processing (1000 μg/100 g) (Table 2). Compared with raw broccoli, blanching and steaming resulted in a significant ($p < 0.05$) increase in folate content of 31% and 41%, respectively on DM basis, whereas corresponding figures based on FW values showed no folate increase after blanching and 37% increase ($p < 0.05$) after steaming.

Broccoli was held warm for 2 h at 60 °C after steam-boiling and boiling, and this treatment resulted in a folate retention (on DM basis) of 95% and 99%, whereas further reheating of the warm held samples of steam boiled and boiled broccoli showed a retention of 86% and 96%, compared with steamed and boiled samples. These minor losses in the folate content were not significant.

Cooling and reheating of blanched, steam boiled and boiled broccoli resulted in folate retention of 84%, 92% and 87% on DM basis, respectively (Fig. 3). None of these treatments showed a significant decrease in folate retention.

3.3. Folates in raw, cooked, stored and reheated samples of peeled and unpeeled potatoes

The folate content in raw, cooked, stored and reheated peeled and unpeeled potatoes is presented on both dry matter (DM) and fresh weight (FW) in Table 3. Folate content in potatoes refers only to 5-CH_3-H_4folate because no trace of H_4folate was detected in the analysed samples. The DM content of raw potatoes was close to 26%. Boiling and sous-vide processing of peeled potatoes resulted in a DM content of 21% and 20%, respectively. Oven baking and boiling of unpeeled potatoes resulted in a DM content of 35% and 22% respectively.

Overall, the moisture content varied between 75 and 80% for cooked potatoes, except for oven baking that resulted in 65% moisture.

Boiling significantly ($p < 0.05$) reduced folate retention in both peeled potatoes (59% on DM basis) and unpeeled potatoes (72% DM) when compared to raw potatoes (Figs. 4 and 5). This means that there were no significant differences in folate retention between unpeeled and peeled potatoes on DM basis, after boiling (Fig. 4). Sous-vide processing of peeled potatoes resulted in a somewhat higher, but not statistical significant, concentration of folate compared with raw potatoes (103% on DM) (Fig. 4). However, the retention of folate was significantly ($p < 0.05$) higher (58% on DM basis) after sous-vide processing, compared to the boiling of peeled potatoes (Fig. 4). A significantly reduced folate content ($p < 0.05$) was shown after oven baking of unpeeled potatoes (63% on DM basis) when compared with raw potatoes (Figs. 4

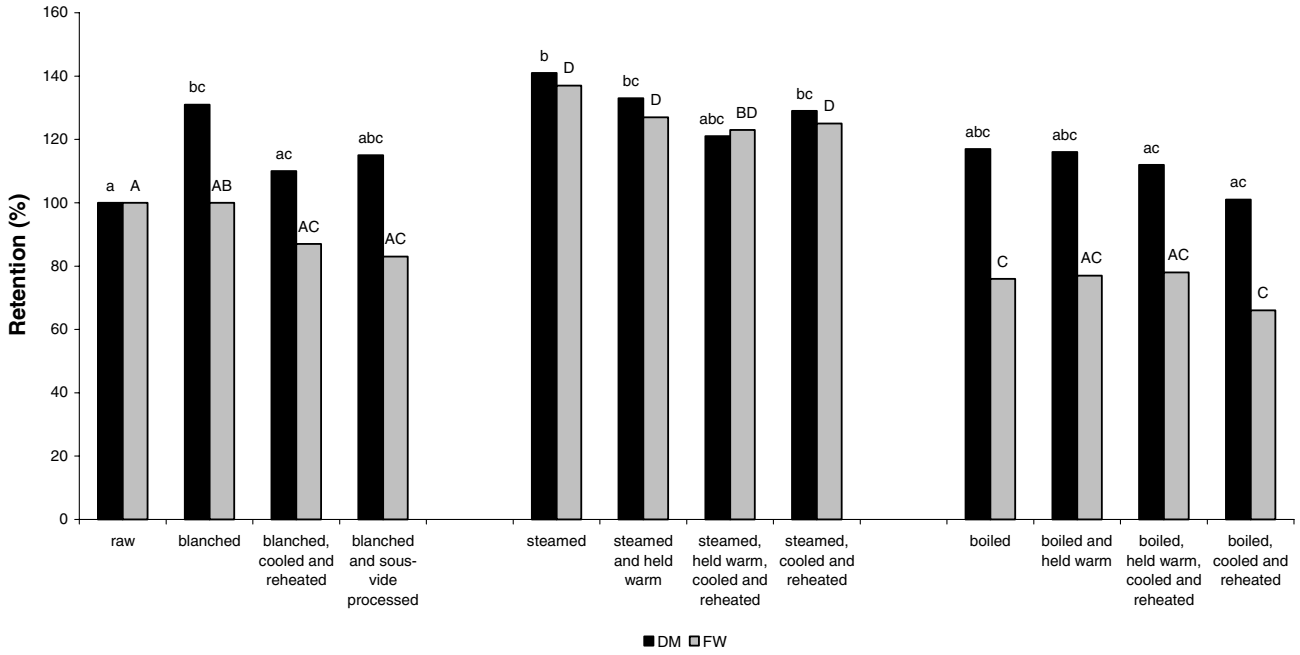

Fig. 3. Retention (%) of folate in broccoli. Values are means of duplicates, and the difference between two separate values for a duplicate was less than 15% for all samples. Different letters show statistically significantly ($p < 0.05$) differences in folate content after processing of broccoli compared with raw broccoli. DM: dry matter, FW: fresh weight.

Table 3
Mean content of individual and total folate in raw cooked, stored and reheated potatoes[a]

Process	DM (g/100 g)	Folate content (μg/100 g DM)			Folate content (μg/100 g FW)
		H_4folate[b]	5-CH_3-H_4folate[c]	Total folate[d]	Total folate[d]
Peeled potato					
Raw	25.8	nd	65.5	63.2	16.3
Boiled	20.9	nd	38.8	37.3	7.8
Boiled and held warm	22.6	nd	61.1	58.8	13.3
Boiled, held warm, cooled and reheated	21.8	nd	37.5	36.2	7.9
Boiled, cooled and reheated	21.1	nd	38.6	37.1	7.8
Sous-vide processed	19.9	nd	67.4	64.9	12.9
Sous-vide processed, cooled and reheated	23.6	nd	75.1	72.4	17.1
Unpeeled potato					
Raw	25.8	nd	65.4	63.2	16.3
Boiled	22.3	nd	47.5	45.7	10.2
Boiled and held warm	22.6	nd	43.8	42.2	9.5
Boiled, held warm, cooled and reheated	21.7	nd	52.3	50.2	10.9
Boiled, cooled and reheated	23.8	nd	46.3	44.5	10.6
Oven-baked	35.0	nd	41.4	39.8	13.9
Oven-baked and held warm	37.4	nd	31.6	30.5	11.4
Oven-baked, held warm, cooled and reheated	28.6	nd	35.5	34.2	9.8
Oven-baked, cooled and reheated	33.4	nd	33.5	32.3	10.8

DM = dry matter; FW = fresh weight; nd = not detected.
[a] All folate results are means of duplicates. The difference between two separate values for a duplicate was less than 10% for all samples.
[b] H_4folate = tetrahydrofolate.
[c] 5-CH_3-H_4folate = 5-methyltetrahydrofolate.
[d] Total folate = measured as the sum of H_4folate and 5-CH_3-H_4folate, expressed as folic acid.

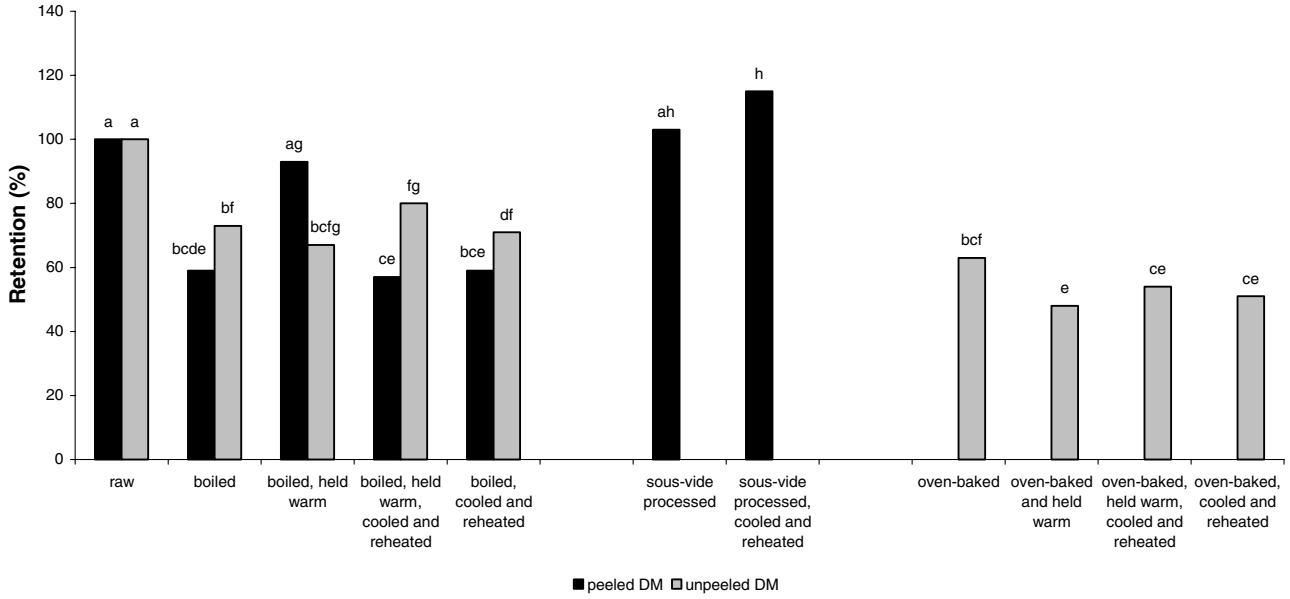

Fig. 4. Retention (%) of folate in potatoes on dry matter basis (DM). Values are means of duplicates, and the difference between two separate values for a duplicate was less than 10% for all samples. Different letters show statistically significantly ($p < 0.05$) differences in folate content after different processes compared with raw potatoes.

and 5). The retention of folate was significantly higher ($p < 0.05$) after oven baking than after boiling of unpeeled potatoes, on FW basis (Fig. 5) but not on DM basis (Fig. 4).

The boiled, peeled and unpeeled, potatoes and oven baked potatoes were held warm for 2 h at 60 °C and this treatment resulted in retention of 157%, 92%, and 76%, respectively on DM basis compared with the boiled and baked potatoes. The folate retention in potatoes held warm after oven baking was significantly reduced on DM basis compared with folate content of potatoes after oven baking ($p < 0.05$) (Fig. 4). Reheating of boiled, held warm and cooled potatoes revealed folate retention, on DM basis, of 97% (peeled) and 110% (unpeeled), respectively compared with boiled potatoes. These changes in folate content were not statistically significant. Reheating of oven-baked,

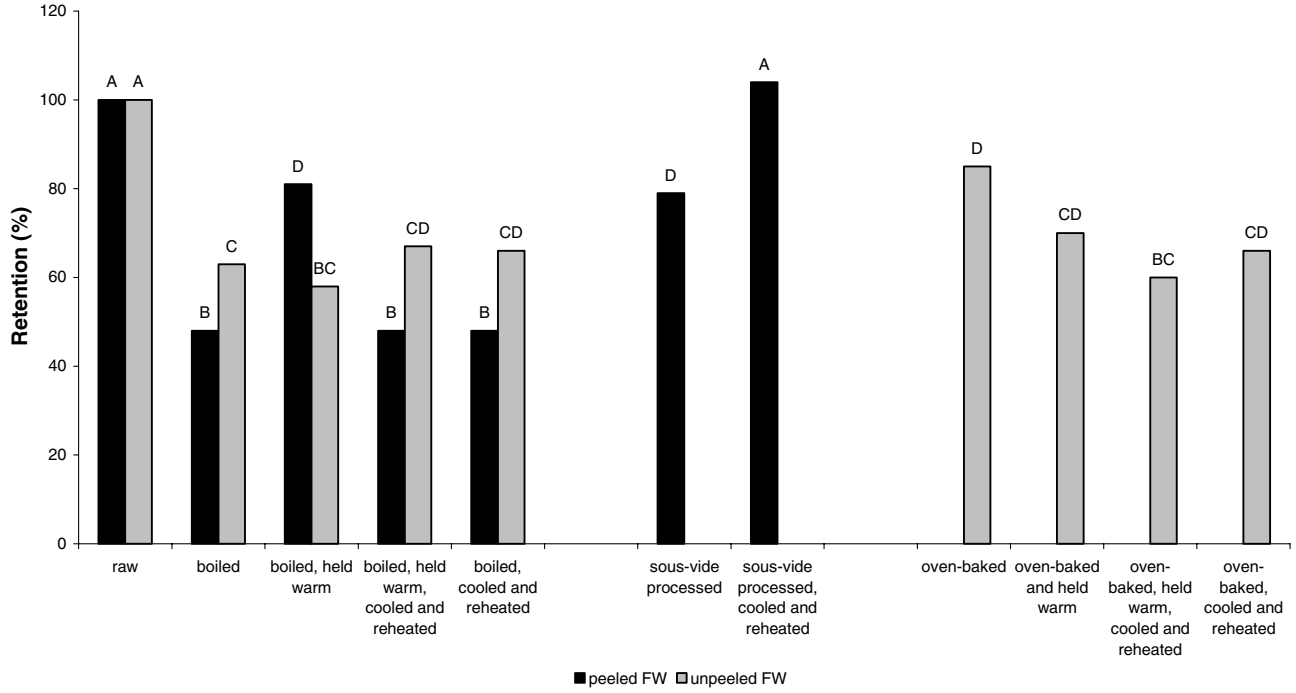

Fig. 5. Retention (%) of folate in potatoes on fresh weight basis (FW). Values are means of duplicates, and the difference between two separate values for a duplicate was less than 10% for all samples. Different letters show statistically significantly ($p < 0.05$) differences in folate content after different processes compared with raw potatoes.

held warm and cooled potatoes resulted in (not significant) folate retention of 86% on DM basis, respectively. Cooling and reheating revealed folate retention of 99%, 111%, 97% and 81% on DM basis compared with boiled and sous-vide processed peeled potatoes and boiled and oven baked unpeeled potatoes, respectively (Fig. 4). None of these changes in folate content was statistically significant.

3.4. Evaluation of the HPLC method

The detector response was linear over the concentration ranges tested for H_4folate, 5-CH_3-H_4folate and 5-HCO-H_4folate and calibration curve has a correlation coefficient equal or higher than 0.9999. The limits of quantification were 0.3, 0.2 and 4.0 ng/ml for H_4folate, 5-CH_3-H_4folate and 5-HCO-H_4folate, respectively.

The repeatability of the analytical procedure was checked using certified reference material CRM 485 on different extraction days. Mean value of 5-CH_3-H_4folate was measured to 228.8 μg/100 g ± 24.4 ($n = 14$) and the mean value of H_4folate was measured to 17.2 μg/100 g ± 5 ($n = 14$). No traces of 5-HCO-H_4folate were found in the sample.

Accuracy of the HPLC method was determined by recovery tests. Peas and the certified CRM 485 were spiked with a standard solution of H_4folate and 5-CH_3-H_4folate. The method showed good recoveries that were close to 100%, which indicates that the method is accurate (Table 4).

A typical chromatogram of folates in peas is shown in Fig. 6. 5-CH_3-H_4folate was the major form in all three vegetables, while H_4folate was the minor form representing <7% and 6–20% in green peas and broccoli respectively.

Table 4
Recoveries of added folate to certified reference material CRM 485 and green peas

Folate form	CRM 485				Green Peas			
	Amount in sample (μg/100 g)	Added (μg/100 g)	Found (μg/100 g)	Recovery (%)	Amount in sample (μg/100 g)	Added (μg/100 g)	Found (μg/100 g)	Recovery (%)
H_4folate[a]								
Day1	14.7	6.19	20.5	92 ($n = 3$)	0.70	0.99	1.51	83 ($n = 2$)
5-CH_3-H_4folate[b]								
Day1	226.9	225.8	444.0	96 ($n = 2$)	41.6	40.6	77.8	89 ($n = 3$)
		112.9	335.0	96 ($n = 3$)				
Day2	204.8	225.8	422.5	96 ($n = 3$)	42.6	40.6	75.8	82 ($n = 3$)
		112.9	312.6	95 ($n = 2$)		20.3	61.0	90 ($n = 2$)

[a] H_4folate = tetrahydrofolate.
[b] 5-CH_3-H_4folate = 5-methyltetrahydrofolate.

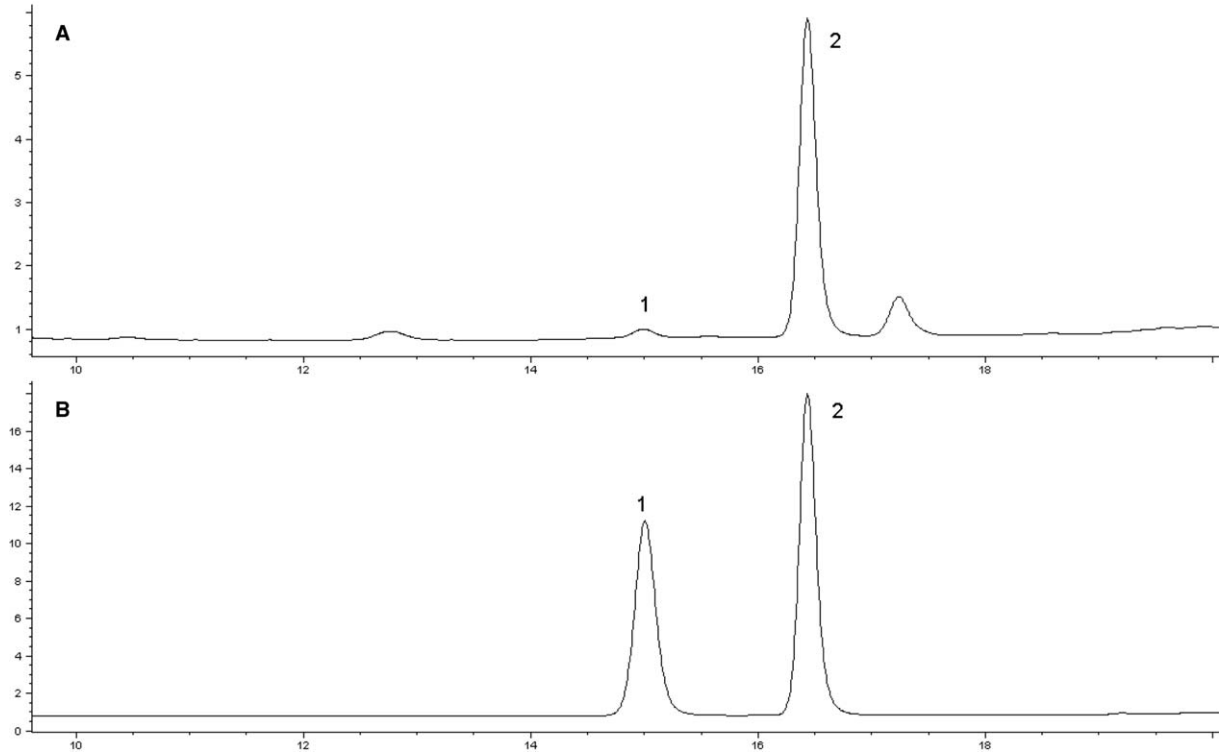

Fig. 6. Chromatograms of reduced folate monoglutamates detected by fluorescence ($\lambda_{ex} = 290$ nm, $\lambda_{em} = 360$ nm). Sample A: extract of green peas. Sample B: standard mixture containing H_4folate 50 ng/ml and 5-CH_3-H_4folate 30 ng/ml in elusion buffer. Peaks: 1 = H_4folate and 2 = 5-CH_3-H_4folate.

Table 5
Comparison of total folate content (expressed in μg/100g FW) determined by our liquid chromatography-fluorescence detection (LC/FD) with literature data based on LC/FD, liquid chromatography-mass spectrometric detection (LC/MS) or microbiological assay (MA)

Vegetable	LC/FD (own results)	LC/FD & LC/MS (literature data)	MA (literature data)
Broccoli, raw	115	27–42[a] 65[b], 111[c], 114[d],102[e]	65–187[f,g,h], 177[i]
Green peas, raw	48	33[j]	25–53[f,g,h], 102[k], 93–110[l]
Potatoes, raw	16	12[b], 23[j]	10[m], 19–22[f,g,h], 125[i]

[a] Rychlik (2004).
[b] Konings et al. (2001).
[c] Müller (1993).
[d] Vahteristo et al. (1997).
[e] DeSouza and Eitenmiller (1986).
[f] National food table of Sweden (SLV, 1996).
[g] National food table of Norway (Rimestad et al., 2001) whatever happened to g, h and i?
[h] National food table of Denmark (Moller, 1996).
[i] McKillop et al. (2002).
[j] National food table of Finland (Rastas et al., 1997).
[k] Dang et al. (2000).
[l] Puupponen-Pimiä et al. (2003).
[m] Augustin et al. (1980).

There were no detectable amounts of H_4folate in potatoes and no 5-HCO-H_4folate in the raw and processed samples of peas, broccoli or potatoes.

Table 5 presents folate contents in raw peas, broccoli and potatoes compared with literature data based on HPLC quantification with fluorescence (LC/FD) or mass spectrometric (LC/MS) detection or by microbiological assay (MA).

4. Discussion

The present study investigated the influence of different cooking procedures used in small-and large-scale service system (boiling, microwave cooking, steam boiling and oven boiling) and the food industry (blanching and sous-vide processing) on folate retention in green peas, broccoli and potatoes.

Blanching is a first regular heat treatment of green vegetables aimed for marketing as frozen items. It takes place during short times (seconds or a few minutes), and usually below boiling temperatures in excess of water. The folate content in peas decreased (31%) during blanching on FW basis. This is in agreement with Puupponen-Pimiä and coworkers (2003), who reported a folate loss between 12% and 35% on FW basis after blanching peas for 2 min. Similarly, the loss of folate in blanched broccoli was 27% ($p < 0.05$) compared with steamed on FW basis. DeSouza and Eitenmiller (1986) also showed a higher loss of folates in blanched broccoli compared with steamed with a 67% loss on wet weight basis. Jägerstad et al. (2004) reported losses of up to 50% of folate in grated root vegetables after blanching.

Our present results showed that *boiling* of frozen, blanched, brine graded green peas significantly ($p < 0.05$) reduced the folate content by 20% on FW basis and significantly ($p < 0.05$) reduced the folate content of boiled peeled and unpeeled potatoes compared with raw values by 52% and 37% on FW basis, respectively. Our study also showed that boiling of broccoli significantly ($p < 0.05$) reduced the folate content by 45% compared with steam boiling, on FW basis. Boiling of green peas did significantly ($p < 0.05$) affect the folate retention on FW basis, with a retention of 84% compared with steam-boiling and a retention of 74% compared to microwave cooking. However, neither broccoli nor green peas showed any significant changes in retention figures for folates after different cooking procedures when calculated on DM. In the present study we have calculated the folate loss on DM or FW basis. However, the differences in DM content after different processes will also affect the folate content in the different vegetables.

Boiling of peeled potatoes resulted in greater loss of folate than boiling of unpeeled potatoes. This result is confirmed by a study of Augustin et al. (1980), which indicates that the skin gives protection against folate losses. On the other hand, McKillop et al. (2002) observed a non-significant reduction of folates in both peeled potatoes and unpeeled potatoes after boiling for 60 min. However, compared to green vegetables, few studies have examined the folate loss associated with processing of potatoes although potatoes is an important contributor to folate in the Nordic European diet, accounting for 8–15% (Becker, 1999; de Bree et al., 1997). On DM basis, oven-baked potatoes retained 63% ($p < 0.05$) of the folates compared with unpeeled raw potatoes. Augustin et al. (1980) show non-significant folate retention of 87% (on DM basis) after oven baking of unpeeled potatoes for 60 min at 204 ° C. The somewhat larger loss in this present study might be explained by the slightly higher oven temperature (225 °C) and longer baking time (80 min).

The highest content of folate (both on DM basis and as FW) appears in broccoli prepared by *steam boiling*, a method that involves less contact with water compared with boiling and blanching. Similar to our results, other studies also show greater losses of folate after boiling compared with steam boiling of broccoli (McKillop et al., 2002; Peterson, 1993). This confirms previous studies that folate is primarily lost due to leaking into the cooking water and not oxidation or other pathways of degradation (Dang et al., 2000; Hawkes and Villota, 1989; McKillop et al., 2002; Petersen, 1993; Scott, Rébeille, and Fletcher, 2000).

Folate retention was studied using the *sous-vide* system of processing. In this method vegetables are heated in a vacuum-packed container; therefore the lack of exposure to exogenous water would be expected to prevent losses due to leaching. This was confirmed by our results since the sous-vide processed potatoes showed significantly higher folate retention compared with boiled samples of peeled potatoes. The sous-vide processing of broccoli in our study involved an additional pre-treatment with blanching, which might explain the unexpected low content of folate in sous-vide processed broccoli. This two-step treatment of broccoli also resulted in less attractive appearance compared with the broccoli processed with other methods. For these reasons, sous-vide processing of broccoli might not be a desirable choice of treatment when preparing this vegetable.

After preparation in institutional settings, food may be held warm for significant periods of time before consumption. Therefore, one of the main objectives of this study was to observe folate losses during storage and reheating of food. Steamed broccoli and boiled unpeeled potatoes and broccoli were held warm for 2 h at 60 °C, resulting in a small loss of folate of 0.6–8% on DM basis. However, oven baked unpeeled potatoes that were held warm showed a significant ($p < 0.05$) loss 24% on DM basis and warm holding of peeled potatoes resulted in an unexpected higher folate content when compared with the folate content of potatoes directly after boiling. Williams et al. (1995) reported a folate loss of 8–28% after 2 h warm holding at 72 °C of several cooked vegetables. They concluded that warm holding would ensure better folate retention than a cooked/chill system. However, we could not confirm this since with the conditions in our study there were no significant differences in steamed and held warm and steamed, cooled and reheated broccoli or boiled and held warm and boiled, cooled and reheated broccoli. Furthermore there were no significant differences in folate content between oven-baked and held-warm potatoes or boiled and held-warm unpeeled potatoes and boiled, cooled and reheated potatoes.

Sous-vide processed potatoes were stored for 2 weeks before reheating, which resulted in an increased content of folate. We cannot see any reasonable explanation for this result and we therefore think there is a need for further studies to investigate the folate retention of sous-vide processed potatoes both before and after storage.

Folate retention in peas after undergoing commercial processing (process 1, Table 1) was also studied. The frozen pea samples lost 12% compared with newly harvested (raw) material. Similar losses, of 5–15%, have previously been

reported (Scott et al., 2000). Our study indicates that at most one third of total folate was lost in green peas between soil and table.

Because the results of folate content of raw broccoli were lower than the processed broccoli, it was not possible to compare the retention of the different processing methods with raw broccoli. Moreover, the retention of folates in peeled-boiled and warm-held potatoes and sous-vide processed, cooled and reheated potatoes were unexpectedly high. The reasons for these values are unclear especially since the accuracy of the method was good (Table 4) and the obtained mean value of 228.8 ± 24.4 μg 5-CH_3-H_4folate/100 g for the certified reference material CRM 485 was close to the previously reported 214 ± 42 μg/100 g (Finglas et al., 1999), 210 ± 19 μg/100 g (Konings et al., 2001) and 247 ± 2 μg/100 g (Jastrebova et al., 2003). Jastrebova et al. (2003) also reported an H_4folate value of 7.86 ± 0.64 μg/100 g in this reference material, which is somewhat lower than our results.

Furthermore, when comparing our results for raw broccoli, green peas and potatoes with those previously reported (Table 5) the value for potatoes is somewhat lower whereas the others are in similar range. The use of the different analytical methods as well as the variation within- and between laboratories can explain some of the variation. In addition, different cultivars (Puupponen-Pimiä et al., 2003; Strålsjö, Åhlin, Witthöft, and Jastrebova, 2003) and time of harvesting (Vahteristo, Lehikoinen, Ollilainen, and Varo, 1997) have shown variation in folate content. It is also suggested that the differences in results of folate content in processed potatoes might be due to difference in distribution of folate dependent on maturity and possibly on the variety of potatoes (McKillop et al., 2002).

Because the level of H_4folate increases and the level of 5-CH_3-H_4folate decreases after processing of peas, processing might be a result of an interconversion of 5-CH_3-H_4folate to H_4folate. However, the same pattern was not observed in the broccoli samples. Potato samples did not contain H_4folate, which has previously been confirmed by Konings et al. (2001). It should be emphasized that the HPLC method used in the present study includes the use of BAL as antioxidant, which has been shown to be the best stabilizer of H_4folate in phosphate buffer (Patring, Johansson, Yazynina, and Jastrebova, 2005b).

5. Conclusions

This study demonstrates that retention of folate in various food items is dependent on both the food in question and the method of processing. For potato samples only boiling of potatoes (59%) and oven-baking of unpeeled potatoes (63%) caused a significant ($p < 0.05$) reduction of the folate content compared with raw potatoes on DM basis. For peas no method used for processing in service systems (boiling, steam-boiling or microwave heating) caused any significant ($p < 0.05$) losses of folates. However, folates were significantly ($p < 0.05$) reduced with 29% after industrial blanching. No significant ($p < 0.05$) differences in folate content could be observed in broccoli processed by traditional cooking systems such as boiling or by minimal processing such as steam boiling.

Warm holding has previously been suggested to ensure better folate retention than a cook/chill system. This could not be confirmed by this study since storage at various temperatures and times followed by reheating caused no further significant ($p < 0.05$) losses of total folate.

Acknowledgements

This work was partly supported by BAMA AS, Norway. We thank Norconserv AS, Norway for excellent help with processing the broccoli and potato samples. We thank Findus, Bjuv, Sweden, for supplying peas from all steps prior to freezing and a special thanks to Rolf Stegmark from Findus for valuable comments on the manuscript. The folate standards were a kind gift from Merck Eprova AG.

References

Andersen, N. L., Fagt, S., Groth, M. V., Hartkopp, H. B., Möller, A., & Ovesen, L. (1996). *Danskernes kostvaner 1995*. Copenhagen: Levnedsmiddelstyrelsen.

Augustin, J., Marousek, G., Tholen, L., & Bertelli, B. (1980). Vitamin retention in cooked, chilled and reheated potatoes. *Journal of Food Science, 45*, 814–816.

Becker, W. (1999). Svenskarna äter nyttigare- allt fler väljer grönt. *Vår Föda*, 24–27.

Becker, W. (2000). Vilka är källorna till våra näringsämnen?. *Vår Föda 3*, 16–20.

Becker, W., Lyhne, N., Pedersen, A., Aro, A., Fogelholm, M., Thórsdottir, I., et al. (2004). Nordic nutrition recommendations 2004 – integrating nutrition and physical activity. *Scaninavian Journal of Nutrition, 48*, 178–187.

Dang, J., Arcot, J., & Shrestha, A. (2000). Folate retention in selected processed legumes. *Food Chemistry, 68*, 295–298.

de Bree, A., van Dusseldorp, M., Brouwer, I., van het Hof, K., & Steegers-Theunissen, R. (1997). Folate intake in Europe: Recommended, actual and desired intake. *European Journal of Clinical Nutrition, 51*, 643–660.

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

Eitenmiller, R. R., & Landén, W. O. (1999). Folate. In R. Eitenmiller & W. O. Landén (Eds.), *Vitamin analysis for health and food sciences* (pp. 411–466). Boca Raton, FL: CRS Press.

Finglas, P. M., Wigertz, K., Vahteristo, L., Witthöft, C., Southon, S., & de Froidmont-Görtz, I. (1999). Standardisation of HPLC techniques for the determination of naturally-occurring folates in food. *Food Chemistry, 64*, 245–255.

Hawkes, J., & Villota, R. (1989). Folates in foods: Reactivity, stability during processing, and nutritional implications. *Critical Review of Food Science Nutrition, 28*, 439–538.

Holland, B., Welch, A., Unwin, I., Buss, D., Paul, A., & Southgate, D. (1991). *McCance and Widdowsson's the composition of foods* (5th ed.). Cambridge: The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

Jägerstad, M., Jastrebova, J., & Svensson, U. (2004). Folates in fermented vegetables-a pilot study. *Lebensmittel-Wissenschaft und-Technologie, 37*, 603–611.

Jastrebova, J., Witthöft, C., Grahn, A., Svensson, U., & Jägerstad, M. (2003). HPLC determination of folates in raw and processed beetroots. *Food Chemistry, 80*, 579–588.

Johansson, M., Witthöft, C., Bruce, Å., & Jägerstad, M. (2002). Study of wheat breakfast rolls fortified with folic acid. The effect on folate status in women during a 3-month intervention. *European Journal of Nutrition, 41*, 279–286.

Konings, E. J., Roomans, H. H., Dorant, E., Goldbohm, R. A., Saris, W. H., & van den Brandt, P. A. (2001). Folate intake of the Dutch population according to newly established liquid chromatography data for foods. *American Journal of Clinical Nutrition, 73*, 765–776.

McKillop, D., Pentieva, K., Daly, D., McPartlin, J., Hughes, J., Strain, J., et al. (2002). The effect of different cooking methods on folate retention in various foods that are amongst the major contributors to folate intake in the UK diet. *British Journal of Nutrition, 88*, 681–688.

Moller, A. (1996). *The composition of food (Levnedsmiddeltabeller)* (4th ed.). Copenhagen: Levnedsmiddelstyrelsen.

Müller, H. (1993). Determination of folic acid contents in vegetables and fruits by HPLC. *Zeitschrift für Lebensmittel Untersuchung und Forschung, 196*, 137–141.

Nilsson, C., Johansson, M., Yazynina, E., Strålsjö, L., & Jastrebova, J. (2004). Solid-phase extraction for HPLC analysis of dietary folates. *European Food Research and Technology, 219*, 199–204.

NMKL (1991). *Moisture and ash. Method 23* (3rd ed.). Oslo: Nordic Committee on Food Analysis (NMKL).

Patring, J., Jastrebova, J., Hjortmo, S., Andlid, T., & Jägerstad, M. (2005a). Development of a simplified method for the determination of folates in baker's yeast by HPLC with ultraviolet and fluorescence detection. *Journal of Agriculture and Food Chemistry, 53*(7), 2406–2411.

Patring, J. D. M., Johansson, M. S., Yazynina, E., & Jastrebova, J. A. (2005b). Evaluation of impact of different antioxidants on stability of dietary folates during food sample preparation and storage of extracts prior to analysis. *Analytica Chimica Acta, 553*, 36–42.

Petersen, M. (1993). Influence of sous vide processing, steaming and boiling on vitamin retention and sensory quality in broccoli florets. *Zeitschrift für Lebensmittel- Untersuchung und Forschung, 197*, 375–380.

Phillips, K., Wunderlich, K., Holden, J., Exler, J., Gebhardt, S., Haytowitz, D., et al. (2005). Stability of 5-methyltetrahydrofolate in frozen fresh fruits and vegetables. *Food Chemistry, 92*, 587–595.

Puupponen-Pimiä, R., Häkkinen, S., Aarni, M., Suortti, T., Lampi, A.-M., Eurola, M., et al. (2003). Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. *Journal of the Science of Food and Agriculture, 83*, 1389–1402.

Rastas, M., Seppanen, R., Knuts, L., Hakala, P., & Karttila, V. (1997). *Nutrient composition of foods*. Turku & Helsinki: Kansaneläkelaitos.

Rimestad, A., Borgejordet, A., Verterhus, K., Sygnestveit, K., Løken, E., Trygg, K., et al. (2001). *Den store matvaretabellen* (2nd ed.). Oslo: Gyldendal Norsk forlag ASA.

Rychlik, M. (2004). Revised folate content of foods determined by stable isotope dilution assays. *Journal of Food Composition and Analysis, 17*, 475–483.

Scott, J., Rébeille, F., & Fletcher, J. (2000). Review Folic acid and folates: the feasibility for nutritional enhancement in plant foods. *Journal of the Science of Food and Agriculture, 80*, 795–824.

Selman, J. (1994). Vitamin retention during blanching of vegetables. *Food Chemistry, 49*, 137–147.

SLV (1996). *The composition of food (Livsmedelstabell)*. Uppsala: Swedish National Food Administration (SLV).

Strålsjö, L., Åhlin, H., Witthöft, C., & Jastrebova, J. (2003). Folate determination in Swedish berries by radioprotein-binding assay (RPBA) and high performance liquid chromatography (HPLC). *European Food Research and Technology, 216*, 264–269.

Vahteristo, L., Lehikoinen, K., Ollilainen, V., & Varo, P. (1997). Application of an HPLC assay for the determination of folate derivatives in some vegetables, fruits and berries consumed in Finland. *Food Chemistry, 59*(4), 589–597.

van den Berg, H., Finglas, P. M., & Bates, C. (1994). FLAIR intercomparisons on serum and red cell folate. *International Journal of Vitamin and Nutrition Research, 64*, 288–293.

Williams, P., Ross, H., & Miller, J. B. (1995). Ascorbic acid and 5-methyltetrahydrofolate losses in vegetables with cook/chill or cook/hot-hold foodservice systems. *Journal of Food Science, 60*, 541–546.