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

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Folate intake is strongly influenced by various methods of cooking that can degrade the natural forms of the vitamin in foods. The aim of the present study was to determine the effect of different cooking methods on folate retention in various foods that contribute to folate intake in the UK diet. Typical purchasing and cooking practices of representative food folate sources were determined from a questionnaire survey of local shoppers (*n* 100). Total folate was determined by microbiological assay (*Lactobacillus casei* NCIMB 10463) following thermal extraction and tri-enzyme (α-amylase, protease and conjugase) treatment in raw foods and after typical methods of cooking. Boiling for typical time periods resulted in only 49 % retention of folate in spinach (191·8 and 94·4 μg/100 g for raw and boiled spinach respectively; $P < 0.005$), and only 44 % in broccoli (177·1 and 77·0 μg/100 g for raw and boiled broccoli respectively, $P < 0.0001$). Steaming of spinach or broccoli, in contrast, resulted in no significant decrease in folate content, even for the maximum steaming periods of 4·5 min (spinach) and 15·0 min (broccoli). Prolonged grilling of beef for the maximum period of 16·0 min did not result in a significant decrease in folate content (54·3 and 51·5 μg/100 g for raw and grilled beef respectively). Compared with raw values, boiling of whole potatoes (skin and flesh) for 60·0 min did not result in a significant change in folate content (125·1 and 102·8 μg/100 g for raw and boiled potato respectively), nor was there any effect on folate retention whether or not skin was retained during boiling. These current results show that the retention of folate in various foods is highly dependent both on the food in question and the method of cooking. Thus, public health efforts to increase folate intake in order to improve folate status should incorporate practical advice on cooking.

Food folate retention: Cooking methods: Food folates: Dietary folate intake

Optimal folate status may have a role in the prevention of cardiovascular disease via plasma homocysteine-lowering (Boushey *et al.* 1995), and possibly in the prevention of certain cancers (Branda & Blickenderfer, 1993; Kim *et al.* 1997; Jacob *et al.* 1998; Choi & Mason, 2000). However, the most compelling evidence for the benefit of optimal folate status is its link with the prevention of neural tube defects (Medical Research Council Vitamin Study Research Group, 1991; Czeizel & Dudas, 1992; Botto *et al.* 1999). The national committees tasked with public health in the UK, USA and Australia (Department of Health, 1992; Public Health Service Centers for Disease Control and Prevention, 1992; National Health and Medical Research Council, 1993) have recommended an extra 400 μg folic acid/d in addition to normal dietary folate intake to prevent the occurrence of neural tube defects. However, more recent studies suggest that an additional intake of 200 μg folic acid/d may be optimal both for the prevention of neural tube defect occurrence (Daly *et al.* 1997) and for the lowering of plasma homocysteine (Ward *et al.* 1997). This level could potentially be achieved by doubling the current dietary folate intake of about 190 μg/d in the UK (Ball, 1998) through the increased consumption of folate-rich foods.

Natural folates are a group of water-soluble compounds with similar biological activity to the synthetic vitamin, folic acid. Unlike folic acid, which is a fully oxidised molecule, natural folates are reduced at the 5, 6, 7 and 8

positions of the pteridine ring and so are prone to oxidative cleavage at the C_9-N_{10} bond producing two degradation products, a pteridine and *p*-aminobenzoylglutamate (Murphy *et al.* 1976, 1978). Both of these molecules are inactive in themselves and cannot be biologically converted to any active folate form (Scott, 2001). In addition, natural folates are predominately polyglutamates, containing up to seven L-glutamate residues attached to the *p*-aminobenzoic group by γ-peptide linkage. The degree of glutamation does not appear to impact on folate stability (Gregory, 1996). However, the introduction of a group onto the N_5 has a stabilising effect on the oxidative cleavage process (Eitenmiller & Landen, 1999).

Compared with other water-soluble vitamins, in particular ascorbic acid, literature on the extent and mechanisms of folate loss during processing is limited (Ball, 1998). Folate losses during cooking and preparation are the result of a combination of thermal degradation and leaching of the vitamin into the cooking water (Eitenmiller & Landen, 1999). The degree of loss can be influenced by environmental factors, including pH, O_2 content, metal ion concentrations, antioxidant levels, duration and product:water ratio (Keagy, 1985; Gregory, 1989; Hawkes & Villota, 1989). Extensive losses of folate have been reported in boiled vegetables (Hurdle *et al.* 1968; Lin *et al.* 1975; Leichter *et al.* 1978; Dang *et al.* 2000) and baked meats (Vahteristo *et al.* 1998). However, studies specifically designed to investigate the impact of different cooking methods on folate content are limited. Furthermore, most studies comparing folate losses between methods are based on industrial, rather than domestic processes. The aim of the present study was to determine 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.

Materials and methods

The foods selected and the methods of cooking chosen in the present study were determined on the basis of an initial examination of purchasing and cooking practices of a sample of the general public. Thus, the overall study design comprised two separate components. Part 1 consisted of a consumer questionnaire focusing on food folate sources; part 2 was the determination of folate retention in foods following cooking, under conditions considered to be 'typical' on the basis of the survey in part 1.

Consumer questionnaire

A questionnaire survey of local shoppers (*n* 100) was conducted with the objective of establishing typical purchasing and cooking practices of consumers of foods representing major dietary sources of folate. The questionnaire comprised a series of open questions to ascertain the form of purchase (e.g. fresh or frozen) and typical cooking practices of four foods representing the major contributors to folate intake in the UK diet (Ministry of Agriculture, Fisheries and Food, 1994). Cereal products, the largest folate contributors (accounting for 21 % of total folate intake), were not included in this present study since they do not normally require post-purchase cooking. The study focused on vegetables, the next most important category, accounting for 16 % of total folate intake, with leafy green vegetables being the largest sub-category (Ministry of Agriculture, Fisheries and Food, 1994), of which spinach and broccoli were selected for investigation as representative items. Potato (which is classified independently of vegetables) is reported to be the next most important source (Ministry of Agriculture, Fisheries and Food, 1994), providing 14 % of total folate and was, therefore, selected for investigation. Finally, beef was selected, as it represents one of the major sources of meat folates, which account for 9 % of total folate intake (Ministry of Agriculture, Fisheries and Food, 1994). The questionnaire did not attempt to determine typical cooking times as it was felt that consumers do not normally measure this and would therefore not be able to recall it with any degree of accuracy.

A large local retail outlet, Tesco, was the location selected for administration of the questionnaire. The questionnaire was administered over three different days (two weekdays plus a Saturday) and at three different times of the day (morning, afternoon or evening) in order to obtain an average sample of 100 shoppers.

Food samples

Purchase of food samples. Cooking stability experiments were performed on three separate occasions; on each occasion foods were sourced from different local retail outlets (Tesco, Safeway and Sainsbury). Samples of broccoli, spinach, potato and beef (steak) were purchased on the evening prior to analysis. Spinach, broccoli and beef were stored overnight at $2-8°C$, and potatoes were stored at room temperature.

Preparation of food samples. On each day of preparation of food samples, three packets of spinach (300 g each) and three heads of broccoli were removed from supermarket packaging and condensation removed by blotting dry on paper towels. Fourteen (25 g) homogenous samples were prepared for both spinach (leaves were taken in random order from each packet of spinach) and broccoli (florets were cut from each part of the broccoli head). Two samples were retained for duplicate determination of folate content in the raw food and the remaining twelve were divided for duplicate examination of two cooking methods at three time points. Twelve average sized potatoes were washed and blotted dry and allocated to duplicate analysis of four treatments (raw with skin retained, peeled then boiled for 60·0 min, boiled for 60·0 min in skin, boiled for 60·0 min then peeled). Three potatoes were used for each treatment. For the samples of raw potato with skin retained, small pieces of each potato were cut to prepare the two individual samples of 25 g. In the case of the cooked potato samples, the whole potatoes were mashed after cooking and from the mashed material, individual samples of 25 g were prepared. Potatoes requiring peeling prior to cooking were peeled with a traditional potato peeler, whereas those peeled after cooking were peeled with a butter knife. Four lean beef steaks were blotted dry on paper towels, any visible fat was carefully removed and the remaining meat was cut into eight

25 g portions. Two were retained for duplicate determination of folate content in the raw food and the remaining six were allocated to duplicate examination of the effect of grilling at three time points.

Cooking of food samples. Preliminary experiments were conducted to determine the cooking time (min) required to achieve consistencies corresponding to 'undercooked', 'cooked' and 'overcooked' for the various food samples and cooking methods. In the case of spinach, broccoli and beef, all three time points (i.e. corresponding to 'undercooked', 'cooked' and 'overcooked') were considered, whereas for potato samples, only one time period ('cooked', i.e. intact but tender enough to mash) was examined. Instead, different methods of preparation of potatoes for boiling were examined.

Samples for spinach and broccoli were placed in covered stainless-steel saucepans containing 100 ml boiling water (unsalted). In order to ensure that the cooked samples would correspond to the raw ones, each sample was cooked in an individual saucepan and the pieces collected through a fine sieve. The effect of duration of boiling was investigated in duplicate at 0·0, 1·5, 3·5 or 5·0 min for spinach, and at 0·0, 5·0, 10·0 or 15·0 min for broccoli. Duplicate potato samples for each treatment (raw with skin retained, peeled then boiled for 60·0 min, boiled for 60·0 min in skin, boiled for 60·0 min then peeled) were placed in covered stainless-steel saucepans containing 250 ml boiling water (unsalted) and simmered for 60·0 min.

Steaming of spinach and broccoli was conducted in a domestic split-level steamer. Unsalted water was taken to boiling temperature prior to the addition of the food samples. The effect of duration of steaming was investigated in duplicate at 0·0, 1·5, 3·0 or 4·5 min and at 0·0, 5·0, 10·0 or 15·0 min for spinach and broccoli respectively. Beef samples were placed on the middle shelf of a preheated domestic electric-cooker grill. The effect of duration of cooking on folate content was investigated at 0·0, 6·0, 11·0, and 16·0 min. Sample weight was based on a raw sample that took account of the weight changes as a result of cooking.

Sampling. Cooking (all samples) was terminated by placing samples in an ice-water bath. In order to avoid differences in the moisture contents of food samples cooked by different methods of cooking for different time periods, samples of spinach, broccoli and steak were weighed raw before cooking. Food samples (corresponding to 25 g raw weight) were strained and blotted dry after cooking. Potato samples for each preparation group were blotted dry prior to mashing and removal of a 25 g sample (cooked weight). All cooked samples of spinach, broccoli, beef and potato were homogenised in 100 ml *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid) (Hepes)/2-(*N*-cyclohexylamino)ethanesulfonic acid (Ches) buffer (50 mM-Hepes, 50 mM-Ches, 20 g ascorbic acid/l and 10 mM-2-mercaptoethanol, pH 7·85) in a domestic food processor. The homogenates (500 μl aliquots) were stored at −80°C for subsequent analysis.

Determination of folate content of food samples

All preparative and analytical procedures were performed under subdued light and contact with air was minimised. Rat serum obtained from Sigma Chemical Co. (Poole, Dorset, UK) was dialysed for 24 h at 2–4°C in 1 litre Hepes/Ches buffer, pH 7·85 (50 mM-Hepes, 50 mM-Ches, 20 g ascorbic acid/l and 10 mM-2-mercaptoethanol, pH 7·85) containing 2 g acid-washed C powder (Sigma Chemical Co.). The crude rat serum conjugase was filtered through a 0·2 μm syringe filter (Sartorius AG, Göttingen, Germany) and divided into 1 ml portions and stored at −80°C. Solutions of α-amylase and protease (Sigma Chemical Co.) were prepared fresh each day by dissolution in ultrapure water at concentrations of 10 and 20 mg/ml respectively. Trace levels of folate were removed from the enzyme solutions by treatment with activated charcoal, as described by Tamura (1998). All enzyme solutions were tested for folate content and no measurable folate was detected.

Thawed samples (500 μl) were mixed in 50 ml Oak Ridge centrifuge tubes (Nalgen, Rochester, NY, USA) with 4·5 ml Hepes/Ches buffer, pH 7·85, which had been taken to 100°C by immersion in a boiling water-bath. Samples were maintained at 100°C for 10 min. After rapid cooling on ice, 500 μl α-amylase was added to each tube and incubated for 2 h at 37°C. Prior to the addition of conjugase, the α-amylase was thermally deactivated by immersion in a boiling water bath for 5 min followed by rapid cooling on ice. Rat serum conjugase (100 μl) was added to each sample and incubated for 2 h at 37°C. Conjugase was thermally deactivated (100°C for 5 min followed by rapid cooling on ice) prior to the addition of 500 μl protease and incubation for 2 h at 37°C. Protease was deactivated by immersion of sample tubes in a boiling water bath for 5 min followed by rapid cooling on ice and centrifugation at 3000 **g** for 15 min. The resulting supernatant fraction was divided into portions in 3 ml tubes, flushed with N_2 and stored for up to 3 months at −80°C until folate determination.

Total folate was determined by microbiological assay with *Lactobacillus casei* NCIB 10463 (Molloy & Scott, 1997) following thermal extraction and tri-enzyme (α-amylase, protease and conjugase) treatment. The calibration of the assay was performed using folic acid (Sigma Chemical Co.). Under the conditions of the assay in our laboratory (pH 6·7 of the assay medium), *L. casei* showed equivalent responses to the main folate derivatives (folic acid, 5-methyl tetrahydrofolate, 5-formyl tetrahydrofolate, 10-formyl tetrahydrofolate and tetrahydrofolate). A similar response by *L. casei* was observed when the pH of the assay medium was adjusted to 6·2. For quality control purposes, spinach-extract samples were prepared by thermal extraction and tri-enzyme treatment. The inter-assay CV of the folate content of spinach extract quality control samples was 5·5 % (*n* 48). Recovery studies were performed by spiking the spinach quality control samples with folic acid, (6*S*)-5-methyl tetrahydrofolate and (6*S*)-5-formyl tetrahydrofolate, at three different levels: 0·25, 0·50 and 1·00 μg. The spiked samples underwent the entire procedure of food folate analysis (thermal extraction, tri-enzyme treatment and microbiological assay). The recovery for different folate derivatives was between 87 and 101 %. A linear response was demonstrated corresponding

to the increased concentration of folate content of the spiked spinach samples. Samples for all treatment groups for each food were analysed together. All dilutions were carried out in an ascorbate solution (5 g/l) using an automated dilutor (Hamilton, Bonaduz AG, Bonaduz, Switzerland).

Statistical methods

The consumer questionnaire was analysed by SPSS (version 9.0.1; SPSS UK Ltd, Chertsey, Surrey, UK). Statistical evaluation of folate values was analysed by Data Desk (version 6; Data Description Inc., Ithaca, NY, USA). Differences in folate content between time points and between cooking methods were established by ANOVA and paired t test. Differences were considered significant at $P < 0.05$.

Results

Consumer questionnaire

Of 190 people approached at random, 100 completed the questionnaire, of which 71 % were female and 29 % were male. The age profile ranged from 18 to >85 years with a median age range of 36–45 years. The proportion of the survey population that regularly consumed each of the four food items under examination is presented in Fig. 1. Spinach was the food item that was least popular amongst the sample surveyed. Most respondents purchased fresh vegetables (84 %), with 25 % reporting purchase of frozen vegetables. Steak was the most popular form of beef purchased (83 % of the sample). Microwaving as a method of cooking (as opposed to a means of reheating) was not popular with our respondents, with only 3, 9 and

6 % reporting a preference for this method of cooking of spinach, broccoli and potatoes respectively. The two most popular cooking methods employed for green vegetables were boiling (49 % of respondents) and steaming (45 % of respondents). Boiling was the preferred method of cooking potatoes, with 79 % of the sample reporting a preference for this method. Grilling was the most popular method of cooking beef (steak), with 48 % of respondents stating a preference for this method.

The effect of different cooking methods on folate retention in various foods

Results showing folate in foods following typical cooking procedures are presented in Table 1. Boiling resulted in a significant decrease in the folate content of spinach (51 %, $P < 0.005$) and broccoli (56 %, $P = 0.0001$) compared with the raw food. Compared with raw spinach or broccoli, no significant losses of folate were observed as a result of steaming. Overall, steaming compared with boiling (for typical cooking times) resulted in significantly greater retention of folate for both spinach (100 v. 49 %, $P < 0.0001$) and broccoli (91 v. 44 %, $P < 0.0001$). Boiling of whole potatoes (skin and flesh) for 60·0 min did not result in a significant reduction in folate content compared with the raw value. Grilling of beef for 11·0 min did not significantly change its folate content compared with the raw sample.

Folate retention during prolonged duration of cooking

The effects of duration of cooking on folate retention are presented in Fig. 2. Significant decreases in folate retention were observed with increasing duration of boiling of spinach and broccoli (Fig. 2). Steaming, in contrast, did not
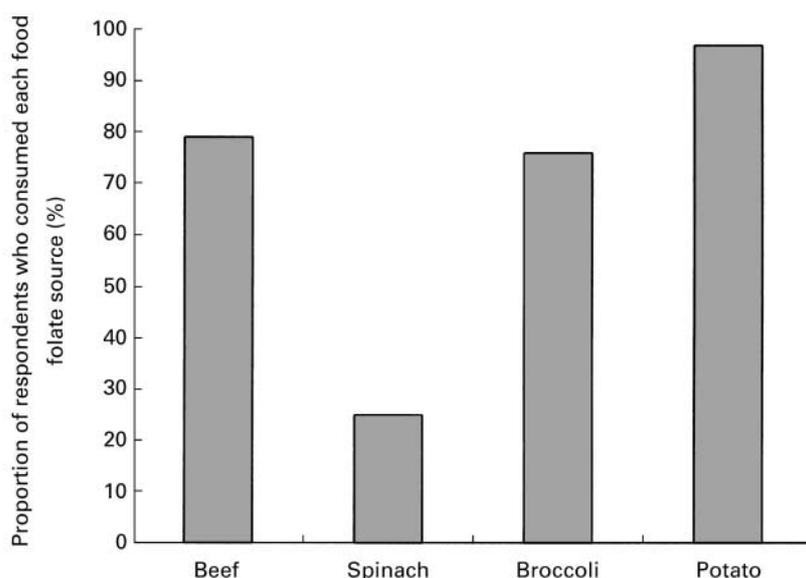

Fig. 1. Consumer questionnaire respondents (%) who reported consuming four foods representing major food folate sources in the UK diet (Ministry of Agriculture, Fisheries and Food, 1994). The sample comprised shoppers (*n* 100) at a local retail outlet and the results were collected over three different days, at three different times of day. For details of questionnaire and procedures, see p. 682.

Table 1. Folate retention in major food folate sources following typical cooking procedures†

(Mean values with their standard errors for duplicate samples in three independent experiments)

Food	Cooking method	Cooking duration (min)‡	Raw food§		Cooked food§	
			Folate (μg/100 g)	SEM	Folate (μg/100 g)	SEM
Spinach	Boiled	3·5	191·8	5·8	94·4***	13·3
Spinach	Steamed	3·0	189·5	9·0	218·5	22·6
Broccoli	Boiled	10·0	177·1	8·5	77·0****	3·6
Broccoli	Steamed	10·0	172·0	9·4	156·2	14·5
Potato	Boiled	60·0	125·1	11·7	102·8	15·0
Beef	Grilled	11·0	54·3	4·8	50·6	5·8

Mean values were significantly different from those of the raw food (paired *t* test: ***$P < 0.005$, ****$P < 0.0001$.

† 'Typical' cooking procedures were established from the results of a consumer questionnaire (for details see p. 682).

‡ Times shown reflect the duration required to cook the food item (determined from preliminary experiments, i.e. not 'undercooked' or 'overcooked'; for details, see p. 683).

§ To avoid differences in moisture content as a result of different cooking procedures, spinach, broccoli and beef were weighed raw before cooking and values therefore given per 100 g raw weight. Values for potatoes relate to whole potatoes (skin and flesh) and are given per 100 g raw or cooked weight as appropriate (for details of procedures see p. 682).
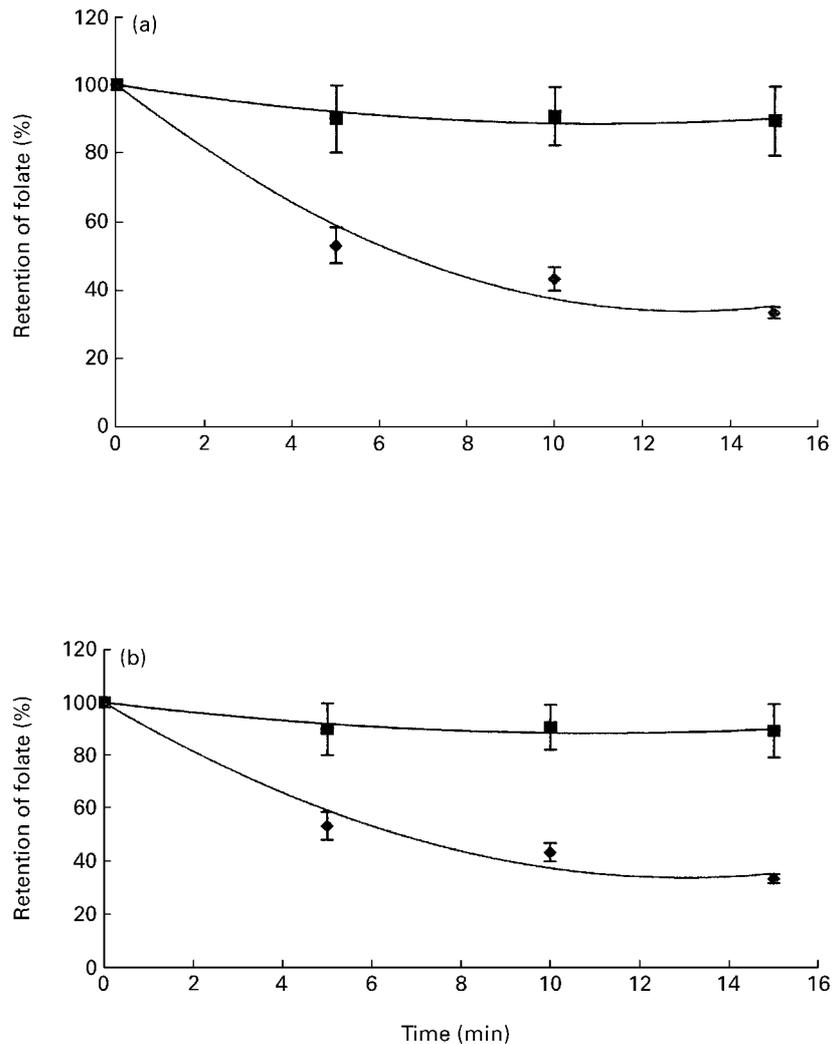

Fig. 2. The effect of duration and method of cooking on folate retention in: (a), spinach; (b), broccoli. ◆, Boiled; ■, steamed. For details of samples and procedures, see p. 682. Values are means for six samples, with their standard errors shown by vertical bars.
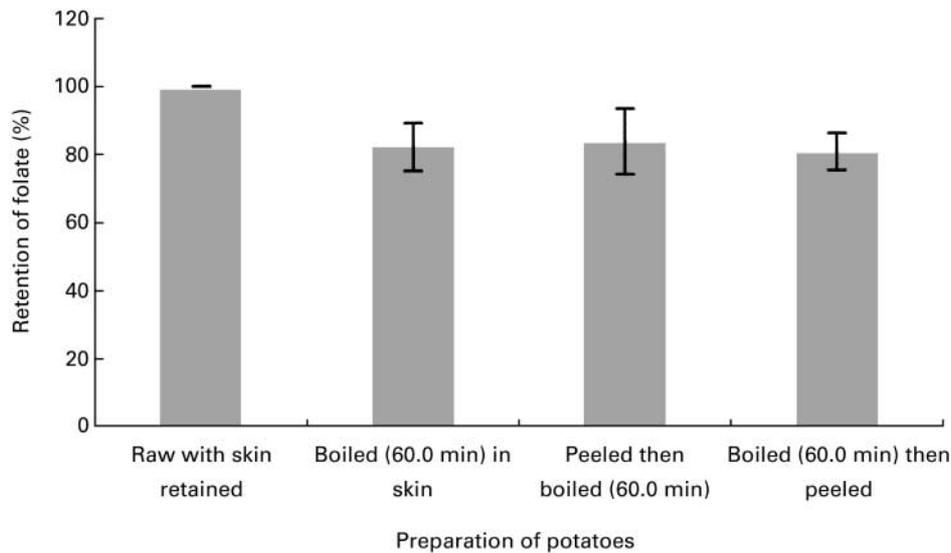
Fig. 3. Impact of preparation of potatoes on folate retention during boiling. For details of samples and procedures, see p. 682. Values are means for six samples, with their standard errors shown by vertical bars. No significant differences were observed for folate content when various cooking procedures were compared (ANOVA).

result in a significant decrease in folate content, even after the maximum steaming periods of 4·5 min and 15·0 min for spinach and broccoli respectively. Grilling of beef for the maximum period of 16·0 min did not result in a significant loss in folate content (54·3 *v.* 51·5 μg/100 g, *P* = 0·5).

The effect of preparation of potatoes on folate retention during boiling

The effects of different preparation techniques on folate retention in potatoes during boiling are presented in Fig. 3. The presence or absence of potato skin had no significant impact on folate retention during boiling.

Discussion

The present study investigated the influence of typical cooking methods on folate retention in foods that are amongst the major contributors to folate intake in the UK diet. The consumer questionnaire survey revealed a preference for fresh produce and traditional modes of cooking (boiling, steaming and grilling). Potatoes were consumed by three times as many respondents as spinach and were cooked predominantly by boiling (almost 80 % of the respondents). Green vegetables (spinach and broccoli) were typically prepared by boiling (49 % of respondents) and steaming (45 % of respondents). Grilling was reported to be the most popular method of cooking beef, with almost half (48 %) of the respondents preferring this method.

Our present results showed significant reductions of 51 and 56 % in the folate content of spinach and broccoli compared with raw values respectively, as a result of boiling for typical time periods. In contrast, steaming of spinach or broccoli did not result in a significant loss of folate content, even after the maximum steaming periods of 4·5 min and 15·0 min respectively, which produced overcooked consistencies. Our present results, showing markedly greater

folate retention following steaming compared with boiling of green vegetables under domestic conditions, are in good agreement with the findings of De Souza & Eitenmiller (1986) and Lund (1988), based on industrial processing. They reported that steam blanching resulted in greater retention of folate compared with water blanching. Likewise, other forms of cooking (not examined here) that minimise the direct contact of food with the cooking water such as pressure cooking (Dang *et al.* 2000) and microwave cooking (Chen *et al.* 1983; Klein, 1989) have been found to be preferable to boiling in terms of folate retention.

These results are in good agreement with Leichter *et al.* (1978), who reported a folate loss of 62 % for broccoli boiled for 10·0 min, compared with 56 % for the same duration in the current study. Likewise, DeSouza & Eitenmiller (1986) reported a 62 % loss in the folate content of broccoli after water blanching (i.e. treated in boiling water for 3·0 min) and Hurdle *et al.* (1968) reported a 59 % reduction in folate content as a result of boiling broccoli for an unspecified time. The strong relationship between folate loss and duration of boiling demonstrated here may in part explain the discrepancy between the Leichter *et al.* (1978) findings of 78 % loss in the folate content of spinach after 10·0 min of boiling, compared with the 51 % folate loss after 3·0 min of boiling shown in the current study. However, De Souza & Eitenmiller (1986) reported an 83 % loss in spinach folate after water blanching for only 3·0 min, a result that may be explained by the large water:product ratio associated with industrial blanching procedures. The large surface area of spinach is likely to make it more susceptible to the influence of water:product ratio (Klein, 1989) on leaching of folate from it compared with that of other vegetables.

Compared with green vegetables, relatively few studies have examined the folate loss associated with the preparation and cooking of potatoes. Although not considered to be particularly rich in folate, their high level of

consumption means that they are major contributors to folate in the UK diet, accounting for 14 % of total folate intake (Ministry of Agriculture, Fisheries and Food, 1994). Our present results show that compared with raw values, boiling of whole potatoes (skin and flesh) for 60·0 min did not result in a significant reduction in folate content. Furthermore, our present results indicate that folate is not concentrated in the skin region, as the folate content of peeled potatoes did not differ significantly from whole potatoes. In addition, the skin did not appear to impede leaching, as there was no significant difference in folate retention of potatoes boiled whole (with skin) compared with potatoes peeled prior to boiling. In contrast, Augustin et al. (1987) found that retention of potato skins significantly retarded the loss of folate during boiling. The precise distribution of folate in potatoes is unclear; however, it is likely to be dependent on maturity (Arthey, 1975) and possibly on the variety of potato.

The difference in folate losses during boiling between foods in the current study is worth noting. No loss in folate was observed for potato despite being boiled for 60·0 min, compared with 51 % folate loss from spinach boiled for only 3·0 min. In addition, the folate loss from broccoli is comparable with that from spinach, despite being boiled for three times longer. Our findings, therefore, support the theory of Malin (1977) that surface area and geometric shape significantly affect the folate loss resulting from leaching. Leichter et al. (1978) reported that folate loss as a result of boiling was principally as a result of leaching, and not as a result of degradation of the folate molecule. In the current study, water samples were collected during one of the experiments. All of the folate lost from the food during cooking (both steaming and boiling) was accounted for in the cooking water (results not shown), which supports the view that folate losses during cooking are not related to folate degradation, but to leaching.

Folates of animal origin are reported to be stable during boiling and frying (Ball, 1998). In the current study we showed that cooking of beef (steak) by direct heat (grilling) also results in negligible folate loss, even after a prolonged period of grilling for 16·0 min. However, Aramouni & Godber (1991) reported decreases in the folate contents of beef liver of 41 and 50 % as a result of broiling (grilling) and frying respectively. A possible explanation for this inconsistency may be that it relates to differences in cofactor forms of folate found in beef liver compared with muscle (Vahteristo et al. 1998), some of which may be more labile than others. In addition, the reported folate content in beef muscle (9·0 µg/100 g) is considerably less than that in liver (240·0 µg/100 g); therefore, losses are likely to be more marked in the latter.

It is well established that reported food folate values are affected by the method of analysis employed. The tri-enzyme methodology (α-amylase, protease and conjugase) used in the present study has been reported by a number of investigators to result in substantial increases in measurable folate, compared with traditional methods involving treatment with conjugase alone prior to quantification (Tamura, 1998). In the current study, we found folate values (µg/100 g raw food) to be between 1·3- and 6·0-fold

higher than the published values (Holland et al. 1991): spinach 191 v. 150, broccoli 175 v. 90, potato 125 v. 35, beef 54 v. 9. The increase of measurable folate after tri-enzyme treatment cannot be attributed to any folate contribution by the enzymes, because all the enzyme solutions used were purified and analytically proven to be free of folate. Thus, the reasonable explanation for this finding is that the bound folates were liberated from the food matrix as a result of enzyme treatment. In support of this, the difference in folate content was found to be much greater in the case of potato and beef compared with green vegetables, which might be expected given that potatoes and beef are rich in starch and protein, the target molecules of tri-enzyme digestion, in comparison with green vegetables, which are not rich in these substrates. In addition, the degradation of ascorbic acid (added to protect folates during analysis) at neutral pH can be prevented by the addition of the antioxidant, 2-mercaptoethanol (Wilson & Horne, 1984), or by naturally occurring thiols and antioxidants in the foods. Thus, the impact of the introduction of 2-mercaptoethanol in addition to ascorbate in the extraction buffers in the present study (not typically used in the traditional methods) may have had a greater impact on recovery of intact folate in foods that are not rich in antioxidants, such as beef and potatoes, compared with antioxidant-rich foods such as green vegetables. Whatever the explanation, such marked differences in folate values using newer methodology compared with published values have major implications for the calculation of dietary folate intake (and therefore folate recommendations) and require further investigation.

In conclusion, we show that the retention of folate in various foods is highly dependent both on the food in question and the method of cooking. Folates of animal origin (i.e. beef) were found to be stable to cooking even for prolonged periods. Likewise, folate was well retained in potatoes during boiling and we found no evidence (from a folate perspective) to support the popular view that: 'the goodness is in the skin'. However, the method and duration of cooking of green vegetables were found to have marked effects on folate retention from this major food folate source. Public health efforts to increase folate intakes should, therefore, incorporate practical advice on cooking. For example, steaming in preference to boiling could be promoted as a means of doubling the folate content of cooked green vegetables. In addition, consumers choosing to boil green vegetables should be strongly discouraged from doing so for prolonged periods and should be advised to minimise the cooking water and utilise it for soups or gravy. These practical measures could make a substantial impact on folate intake from natural food sources and play a potential role in the prevention of folate-related diseases by helping to optimise folate status.

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