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Folate contents of some selected Fijian foods using tri-enzyme extraction method

Riteshma Devi [a], Jayashree Arcot [b], Subramanium Sotheeswaran [a,*], Sadaquat Ali [a]

[a] School of Biological, Chemical and Environmental Sciences, Faculty of Science and Technology, The University of the South Pacific, Suva, Fiji
[b] Food Science and Technology, School of Chemical Sciences and Engineering, The University of New South Wales, Sydney 2052, Australia

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Abstract

Folic acid and total folate contents of 18 common foods in the Fijian diet were assayed. Foods were purchased from the central and eastern parts of Viti Levu in Fiji. Tri-enzyme treatment was performed to release bound folates using protease and α-amylase, with chicken pancreas as the conjugase. The highest total folate content was recorded for egg yolk (*Gallus domesticus*) at 256 μg/100 g, followed by long beans (*Vigna sesquipedalis*) which contained 130 μg/100 g of total folate (fresh weight basis). The local leafy vegetable called *Bele* (*Abelmoschus manihot*) and the Drumstick leaves (*Moringa oleifera*) available in Fiji also had high total folate contents, above 100 μg/100 g (fresh weight basis). For the 18 foods studied, the content of folic acid ranged from 3 to 189 μg/100 g and the total folate content was in the range of 3–256 μg/100 g, indicating a very wide range of folate content in the foods studied.

Keywords: Fresh Fijian vegetables; Folic acid; Total folate; Chinese cabbage (*Brassica chinesis*); *Bele* (*Abelmoschus manihot*); Long bean (*Vigna sesquipedalis*); Tri-enzyme method; Potease; α-Amylase and chicken pancreas

1. Introduction

Folic acid is a water-soluble vitamin, acting as a coenzyme in many single carbon transfer reactions in the synthesis of DNA, RNA and protein components (Ruggeri, Vahteristo, Aguzzi, Finglas, & Carnovale, 1999). Folic acid is *N*-[4(2-amino-4-hydroxypteridin-6-ylmethylamino) benzoyl]-L (+)-glutamic acid, pteroyl-L-glutamic acid (PGA). Obsolete names are vitamin B8, vitamin B9, vitamin Bc and vitamin M (Kohlmeier, 2003). The occurrence of folic acid in nature is not in appreciable amounts, though it is assimilated in the body and is converted to the active cofactor forms of the vitamin (Brody, 1999).

The term 'folate' is used in this study to refer to natural folates and folic acid, all possessing vitamin activity. In this paper we report the folic acid and total folate contents of 18 common Fiji foods. Total folates were measured using the tri-enzyme technique, which ensures the extraction of folates bound to the matrices of proteins and carbohydrates in foods (see Fig. 1).

In the 1990s, it became evident that low levels of folate played an important role in the development of neural tube defects (Czeizel & Dudás, 1992). *Spina bifida* is one of the forms that results in the new born due to a deficiency of folic acid in the maternal diets. Fiji has a rich source of green vegetables. Nevertheless, statistical data have shown that *S. bifida* has a probability of occurring in three people per 200,000 of the population surveyed (Boila, 2005).

Folates are mostly found in vegetables and fruits, such as legumes, spinach, citrus fruits (oranges and grapefruit juices) (Thomas, Flanagan, & Pawlosky, 2003) bananas, cabbage (raw/boiled), broccoli (raw/boiled), apples, tomatoes (fresh) and avocados, and other foods, such as hard-boiled egg yolk, liver, canned tuna, cheddar cheese and whole wheat bread (Machlin, 1991).

* Corresponding author. Tel.: +679 323 2501; fax: +679 323 1513.
E-mail address: sotheeswaran@usp.ac.fj (S. Sotheeswaran).

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Folic (pteroyl-L-glutamic) acid

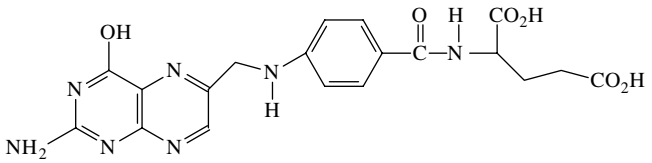

Fig. 1. Structure of folic acid.

Most of the Fijian population do not consume fortified grains or cereals and therefore it is important that people are made aware of the sources of folates in their diet. There is an urgent need in Fiji to generate folate composition data for the foods to reveal those foods that are abundant in folates. Analysis of folate in foods is not easy, due to its multiple forms, lower stability, and presence at low concentrations in biological systems, and complex extraction and detection techniques. Although chromatographic methods and other biological methods, such as protein binding assays, are available, the most commonly used method, namely the microbiological method that is currently used for food composition purposes throughout the world, was adopted for this study. The method follows a tri-enzyme technique, as suggested by Tamura, Mizuno, Johnston, and Jacob, 1997. The study provides new data on folic acid and total folate contents of eighteen commonly consumed foods in Fiji, including vegetables and poultry, which can be incorporated into the national food databases. Availability of such data will be very useful for future dietary and bioavailability studies.

2. Materials and methods

2.1. Materials

Fresh vegetables and poultry products listed in Table 1 were purchased from the markets located in the central and eastern divisions of the Fiji islands.

2.2. Moisture determination

Triplicates of each composite food sample were separately analysed for moisture content according to the AOAC (2002) method. Samples were initially weighed, kept overnight in a vacuum oven at 70 °C and then weighed. The moisture contents of the samples were determined within 24 h of purchase.

2.3. Sample preparation, extraction and the determination of folic acid

Edible portions of the food samples (about 300 g) were individually homogenised in a domestic blender. All food samples were freeze-dried and kept in airtight plastic bags. For ease of transport of samples to the University of New South Wales (UNSW), the freeze-dried samples were stored at −20 °C. All sample extractions described below were carried out in subdued light and all the glassware was wrapped with aluminium foil. Prior to the extractions, the freeze-dried samples were first milled using a mortar and pestle. Each sample (1 g) was suspended in 25 ml of extraction buffer (0.1 M potassium phosphate, 1% ascorbic acid and pH 6.1) in a 250 ml Erlenmeyer flask, autoclaved at 121 °C for 10 min, immediately cooled, centrifuged (Beckman J2-MC with a JA 14 Fixed Angle Rotator, Beckman Instruments, Inc., Paulo Alto, CA, USA) at 3000 rpm for 15 min at 4 °C and filtered, if necessary. The supernatant was transferred to small brown bottles and stored at −20 °C. The extracts were

Table 1
The folate content of selected Fijian foods (μg/100 g ± SD) on a fresh weight basis

Foods	Moisture (g/100 g)	Folic acid[a]	Total folate[a]
Sample			
Green leafy vegetables			
Chinese cabbage (*Brassica chinensis.*)	95	65 ± 9	81 ± 2
English cabbage (*Brassica oleracea* var. *bullata.*)	90	30 ± 5	33 ± 2
Drumstick leaves (*Moringa oleifera*)	81	86 ± 6	101 ± 13
Amaranth leaves (*Amaranthus* sp.)	89	40 ± 3	57 ± 7
Bele (*Abelmoschus manihot*)	89	131 ± 14	177 ± 5
Fern (Ota) (*Athyrium esculenta*)	91	3 ± 0.2	3 ± 0.09
Roots and tubers			
Carrots (*Daucus carota*)	89	13 ± 1	15 ± 1
Cassava/ tapioca (*Manihot esculenta*)	60	36 ± 1	48 ± 4
Taro shoots (*Colocasia esculenta*)	83	5 ± 1	6 ± 0.34
Other vegetables			
Pumpkin (*Cucurbita maxima.*)	87	17 ± 2	25 ± 0.26
Okra, (ladies Finger) (*Abelmoschus esculentus* L.)	88	76 ± 6	81 ± 6
Green Bananas (*Musa paradisiaca* var. *sapientum*)	73	10 ± 1	12 ± 1
Long beans (*V. sesquipedalis*)	89	123 ± 2	130 ± 10
Tomatoes (fresh) (*Lycopersicon esculentum* var. *commune*)	94	10 ± 1	17 ± 2
Tomatoes (canned) (*Lycopersicon esculentum* var. *commune*)	94	8 ± 1	10 ± 1
Poultry products			
Egg white dried (*G. domesticus*)	62	7 ± 1	11 ± 0.38
Egg Yolk (*G. domesticus*)	52	189 ± 28	256 ± 8
Chicken breast (*G. domesticus*)	60	31 ± 4	33 ± 6

[a] All values are means of triplicate determinations.

directly used for the determination of the folic acid content of the foods, using the method suggested by Tamura (1998). The experimental method for this determination is given below in Section 2.6. For the determination of total folates, the above extracts were subjected to the methods described in Sections 2.4–2.6.

2.4. Determination of total folates

To release the folates that may be trapped in or bound to the matrices of protein and carbohydrate in the food, the tri-enzyme technique described by Iwatani, Arcot, and Shrestha (2003) was used, based on the method suggested by Tamura et al. (1997) and Rader, Weaver, and Angyal (1998). The tri-enzymes were: (a) protease (Megazyme, subtilisin A from *B. licheniforms*) (2 mg/ml), which was prepared according to the method described by Rader et al. (1998), (b) α-amylase (A-3176, Sigma Chemical Co., St. Louis, MO 63178) (20 mg/ml) and (c) desiccated chicken pancreas conjugase (CP) (No. 0459-12-2, Difco Laboratories, Detroit, MI 48232-7058) prepared according to Kirsch and Chen (1984) (5 mg/ml).

2.5. Tri-enzyme treatment

A 5 ml aliquot of the sample, obtained in Section 2.3, was taken for the determination of the total folate content from each of the food extracts. The pH was adjusted to 4.5 using 0.1 M HCl. To this sample, 0.8 ml of protease was added, vortexed and incubated at 37 °C for 16 h. The sample was then placed in a 100 °C water bath for 5 min to deactivate the enzyme. The sample was then cooled and 0.8 ml of α-amylase was added and incubated at 37 °C for 4 h. The sample was then placed in a boiling water bath again for 5 min to deactivate the enzyme and the pH was adjusted to 7.2 using 0.1 M sodium hydroxide. A 1 ml aliquot of CP was added and incubated at 37 °C for 3 h. The enzyme was deactivated at 100 °C for 5 min and cooled, following the method of Tamura (1998). The contents were then centrifuged at 3000 rpm for 10 min. The supernatant was kept in brown bottles at −20 °C prior to the microbiological assay.

2.6. Microbiological assay of folic acid/total folate

Lyophilized *L. casei* subsp. *Rhamnosus* was obtained from the Department of Microbiology, University of New South Wales, Sydney, Australia. Glycerol-cryoprotected *L. casei* culture was prepared according to Wilson and Horne (1982). Before the assay, 0.5 ml of culture was diluted to 50 ml with 0.85% sterile saline solution. A folic acid (F-7876, Sigma Chemical Co., St. Louis MO, 63178) standard solution with final concentration of 1 ng/ml was prepared with the dilution buffer (0.05 M potassium phosphate, 0.15% ascorbic acid, pH 6.1). The standard folic acid solutions of 0.2–1.0 ml were pipetted into 12 mm × 110 mm test tubes and made up to 1.5 ml with dilution buffer, in triplicate. In another set of test tubes, 0.5 ml of the suitably diluted vegetable extract was added, and volume was made up to 1.5 ml as before. A total assay volume of 3 ml was achieved using 1.5 ml of the folic acid *L. casei* subsp. *Rhamnosus* medium (prepared as per label instructions) in all tubes. The tubes were autoclaved at 121 °C for 5 min and cooled immediately. An innoculum volume of 50 µl was added to each tube and incubated at 37 °C for 16–18 h. Growth (turbidity) of *L. casei* subsp. *Rhamnosus* was measured as absorbance in a spectrophotometer set at a wavelength 540 nm.

2.7. Quality control

The certified reference material (CRM 485, lyophilised mixed vegetables) was obtained from the Institute for Reference Materials and Measurements, Geel, Belgium, to validate the method used for the determination of total folate in foods. Recovery of folic acid spiked at the level of 0.2 ng to the sample tube (1 set) was also determined. The recovery studies were carried out throughout the assay procedure. Recovery of added folic acid was calculated as:

$$\%\text{Recovery} = \frac{\text{ng folic acid in spiked sample tube} - \text{ng folic acid in unspiked sample tube}}{\text{ng folic acid added in spiked sample}} \times 100$$

The assays with percentage recoveries of added folic acid outside the range 95–105% were deemed unacceptable and not included.

3. Results and discussion

3.1. The selection of Fiji foods

In an effort to ascertain the knowledge of the people of the central and eastern parts of Viti Levu, in Fiji, about the importance of folates in their diet, a short food frequency questionnaire was administered to 200 people living in these areas of Fiji. From this study, 18 common foods in the Fijian diet were selected for the analyses of folic acid and total folate contents.

Six of the foods analysed, such as long beans (*Vigna sesquipedalis*), drumstick leaves (*Moringa oleifera*), fern (Ota) (*Athyrium esculenta*), *bele* (*A. manihot*), pumpkin (raw) (*Cucurbita maxima*) and raw taro shoots (*Colocasia esculenta*) did not have any literature data available on their

folate levels. However, folate levels are reported for the other 12 food varieties studied, elsewhere (Combs, 1998). This is the first report on the total folate levels of the 18 fresh food varieties consumed in Fiji.

3.2. Folic acid and total folate contents of the foods

The folic acid content of the foods analysed (Table 1) was in the range 3–189 μg/100 g (fresh weight). Bound folates were released using the tri-enzyme extraction (protease, α-amylase and chicken pancreas) method to yield total folates. The total folate content of the different food samples studied (Table 1) varied from 3–256 μg/100 g (fresh weight). The coefficient of variation for the total folate was 1.3–18.6%. Among the green leafy vegetables, *bele* had the highest amount of total folate (177 μg/100 g), followed by drumstick leaves (101 μg/100 g) and Chinese cabbage (81 μg/100 g). Among the roots and tubers analysed, cassava had the highest level of folate (48 μg/100 g). Long beans had the highest folate level (130 μg/100 g) among the other green vegetables. Egg yolk had the highest total folate content (256 μg/100 g) of the foods studied; dried egg white contained only 7 μg/100 g of folate.

Only two samples studied *Bele* (*A. manihot*) and egg yolk (*Gallus domesticus*) had total folate contents in the range 177–256 μg/100 g. Five foods had folate contents in the range 57–176 μg/100 g. They were: amaranth leaves (*Amaranthus* sp.), Chinese cabbage (*Brassica chinesis*), *okra* (ladies finger) (*Abelmoschus esculentus*) (L.), long beans (*V. sesquipedalis*) and drumstick leaves (*M. oleifera*). Most of the foods analysed (11 samples) had folate contents in the range of 3–48 μg/100 g. They were: English cabbage, fern (*Ota*) (*Athyrium esculenta*), *taro* shoots (*Colocasia esculenta*), tomatoes (canned) (*Lycopersicon esculentum* var. *commune*), dried egg white (*G. domesticus*), bananas (*Musa paradisiaca* var. *sapientum*), carrots (*Daucus carota*), tomatoes (fresh) (*Lycopersicon esculentum* var. *commune*), pumpkin (*Cucurbita maxima.*), chicken breast (*G. domesticus*) and cassava/tapioca (*Manihot esculenta*). The results show that the amounts of folate present in some of the vegetables were quite substantial.

The average folic acid to total folate ratio in the 18 foods analysed was 0.62, ranging from 0.59 to 0.95. These results indicated that tri-enzyme method used improved the extractions of folates in the foods studied.

Microbiological assay, for the determination of folates, has been well documented in previous studies using foods from other countries. Combs (1998) derived the vitamin contents of foods from the National Nutrient Database (USDA National Nutrient Database for Standard Reference 19, 2006).

A comparison of folate values (Table 1) between the present study and the reported data on fresh foods in the literature (Combs, 1998) is as follows; tomatoes (fresh) (*Lycopersicon esculentum* var. *commune*) (15 μg/100 g), carrots (*Daucus carota*) (14 μg/100 g), cassava/ tapioca (*M. esculenta*) (27 μg/100 g), green bananas (*Musa paradisiaca* var. *sapientum*) (19.1 μg/100 g), egg yolk (*G. domesticus*) (146 μg/100 g), egg white dried (*G. domesticus*) (18 μg/100 g) and cabbage (*Brassica oleracea* var. *bullata.*) (43 μg/100 g). All of these foods, except cabbage, green bananas and dried egg white had lower folate levels than those in the present study. These folate values for the foods reported above have also been analysed using the tri-enzyme microbiological procedure (USDA National Nutrient Database for Standard Reference 19, 2006) and thus the data are comparable with the results presented in this paper.

Combs (1998) did not report the folate levels in the following fresh vegetables but reported the folate levels in these processed foods, as indicated in parentheses in μg/100 g : amaranth leaves (*Amaranthus* sp.) (56.8), Chinese cabbage (*Brassica chinensis*) (40.6), taro shoots (*Colocasia esculenta*) (2.6), pumpkin (*Cucurbita maxima*) (12.3), *okra* (ladies Finger) (*Abelmoschus esculentus* L.) (45.7), and tomatoes (canned) (*Lycopersicon esculentum* var. *commune*) (22.4).

No literature data are available for fresh or processed local foods such as long beans (*V. sesquipedalis*), drumstick leaves (*M. oleifera*), *bele* (*A. manihot*), Fern (*Ota*) (*Athyrium esculenta*) and chicken breast (*G. domesticus*).

The results clearly indicate that vegetables, which are green in colour, are very good sources of total folates and that the folate levels also depend on the sources of the foods.

It was very essential to use ascorbic acid in the extraction, as well as in the microbiological assay (Wilson and Horne 1983 and 1984). Ascorbic acid acts as a reducing agent, which protects some of the relatively labile reduced folates against oxidation, thus increasing the accuracy of the estimation of food folate content (Baker-man, 1961). Various factors affect the level of folate in vegetables such as the environmental aspects, e.g. geographical and geological conditions, season, climate and species variation (Mullin, Wood, & Howsam, 1982). Type of conjugase used, enzyme treatment, incubation time and extraction pH also play important roles in determining the folate levels in particular foods (Tamura, 1998).

3.3. Quality control

The accuracy of the results was verified by analyzing a commercially available reference sample (CRM 485). The total folate content of CRM 485, as determined in this study, was 368 ± 24 μg/100 g (dry weight) using the tri-enzyme (protease, α-amylase and chicken pancreas) method. This result agrees with the recorded total folate content of the reference sample, which was 315 ± 44 μg/100 g (dry matter). The assays with recovery values beyond 95–105% were not included.

4. Conclusions

Folic acid and total folate contents of 18 common Fijian foods were analysed with the microbiological assay, using the tri-enzyme extraction method. In the foods studied, the values for folic acid ranged from 3 to 189 μg/100 g and the total folate contents were in the range 3–256 μg/100 g on a fresh weight basis. It is notable that egg yolk from the Fijian poultry had the highest folate level (256 μg/100 g). The Fijian vegetables that had reasonably high folate contents were: *Bele* (177μg/100 g); long beans (130μg/100 g); drumstick leaves (101 μg/100 g); Chinese cabbage and Okra (81 μg/100 g). All foods analysed were predominant in folic acid excepting in egg yolk. The data obtained indicate that the Fijian population has access to foods with high folate levels and it is possible to meet the recommended dietary intake for folate of 300–400 μg/day (Gregory, Quinlivan, & Davis, 2005) by the population.

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