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Total folate: diversity within fruit varieties commonly consumed in India

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Abstract

Folate concentrations in selected fruits were measured using the trienzyme extraction and microbiological assay with *Lactobacillus casei* (subsp. *Rhamnosus*) as an assay organism. Fruits were purchased from different retail outlets at Coimbatore, Tamilnadu, India and were analyzed for total folate content. The folate content in all fruits varied considerably on a fresh weight basis from 10 to 328 μg/100 g, with tropical fruits ranging between 10 and 211 μg/100 g, temperate fruits from 11 to 328 μg/100 g, and the subtropical fruits in the range of 9–237 μg/100 g. Amongst all fruits, plum had the highest content of folate (328 μg/100 g). Data analyzed will assist dietary studies to estimate and evaluate the adequacy of folate intakes of the population, to formulate experimental diets for folate bioavailability studies, and to revise dietary recommendations for the population. In addition, the data will assist the health authorities in planning and executing strategies for intervention programs.

Keywords: *Folate, fruits, trienzyme treatment, microbiological assay, Lactobacillus casei*

Introduction

In the past, several studies have shown that a high consumption of fruits is associated with a decreased number of human cancers (Miller 1990; Negri et al. 1991; Block et al. 1992; Steinmetz and Potter 1991; Weisburger 1991), cardiovascular disease and many other diseases (Palgi 1981; Gramenzi et al. 1990; Gey et al. 1993; Dauchet et al. 2006). The essentiality of fruits as food for human beings has been known to mankind since time immemorial (Veeraraghavathatham et al. 2004). It is highly potent in nature and can be attributed to its specific effects due to the presence of compounds such as vitamins, minerals, dietary fiber and a wide range of secondary metabolites (phytochemicals) responsible for characteristics such as flavor, color and taste and, above all, nutritional value. The important role of fruit is in the defense mechanism in the body because of the antioxidant capacity of several components like carotenoids, vitamin C and vitamin E (Tomás-Barberán and Robins, 1997). Another possible mechanism is its

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potential influence on homocysteine metabolism by folate (De Bree et al. 1997; Brouwer et al. 1999).

Human life cannot exist without folate because it is involved in essential functions of cell metabolism such as DNA replication, repair, methylation and synthesis of nucleotides, vitamins and some amino acids (LeBlanc et al. 2007). The term folate (water soluble vitamin) refers to all forms of the vitamin including the naturally occurring forms (polyglutamates), whereas folic acid refers to the most oxidized, stable, and easily absorbable synthetic form (monoglutamate) (Hoffpauer and Bonnette 1998; Arcot and Shrestha 2005) and is synthesized from the precursor GTP, *p*-aminobenzoate, and glutamate. These three building blocks are modified in a number of enzymatic steps and used for the further production of various folate derivatives that are necessary for one carbon metabolism, such as tetrahydrofolate, 5-formyltetrahydrofolate, 5,10-methenyltetrahydrofolate, 10-formyltetrahydrofolate, and 5,10-methylenetetrahydrofolate (Stover and Schirch 1993; Green et al. 1996; Sybesma et al. 2003). Folate is involved as a cofactor in many reactions and mainly building blocks of DNA, RNA and nucleotides (Wald 1991; Vanderput et al. 2001). Thus the deficiency states lead to impaired cell division manifested as megaloblastic anemia and fetal development defects in humans, certain types of cancer and Alzheimer's disease (Pandrangi and LaBorde 2004). Folate deficiency is high in India, where the prevalence of neural tube defects is 6.27–8.31/1,000 live births (Salvia and Damania 2005). The Food Composition Database does not include any data on the folate composition of fruits (Gopalan et al. 2006). The fruit consumption in India is 13–49 g/consumption unit/day.

Food composition data are necessary before nutrition recommendations are given and before trends in nutrient intakes are interpreted (Leclercq et al. 2001). The available data on folate content in foods, particularly in India, are now known to underestimate folate content (Vishnumohan et al. 2009). The ineffectiveness of common extraction techniques such as the single enzyme treatment also indicates lower folate values. Improvements in the extraction and analytical techniques over the past decade have led to more accurate determination of not only total folate contents in foods, but naturally occurring individual forms of folate in varied matrices (Pfeiffer et al. 1997; Tamura et al. 1997; Tamura 1998; Konings 1999; Finglas et al. 1999; Stralsjo et al. 2002; Freisbleben et al. 2003; Jastrebova et al. 2003). A great deal of evidence indicates that the use of protease and amylase, in addition to deconjugation with folate conjugase, enhances the yield of measurable folate from foods. It is apparent to many that this 'trienzyme' approach is the method of choice for measurement of folate in foods (Gujaska and Kuncewicz 2005). The objective of the present study was to determine the folate content of fruits using the trienzyme extraction technique.

Materials and methods

Sampling of fruits

Thirty-three different types of fruits with at least two varieties in each were purchased from the retail markets in different parts of Coimbatore, Tamilnadu, India. Five lots of each fruit type were bought from at least five different locations. The fruits were purchased during June–August 2007 in the form they are usually consumed and ripe

Table I. Folate content (μg/100 g fresh weight) of tropical Indian fruits.

Fruit variety and common name	Moisture content (%)	Total folate (μg/100 g)
Banana and its varieties		
(*Musaparadisiaca* spp.)		
Rasthali	73.4	15 ± 0.5
Poovam	72.0	10 ± 1.4
Sevvalai	73.0	21 ± 0.5
Karpuravalli	73.1	10 ± 1.7
Nendram	75.8	39 ± 6
Moris	74.0	47 ± 1.4
Malaipazham	68.9	88 ± 7
Nadampazham	61.6	188 ± 22
Mango varieties (*Mangifera indica*)		
Vadu (unripe)	65.0	138 ± 22
Kilimuku	68.0	121 ± 1.2
Palghat	67.8	71 ± 1.5
Senduram	81.2	88 ± 7
Nilam	88.1	120 ± 72
Rumani	86.3	60 ± 7
Orange variety (*Citrus sinensis*)		
Kamala	87.0	20 ± 3
Delhi	87.6	43 ± 4.5
Papaya variety (*Carica papaya*)		
Co-2 (seeded)	90.8	11 ± 1.5
Sunrise solo (seedless)	90.1	23 ± 0.6
Grape varieties (*Vitis vinifera*)		
Paneer draksha	88.5	11 ± 1.4
Thomson	83.6	13 ± 3
Bangalore blue	87.6	19 ± 4
Pusa seedless	87.3	57 ± 33
Tomato variety (*Lycopersicon esculentum*)		
PKM-1	94.0	100 ± 5.6
Lakshmi	93.0	22 ± 2
Sapota variety (*Achrus sapota*)		
Cricket ball	73.6	21 ± 2.5
PKM-4	73.2	20 ± 3.5
Guava variety (*Psidium guvajava*)		
Lucknow 46	81.7	49 ± 1.5
Chittipar	85.1	211 ± 7.2
Pomegranate variety (*Punica granatum*)		
Dholka	83.0	20 ± 10
Kabul	75.0	64 ± 8
Watermelon (*Citrullus vulgaris*)	95.8	64 ± 8
Muskmelon (*Cucumis L. melon*)	95.1	63 ± 1.5
Dates (*Pheonix dactylifera*)	55.3	151 ± 114
Korukkapalli (*Pithachellobium dulce*)	75.6	10 ± 1.7
Amla (*Emblica officinalis*)	76.2	25 ± 4
Harfarworie (*Phyllanthus distichus*)	89.3	35 ± 1.5
Citrus variety		
Bablimas (*Citrus grandes*)	85.6	17 ± 0.1
Lemon (*Citrus lemon*)	90.5	11 ± 1.5
Lime (*Citrus aurantifolia*)	84.6	22 ± 1.5

Folate values are expressed as mean ± standard deviation of triplicate determinations.

fruits were used for analysis. The fruits selected are presented in Table I along with their botanical names.

Sample preparation

The edible portion from each of the five replicate purchases of each fruit sample was homogenized in a waring blender and composites prepared. All fruit samples were kept individually in airtight aluminum bags, sealed and stored in the refrigerator at –20°C for analysis.

Extraction of total folate

Five grams of each homogenate was mixed with 100 ml extraction buffer (0.1 M potassium phosphate, 1% ascorbic acid and pH 6.1) according to Tamura et al. (1997) with slight modification, where the centrifugation of the food extracts was eliminated from the procedure before trienzyme treatment, and the final enzyme-treated samples were only centrifuged (RM-12C; Remi Instruments, Maharashtra, India) at 5,000 rpm for 20 min and filtered. A cold extraction procedure was followed because the food type (fruits) analyzed have negligible protein and hence heat treatment was considered unnecessary (Hyun and Tamura 2005). The supernatant was transferred to small brown bottles and stored at –18°C. The extract was directly used for determination of total folate content of foods.

Determination

Lyophilized *Lactobacillus casei* subsp. *rhamnosus* (ATCC 7469) was used for the analysis. Glycerol cryoprotected *L. casei* was prepared according to the method developed by Wilson and Horne (1982). Before the assay, 1.0 ml culture was diluted to 10 ml of 0.85% sterile solution.

Folic acid (Sigma Chemical, St Louis, MO, USA) standard solution with a final concentration 1 ng/ml was prepared with the dilution buffer (0.05 M potassium phosphate buffer, 0.15% vitamin C, pH 6.1). The standard folic acid solutions of 0.2, 0.4, 0.6, 0.8, and 1.0 ml were pipetted into test tubes and made up to 1.5 ml with dilution buffer in triplicates. In another test tube, 0.5–1.0 ml diluted fruit extract was added and the volume was made up to 1.5 ml with dilution buffer as before. Folic acid *L. casei* medium (prepared as per label instructions; Difco Laboratory, Detroit, MI, USA) was added to each test tube, making the total assay volume to 3 ml. The tubes were autoclaved at 121°C for 5 min and cooled immediately. A 60 μl diluted inoculum was added to each tube, vortexed and incubated at 37°C for 16 h. The growth of *L. casei* was measured as absorbance in a spectrophotometer set at wavelength 540 nm.

Quality control

The certified reference material (CRM 485, lyophilised vegetable mix) obtained from the BRC (Commission of the European Communities. Community Bureau of Reference) was analyzed with the samples to ensure accuracy of data. Recovery studies were carried out with every batch of analysis. Assays with recovery levels below or above the range of 90–105% were discarded.

Moisture determination

Five replicates of each homogenized fruit sample were separately analyzed for moisture content in a hot air oven at 100°C (National Institute of Nutrition 2003).

Results and discussion

In the fruits analyzed, the total folate content was found to vary from 10 to 328 μg/100 g. All values are reported on a wet weight basis. Among the tropical fruits the folate content was in the range 10–211 μg/100 g. The banana varieties (*Musaparadisiaca* spp.) (Figure 1) had a total folate content in the range 10–188 μg/100 g. *Nadampazham* had 188 μg/100 g followed by *malaipazham* with 88 μg/100 g, and *poovam* and *karpuravalli* had the least of 10 μg/100 g. Figure 1 shows the differences in total folate contents between varieties for the same fruit. An earlier study by Vishnumohan et al. (2009) reported a value of 13 ± 8 μg/100g for banana (*moris* variety). The differences in extraction methods used (cold in the present study) indicate a higher extraction of folate.

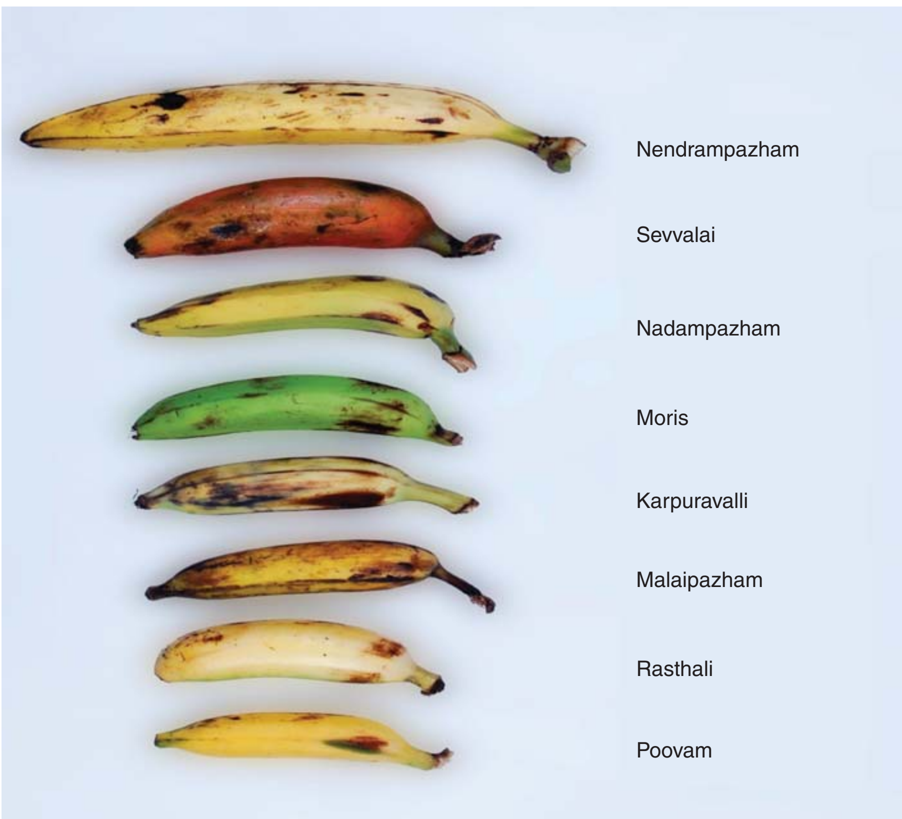

Figure 1. Banana varieties (*Musaparadisiaca* spp.).

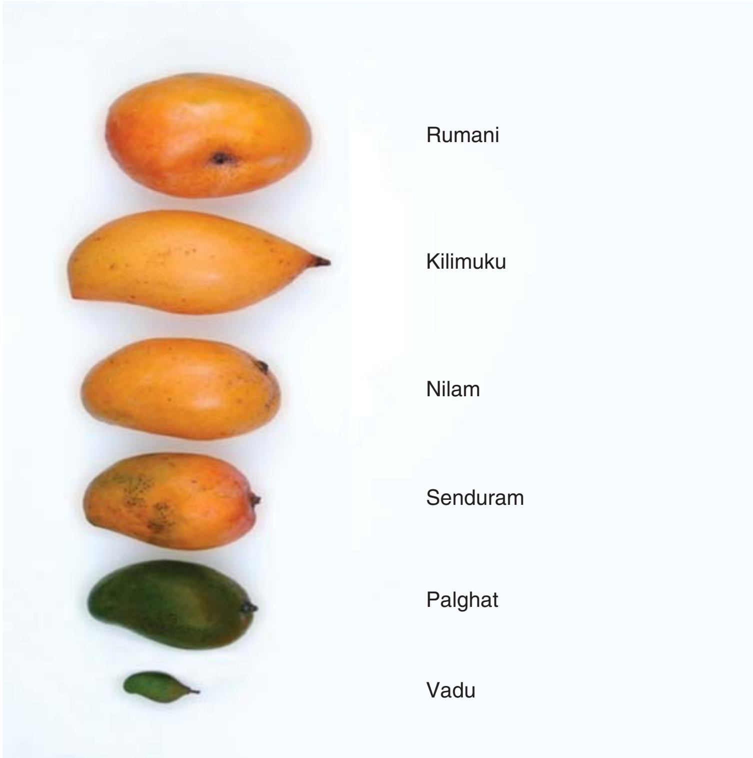

Figure 2. Mango varieties.

Mango is also known as the king of fruits. It has been grown in India for over 400 years and more than 1,000 varieties exist today (Veeraragavathaham et al. 2004). Ripe mangoes eaten as fruit were analyzed in the present study along with one variety (Vadu), which is normally consumed in the unripe form. In the mango varieties (*Mangifera indica*) (Figure 2), the total folate content ranged between 60 and 138 μg/100 g. The *vadu* variety had the highest (138 μg/100 g on a fresh weight basis). The *rumani* variety had a low folate level of 60 μg/100 g on a fresh weight basis. The value reported by the US Department of Agriculture (USDA 2009) is 14 μg/100 g, a value much lower than reported here for the same species.

The grape variety (*Vitis vinifera*) Paneer draksha had a low value 11 μg/100 g and *pusa* seedless had 57 μg/100 g. The USDA (2009) reported a value of 2 μg/100 g for the Thompson seedless variety, which is lower than reported here (13 μg/100 g). Among the other tropical fruits, dates (*Pheonix dactylifera*) had a total folate value of 151 μg/100 g. The USDA (2009) value was 19 μg/100 g. It is interesting to note that watermelon (*Citrullus vulgaris*) had 64 μg/100 g in spite of its high moisture content being 95.8%. The USDA has also reported a lower value of 3 μg/100 g. Lemon (*Citrus lemon*) had 11 μg/100 g. A similar value of 11 μg/100 g was reported by USDA (2009). In the present study, the orange (Delhi variety) fruit had a folate content of (43 μg/100 g). Seyoum and Selhub (1998) reported that folates in orange juice and egg yolk were the most stable, whereas those in the other foods exhibited intermediate stability.

Table II. Total folate content (μg/100 g fresh weight) in temperate fruits.

Fruit variety and common name	Moisture (%)	Total folate (μg/100 g)
Plum (*Prunus persica*)	85.1	328 ± 26
Avocado (*Persea Americana*)	80.6	306 ± 17
Strawberry (*Fragaria annanasa*)	87.2	11 ± 0.5
Cherry (*Prunus avium*)	73.0	73 ± 5
Pear (*Pyrus communis*)		
Gola	86.0	147 ± 25
Barlett	85.0	84 ± 8

Folate values expressed as mean ± standard deviation of triplicate determinations.

Canene-Adams et al. (2005) reported the folate content of raw tomatoes as 15 μg/100 g while tomato juice, soup, and sauce had 20 μg/100 g, 7 μg/100 g and 9 μg/100 g, respectively. In the present study, the tomato PKM-1 variety had 100 μg/100 g and the *lakshmi* variety had 22 μg/100 g.

Amongst temperate fruits (Table II), strawberry had folate of 11 μg/100 g; Stralsjo et al. (2002) found the folate content of strawberry to be between 70 and 100 μg/100 g on a fresh weight basis, and plum was 328 μg/100 g, cherry was 73 μg/100 g, pear-gola was 147 μg/100 g, avocado was 306 μg/100 g and pear-barlett was 84 μg/100 g.

The folate content of subtropical fruits (Table III) was in the range 9–237 μg/100 g. The folate content of apple reported earlier was found to be 3 μg/100 g (Lee et al. 2003). The palmyra contained folate of 103 μg/100 g, jamun of 167 μg/100 g, figs of 149 μg/100 g, custard apple of 237 μg/100 g, apple-simla of 9 μg/100 g, apple-china of 74 μg/100 g, woodapple of 22 μg/100 g, jackfruit of 35 μg/100 g, and pineapple of 25 μg/100 g. Data obtained clearly demonstrate that fruits are fairly good sources of folate depending on the variety and type.

In general, the folate values obtained from the current study for a majority of fruits analyzed were higher than the folate values reported in literature. This could possibly be due to various environmental factors such as season, climate, soil conditions and

Table III. Total folate content (μg/100 g fresh weight) in subtropical fruits.

Fruit variety and common name	Moisture (%)	Total folate (μg/100 g)
Figs (*Ficus carica*)	81.8	149 ± 39
Palmyra (*Borassus flabellifer*)	92.8	103 ± 5.5
Jamun (*Syzygium cumini*)	83.3	167 ± 30
Custard apple (*Annona squamosa*)	69.3	237 ± 9
Woodapple (*Limonia acidissima*)	60.8	22 ± 1.15
Jackfruit (*Artocarpus heterophyllus*)	76.0	35 ± 1.5
Pineapple (*Ananas cosmosus*)	86.8	25 ± 1.7
Apple (*Malus sylvestris*)		
Simla	80.1	9 ± 1.4
Washington	79.2	37 ± 1.7
China	82.6	74 ± 1.5
Ber (*Zizyphus jujuba*)		
Banarsi	81.0	30 ± 5.5
Elaichi	81.6	40 ± 1.7

Folate values are expressed as mean ± standard deviation of triplicate determinations.

geographical variations, which can affect the levels of folate in fruits. Besides, the folate value of a particular food can also be influenced by variations in the method of folate extraction (type of conjugase, enzyme treatment, incubation period, etc.) and detection methods (Tamura 1998).

A South Indian study revealed that only less than 10% of the selected population consumed fruits daily. This was based on a 24-h dietary recall conducted in 200 respondents (Vishnumohan et al. 2009). The present study provides valuable information on the folate content in various fruits that can be used as a guideline to promote foods rich in folate that might be beneficial in reducing folic acid deficiency in the population.

Quality control

In order to ensure the quality of the assay performed, a standard reference material (CRM 485, lyophilised vegetable mix) was also analyzed for folate content following the same procedure of extraction and determination. The values obtained were within the acceptable range of the certified value, ensuring accuracy of the data.

Conclusion

The results clearly demonstrated that fruits are fair to good sources of folate. The recommended daily intake of folate for the adult population in India is 100 μg/100 g (Gopalan et al. 2006). This study on folate content in fruits in India is fundamentally important for the estimation of appropriate dietary folate intake. Recent investigations have indicated that certain diseases can be prevented by increasing dietary folate intake and a significant segment of a certain population appears to have inadequate folate nutrition in India. Further investigation in India should focus on analysis of the folate content of other food groups and then on bioavailability of folate.

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