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Determination of folate content in commonly consumed Malaysian foods

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Abstract: Currently, data concerning the content of naturally occurring dietary folate in Malaysian foods is scarce. The aim of this study was to determine the folate content of vegetables, fruits, legumes and cereals that were commonly consumed among Malaysians. The total folate content of 156 samples (51 vegetables, 33 fruits, 22 legumes and legume products, and 50 cereals and cereal products) available in Malaysia was determined by microbiological assay using *Lactobacillus casei* (*L. casei*) after trienzyme treatment with protease, α-amylase and folate conjugase (from rat serum). An internal quality control system was used throughout the study by analyzing CRM 121 (wholemeal flour) and CRM 485 (lyophilized mixed vegetables); percent recovery (as mean ± SD) of 97 ± 2.0 and 101 ± 4.0 was obtained. The range of folate content in vegetables, fruits, legumes and cereals were 1-11 µg/100 g and 1-31on the basis of fresh weight and 1-31 µg/100 g and 2-156 µg/100 g on the basis of dry weight, respectively. This study has shown that some of these underutilized vegetables and fruits are good sources of folate and could fulfill the recommended dietary intake of total folate.

Keywords: Folate, Malaysia, vegetable, fruit, legume, cereal

Introduction

Folic acid (folate) plays an important role in fetal development and maintenance of health. An adequate intake of folate helps in reducing the risk of early embryonic brain development, specifically neural tube defects (NTDs) (Ashfield-Watt *et al*., 2002), colon cancer (Su and Arab, 2001), and brain disorders such as depression, reduced cognition, and Alzheimer's disease (Yoo *et al*., 2000). Folate deficiency is associated with an elevation of the homocysteine level, which is an independent risk factor for cardiovascular disease (Hao *et al*., 2003). Several factors contribute to folate deficiency including inadequate dietary intake, smoking, alcohol consumption, and abnormal folate metabolism (Le Marchand *et al*., 2005; Ren *et al*., 2007). Folate is present in a wide range of foods including fruits, vegetables, cereals and legumes. The amount of folate in food is affected by many factors including processing procedures, sample preparation, storage time and conditions (McKillop *et al*., 2002; Han *et al*., 2005; Witthoft *et al*., 2006).

A wide variety of tropical and temperate vegetables and fruits are available in Malaysia all year round. Based on the national food consumption survey involving adults aged 18-59 years in 2002-2003, 40% and 54% said they consumed green leafy vegetables daily and weekly, respectively (Norimah *et al*., 2008). Almost three-quarters (73%) reported consuming bean vegetables (such as long beans, French beans) on a weekly basis. However, the folate intake was not reported in this national survey (Mirnalini *et al*., 2008). The main reason is because the Malaysian food composition table (Tee *et al*., 1997) does not include data on folate content. One study by Khor *et al*. (2006) did report on the folate intake by Malaysian women of childbearing age but their data was analyzed using the USDA National Nutrient Database. In the same study, about 15.1% of the women showed plasma folate deficiency (< 6.8 nmol/L), while another 84.8% had red blood cell folate below 906 nmol/L, which is indicative of inadequate blood folate for protection from neural tube defects in pregnancy.

In light of the lack of data on the folate content in Malaysian foods and the high prevalence of sub-optimal blood folate concentrations among the women, it is important to provide data on the folate content of commonly consumed foods in Malaysia. The folate contents of 156 types of food items including vegetables, fruit, cereals and legumes were determined by using microbiological assay.

Microbiological assay is a method which uses the *L. casei* and the growth of bacterial is compared by measuring the turbidity of different samples after incubation period (Pandrangi and LaBorde, 2004). *L. casei* is the most frequently used since it responds well to most metabolic forms of folate (Rader *et al*., 1998). Generally, in microbiological

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assay, the trienzyme treatment is more preferable and the use of conjugase prior the assay helps in cleaving the polyglutamyl forms of folate in food into monoglutamyl forms, which is the form utilized by the *L. casei* (Bagley and Selhub, 2000; de Bree *et al*., 1997). This is because the accuracy of the results is depends on the preparative steps which consist of the extraction of the polyglutamates and the enzymatic deconjugation (de Bree *et al*., 1997). These enzymes; α-amylase and protease, degrade the matrix of carbohydrates and proteins before deconjugating the terminal glutamyl peptides of folate to monoglutamyl or diglutamyl derivatives (Chen and Eitenmiller, 2007; Pandrangi and LaBorde, 2004; Doherty and Beecher, 2003). Besides microbiological assay, there are other methods to measure folate such as by high-performance liquid chromatography (HPLC) and ligand binding (de Bree *et al*., 1997).

Materials and Methods

Sample preparation

Four major food groups, namely, vegetables, fruits, cereal and cereal products, and legume and legume products were analyzed in this study. The final selection of 156 food items was based on the Nutrient Composition of Malaysian Foods (Tee *et a*l., 1997), which consists of foods frequently consumed in Malaysia. Figure 1 shows the sampling process of food samples from various markets. About one kilogram of each dry and fresh food was purchased from each of three sources – hypermarkets, markets and local farmer's markets - located in Selangor. All the samples were collected in calendar year 2009. All the food samples were kept in airtight plastic bags and stored at -20°C upon purchasing.

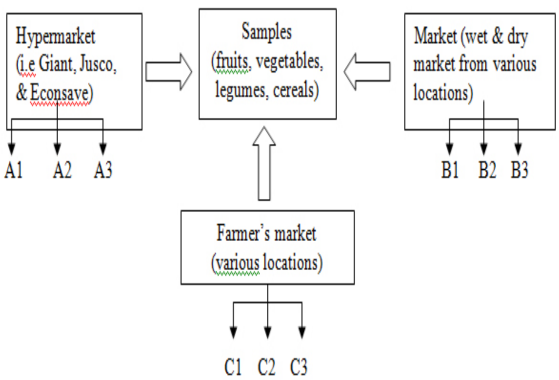

Figure 1. Food sampling process
(One kilogram of food samples obtained from each location (A1, A2, A3, B1, B2, B3, C1, C2 and C3)

The vegetables and fruits were extracted for folate analysis within two days of purchase. All the edible portions of each food was minced, ground and homogenized individually in a domestic blender (Dual Speed Laboratory Blenders, 90-7010G, Waring, Torrington, CT). The prepared samples were individually analyzed for total folate. Approximately 0.25-1 g (containing 1 µg folic acid) of all various kind of sample was suspended with 20 ml extraction buffer (pH 7.8 phosphate buffer containing ascorbic acid) in a 125 Erlenmeyer flask, autoclaved at 121°C for 15 min, and cooled immediately (AOAC, 2006). In order to release the folate that may be trapped or bound to the matrices of protein and carbohydrate in the food, the trienzyme extraction technique was used (AOAC, 2006). It included the usage of three enzymes; protease (EC 3.4.24.31, P-5147, Sigma Chemical Co., St. Louis, MO) (2 mg/ml), α-amylase (EC 3.2.1.1, A-6211, Sigma Chemical Co., St. Louis, MO 63178) (20 mg/ml), and a conjugase from rat serum (R9759, Sigma Chemical Co., St. Louis, MO) (5 mg/ml), which were prepared according to the AOAC method. First, the sample was incubated with protease for 3 hours at 37°C, heated at 100°C for 3 min to inactivate the protease before cooling at room temperature. After that, the mixtures were incubated again with α-amylase for 2 hours at 37°C. Finally, the sample was treated with rat serum for 16 hours at 37°C before the enzyme was inactivated. All sample extractions were carried out in subdued light and all the glassware was wrapped with aluminium foil. These homogenates were stored at 4°C until ready for the assay.

Preparation of cryoprotected inoculum

For use in the microbiological analysis of the extracted folate according to AOAC method (2006), a cryoprotected inoculum of *L. casei* was prepared as follows. A medium containing folic acid casei medium (4.7 g) (Difco, Merck, Darmstadt, Germany) was dissolved in 50 mL distilled water. It was then heated to boiling, cooled in ice before adding 50 mL water and 0.025 g of sodium ascorbate (Sigma Chemical, St Louis, MO, USA). A diluted folic acid stock (Sigma Chemical, St Louis, MO, USA) solution (100 ng/mL) (0.5 mL) was mixed. *L. casei* (0.1 g) (JCM 1136, Riken, Japan) was then suspended in 1 mL of above solution. After the addition, about 0.15 mL of this suspension was transferred to newly prepared folic acid casei medium (0.85 mL) to be incubated for 18 hours under 37°C. Meanwhile, cold glycerol was prepared by adding 120 mL glycerol (Sigma Chemical, St Louis, MO, USA) and 30 mL water before autoclaving it (121°C, 15 min), and cooled in an ice bath. After that, 100 mL of 80% cold glycerol was added and mixed gently. The mixtures were transferred into sterile tubes and stored in -70°C.

96-well Microtiter plate assay

The medium for the assay, which contained phosphate buffer pH 6.8 (1.42 g sodium phosphate dibasic and 1 g ascorbic acid) (Sigma Chemical, St Louis, MO, USA), as an assay buffer was prepared and pipetted into wells. After pipetting the samples, standards, and working inoculum to appropriate wells, the plate was then put into a plastic bag and sealed before incubating it for 22 hours at 37°C. In the mean time, a pan containing water was placed in the incubator to ensure adequate humidity in the incubator to avoid the evaporation of water from the outer wells. After the incubation, the plate was removed and allowed to stand at room temperature for 30 min before the reading was taken at 595 nm using a microplate reader.

Serum and enzymes

Rat serum was used as folate conjugase (γ-glutamyl hydrolase) and was purchased from Sigma-Aldrich (USA). In order to remove the endogenous folate, the rat serum was mixed with one tenth volume of charcoal, stirred for 1 hour on ice and filtered through a microfilter (0.22 µm, Fisher Scientific). Aliquots of rat serum folate conjugase were stored at -70° until used. α-amylase (EC 3.2.1.1, A-6211) and protease (EC 3.4.24.31, P-5147) were freshly prepared and filtered through a microfilter immediately before using to remove the microbes that can synthesize folates during the incubations (Tamura, 1998; Yon and Hyun, 2003).

Quality control

Two certified reference materials, CRM 485, lyophilized mixed vegetables and CRM 121, wholemeal flour, with the folate contents of 315 ± 44 µg/100 g dry matter and 50 ± 11 µg/100 g dry matter, respectively, were used to validate the method for the determination of the total folate in the foods and for daily quality control. Reference samples (CRM 121 and CRM 485) were included in each analytical run. The CRM 485 is a preparation which contain canned chopped tomatoes, frozen carrots and sweet corn that has been packaged into food-grade, heat-sealed, and in aluminium laminate sachets under an inert atmosphere. The recovery of folic acid spiked at the level of 0.2 ng to the CRMs was also determined. It was carried out throughout the assay procedure. The formula for the calculated recovery was as follows:

The assays with percentage recoveries of added folic acid outside the range 88-110% for CRM 485 (mixed vegetables) and 95-105% for CRM 121 (wholemeal flour) were unacceptable and not included.

Statistical analysis

Data were analyzed using Statistical Package for Social Science (SPSS) version 17, and presented as means ± SD.

Results

Recovery studies were conducted by CRM 121 (wholemeal flour) and CRM 485 (lyophilized mixed vegetables) with 96 ± 5.0 and 99 ± 11.0, respectively. The total folate content of CRM 121 measured was 48.4 ± 4 µg/100 g dry matter which is still within the range mentioned on the certificate of the reference material which was 50 ± 11 µg/100 g dry matter. The total folate content of CRM 485 as determined in this study was 317.6 ± 22 µg/100 g dry matter. This result was in the range with the total folate content of the certified reference material which was 315 ± 44 µg/100 g dry matter.

Based on Table 1, the overall folate content of the fruits ranges widely from 2 to 31 µg/100 g on the basis of fresh weight. Among the fruits, papaya (*Carica papaya*) had the highest folate contents (31 µg/100 g), followed by Sapodilla (*Manilkara achras*) (30 µg/100 g), kiwi (*Actinidia deliciosa*) (29 µg/100 g) and orange (*Citrus nobilis*) (24 µg/100 g). Other fruits showed lower folate contents, that is, less than 20 µg/100 g with lemon (*Citrus medica*) showing the lowest amount (2 µg/100 g). Comparing folate content for legumes, soya sauce was found to contain the highest amount of folate, whereas soya bean curd (Tau kua) contains the least (Table 2). The folate content of the legume samples were generally lower than those compared in Table 2. In Table 3, it shows the comparison of the folate content in the analyzed samples of vegetables with the reported data. The highest folate content was in Kesom (*Polygonum minus*) (10 µg/100 g wet weight) and pumpkin (*Cucurbita maxima*) (8 µg/100g wet weight). The folate content in the cereals and cereal products is shown in Table 4. The values range from the lowest in rice flour (2 µg/100 g dry weight) to the highest in corn flakes (156 µg/100 g dry weight).

Discussion

This study gives some preliminary values of total folate content of commonly consumed food found in Malaysia. As shown in Table 1, most of our values for the fruits analyzed were similar to those reported by other references with only some having a lower value compared to the references such as mango, Mandarin orange, and strawberry. Legumes are a good source of folate and, in Malaysia, they are relatively

Table 1. Folate content (µg/100 g wet weight) of fruits in the present study compared with other literature

Samples (n=9)	Our results	USDA[1]	AUS/ NZ[2]	Philippine[3]	East Asia[4]
Apple, green (Epal hijau); *Pyrus malus*	4 ± 0.1				
Apple, red (Epal merah); *Pyrus malus*	3 ± 0.1	2.9		2	2
Banana (Pisang abu); *Musa paradisiaca*	7 ± 0.2				
Banana (Pisang brangan); *Musa acuminata*	5 ± 0.4				
Carambola/Star-fruit (Belimbing manis/besi); *Averrhoa Carambala*	6 ± 0.4	12			
Custard apple (Buah nona); *Annona squamosa*	3 ± 0.2				
Dragon fruit (Buah pitaya); *Hylocereus undatus*	3 ± 0.1				
Green grape (Buah anggur); *Vitis vinifera*	5 ± 0.2	2		5.2	5.2
Guava (Jambu batu); *Psidium guajava*	2 ± 0.0			6.8	6.8
Hog plum/Ambarella (Kedondong); *Spondias cytherea*	5 ± 0.3				
Honeydew (Tembikai susu); *Cucumis melo*	9 ± 0.6	17.6			
Kiwi fruit (Buah kiwi); *Actinidia deliciosa*	29 ± 0.6	25	26		
Korean persimmon (Pisang kaki); *Diospyros kaki*	6 ± 0.5				
Lemon (Limau susu); *Citrus medica*	2 ± 0.0	10.3	11		
Lime, wild (Limau purut); *Citrus hystrix*	2 ± 0.0				
Mango (Mangga); *Mangifera indica*	4 ± 0.1	14		6.5	6.5
Mangosteen (Manggis); *Gardinia mangostana*	3 ± 0.1				
Mini orange, Mandarin/Tangerine (Limau Cina); *Citrus Reticulate*	2 ± 0.2	15.5			
Orange (Limau manis); *Citrus nobilis*	24 ± 1.5	30			
Orange, Mandarin/Tangerine (Limau Cina); *Citrus reticulate*	3 ± 0.1	15.5		5.1	5.1
Papaya (Betik); *Carica papaya*	31 ± 1.1	38		1.1	1.1
Pear, green (Buah pir hijau); *Pyrus communis*	5 ± 0.1				
Pear, yellow, Chinese (Buah lai); *Pyrus sinensis*	5 ± 0.1	7.2			
Pineapple (Nenas); *Ananas comosa*	4 ± 0.3	14.8		6	6
Plums (Buah plum); *Prunus spp.*	6 ± 0.2	4.6			
Red grape (Buah anggur); *Vitis vinifera*	7 ± 0.2	2		5.2	5.2
Sapodilla (Ciku); *Manilkara achras*	30 ± 0.4				
Snake fruit (Salak); *Zalacca edulis*	6 ± 0.1				
Soursop (Durian belanda); *Annona muricata*	19 ± 0.6				
Strawberry (Strawberi); *Fragaria grandiflora*	8 ± 0.2	22.2	74		
Tamarind, fresh pods (Buah Asam Jawa); *Tamarindus indica*	3 ± 0.1				
Water apple (Jambu air); *Eugenia aquea/Syzygium aqueum*	4 ± 0.3				
Watermelon (Tembikai); *Citrullus vulgaris*	3 ± 0.0	3.3		6	6

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[2] NUTTAB (2006). Australian Food Composition Tables. Food Standards Australia New Zealand.
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[4] Food and Agricultural Organization (FAO). (1972). Food composition table for use in East Asia. Rome. FAO.

affortable the market and consumed by different ethnic groups. Based on Table 2, there was a huge difference between the folate content in this study with other countries such as kidney bean and chickpea. Besides, legumes are often processed into foods prior to consumption, such as canned baked bean, which resulting in the loss of folate in the end product due to the characteristic of folate, which is water soluble and sensitive to light, air, heat, and pH (Ginting *et al*., 2003). From Table 3, among most of the From Table 3, among most of the vegetables studied, the folate content was found to be lower than that shown in the literature with alarming big difference in asparagus, cabbage, cauliflower, and green bean sprout. The differences in the values of samples in this study with other references may be due to various factors such as differences in cultivation conditions (season and climate), agronomic condition, ripeness, cultivar and species (Holasova *et al*., 2008; Iniesta *et al*., 2009; Akilanathan *et al*., 2010; Soongsongkiat *et al*., 2010).

Table 2. Folate content (µg/100 g dry weight) of legumes and legumes products in the present study compared with other literature.

Samples (n=9)	Our results	USDA[1]	AUS/ NZ[2]	Philippine[3]	East Asia[4]
Appalam	4 ± 0.3				
Baked bean, canned (Kacang panggang dalam tin)	27 ± 0.9		54		
Chickpea/Common gram (Kacang kuda); *Cicer arietinum*	10 ± 0.7			163	
Dhal, yellow (Dal kuning)	4 ± 0.4				
Gram, black (Kacang hitam); *Phaseolus mungo*	5 ± 0.9				
Gram, green/Mung bean (Kacang hijau); *Phaseolus Aureus*	5 ± 1.8			121	
Gram, red (Kacang merah); *Phaseolus angularis*	4 ± 0.3				
Kidney bean/Hyacinth bean (Kacang sepat); *Dolichos lablab*	11 ± 2.7	394			
Lima beans (Kacang cina); *Phaseolus lunatus*	3 ± 0.7			125	36
Malavi soya bean	8 ± 0.4				
Soya bean cake, fermented (Tempeh)	10 ± 0.8				
Soya bean curd (Tau-kua)	1 ± 0.1				
Soya bean curd, fried (Tau-kua goreng)	10 ± 2.1				
Soya bean curd, round (Tau-hoo)	8 ± 1.1				
Soya bean curd, sheet/film (Fucok)	13 ± 2.4	15			
Soya bean curd, strands (Fucok)	14 ± 2.3	15			
Soya bean curd, square (Tau-hoo)	6 ± 0.8				
Soya bean milk, unsweetened (Susu kacan soya, tanpa gula)	23 ± 1.6	2			
Soya bean, paste, fermented (Tau-ceo)	7 ± 0.9				
Soya bean, white (Kacang soya putih)	4 ± 0.4			210	
Soya sauce, 'thick' (viscous) (Kicap pekat)	31 ± 4.6				
Soya sauce, 'thin' (Kicap cair)	31 ± 3.0				

[1] US Department of Agriculture (USDA) (2005). National Nutrient Database for Standard Reference, Release 18. Nutrient Data Laboratory.
[2] NUTTAB (2006). Australian Food Composition Tables. Food Standards Australia New Zealand.
[3] Food composition table (1990). Recommended for use in the Philippines. 6th Revision. Manila, Philippines.
[4] Food and Agricultural Organization (FAO). (1972). Food composition table for use in East Asia. Rome. FAO.

In addition, sample-to-sample variation, variety in the method used, which includes the extraction (conjugase, enzyme treatment, incubation periods) and deconjugation procedure, might also explain the differences in the folate value in the foods (Yon and Hyun, 2003; Holasova *et al*., 2008; Devi *et al*., 2008). The use of ascorbic acid during extraction and microbiological assay help in protecting the labile folate against oxidation since it is a reducing agent; hence, this will increase the accuracy of the measurement (Devi *et al*., 2008). Furthermore, the selection of treatment also influences the folate content obtained. This is because, using a conventional method, which only involves the conjugase treatment, the conjugase itself helps in the hydrolysis of polyglutamyl folates to monoglutamyl folates, which can then be utilized by *L. casei*. According to Akilanathan *et al*. (2010), the use of conjugase treatment causes lower folate values. However, since folate might also be bound to the protein or polysaccharides, the introduction of trienzyme treatment, with the inclusion of α-amylase and protease in the folate analysis, is often used to enhance the release of the folate that is bound or trapped in the food matrix of proteins and oligosaccharides besides the deconjugation with the folate conjugase, thus, enhancing the yield of folate from foods (Johnston *et al*., 2001). Holasova *et al*. (2008) reported that the incubation of α-amylase and conjugase for 3hr resulted in a moderate increase in the folate readings. They further found that the period of incubation inversely contributed to the increment in folate values. This is supported by many researchers who opt for the trienzyme approach as the method of choice for folate measurement in food rather than conjugase alone (Gujska and Kuncewicz, 2005). In contrast, some researchers disagreed with this as they claimed that the addition of α-amylase reduced the amount of folate in the food (Pandrangi and LaBorde, 2004). Besides the value differences observed in our study compared to other literature might also be due

Table 3. Folate content (µg/100 g wet weight) of vegetables in the present study compared with other literature

Samples (n=9)	Our results	USDA[1]	AUS/NZ[2]	Philippine[3]	East Asia[4]
Asparagus, fresh (Asparagus); *Asparagus officinalis*	3 ± 0.8		114	109	109
Bamboo shoot (Rebung); *Dendrocalamus* and *Bambusa spp.*	3 ± 1.7			7.1	7.1
Bean, four-angled (Kacang botor); *Psophocarpus tetragonolobus*	3 ± 0.3				
Bean, French (Kacang buncis); *Phaseolus vulgaris*	3 ± 0.5				
Bean, string (Kacang panjang); *Vigna sinensis*	2 ± 0.0				
Beetroot (Akar bit); *Beta vulgaris*	3 ± 0.1				
Broccoli (Brokoli); *Brassica oleracea*	5 ± 0.4	6.5	49		
Cabbage, Chinese (Pak-coy); *Brassica chinensis*	4 ± 1.3		40		
Cabbage, common (Kobis); *Brassica oleracea*	3 ± 0.7	43		46.1	46.1
Carrot (Lobak merah); *Daucas carota*	4 ± 1.7	14	18	8	8
Cauliflower (Bunya kobis); *Brassica oleracea*	5 ± 0.2	53.9	62	22.2	22.2
Celery (Daun seladeri); *Apium graveolens*	4 ± 0.8	35	13	7	7
Chili, green (Lada hijau); *Capsicum annuum*	5 ± 0.1		23		
Chili, red (Lada merah); *Capsicum annuum*	4 ± 1.8		23		
Chili, small (Cili padi); *Capsieum frutescens L.*	6 ± 3.6				
Chinese mustard (Sawi pahit); *Brassica juncea*	3 ± 0.5				
Cucumber (Timun); *Cucumis sativus*	3 ± 0.8	6.7	7	6	6
Cucumber, hairy (Timun bulu); *Cucumis spp.*	3 ± 0.2				
Cymbopogon (Serai); *Cymbapogon atratus*	3 ± 0.5				
Egg plant/Brinjal (Terung); *Solanum melongena*	5 ± 1.7	19		15.7	15.7
Fern shoots (Pucuk paku); *Diplazium esculentum*	3 ± 0.1				
Garlic Chives (Kucai); *Mulgedium tataricum*	4 ± 1.6				
Ginger flower (Bunga kantan); *Phaeomeria speciosa*	5 ± 0.6				
Gourd, bitter/Balsam pear (Peria); *Momordica charantia*	3 ± 0.4				
Gourd, snake (Ketola ular); *Tricosanthes anguina*	2 ± 0.1				
Gourd, wax/Winter melon (Kundur/Tong-kuah); *Benincasa Hispida*	4 ± 0.4				
Green bean sprout (Taugeh); *Phaseolus sp.*	6 ± 0.6		25		
Indian Pennywort (Pegaga); *Hydrocotyle asiatica*	6 ± 0.5				
Japanese cucumber (Timun Jepun); *Cucumis sativus*	5 ± 0.8				
Lady finger/Okra (Kacang bendi); *Hibiscus esculentus*	4 ± 0.6			22.7	22.7
Lotus root (Akar teratai); *Nelumbo nucifera*	4 ± 0.3				
Mustard cabbage (Sawi putih); *Brasica rapa chinensis*	2 ± 0.0				
Paprika/Bell peppers (Lada besar hijau); *Capsicum annuum*	5 ± 2.4			15.8	
Parsley (Daun sup); *Petroselinum crispum*	3 ± 0.7				
Pea shoot (Dau Miao); *Pisum sativum*	3 ± 0.2				
Pithecolobium (Jering); *Pithecellobium jiringa*	2 ± 0.5				
Plantain, flower (Jantung pisang); *Musa sapientium*	3 ± 1.2				
Pumpkin (Labu merah); *Cucurbita maxima*	8 ± 4.0	16		9.3	9.3
Romaine lettuce (Yau Mak Tam); *Lactuca sativa L. var. longifolia*	3 ± 0.6				
Spinach, red (Bayam merah); *Amaranthus gangeticus*	3 ± 0.4				
Spring onion (Daun bawang); *Allium fistulosum*	5 ± 0.2				
Swamp cabbage/Water convulvolus (Kangkung); *Ipomoea aquatica/I. reptans*	4 ± 0.5				122
Sweet potato shoots (Pucuk ubi keledek); *Ipomoea batatas*	6 ± 0.2			88.4	88.4
Tapioca shoots (Pucuk ubi kayu); *Manihot utilissima*	6 ± 0.3				
Tomato (Tomato); *Lycopersicum esculentum*	4 ± 1.4	15		6.3	
Turmeric (Kunyit); *Cucuma domestica (Zingeberaceae)*	3 ± 0.4				
Vietnamese mint (Kesom); *Polygonum minus*	10 ± 0.9				
Watercress (Semanggi/Sai-yong-coy); *Nashturtium officinale*	4 ± 0.5				
Wolfberry leaves/Chinese box thorn (Kau-kei); *Lycium Chinese*	3 ± 0.5				
Yam bean (Sengkuang); *Pachyrrhizus erosus/P. bulbosus*	4 ± 0.2				
Yam stalks (Batang keladi); *Colocasia esculentum*	3 ± 1.2				

[1] US Department of Agriculture (USDA) (2005). National Nutrient Database for Standard Reference, Release 18. Nutrient Data Laboratory.
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to the reason that *L. rhamanosus* respond differently to natural monoglutamate (Philips and Wright, 1982) as well as in the presence of high level of methyl-folate (Finglas *et al*., 1996)

Besides the factors mentioned above, for cereals, generally, most of our values are lower than those in the other references. This is due to fact that most of the locally produced cereal products in Malaysia are not enriched or fortified with folic acid unlike the mandatory fortification in other countries such as the United States. Malaysia is currently following the footsteps of other countries in implementing

Table 4. Folate content (µg/100 g dry weight) of cereals and cereal products in the present study compared with other literature.

Samples (n=9)	Our results	USDA[1]	AUS/NZ[2]	Philippine[3]	East Asia[4]
Atta for making capatti (Tepung atta untuk membuat capati)	8 ± 0.7				
Beras Basmathi	25 ± 0.7				
Beras perang (brown rice)	45 ± 5.7		44		
Beras perang Kemboja	20 ± 1.7				
Beras Siam pulut susu	16 ± 1.6				
Beras wangi Thai	18 ± 0.5				
Biscuit, coconut (Biskut kelapa)	10 ± 0.5				
Biscuit, cream crackers (Biskut krim kraker)	8 ± 0.6				
Biscuit, finger cream (Biskut jejari berkrim)	14 ± 1.9				
Biscuit, lemon (Biskut lemon)	3 ± 0.6				
Biscuit, marie (Biskut marie/Biskut manis)	14 ± 1.6				
Biscuit, peanut (Biskut kacang)	9 ± 1.3				
Biscuit, sultana (Biskut sultana)	11 ± 0.5				
Bread, white (Roti putih)	18 ± 3.8		30		
Cookies, cashewnut (Biskut gajus)	15 ± 0.1				
Cookies, oats (Biskut oat)	3 ± 0.3				
Cookies, sesame seed (Biskut bijian)	6 ± 0.6				
Corn flour; maize flour (Tepung jagung)	46 ± 6.9		1		
Corn snack, cheese flavored (Snek jagung berperisa keju)	8 ± 1.4				
Corn snack, chicken flavored (Snek jagung berperisa ayam)	11 ± 0.7				
Custard powder (Tepung kastad)	3 ± 0.4				
Cocoa ball	155 ± 7.4				
Corn flakes	156 ± 8.4		703		
Honey star	154 ± 6.4				
Koko krunch	155 ± 7.2				
Macaroni (Makaroni)	15 ± 1.0				
Maize (jagung); *Zea Mays*	3 ± 1.5				
Mee soya	11 ± 1.4				
Milk cereal (wheat) for infants (Bijirin bersusu untuk bayi)	5 ± 0.2				
Millet (Sekoi); *Eleusine coracana*	11 ± 2.1		85		
Noodle, dry (Mee kering)	3 ± 0.3	14			
Noodle, dry, instant (Mee kering, segera)	15 ± 1.8				
Noodle, rice (Mee-sua)	10 ± 0.5				
Noodle, rice (Mee-hoon)	11 ± 0.7				
Noodle snack, chicken flavoured (Snek mee berperisa ayam)	8 ± 0.7				
Oats, processed, tinned (Oat dalam tin); *Avena sativa*	6 ± 0.4				
Red rice	14 ± 1.4				
Rice bran, coarse (Dedak kasar)	6 ± 0.2				
Rice bran, fine (Dedak halus)	5 ± 0.2				
Rice, broken (Beras hancur)	18 ± 1.0				
Rice flour (Tepung beras)	2 ± 0.4				
Rice, glutinous, black (Pulut hitam)	8 ± 0.5				
Rice, glutinous, white (Pulut putih)	7 ± 1.1				
Rice, parboiled (Beras, 'parboiled')	26 ± 4.1				
Rice, uncooked	16 ± 0.6		10		
Sushi rice	16 ± 0.5				
Wheat cracker	4 ± 0.1				
Wheat flour (Tepung gandum)	2 ± 0.3	26			
Wheat, whole grain (Gandum, biji)	14 ± 3.1				
Wholemeal biscuit	11 ± 2.2				

[1] US Department of Agriculture (USDA) (2005). National Nutrient Database for Standard Reference, Release 18. Nutrient Data Laboratory.
[2] NUTTAB (2006). Australian Food Composition Tables. Food Standards Australia New Zealand.
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[4] Food and Agricultural Organization (FAO). (1972). Food composition table for use in East Asia. Rome. FAO.

mandatory fortification of folic acid in all the flour in the very near future. We hope that this data could give some light to the authorities on the necessity as well as possible drawback of implementing mandatory fortification of folic acid. Looking at our data, it is not impossible to achieve the required amount of folic acid from foods alone.

Conclusions

This study provides total folate values for some of the commonly consumed foods representing key foods in the diet of the Malaysian sampled from a small location in Selangor by microbiological assay. Despite the advantages of using microbiological assays on folate analysis where it is considered as a reference method for folate with low cost and acceptable specificity, it is tedious and time consuming (Karmi *et al*., 2011). This study also showed that food like vegetables, fruits, legumes and cereals which are commonly and easily available in Malaysia can be sources of folate intake. Hence, it provides preliminary data that might be useful in looking at the normal and habitual folate intake among the Malaysian population. This further could justify or help authorities in looking at the need to have mandatory fortification of folic acid implemented. The trienzyme treatment used in this study seems to provide higher folate values in some samples although not all. Beside this factor, other reason such as different growing condition and cultivar could also have an impact on the differences seen with other literatures. Finally, we hope that this study will provide useful information to Malaysians, concerning the amount of folate found in foods so that they will make informed decisions on the selection of food to be consumed by their families.

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