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Folate Content and Availability in Malaysian Cooked Foods

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ABSTRACT

Introduction: Data on folate availability of Malaysian cooked foods would be useful for estimation of dietary folate intake; however such information is scarce. **Methods:** A total of 53 samples of frequently consumed foods in Malaysia were selected from the Nutrient Composition of Malaysian Foods. Folate content was determined using HPLC method hyphenated with a stainless steel C_{18} column and ultraviolet detector (λ = 280 nm). The index of folate availability was defined as the proportion of folate identified as monoglutamyl derivatives from the total folate content. **Results:** Total folate content of different food samples varied from 30-95 μg / 100g fresh weight. Among rice-based dishes, the highest and the lowest total folate was in coconut milk rice (*nasi lemak)* and ghee rice (*nasi minyak*), respectively. In noodle dishes, fried rice noodle (*kuey teow goreng*) and curry noodle (*mee kari*) had the highest folate contents. The highest index of folate availability was in a flat rice noodle dish (*kuey teow bandung*) (12.13%), while the lowest was in a festival cake (*kuih bakul*) (0.13%). Folate content was found to be negatively related to its availability. **Conclusion:** This study determined folate content and folate availability in commonly consumed cooked foods in Malaysia. The uptake of folate from foods with high folate content may not be necessarily high as folate absorption also depends on the capacity of intestinal deconjugation and the presence of high fibre in the foods.

Keywords: Folate content, availability, HPLC

INTRODUCTION

Folates are mainly found in vegetables, fruits, legumes and cereal products. It is a B vitamin which constitutes various mono- and pteroylpolyglutamates (two to seven glutamyl residues) of pteroic acid and has nutritional benefits similar to folic acid besides its chemical structures (Gregory, 2012). The only difference between folic acid and a folate is that folate is present naturally in the food whereas folic acid is in the form of vitamin applied mainly during fortification. Natural food folates are highly sensitive to heat, hence, cooking can result in destruction of 50-95% of the folate contents of food.

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Folate plays a crucial function in synthesising components of DNA, RNA and proteins. Besides, it is essential for hematopoiesis as well (Klee, 2000). Hence, deficiency in this vitamin causes the occurrence of megaloblastic anaemia and neural tube defects (Gujska & Kuncewicz, 2005). Further, the elevation of plasma homocysteine could be due to folate deficiency (Gujska & Kuncewicz, 2005). Worldwide, attention and programmes have been initiated to encourage a higher intake of folate. However, in many countries including Malaysia, the intake of folate has been found to be below the recommended intake (Khor *et al.*, 2006, Chew, Khor & Loh, 2011).

It has been reported that analysis of folate in foods is not easy, due to its multiple forms, lower stability, presence at low concentrations in biological systems, and complex extraction and detection techniques (Devi *et al.*, 2008). Generally, the most common and widely used method for folate analysis is microbiological assay. However, the disadvantage of using this method is it only provides a total figure for all the derivatives of folic acid and does not distinguish between individual forms of folate (Johnsson, Jagerstad & Frolich, 2007; Rychlik *et al.*, 2007; Poo-Prieto *et al.*, 2006). Another approach is using affinity chromatography in combination with a high-performance liquid chromatography (HPLC) diode array ultraviolet (UV) detection system. This has been used lately for folate determination to distinguish the single vitamers. Compared to microbiological assay, HPLC is a simple and more rapid procedure in determining folate content. The downside of using HPLC is the need to identify all the known forms of folates in order to be able to quantify total folates in a given sample, where some forms are hard to find or costly (LeBlanc *et al.*, 2007).

Knowledge about folate bioavailability from food is essential for the estimation of dietary requirements. Folate bioavailability is defined as the fraction of ingested folate that is absorbed and can be used for metabolic processes (Melse-Boonstra, Verhoef & West 2004). The bioavailability of folate from the diet is less than 100% due to several factors including the species of folate, linkages at molecular level, amount of folate and folic acid consumed, matrix, effect of modifiers, nutrient status, genetic factors, host-related factors, and mathematical interactions between the various factors (Melse-Boonstra *et al.*, 2004). Currently data on folate content and availability of cooked foods consumed by Malaysians are scarce. This data would be seen as necessary to help in evaluating the adequacy of folate intake especially among Malaysians. Therefore, the objective of this study was to determine folate content and availability of commonly consumed foods among Malaysians, using the HPLC method.

METHODS

Chemicals and reagents

Sodium ascorbate, pepsin, sodium chloride, tetrabutyl ammonium phosphate, dithio-erythritol, sodium acetate, hydrochloric acid, mannitol, calcium chloride, HEPES, zinc acetate, 5-methyltetrahydrooflate (disodium salt), folic acid, dimethyl glutaric acid and bis (2-hydroxyethyl) imino-tris (hydroxy-methyl) methane (Bis-Tris) were obtained from Sigma Chemical (St. Louis, MO, USA). Acetonitrile and water were of HPLC grade (Merck, Darmstadt, Germany).

Preparation of jejunal brush border membrane from pig intestine

Pig intestine was chosen as the source of pteroylpolyglutamate hydrolase due to the similarities between the enzymes present in human and pig intestine (Gregory *et al.*, 1987). The mucosa was scraped and the brush border membrane vesicles were prepared according to Selhub & Rosenberg (1981). Firstly, the mucosal scrapings were

homogenised for 30 s at 0°C in 15 volumes (w/v) of 0.05 M mannitol. Then, the homogenate was centrifuged for 10 min at 10000 x g. The pellet underwent a second homogenisation for 1 min by using fresh 15 ml mannitol solution before being combined with the first supernatant. The whole mixture was then filtered. Solid calcium chloride was added to the filtrate to make a final concentration of 10 mM before being centrifuged again at 4000 x g for 10 min and 40000 x g at 15 min. The pellet was then re-suspended in 100mM mannitol containing 10 mM potassium / HEPES at pH 7.4. Finally, the homogenate was centrifuged again (40000 x g, 15 min) and the pellet fraction was re-suspended in 2 ml mannitol potassium / HEPES solution, divided into aliquots and stored at -70°C.

Sample preparation

A total of 53 samples of frequently consumed cooked foods in Malaysia were selected based on the foods analysed in the Nutrient Composition of Malaysian Foods (Tee *et al*., 1997). The foods chosen were rice-based, noodles, local cakes (*kuih-muih)*, and breads. All the samples were purchased from different types of stalls in Serdang, Selangor as described in Chew, Loh & Khor (2012). The foods were ground and homogenised before analysis. Preparation was carried out under subdued light. Due to the physical characteristic of folate which is easily destroyed by heat, air and light during food preparation, the foods were analysed as ready for consumption to determine the actual folate contents. The grounded food was divided into two equal portions; the control group in which the folate was extracted under protective conditions and analysed as such without treatment and the experimental group where the extraction and treatment was performed. For control group, the foods was extracted by autoclaving for 30 min at 120°C in 10 vol 20g / L sodium ascorbate and 100 mmol / L Bis-Tris buffer, pH 7.8 (Seyoum & Selhub, 1993). After cooling in an ice bath, the extract was further centrifuged (40000 x g, 20 min, 4°C) before storing the supernatant at -80°C. The folate content for each food was determined by the HPLC method according to Seyoum & Selhub (1993). For the experimental group, it first underwent digestion with acid / peptic mixture. In this step, the homogenate was acidified to pH 2 with 1 mol / L HCL and mixed thoroughly with pepsin (0.5 g / 100g food). Then, it was incubated for 2 h at 37°C in a shaking water bath before adjusting the mixture to 6.5 with 1mol / L NaOH. The mixture was then boiled at 120°C for 30 min in equal volume of extraction buffer (20 g / L sodium ascorbate and 100 mmol / L Bis-Tris buffer, pH 7.8) in order to extract the folates. The extract was centrifuged at 40000 x g for 20 min at 4°C after cooling in an ice bath and the supernatant was then divided into two equal portions in order to determine susceptibility of folates to pteroylpoly-glutamate hydrolase action before and after purification by high-performance liquid chromatography.

Incubation before purification

One portion of the supernatant was incubated (37°C, 4h) with pig intestine (5 mg protein), 33 mmol / L dimethylglutarate buffer (pH 6.5), 0.25 mmol / L sodium chloride, 0.1 mmol / L zinc acetate and 2.5 g / L sodium acetate solution in a shaking water bath. The mixtures were determined by HPLC after the incubation as outlined by Seyoum & Selhub (1993). The absorbance of eluted folates was monitored at 280 nm using a diode array detector.

Incubation after purification

Another portion of the supernatant was subjected to purification on affinity column before incubating with the brush border membrane preparation as described above. After purification by affinity chromatography, folate monoglutamates were measured

using HPLC method with diode array detection. All analyses were performed under subdued light. This procedure was used to determine the most abundant folate forms naturally present in foods including 5-methyltetrahydrofolate. The calibration curves were constructed by plotting peak area against concentrations.

HPLC condition

The HPLC system (Hewlett Packard HPLC Series 1100) was equipped with a stainless steel column C_{18}, 250 mm x 4.6 mm I.D (Hewlett Packard, USA). The chromatographic conditions were as follows: column temperature, 23°C; autosampler temperature, 8°C; flow rate, 1ml / min; volume injected, 20 μL, and untraviolet detection (UV), 280 nm. The mobile phase consisted of variable mixtures of 5mmol / L tetrabutyl ammonium phosphate, 25 mmol/L NaCI, 5 mmol/L dithioerythritol, and 10% acetonitrile. The index of folate availability was assessed by comparing the concentration of the monoglutamylfolate in the experimental group to the folate concentration in the control group as follows:

$$\text{Folate availability index} = \frac{M1}{T1} \times 100$$

where M1 is the monoglutamylfolate concentration after treatment and T1 is the total folate concentration in the control group.

Data analysis

All data are reported as mean ± SD. The statistical analyses were carried out by using SPSS 17. The differences in the release of monoglutamylfolate before and after the purification were compared by using paired sample T-test. The value was significant at $p < 0.05$ level. Besides, the mean differences between the folate content with availability was compared using independent sample *T*-test and their relationships were computed using the Pearson correlation test.

RESULTS AND DISCUSSION

Folate content quantification using HPLC method

Total folate content of different food samples in this study varied from 30-95 μg / 100g fresh weight (Table 1). Among the samples, *roti canai* with egg (*roti telur*) had the highest amount of total folate, followed by fried kueyteow (*kuey teow goreng*) and popiah (*popiah*). Among the rice group, coconut milk rice (*nasi lemak*) had the highest level of folate. Besides fried kuey teow (*kuey teow goreng*), curry noodle (*mee kari*) had the second highest folate level among the other noodles. The highest total folate content in the *kuih-muih* group was unleavened burnt sugar (*apam gula hangus*), followed by popiah (*popiah)*. For *roti* (bread) group, *roti canai* showed the second highest folate level after *roti canai* with egg (*roti telur*). Among the foods studied, the lowest folate levels were found in steamed tapioca parcels (*lepatubi*) in the *kuih-muih* group. Eight foods had folate levels in the range of 50-100 µg / 100g fresh weight; they were coconut milk rice (*nasi lemak*), fried kuey teow (*kuey teow goreng*), curry noodle (*mee kari*), and *popiah*, Chinese crullers (*yau cha kuai*), unleavened burnt sugar (*apam gula hangus*), *roti canai* with egg (*roti telur*), and *roti canai*. The remaining had lower concentrations with a range of 30-50 µg / 100g fresh weight. Some of the foods studied showed high coefficient variations for the total folate using HPLC method. The variability observed in this study could be due to several factors. First, the ingredients used for the preparation of certain foods might differ from one location to another. Our study homogenised and pooled samples from nine stalls obtained from different locations around Serdang, Selangor. This could contribute in part to some of the variability found in this study. Additionally, the HPLC method has been shown to be lacking in stability during extraction (Koontz *et al.*,2005) and this might have an effect on the results obtained.

Table 1. Folate content in foods

Sample	*Mean*	*SD*	*% CV*	*Sample*	*Mean*	*SD*	*% CV*
Rice (μg/100g fresh weight)				Kuih-muih (μg/100g fresh weight)			
Chicken rice (*Nasi ayam*)	34.18	1.66	4.9	Unleavened burnt sugar (*Apam gulahangus*)	52.02	3.92	7.5
Briyani rice(*Nasi briyani*)	38.77	7.12	18.4	Bahulu (*Bahulu*)	33.99	2.49	7.3
Fied rice (*Nasi goreng*)	40.1	4.48	11.2	Banana fritter (*Cekodok pisang*)	34.47	0.55	1.6
Kerabu rice (*Nasi kerabu*)	36.83	4.71	12.8	Prawn fritter (*Cucur udang*)	35.39	1.46	4.1
Coconut milk rice (*Nasi lemak*)	61.77	5.79	9.4	Doughnut (*Donut*)	32.13	1.06	3.3
Ghee rice (*Nasi minyak*)	33.54	2.1	6.3	Currypuff (*Karipap*)	38.2	3.14	8.2
Noodle (μg/100g fresh weight)				Fried lekor (*Keropok lekor*)	34.41	1.1	3.2
				Cassava crackers(*Keropok ubi*)	33.97	2.18	6.4
				Dhall gravy (*Kuah dhal*)	43.37	1.2	2.8
Bandung kueyteow (*Kueyteow bandung*)	42.83	4.75	11.1	New year's cake (*Kuih bakul*)	34.72	2.07	6.0
Fried kueyteow (*Kueyteow goreng*)	94.6	8.78	9.3	Onion cracker(*Kuih bawang*)	33.66	2.43	7.2
Bandung noodle (*Meebandung*)	40.72	9.47	23.3	Sweet potato and sesame balls (*Kuih bom*)	39.21	6.26	16.0
Fried noodle (*Mee goreng*)	48.47	6.96	14.4	Kuihkaswi (*Kuih kwaci*)	32.62	2.59	7.9
Curry noodle (*Mee kari*)	53.07	2.34	4.4	Coconut filled crepe (*Kuih ketayap*)	34.6	2.46	7.1
Noodle soup (*Mee sup*)	36.95	6.44	17.4	Black glutinous rice koci (Kuihkocipuluthitam)	37.86	4.92	13.0
Bandung meehoon (*Meehoon bandung*)	32.35	0.35	1.1	Layer cake (*Kuih lapis*)	45.16	2.25	5.0
Fried meehoon (*Meehoon goreng*)	37.76	7.66	20.3	Draw pie cake (*Kuih serimuka*)	34.34	1.47	4.3
Rojak (*rojak*)	48.53	4.06	8.4	Kuih tiram (*Kuih tiram*)	49.15	7.51	15.3
Roti(μg/100g fresh weight)				Steamed banana parcels (*Lepat pisang*)	32.5	3.67	11.3
				Steamed tapioca parcels (*Lepat ubi*)	31.68	0.9	2.8
				Muruku (*muruku*)	38.87	3.75	9.6
Butter cake	41.95	8.89	21.2	Fried dumpling(*Pau goreng*)	32.11	1.22	3.8
Capati (*capati*)	42.84	1.51	3.5	Fried banana(*Pisang goreng*)	33.91	1.64	4.8
Chocolate cake	36.35	0.17	0.5	Popiah (*popiah*)	62.64	0.95	1.5
Banana cake	39.11	3.61	9.2	Grilled glutinous rice (*Pulut panggang*)	33.19	1.98	6.0
Roti canai (*roti canai*)	55.09	5.18	9.4	Savoury peanut cracker (*Rempenyek*)	33.11	0.64	1.9
Roti canai with egg (Roti telur)	94.71	4.7	5.0	Lace pancake (*Roti jala*)	44.26	4.72	10.7
Chocolate swiss roll	36.45	7.28	20	Fried pancake (*Tumpi*)	37.73	3.62	9.6
Tosai (*thosa*i)	36.29	0.04	0.1	Chinese crullers (*Yauchakuai*)	50.98	3.98	7.8

Based on our previous study on the determination of folate content using microbiological assay, the folate content in raw rice was 16 μg / 100 g dry weight, flour (wheat or rice) was 2 μg / 100 g dry weight, dry noodle (3 μg / 100 g dry weight), rice noodle (meehoon) (11 μg / 100 g dry weight), atta for making capati (8 μg / 100 g dry weight), chickpea (10 μg / 100 g dry weight), and yellow dhal (4 μg / 100 g dry weight) (Chew *et al*., 2012. Basically, most HPLC-measured folate contents were lower than values determined by the microbiological assay. It has been suggested that non-folate compounds could influence bacterial growth causing a higher folate content when detected by microbiological assay (Konings *et al*., 2001). Hence, this explains the large variation in the results obtained when comparing these two methods. This is in agreement with the study by Pfeiffer, Rogers & Gregory (1997) where the microbiological assay showed a slightly higher folate content in bread and spaghetti samples compared to HPLC methods.

Folate availability index

5-methyltetrahydrofolate may be produced during the preparation or processing of foods (Konings *et al*., 2001). The results for the experimental group which were digested with pteroylpolyglutamate hydrolase contained in the brush border membrane from the pig intestine is presented in Table 2. Subsequent to the purification steps, a more obvious release of monoglutamyl folate was seen compared to before purification. The highest folate availability index was in *kuey teow bandung* (12.13%) whereas the lowest was found in the traditional cake (*kuih bakul*) (0.13%).

Based on the *t*-test between folate content and availability, the results showed significant differences ($p < 0.05$) whereas based on the Pearson correlation test, folate content was found to be inversely related to folate availability although the strength of relationship was weak (P = -0.336, $p< 0.05$). This may be due to an altered digestion process resulting from either the high quantity of foodstuff (e.g., exceeding the capacity of intestinal deconjugation) or the presence of high fibre in the food.

The combination of affinity chromatography using the immobilised folate binding protein (FBP) in this study had provided for additional benefit in terms of its function in relation to purification and concentration of foods to achieve the final analysis. This is also supported by the study of Kariluoto, Vahteristo & Piironen (2001). This method produced a very specific and efficient purification. Bagley & Selhub (2000) found that folates are efficiently purified by affinity column. As a result, non-folate compounds such as reducing agents, added to the extraction buffer to protect the labile forms of folate, will be washed off in the affinity column before eluting the folates into the analytical column. Moreover, it enables the food extract to be concentrated ten-fold or more, hence, small endogenous folate concentrations present in foods can be determined with sufficient sensitivity using UV detection (Pfeiffer, Rogers & Gregory, 1997). The addition of piperazine to the affinity column helps to neutralise the acidic effluent of the column.

In conclusion, this study provides information about folate content and folate availability in commonly consumed cooked foods in Malaysia. Most of the folate content was found to be negatively related to its availability. This could be caused by alteration in the uptake of folates due to the reduction in the folate carriers or high fibre content. The data obtained are just preliminary results and hopefully in the future, further studies could be carried out to generate data on folate content and its availability from commonly consumed foods.

Table 2. Folate availability index

Sample	Before purification (%)	Purified fraction (%)	p-value
Rice (μg/100g fresh weight)			
Chicken rice (*Nasi ayam*)	2.46	3.66	0.029
Briyani rice (*Nasi briyani*)	3.73	4.41	0.026
Fied rice (*Nasi goreng*)	3.19	8.2	0.026
Kerabu rice (*Nasi kerabu*)	2.53	3.82	0.028
Coconut milk rice (*Nasi lemak*)	1.69	2.61	0.015
Ghee rice (*Nasi minyak*)	8.55	14.27	0.029
Noodle (μg/100g fresh weight)			
Bandung kueyteow (*Kueyteow bandung*)	2.58	14.71	0.024
Fried kueyteow (*Kueyteow goreng*)	2.13	3.66	0.018
Bandung noodle (*Mee bandung*)	3.08	3.54	0.025
Fried noodle (*Mee goreng*)	2.22	2.83	0.021
Curry noodle (*Mee kari*)	1.05	1.26	0.019
Noodle soup (*Mee sup*)	7.33	10.15	0.027
Bandung meehoon (*Meehoon bandung*)	2.99	4.57	0.03
Fried meehoon (*Meehoon goreng*)	3.03	3.7	0.027
Rojak (*rojak*)	1.53	4.1	0.021
Roti(μg/100g fresh weight)			
Butter cake	5.3	7.42	0.025
Capati (*capati*)	3.09	4.22	0.024
Chocolate cake	3.48	7.07	0.028
Banana cake	3.4	6.25	0.026
Roti canai (*roti canai*)	1.85	4.22	0.018
Roti canai with egg (*Roti telur*)	1.19	1.83	0.014
Chocolate swiss roll	2.04	6.07	0.028
Tosai (*thosai*)	2.84	6.14	0.028

Sample	Before purification (%)	Purified fraction (%)	p-value
Kuih-muih (μg/100g fresh weight)			
Unleavened burnt sugar (*Apam gulahangus*)	1.43	3.19	0.02
Bahulu (*Bahulu*)	5.6	12.14	0.029
Banana fritter (*Cekodok pisang*)	2.15	2.9	0.029
Prawn fritter (*Cucur udang*)	3.9	4.29	0.028
Doughnut (*Donut*)	1.58	12.9	0.03
Currypuff (*Karipap*)	3.84	4.82	0.027
Fried lekor (*Keropok lekor*)	2.9	10.32	0.029
Cassava crackers(*Keropok ubi*)	3.01	5.71	0.029
Dhall gravy (*Kuah dhal*)	2.29	11.08	0.024
New year's cake (*Kuih bakul*)	2.19	2.32	0.028
Onion cracker(*Kuih bawang*)	3.03	7.12	0.029
Sweet potato and sesame balls (*Kuih bom*)	1.81	2.12	0.026
Kuihkaswi (*Kuih kwaci*)	3.06	3.9	0.03
Coconut filled crepe (*Kuih ketayap*)	2.67	3.16	0.029
Black glutinous rice koci (*Kuih koci pulut hitam*)	4.33	6.11	0.027
Layer cake (*Kuih lapis*)	3.26	9.06	0.023
Draw pie cake (*Kuih serimuka*)	7.08	8.3	0.029
Kuihtiram (*Kuih tiram*)	2.93	3.86	0.021
Steamed banana parcels (*Lepat pisang*)	3.76	4.44	0.03
Steamed tapioca parcels (*Lepat ubi*)	1.97	2.73	0.03
Muruku (*muruku*)	2.47	3.88	0.026
Fried dumpling(*Pau goreng*)	2.51	7.05	0.03
Fried banana(*Pisang goreng*)	3.01	8.94	0.029
Popiah *(popiah*)	1.63	3.3	0.014
Grilled glutinous rice (*Pulut panggang*)	4.76	6.02	0.03
Savoury peanut cracker (*Rempenyek*)	2.73	6.98	0.03
Lace pancake (*Roti jala*)	1.98	3.25	0.023
Fried pancake(*Tumpi*)	2.61	3.72	0.027
Chinese crullers (*Yauchakuai*)	1.56	2	0.02

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