

ORIGINAL RESEARCH

Identification of the prepared foods promising for dietary folate intake in Beijing, China

Md Shariful Islam  | Shahid Mehmood | Chunyi Zhang | Qiuju Liang 

Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

Correspondence

Chunyi Zhang and Qiuju Liang, Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China.

Email: zhangchunyi@caas.cn (C. Z.); Email: liangqiuju@caas.cn (Q. L.)

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Abstract

The aim of the present study was to analyze folate content and composition in foods consumed daily by Chinese people. The concentration of seven folate derivatives in sixty-four selected foods was determined by a liquid-chromatography tandem-mass spectrometry method. The total folate levels ranged from 0.28 to 129 $\mu\text{g}/100\text{ g}$ fresh weight, with an average of 21.18 $\mu\text{g}/100\text{ g}$. The highest folate content was found in boiled egg yolk and waxy corn ($>120\text{ }\mu\text{g}/100\text{ g}$), abundant folate levels in cooked vegetables such as hot pepper, spinach, soybean sprout, stem lettuce, coriander, and broccoli (44–72 $\mu\text{g}/100\text{ g}$), and lowest in Coca Cola (0.28 $\mu\text{g}/100\text{ g}$). 5-Methyl-tetrahydrofolate was the major folate derivative in various foods, accounting for 72% of the total folates on average, with the highest being 90% in egg yolk. These data will enable estimation of the daily folate intake and allow dietary recommendations to improve folate status in humans.

KEYWORDS

China, folate content, folate derivatives, folates, processed foods

1 | INTRODUCTION

Folates (vitamin B9), comprising tetrahydrofolate (THF) and its derivatives, are vital cofactors for all organisms. By acting as donors and acceptors in one-carbon metabolism, folates provide methyl groups for the biosynthesis of nucleotides, amino acids, formyl-methionyl tRNA, and pantothenate (Blancquaert et al., 2010). Folates are important for maintaining health and preventing disease. Folate deficiency is linked to several human disorders including neural tube defects (NTDs) in infants and megaloblastic anemia, cardiovascular disease, and cancers in adults (Li et al., 2016; Smith & Refsum, 2016; Stover, 2004). Mammals cannot synthesize folates de novo and therefore dependent on dietary intake, mainly in the form of plant-based foods (Hanson & Gregory, 2011). Thus, assessing the natural folates in cooked/prepared foods becomes

necessary for estimation of the daily folate intake. Folate deficiency is a worldwide problem. China has a high prevalence of folate deficiencies, with 20% of the population being considered deficient and 18,000 pregnancies affected by NTDs annually (De Steur et al., 2010; Zhao et al., 2009). The recommended daily allowance (RDA) of folates is 400 $\mu\text{g}/\text{day}$ for adults and 600 $\mu\text{g}/\text{day}$ for pregnant women (Strobbe & Van Der Straeten, 2017). The RDA of folates can be met either by folic acid supplementation or by adequate intake of food-sourced folates. Folic acid supplementation has been reported to be effective in preventing folate malnutrition in humans (Czeizel et al., 2013; Wang et al., 2016); however, an excess of folic acid intake may result in adverse effects, such as increased cancer risk and progression within certain patient groups, insulin resistance in children, interaction with epilepsy medications, and masking vitamin B12 deficiency and hepatotoxicity

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(Patel & Sobczynska-Malefora, 2017). In light of food-sourced folate intake, the extent to which folates are absorbed by general population remains questionable due to the lack of values of folate content in processed foods and lack of information on the folate forms in the diets. Folate levels vary significantly among plant-based foods, and raw fresh vegetables and legumes are considered as good sources of folates (Blancquaert et al., 2014; Delchier et al., 2016). However, as much as 10%–64% of folate loss was observed during storage, industrial processing, and cooking due to the sensitivity of folates to heat, light, or oxidation (Dong et al., 2011; Hefni et al., 2015; Maharaj et al., 2015). Therefore, investigation of the folate retention in processed foods will enable an accurate estimation of dietary folate intake.

Microbiological assay and the high-performance liquid-chromatography coupled with tandem-mass spectrometry (HPLC-MS/MS) are widely adopted for folate determination (Arcot & Shrestha, 2005; Ringling & Rychlik, 2013). The microbiological assay could not distinguish various folate derivatives, revealing only the total amount of folates. Moreover, because of different responses and stabilities of the folate vitamers, the microbiological assay may give inaccurate results (Ringling & Rychlik, 2017). HPLC-MS/MS has been developed for separation and quantification of individual folate derivatives in diverse plant, animal, and food matrices, such as plant leaf (Shohag et al., 2017), tomatoes (Upadhyaya et al., 2017), cereals (Wan et al., 2019), fruits (Striegel et al., 2019), egg yolks (Sun et al., 2020), and foodstuffs (Loznjak et al., 2020; Loznjak svarc et al., 2020), and showing great advantages due to the high sensitivity and accuracy.

In the present study, we have investigated the folate content and composition in 64 foods commonly consumed in China, including cereals, vegetables, fruits, eggs, meat, fish, and beverages. Seven folate derivatives in the foods were determined by LC-MS/MS.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

10-Formyl-folic acid (10-F-FA), 5,10-methenyl-tetrahydrofolate (5,10-CH = THF), 5-formyl-tetrahydrofolate (5-F-THF), 5-methyl-tetrahydrofolate (5-M-THF), dihydrofolate (DHF), folic acid (FA), tetrahydrofolate (THF), and methotrexate (MTX) standards were purchased from Schircks Laboratories. The purity of all folate standards was >95%. Sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), sodium ascorbate, β -mercaptoethanol, and α -amylase (from *Aspergillus oryzae*, ~ 30 units/mg) were purchased from Sigma-Aldrich. Ultra-pure water was purified on a Heal Force ultra-pure water system. Acetonitrile and formic acid (LC-MS grade) were purchased from Fisher Scientific. Rat serum was purchased from Solarbio.com and was aliquoted in 1 ml portions into 1.5 ml tubes upon arrival, then stored at -80°C freezer before use. α -Amylase was freshly

prepared in water with the concentration of 40 mg/ml and used on the same day. The endogenous folates in rat serum were removed by incubation with one-tenth (w/w) of activated charcoal for 1 hr on ice, followed by centrifuged at 15,000 g at 4°C for 30 min (Sigma 3K15), and the supernatant was used for the following incubation experiment.

2.2 | Selection of foods

A total of 64 frequently consumed ready-for-consumption foods were selected based on the China Food Composition 2018 (main food category and composition of Chinese foods included) and were categorized into five groups: cereal/carbohydrate-based foods (11), vegetables (22), fruits (13), eggs/meat/fish (9), and beverages (9). The foods were purchased from three canteens in Beijing, China. Fruits and beverages were purchased from a local supermarket in Beijing. Sampling and analysis of the foods and beverages were performed in August 2019, and there were three replicates per sample. All of the foods were ground and homogenized before analysis.

2.2.1 | Cereal/carbohydrate-based foods

Cooked plain rice, boiled and drained noodles, boiled waxy corn (fresh corn variety), boiled sweet corn (fresh corn variety), instant rolled oats, bread, steamed bun, fermented pancake, plain pancake, steamed sweet potato, and boiled and drained gluten.

2.2.2 | Vegetable-based foods

Boiled, drained, and salted—spinach, cauliflower and carrot; green salad—coriander, tomato, cucumber; dried peanut, cooked with oil and other ingredients—stem lettuce, cabbage, soybean sprout, brinjal, mungbean sprout, hot pepper, green pepper, bitter melon, onion, tomato, broccoli, mushroom, potato, ginger, and garlic.

2.2.3 | Fresh fruits

Mandarin orange, apple, lemon, seedless grape, melon, navel orange, peach, dragon fruit (white flesh), cherry, pomegranate, kyoho grape, banana, and watermelon.

2.2.4 | Eggs/meat/fish

Stewed with salt, drained—beef shank, mutton shank, and pork rump; cooked chicken breasts, steamed saltwater fish, cooked shrimp, and boiled tofu and boiled egg (yolk and albumen separated).

2.2.5 | Beverages

Soya milk (15 g of soya powder in 100 ml of hot water), whole milk, skimmed milk, yoghurt, orange juice, Nescafe (15 g of Nescafe dissolved in 250 ml of hot water), Coca Cola, Pepsi, and Sprite.

2.3 | Sample preparation and extraction

Cooked rice, noodles, egg yolk, and egg albumen were ground into a fine paste using a mortar and pestle. The other foods were homogenized individually in a domestic kitchen blender. Half of the composite samples (~6 g) were used for determination of moisture content and the rest for folate analysis. Folate extraction was performed as described previously with slight modification (Riaz et al., 2019). The extraction buffer (50 mM phosphate buffer, pH 7.0; 0.5% [w/v] sodium ascorbate; 0.2% β -mercaptoethanol) was freshly prepared. MTX at a final concentration of 20 ng/ml was used as internal standard and added to extraction buffer at the beginning of the extraction procedure.

For vegetables and fruits, 1 ml of extraction buffer was added to 50 mg of fine paste/powder and mixed; for beverages, 500 μ l of 2 \times extraction buffer was mixed with 500 μ l of sample. The mixture was immediately boiled for 10 min in a water bath, cooled on ice, 30 μ l of rat serum was added, and incubated at 37°C for 4 hr to convert polyglutamated into monoglutamated folates. The samples were boiled for 10 min to inactivate rat conjugase, cooled on ice for 10 min, centrifuged at 15,000 g at 4°C for 10 min, and the supernatants were transferred to 3 kDa ultra-filtration tubes (Millipore) for clean-up and centrifuged at 15,000 g at 4°C for 20 min. The resulting solution was used directly for folate analysis.

For cereal/carbohydrate-based foods, additional α -amylase treatment was used. 1 ml of extraction buffer was added to 50 mg of fine powder, mixed, boiled for 10 min, and cooled on ice to room temperature. Next, 20 μ l of α -amylase (40 mg/ml) was added, mixed, and incubated at 37°C for 30 min. Subsequently, the samples were boiled for 5 min to deactivate α -amylase, cooled on ice, 30 μ l of rat serum was added, and incubated at 37°C for 4 hr. The samples were boiled for 5 min, cooled on ice for 10 min, and centrifuged at 15,000 g at 4°C for 10 min. The supernatants were transferred to 3 kDa ultra-filtration tubes (Millipore) for clean-up and centrifuged at 15,000 g at 4°C for 20 min. The filtrate was used for folate analysis.

2.4 | LC-MS/MS analysis

Folate separation and quantification were performed as described previously (Wan et al., 2019). Chromatographic separation was performed in an Agilent 1260 HPLC system using an Agilent analytical column (Poroshell 120SB-C18, 2.1 \times 75 mm, 2.7 μ m particle size) and an Agilent SB-C18 precolumn (2.1 \times 5 mm, 2.7 μ m particle size). The mobile phases were 0.1% (v/v) formic acid in water (phase A)

and 0.1% (v/v) formic acid in acetonitrile (phase B) with a gradient program of 20 min. The initial mobile phase B was set at 5% at a flow rate of 0.3 ml/min. The proportion of mobile phase B increased linearly from 5% to 9% over 2 min. In the following 7 min, phase B increased to 9.6%, then sharply increased to 20% over 0.2 min. After holding at 20% for 4 min, the proportion of phase B decreased to 5% in 0.8 min followed by an equilibration time of 6 min. Mass analysis and folate quantification were operated in an Agilent 6420 triple-quadrupole MS coupled to an electron spray ionization interface system. The mass spectrometer was operated in a positive ion mode. The multiple reaction monitoring (MRM) parameters of each folate derivative including the precursor ion, the product ion, and collision energy (eV) were optimized with a gas temperature of 320°C, drying gas flow at 11 L/min, nebuliser pressure at 35 psi, and capillary voltage at 3,500 V. One major product ion for each folate was selected for the subsequent analysis. The retention time and MRM parameters of folate derivatives were as follows: THF (2.668 min, 446 \rightarrow 299, 30 eV), 5-M-THF (3.754 min, 460 \rightarrow 313, 20 eV), 5,10-CH = THF (5.830 min, 456 \rightarrow 412, 30 eV), 10-F-FA (6.832 min, 470 \rightarrow 295, 20 eV), 5-F-THF (7.072 min, 474 \rightarrow 327, 20 eV), FA (8.131 min, 442 \rightarrow 295, 20 eV), DHF (8.202 min, 444 \rightarrow 178, 20 eV), and MTX (8.983 min, 455 \rightarrow 308, 20 eV). System operation, data acquisition, and data analysis were performed using the Agilent Mass Hunter Software.

2.5 | Determination of moisture content

Moisture content was determined in triplicate using a vacuum oven at 70°C overnight (AOAC, 2007). The foods (2 g) or beverage (2 ml) were initially weighed, incubated overnight in a vacuum oven at 70°C, and weighed. The moisture content was calculated as the difference between the two weights.

2.6 | Statistical analysis

All data were presented as the mean and standard deviation (SD) of three biological replicates. The folate data were subjected to analysis of principal component analysis (PCA) and Pearson's correlation coefficients (r) to find differences in folate content of different foodstuffs, identify folate vitamers with high discriminative properties, and the association between the various folate derivatives. Data analysis and visualization were performed by using R 3.6.2 (R Foundation for Statistical Computing).

3 | RESULTS AND DISCUSSION

3.1 | Total folates in selected foods

A total of 64 foods/preparations commonly consumed by Chinese population were selected for folate analysis. Among them, 11 were

cereal/carbohydrate-based preparations, 22 vegetables, 13 fresh fruits, 9 egg/meat/fish items, and 9 beverages. All of the foods were in ready-for-consumption forms.

The folate levels of these foods ranged from $0.28 \pm 0.08 \mu\text{g}/100 \text{ g}$ to $129 \pm 10.4 \mu\text{g}/100 \text{ g}$ fresh weight (FW), with an average of $21.1 \mu\text{g}/100 \text{ g}$ (Table 1). The mean folate contents of cereal/carbohydrate-based foods, vegetables, fruits, and egg/meat/fish were 27.2, 28.8, 15.5, and $19.1 \mu\text{g}/100 \text{ g}$, respectively, and that of beverages was $5.4 \mu\text{g}/100 \text{ ml}$. Therefore, the folate contents varied considerably among different foods/preparations, with vegetables and cereal/carbohydrate-based foods averaging higher in folates than the other foods.

A principal component analysis (PCA) was conducted to examine the variation in folate concentration among all the selected foods. Foodstuffs were scattered in biplots by origin, with folate derivatives indicated as vectors, and five overlapped clusters were illustrated (Figure 1). The first two principal components together explained 59.5% (PC1, 39.3% and PC2, 20.2%) of the total observed variation. 5-M-THF and THF were closely associated, and they were the major contributors to the variation in PC1; 5-F-THF has a close correlation with FA, and they contributed to the variation in PC2. In further, Pearson's correlation analysis was performed to examine the association among individual and total folate concentration (Figure 2). 5-M-THF showed the highest positive correlations with total folates ($r = .96^{***}$), which indicated that 5-M-THF was the major contributor toward the total folate concentration. THF, 5-F-THF, and 10-F-FA showed moderate correlations with total folates ($r = .53^{***}$, $r = .49^{***}$, and $r = .47^{***}$, respectively). 5-M-THF was positively correlated with THF ($r = .53^{***}$); there were significant and positive correlations between 10-F-FA and 5-F-THF ($r = .61^{***}$), 10-F-FA and FA ($r = .49^{***}$), 5-F-THF and FA ($r = .49^{***}$), and 5,10 = CH-THF and FA ($r = .42^{***}$). The positive correlations revealed by the Pearson's correlation analysis were also evident from the PCA, indicating good consistency.

The highest level of folates was observed in egg yolk ($129 \pm 10.4 \mu\text{g}/100 \text{ g}$), followed by waxy corn ($126 \pm 18.5 \mu\text{g}/100 \text{ g}$), hot pepper ($72 \pm 6.36 \mu\text{g}/100 \text{ g}$), spinach ($69.6 \pm 5.11 \mu\text{g}/100 \text{ g}$), and soybean sprout ($64.1 \pm 9.73 \mu\text{g}/100 \text{ g}$) (Tables 2-4). These five types of foods are considered folate-rich foods. Several beverages, including Nescafe, Coca Cola, Pepsi, and Sprite, contained low level of folates (Table 5; $<1 \mu\text{g}/100 \text{ ml}$).

3.2 | Cereal/carbohydrate-based foods

Cereal/carbohydrate-based foods are usually consumed as staple foods. It was observed that the total folates ranged from $126 \pm 18.5 \mu\text{g}/100 \text{ g}$ to $3.29 \pm 0.26 \mu\text{g}/100 \text{ g}$ among the 11 selected cereal/carbohydrate foods (Table 2). Boiled waxy corn contained the highest level of folates ($126 \pm 18.5 \mu\text{g}/100 \text{ g}$), followed by sweet corn ($47.6 \pm 7.69 \mu\text{g}/100 \text{ g}$). The folate contents detected in corns in this study were comparable to that indicated in the USDA database (United States Department of Agriculture, 2020), where waxy corn and sweet corn were shown to contain $100 \mu\text{g}/100 \text{ g}$ and $42 \mu\text{g}/100 \text{ g}$ of folates, respectively.

Wheat is widely planted and commonly consumed as a staple food in North China (Riaz et al., 2019); unfortunately, wheat flour contains folates as low as $4\text{--}20 \mu\text{g}/100 \text{ g}$ (Patring et al., 2009). In the present study, several foods are made of wheat flour, including bread, steamed bun, fermented pancake, plain pancake, noodles, and gluten. Out of these, bread had a highest level of folates ($31.2 \pm 4.2 \mu\text{g}/100 \text{ g}$), followed by steamed bun ($19.9 \pm 4.3 \mu\text{g}/100 \text{ g}$) and pancakes ($12.5 \pm 1.78 \mu\text{g}/100 \text{ g}$ and $15.4 \pm 1.92 \mu\text{g}/100 \text{ g}$), with noodles and gluten being the least ($4.02 \pm 1.51 \mu\text{g}/100 \text{ g}$ and $3.29 \pm 0.26 \mu\text{g}/100 \text{ g}$). The folate level detected in bread was in agreement with the previous study, which reported a folate level of $29.8 \mu\text{g}/100 \text{ g}$ (Pfeiffer et al., 1997). Compared to noodles and gluten, the higher level of folates in bread and steamed bun may attribute to the yeast used for fermentation during food preparation, a good tool to increase folates in foods (Saubade et al., 2017).

The folate level in rolled oats examined in this study was $23.1 \pm 3.7 \mu\text{g}/100 \text{ g}$, similar to that in Norwegian/Swedish food samples ($21 \mu\text{g}/100 \text{ g}$ and $26 \mu\text{g}/100 \text{ g}$, respectively) (Patring et al., 2009). The folate level observed in plain rice was $8.42 \pm 1.4 \mu\text{g}/100 \text{ g}$, not significantly different from the previous report ($10.8 \mu\text{g}/100 \text{ g}$) (Pfeiffer et al., 1997).

Abundant levels of 5-M-THF, 5-F-THF, and 10-F-FA were found in bread, rolled oats, steamed bun, and pancakes, consistent with the previous study on cereal grain products (Pfeiffer et al., 1997). 5-M-THF was found accounting for 88.1% and 91.0% of the total folates in waxy corn and sweet corn, respectively, thus acting as a dominant folate derivative. Likewise, 5-M-THF was also a major contributor to total folates in rolled oats (51.1%), steamed bun (57.2%), fermented pancake (62.4%), plain rice (69.2%), sweet potato (60.3%), and noodles

| Food groups | No. of samples | Range of folate contents | Average |
|---------------------------------|----------------|------------------------------------|---------|
| Cereal/carbohydrate-based foods | 11 | $126 \pm 18.5 \sim 3.29 \pm 0.26$ | 27.21 |
| Vegetables | 22 | $72.0 \pm 6.36 \sim 2.65 \pm 0.51$ | 28.82 |
| Fruits | 13 | $44.6 \pm 0.61 \sim 6.31 \pm 0.91$ | 15.50 |
| Egg/meat/fish | 9 | $129 \pm 10.4 \sim 1.30 \pm 0.22$ | 19.12 |
| Beverages | 9 | $29.6 \pm 4.2 \sim 0.28 \pm 0.08$ | 5.40 |

TABLE 1 Total folates in commonly consumed Chinese foods ($\mu\text{g}/100 \text{ g}$ or $\mu\text{g}/100 \text{ ml}$)

Note: For food samples of cereal/carbohydrate-based foods, vegetables, fruits, egg/meat/fish, and yoghurt, the unit of folate content was $\mu\text{g}/100 \text{ g}$; for most beverages, the unit was $\mu\text{g}/100 \text{ ml}$.

FIGURE 1 Principal component analysis based on concentration of the individual folate derivatives for various foods. Each point on the scatter plot represents a single food item; the food items are color-coded with different symbols to indicate their clustered types. 10-F-FA, 10-Formyl-tetrahydrofolate; 5,10-CH = THF, 5,10-Methenyl-tetrahydrofolate; 5-F-THF, 5-Formyl-tetrahydrofolate; 5-M-THF, 5-Methyl-tetrahydrofolate; DHF, dihydrofolate; FA, folic acid; THF, Tetrahydrofolate

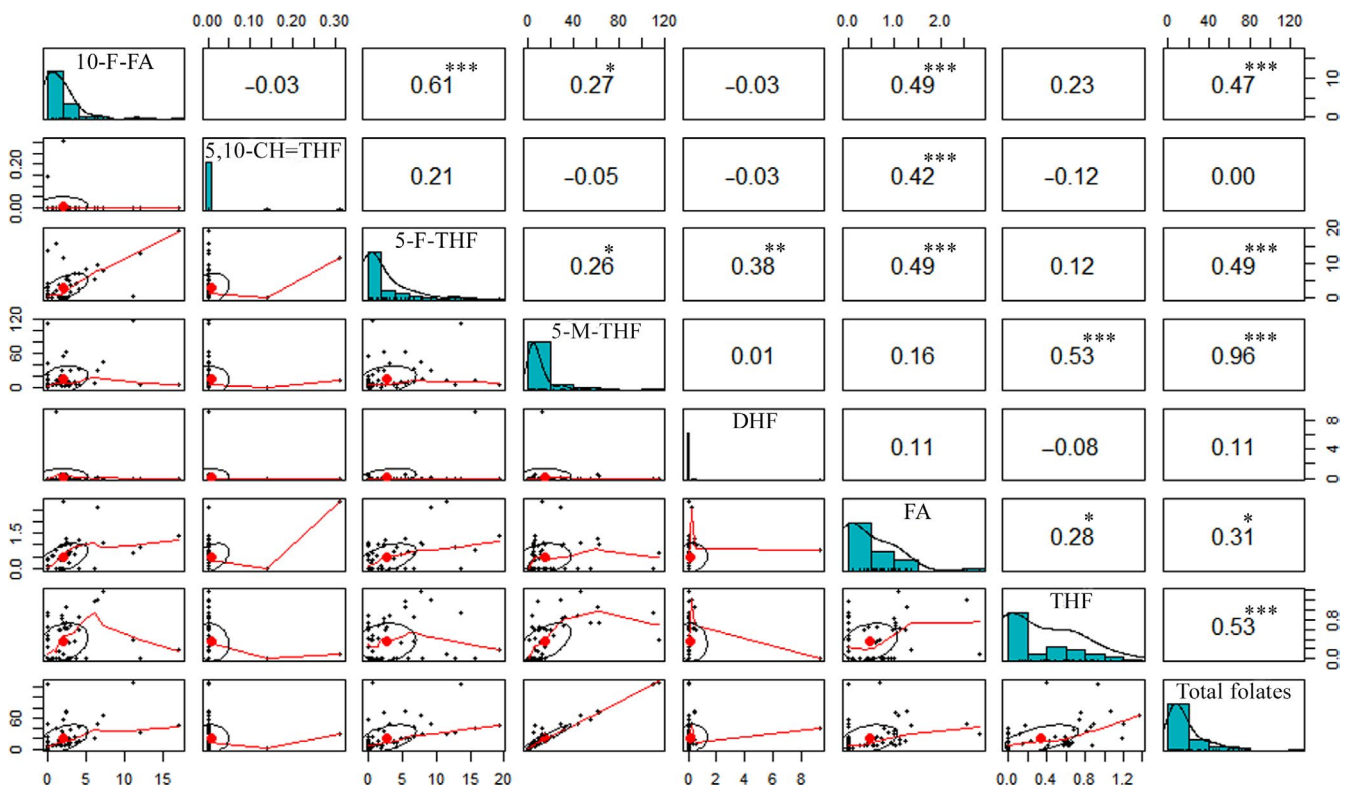
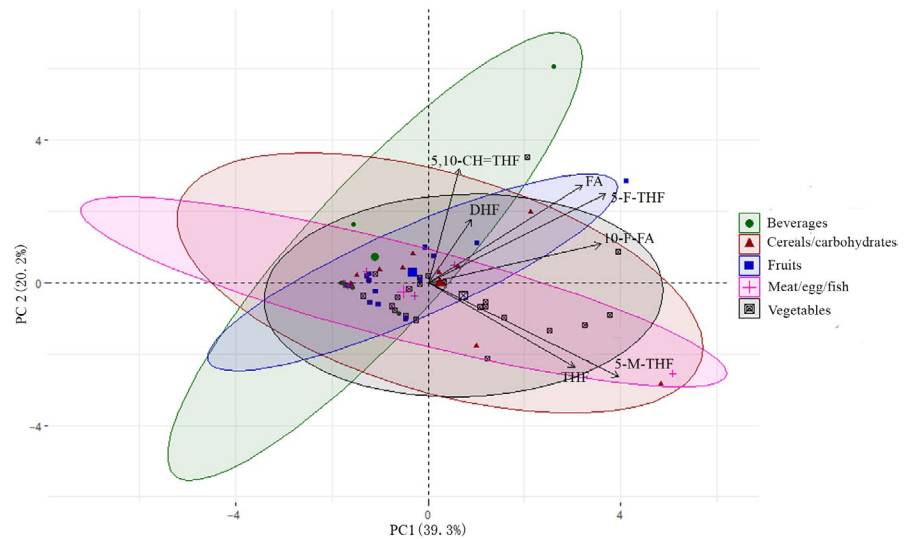


FIGURE 2 Correlation analysis among the individual folate derivatives and total folates of all selected foods. *, **, and *** represent the significance levels at $p < .05$, $.01$, and $.001$, respectively. 10-F-FA, 10-Formyl-tetrahydrofolate; 5,10-CH = THF, 5,10-Methenyl-tetrahydrofolate; 5-F-THF, 5-Formyl-tetrahydrofolate; 5-M-THF, 5-Methyl-tetrahydrofolate; DHF, dihydrofolate; FA, folic acid; THF, Tetrahydrofolate

(82.3%). Unlike 5-M-THF, 5-F-THF contributed most to the total folates in bread and gluten by 40.1% and 59.8%, respectively, and 10-F-FA made a contribution at the level similar to 5-F-THF in bread (38.5%).

Taken together, rice, pancakes, bread, or steamed bun could contribute 4%–16% of the folate RDA, a calculation based on an assumption of 200-gram food consumption per person. In contrast, waxy corn is an optimal food source of folates, providing 62% of the RDA with 200-g consumption.

3.3 | Vegetable-based preparations

The folate levels of 26 fresh vegetables commonly consumed in China were determined previously, ranging from 14.8 $\mu\text{g}/100\text{ g}$ to 146 $\mu\text{g}/100\text{ g}$ and averaging 62 $\mu\text{g}/100\text{ g}$, with the highest in leafy vegetables such as pak choi and spinach (Shohag et al., 2012). However, a severe folate loss occurs very often in vegetables upon industrial processing or cooking. It was reported that 25% of the

TABLE 2 Folate content and composition of cereal/carbohydrate-based preparations ($\mu\text{g}/100\text{ g}$)

| Food samples | Moisture (%) | 10-F-FA | 5.10-CH = THF | 5-F-THF | 5-M-THF | DHF | FA | THF | Total folates |
|-------------------|--------------|------------------|---------------|-----------------|-----------------|-----|-----------------|-----------------|-----------------|
| Waxy corn | 61.5 | N/A | N/A | 13.7 \pm 4.09 | 111 \pm 16.5 | N/A | N/A | 0.94 \pm 0.24 | 126 \pm 18.5 |
| Sweet corn | 78.0 | N/A | N/A | 3.26 \pm 0.20 | 43.3 \pm 7.55 | N/A | N/A | 0.89 \pm 0.11 | 47.6 \pm 7.69 |
| Bread | 24.0 | 12.02 \pm 0.96 | N/A | 12.7 \pm 1.12 | 5.57 \pm 1.12 | N/A | 0.88 \pm 0.24 | N/A | 31.2 \pm 4.24 |
| Rollled oats | 0.00 | 3.44 \pm 0.54 | N/A | 6.81 \pm 1.73 | 11.8 \pm 1.55 | N/A | 0.63 \pm 0.02 | 0.37 \pm 0.01 | 23.1 \pm 3.70 |
| Steamed bun | 38.7 | 2.66 \pm 0.75 | N/A | 4.86 \pm 2.32 | 11.4 \pm 2.32 | N/A | 0.59 \pm 0.20 | 0.34 \pm 0.02 | 19.9 \pm 4.30 |
| Fermented pancake | 46.2 | 1.49 \pm 0.27 | N/A | 3.81 \pm 0.96 | 9.61 \pm 0.96 | N/A | 0.45 \pm 0.07 | N/A | 15.4 \pm 1.92 |
| Plain pancake | 18.5 | 3.01 \pm 0.63 | N/A | 3.84 \pm 0.55 | 4.92 \pm 0.55 | N/A | 0.73 \pm 0.10 | N/A | 12.5 \pm 1.78 |
| Plain rice | 60.5 | 0.68 \pm 0.25 | N/A | 1.34 \pm 1.16 | 5.83 \pm 1.16 | N/A | 0.55 \pm 0.12 | N/A | 8.42 \pm 1.41 |
| Sweet potato | 77.0 | 1.04 \pm 0.19 | N/A | 2.08 \pm 0.24 | 4.76 \pm 0.24 | N/A | N/A | N/A | 7.89 \pm 1.02 |
| Noodles | 75.5 | N/A | N/A | 0.70 \pm 1.03 | 3.31 \pm 1.03 | N/A | N/A | N/A | 4.02 \pm 1.51 |
| Gluten | 72.2 | N/A | N/A | 1.97 \pm 0.28 | 1.22 \pm 0.12 | N/A | 0.10 \pm 0.03 | N/A | 3.29 \pm 0.26 |

Note: N/A, below the limit of detection.

initial folates were lost by wash leaching, 10%–64% of loss by boiling, and 1%–36% of loss by frying (Delchier et al., 2013; Maharaj et al., 2015). Thus, investigation of folate contents in cooked vegetables is necessary for an accurate estimation of folate accessibility to humans.

In this study, folates in 22 cooked/prepared vegetables were examined. It was observed that the folate levels varied from 72 \pm 6.4 $\mu\text{g}/100\text{ g}$ for hot pepper to 2.65 \pm 0.5 $\mu\text{g}/100\text{ g}$ for potato, with an average of 28.82 $\mu\text{g}/100\text{ g}$ (Table 3). Cooked hot pepper, spinach, soybean sprout, lettuce, and broccoli were found containing a considerably high level of folates (>44 $\mu\text{g}/100\text{ g}$), ensuring 11%–25% of the recommended daily folate intake upon 100-g serving.

Previously, it was reported that the folate contents of raw spinach and broccoli were 143.99 and 110.67 $\mu\text{g}/100\text{ g}$, respectively (Shohag et al., 2012). In the present study, the folate levels were 69.6 $\mu\text{g}/100\text{ g}$ for spinach and 44.4 $\mu\text{g}/100\text{ g}$ for broccoli, indicating a 52%–60% folate loss upon cooking. In fact, a similar folate loss upon boiling was also observed in spinach (51%) and broccoli (66%) in a previous study (McKillop et al., 2002). The cooked stem lettuce was also found as a good source of folates in this study (57.7 \pm 6.2 $\mu\text{g}/100\text{ g}$).

Legumes are considered to be rich in folates. Previously, 86 $\mu\text{g}/100\text{ g}$ of folates was detected in raw soybean sprout (Shohag et al., 2012), and 220–267 $\mu\text{g}/100\text{ g}$ (dry matter) in raw soybeans and 96.8–127 $\mu\text{g}/100\text{ g}$ (dry matter) in cooked tofu (Mo et al., 2013). In this study, it was found that the boiled soybean sprout contained folates at a level of 64.05 \pm 9.73 $\mu\text{g}/100\text{ g}$ and the boiled tofu 14.31 \pm 0.59 $\mu\text{g}/100\text{ g}$ fresh weight. Obviously, the big difference of folate levels in tofu was due to the calculations based on dry weight or on fresh weight.

The folate content of tomatoes served in green salad was 11.4 \pm 0.5 $\mu\text{g}/100\text{ g}$, whereas 8.27 \pm 1.1 $\mu\text{g}/100\text{ g}$ in cooked form, both lower than that in red ripe tomatoes (18.1 $\mu\text{g}/100\text{ g}$) (Tyagi et al., 2015). Potato has been grown as a staple crop in certain region, but is a poor source of folates (19 $\mu\text{g}/100\text{ g}$ in raw samples) (Blancaert et al., 2014). In this study, the cooked potato chips had the lowest level of folates (2.65 \pm 0.51 $\mu\text{g}/100\text{ g}$), thus confirming the conclusion mentioned above.

5-M-THF was the dominant folate derivative in almost all the vegetables, accounting for up to 40.9%–95.3% of the total folates, followed by 5-F-THF and 10-F-FA. This result was consistent with the previous report, in which 5-M-THF was also found as the main folate derivative in vegetables (Vishnumohan et al., 2017). Notably, peanut had similar amount of 5-F-THF and 5-M-THF (15.64 \pm 1.99 $\mu\text{g}/100\text{ g}$ vs. 13.0 \pm 1.53 $\mu\text{g}/100\text{ g}$).

3.4 | Fresh fruits

The folate levels in 13 fresh fruits showed a broad range of variations, with the highest being 44.58 \pm 0.62 $\mu\text{g}/100\text{ g}$ in lemon and lowest being 3.78 \pm 0.91 $\mu\text{g}/100\text{ g}$ in mandarin orange, averaging 15.50 $\mu\text{g}/100\text{ g}$ (Table 4). Recently, a range of folate levels of

TABLE 3 Folate content and composition of vegetable-based preparations ($\mu\text{g}/100\text{ g}$)

| Vegetables | Moisture (%) | 10-F-FA | 5,10-CH = THF | 5-F-THF | 5-M-THF | DHF | FA | THF | Total folates |
|------------------|--------------|-----------------|---------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Hot pepper | 22.2 | 2.32 \pm 0.27 | N/A | 5.37 \pm 0.85 | 61.5 \pm 6.94 | 0.49 \pm 0.11 | 1.27 \pm 0.15 | 1.07 \pm 0.04 | 72.0 \pm 6.36 |
| Spinach | 91.2 | 2.46 \pm 0.27 | N/A | 2.28 \pm 0.17 | 62.7 \pm 5.03 | 0.41 \pm 0.02 | 1.02 \pm 0.03 | 0.75 \pm 0.02 | 69.6 \pm 5.11 |
| Soybean sprout | 82.2 | 7.23 \pm 0.89 | N/A | 7.82 \pm 2.20 | 46.6 \pm 6.30 | N/A | 1.07 \pm 0.06 | 1.37 \pm 0.13 | 64.1 \pm 9.73 |
| Stem lettuce | 87.5 | 1.92 \pm 0.24 | N/A | N/A | 55.1 \pm 5.76 | N/A | N/A | 0.74 \pm 0.11 | 57.7 \pm 6.21 |
| Coriander | 83.5 | 6.49 \pm 1.17 | N/A | 9.25 \pm 2.36 | 29.4 \pm 2.18 | 0.22 \pm 0.02 | 2.57 \pm 0.20 | 1.20 \pm 0.25 | 49.2 \pm 8.89 |
| Broccoli | 92.0 | 4.09 \pm 1.51 | N/A | 6.82 \pm 0.18 | 32.6 \pm 4.93 | N/A | N/A | 0.86 \pm 0.13 | 44.4 \pm 3.71 |
| Peanut | 63.5 | 1.14 \pm 0.51 | N/A | 15.64 \pm 1.99 | 13.0 \pm 1.53 | 9.26 \pm 0.84 | 0.77 \pm 0.15 | N/A | 39.8 \pm 4.77 |
| Mung bean sprout | 88.7 | 2.89 \pm 0.03 | N/A | 1.51 \pm 0.48 | 31.1 \pm 3.07 | N/A | 1.03 \pm 0.05 | 0.68 \pm 0.04 | 37.2 \pm 3.39 |
| Green pepper | 47.7 | 1.78 \pm 0.15 | N/A | 2.40 \pm 1.07 | 30.1 \pm 2.57 | N/A | 0.98 \pm 0.03 | 0.82 \pm 0.03 | 36.0 \pm 3.71 |
| Eggplant | 95.2 | 1.70 \pm 0.11 | N/A | N/A | 20.6 \pm 3.09 | N/A | N/A | 0.59 \pm 0.02 | 23.0 \pm 2.99 |
| Bitter gourd | 91.0 | 6.03 \pm 1.34 | N/A | 5.41 \pm 1.24 | 8.77 \pm 0.50 | N/A | N/A | 1.19 \pm 0.27 | 21.4 \pm 1.67 |
| Garlic | 66.5 | 2.45 \pm 0.08 | N/A | N/A | 13.8 \pm 0.75 | 0.45 \pm 0.10 | 1.11 \pm 0.20 | 0.61 \pm 0.02 | 18.4 \pm 0.60 |
| Cauliflower | 95.7 | 1.39 \pm 0.07 | N/A | N/A | 16.3 \pm 2.39 | N/A | N/A | 0.59 \pm 0.04 | 18.3 \pm 2.28 |
| Cabbage | 82.7 | N/A | N/A | 1.42 \pm 0.51 | 13.4 \pm 4.01 | N/A | N/A | 0.43 \pm 0.08 | 15.3 \pm 4.82 |
| Carrot | 93.0 | 1.40 \pm 0.06 | N/A | N/A | 10.1 \pm 0.04 | N/A | N/A | 0.60 \pm 0.03 | 12.1 \pm 0.11 |
| Tomato (salad) | 98.2 | 1.60 \pm 0.05 | N/A | N/A | 8.24 \pm 0.41 | N/A | 0.96 \pm 0.01 | 0.58 \pm 0.03 | 11.4 \pm 0.51 |
| Onion | 70.6 | 1.72 \pm 0.38 | N/A | N/A | 6.06 \pm 2.02 | 0.53 \pm 0.08 | 0.97 \pm 0.04 | 0.59 \pm 0.05 | 9.88 \pm 2.30 |
| Ginger | 51.7 | 2.52 \pm 0.09 | N/A | N/A | 5.13 \pm 0.50 | 0.35 \pm 0.07 | 1.10 \pm 0.13 | 0.63 \pm 0.05 | 9.74 \pm 0.58 |
| Tomato (cooked) | 87.7 | 1.54 \pm 0.10 | N/A | N/A | 5.79 \pm 0.55 | N/A | 0.35 \pm 0.12 | 0.58 \pm 0.03 | 8.27 \pm 1.06 |
| Cucumber | 96.5 | 2.27 \pm 0.08 | N/A | N/A | 3.65 \pm 0.07 | N/A | 0.63 \pm 0.10 | 0.64 \pm 0.02 | 7.19 \pm 0.66 |
| Mushroom | 86.2 | 2.69 \pm 0.53 | N/A | 0.48 \pm 0.07 | 3.07 \pm 0.41 | N/A | 0.25 \pm 0.05 | N/A | 6.52 \pm 1.13 |
| Potato | 67.7 | N/A | N/A | 0.46 \pm 0.19 | 1.79 \pm 0.24 | N/A | N/A | 0.38 \pm 0.08 | 2.65 \pm 0.51 |

TABLE 4 Folate content and composition of fruits ($\mu\text{g}/100\text{ g}$)

| Fruits item | Moisture (%) | 10-F-FA | 5,10-CH = THF | 5-F-THF | 5-M-THF | DHF | FA | THF | Total folates |
|-----------------|--------------|-----------------|---------------|-----------------|-----------------|-----|-----------------|-----------------|-----------------|
| Lemon juice | 93.5 | 17.1 ± 0.50 | N/A | 19.3 ± 0.97 | 6.65 ± 0.97 | N/A | 1.37 ± 0.02 | 0.18 ± 0.01 | 44.6 ± 0.62 |
| Seedless grapes | 85.0 | 4.96 ± 0.31 | N/A | 8.03 ± 0.74 | 15.6 ± 0.74 | N/A | 1.00 ± 0.12 | N/A | 29.5 ± 0.92 |
| Melon kernel | 87.7 | N/A | N/A | 0.56 ± 0.16 | 22.5 ± 4.67 | N/A | N/A | 0.48 ± 0.06 | 23.5 ± 5.02 |
| Apple (peeled) | 83.7 | 2.53 ± 0.23 | N/A | 5.96 ± 1.19 | 11.9 ± 1.19 | N/A | 0.71 ± 0.08 | N/A | 21.1 ± 2.70 |
| Navel orange | 88.5 | N/A | N/A | N/A | 14.8 ± 3.28 | N/A | N/A | 0.24 ± 0.14 | 15.1 ± 3.43 |
| Peach (peeled) | 91.7 | 3.64 ± 0.26 | N/A | 4.13 ± 0.33 | 4.96 ± 0.33 | N/A | 0.93 ± 0.15 | N/A | 13.7 ± 1.09 |
| Dragon fruit | 82.2 | 1.46 ± 0.51 | N/A | 0.56 ± 0.08 | 8.43 ± 1.10 | N/A | N/A | 0.14 ± 0.14 | 10.6 ± 1.94 |
| Pomegranate | 82.0 | 2.16 ± 0.46 | N/A | N/A | 4.43 ± 0.93 | N/A | 1.03 ± 0.01 | 0.62 ± 0.03 | 8.62 ± 0.58 |
| Cherry | 84.2 | 1.10 ± 0.40 | N/A | 1.89 ± 0.17 | 5.48 ± 1.07 | N/A | N/A | N/A | 8.49 ± 2.34 |
| Kyoho grapes | 86.2 | N/A | N/A | 0.73 ± 0.58 | 5.65 ± 0.58 | N/A | 0.39 ± 0.01 | N/A | 6.77 ± 0.37 |
| Watermelon | 83.5 | N/A | N/A | N/A | 6.06 ± 0.93 | N/A | N/A | 0.42 ± 0.05 | 6.48 ± 0.97 |
| Banana | 76.5 | 0.49 ± 0.16 | N/A | N/A | 5.28 ± 0.17 | N/A | 0.53 ± 0.12 | N/A | 6.31 ± 0.43 |
| Orange | 88.5 | N/A | N/A | N/A | 3.78 ± 0.91 | N/A | N/A | N/A | 3.78 ± 0.91 |

TABLE 5 Folate content and composition in meat/egg/fish preparations ($\mu\text{g}/100\text{ g}$)

| Food samples | Moisture (%) | 10-F-FA | 5,10-CH = THF | 5-F-THF | 5-M-THF | DHF | FA | THF | Total folates |
|----------------|--------------|-----------------|---------------|-----------------|-----------------|-----|-----------------|-----------------|-----------------|
| Egg yolk | 47.7 | 11.1 ± 2.24 | N/A | 0.57 ± 0.21 | 116 ± 8.91 | N/A | 0.69 ± 0.17 | 0.40 ± 0.01 | 129 ± 10.4 |
| Egg albumen | 88.0 | N/A | N/A | N/A | 3.74 ± 0.61 | N/A | N/A | N/A | 3.74 ± 0.61 |
| Saltwater fish | 47.7 | N/A | N/A | N/A | 1.57 ± 0.69 | N/A | N/A | N/A | 1.57 ± 0.69 |
| Shrimp | 79.9 | N/A | N/A | 2.68 ± 0.2 | 9.59 ± 1.32 | N/A | 0.44 ± 0.31 | 0.66 ± 0.13 | 13.4 ± 1.78 |
| Tofu | 80.2 | 4.10 ± 0.04 | N/A | 2.47 ± 0.74 | 5.93 ± 0.18 | N/A | 1.23 ± 0.10 | 0.58 ± 0.04 | 14.3 ± 0.59 |
| Chicken | 62.5 | N/A | N/A | 4.21 ± 0.71 | 1.13 ± 0.21 | N/A | N/A | N/A | 5.34 ± 0.93 |
| Mutton | 23.5 | N/A | N/A | 0.21 ± 0.05 | 1.81 ± 0.14 | N/A | N/A | N/A | 2.03 ± 0.20 |
| Beef | 56.7 | N/A | N/A | N/A | 1.44 ± 0.46 | N/A | N/A | N/A | 1.44 ± 0.46 |
| Pork | 48.5 | N/A | N/A | N/A | 1.30 ± 0.22 | N/A | N/A | N/A | 1.30 ± 0.22 |

7.82–271 $\mu\text{g}/100\text{ g}$ was reported among tropical fruits using LC-MS/MS (Striegel et al., 2019). In this study, most of the fruits were temperate or subtropical species, indicating a significant difference in folate levels between nontropical and tropical fruits.

The folate level in the apple was $21.08 \pm 2.7\ \mu\text{g}/100\text{ g}$, in good agreement with that stated in the USDA database 2019 (19 $\mu\text{g}/100\text{ g}$). Melons and grapes are the fruits in market supply in August in the North of China. The folate contents of watermelon and kyoho grape were 6.48 ± 0.97 and $6.77 \pm 0.37\ \mu\text{g}/100\text{ g}$, respectively, and those of the other varieties of seedless grape and green melon were 29.54 ± 0.92 and $23.53 \pm 5.02\ \mu\text{g}/100\text{ g}$, respectively. Thus, the seedless grape and green melon were good sources for folate nutrition improvement compared to watermelon and kyoho grapes. Navel oranges and mandarin oranges were also commonly consumed by general population, the folate levels were $15.1 \pm 3.4\ \mu\text{g}/100\text{ g}$ and $3.78 \pm 0.91\ \mu\text{g}/100\text{ g}$, respectively (Table 4).

5-M-THF was the major folate derivative in most of the fruits, followed by 5-F-THF and 10-F-FA. Interestingly, the level of 5-F-THF was much higher than that of 5-M-THF in lemon

($19.3 \pm 0.97\ \mu\text{g}/100\text{ g}$ vs. $6.65 \pm 0.97\ \mu\text{g}/100\text{ g}$), whereas peach had almost same levels of 5-F-THF and 5-M-THF ($4.13 \pm 0.33\ \mu\text{g}/100\text{ g}$ vs. $4.96 \pm 0.33\ \mu\text{g}/100\text{ g}$).

3.5 | Meat/egg/fish preparations

Usually, plant protein and animal foods, such as meat, egg, and fish, contain high level of good quality proteins. In this study, the cooked/prepared chicken, beef, mutton, pork, fish, shrimp, egg yolk, tofu, soya milk, and egg albumen were analyzed for folates and a big variation was observed among these preparations (Table 5). The boiled egg yolk contained the highest level of folates ($129 \pm 10.4\ \mu\text{g}/100\text{ g}$), consistent with the data of the USDA database (116 $\mu\text{g}/100\text{ g}$). Thus, egg yolk is a good source of folates, with 5-M-THF accounting for up to 90% of the total; similar 5-M-THF abundance was also reported in a recent investigation on folate-enriched egg yolks (Sun et al., 2020). In contrast, egg albumen contained a low level of folates ($1.30 \pm 0.22\ \mu\text{g}/100\text{ g}$). The folate content in shrimp was

TABLE 6 Folate content and composition of beverages ($\mu\text{g}/100\text{ ml}$)

| Beverage items | Moisture (%) | 10-F-FA | 5,10-CH = THF | 5-F-THF | 5-M-THF | DHF | FA | THF | Total folates |
|----------------------|--------------|-----------------|-----------------|-----------------|-----------------|-----|-----------------|------------------|-----------------|
| Soya milk | 96.7 | 2.08 ± 0.23 | 0.31 ± 0.05 | 11.5 ± 1.74 | 12.7 ± 1.88 | N/A | 2.84 ± 0.33 | 0.11 ± 0.001 | 29.6 ± 4.23 |
| Yoghurt ^a | 84.0 | 1.88 ± 0.06 | N/A | N/A | 4.10 ± 0.20 | N/A | N/A | 0.87 ± 0.03 | 6.85 ± 0.27 |
| Orange juice | 88.5 | N/A | N/A | 0.27 ± 0.03 | 5.69 ± 0.69 | N/A | N/A | N/A | 5.96 ± 0.70 |
| Skimmed milk | 78.0 | N/A | N/A | N/A | 2.41 ± 0.42 | N/A | N/A | N/A | 2.41 ± 0.42 |
| Whole milk | 87.2 | N/A | N/A | N/A | 2.12 ± 0.31 | N/A | N/A | N/A | 2.12 ± 0.31 |
| Nescafe | 99.0 | 0.02 ± 0.01 | 0.14 ± 0.02 | 0.09 ± 0.02 | 0.40 ± 0.03 | N/A | N/A | N/A | 0.66 ± 0.10 |
| Pepsi | 89.5 | N/A | N/A | N/A | 0.37 ± 0.04 | N/A | N/A | N/A | 0.37 ± 0.04 |
| Sprite | 88.5 | N/A | N/A | N/A | 0.35 ± 0.10 | N/A | N/A | N/A | 0.35 ± 0.10 |
| Coca Cola | 89.5 | N/A | N/A | N/A | 0.28 ± 0.07 | N/A | N/A | N/A | 0.28 ± 0.08 |

^aValues expressed as $\mu\text{g}/100\text{ g}$.

$13.4 \pm 1.8\ \mu\text{g}/100\text{ g}$. An extremely low level of folates was observed in fish and the selected meat ($1.3\text{--}5.34\ \mu\text{g}/100\text{ g}$), more or less the same as that in the USDA database ($2\text{--}8\ \mu\text{g}/100\text{ g}$). Our results were in agreement with the recent literature, which reported that the folate levels in chicken breast and pork tenderloin were $5 \pm 2\ \mu\text{g}/100\text{ g}$ and $1 \pm 1\ \mu\text{g}/100\text{ g}$, respectively, and therefore, meat is not a good source of folates (Loznjak et al., 2020).

3.6 | Beverages

The folate levels of nine beverages were found to vary dramatically, ranging from $29.6 \pm 4.2\ \mu\text{g}/100\text{ ml}$ to $0.28 \pm 0.08\ \mu\text{g}/100\text{ ml}$ (Table 6). Soya milk made of soybean contained the highest level and Coca cola contained the lowest level. 5-F-THF and 5-M-THF were the two dominant folate derivatives in soya milk, contributing equally to the total folates ($11.5 \pm 1.74\ \mu\text{g}/100\text{ ml}$ vs. $12.7 \pm 1.88\ \mu\text{g}/100\text{ ml}$).

Whole milk and yoghurt contained folates at levels of $2.1 \pm 0.3\ \mu\text{g}/100\text{ ml}$ and $6.85 \pm 0.3\ \mu\text{g}/100\text{ g}$, respectively. A previous study also examined folate contents in milk and yoghurt, with a range of $4\text{--}10\ \mu\text{g}/100\text{ g}$ due to seasonal variations and different starter cultures (Forssen et al., 2000); in comparison, a higher level of folates, $20 \pm 7\ \mu\text{g}/100\text{ g}$, was determined by a microbiological assay in the home-made yoghurt (Vishnumohan et al., 2009). As expected, a low level of folates was observed in Pepsi, Sprite, Nescafe, and Coca Cola ($<1\ \mu\text{g}/100\text{ ml}$) with 5-M-THF only being detected, and in agreement with the data indicated in the USDA database.

4 | CONCLUSION

Dietary folate intake by humans is dependent on food consumption. This is the first report on natural folates presented in cooked foods/preparations in China. The data of this study demonstrate that the level of folates vary greatly among foods, with boiled egg yolk, waxy corn, cooked hot pepper, spinach, soybean sprout, stem lettuce, broccoli, coriander, lemon, and soya milk being optimal sources of folates. Boiled waxy corn has been found most abundant in folates

among the foods examined in this study. Given a high popularity with favorable flavor and nutritious ingredients in Chinese population, waxy corn, including sweet corn, is strongly recommended as daily food for dietary folate intake. To conclude, the data generated in the study will be of help for an accurate estimation of daily folate intake of Chinese people and for establishment of an optimal dietary pattern to meet the daily folate requirement as well.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

This study does not involve any human or animal testing.

ORCID

Md Shariful Islam  <https://orcid.org/0000-0003-2934-3499>

Qiuju Liang  <https://orcid.org/0000-0003-4963-2765>

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