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Folate Vitamers Content of Selected Indian Foods

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Abstract

Rationale of the study: The folate is needed in the body to perform numerous roles. The folate deficiency is high in India (52% - among low income group) and data on cooked food is limiting, the bioavailability of folate is 50%, hence, to plan for intervention trial, data on folate content of cooked and raw foods is needed. The study was planned to estimate the folate vitamers content of cooked foods using HPLC method. This paper highlights the data generated on cooked foods alone.

Methodology: A Survey was conducted among the women population residing at Coimbatore, Tamil Nadu, India. The foods most frequently consumed by the population were selected for the vitamer analysis using HPLC method.

Results and discussion : The results showed the folate vitamers content of foods. The rasam and mixed vegetable Subji had the highest amount of vitamers and least was noted in idli among the preparations.

C**onclusion**: The study analysed the the vitamers content of selected foods further studies are needed to draw conclusion and in India fortification was not practised and in developed countries folate fortification arises many health concern therefore data is needed to prevent the deficiency.

Keywords: folate, tetrahydrofolate, vitamers, cooked foods, population

Introduction

The total folate belongs to the class of water soluble vitamin. Folate is present in foods of animal and plant origin. Folate found in foods consists of polyglutamyl tetrahydrofolate, 10-formyl, 5-methyl and 10-methylene tetrahydrofolate. Folate exist in more than 150 forms in foods. However, only 50 forms are found in plants and animals were identified. All these compounds play a role in various metabolisms in the body to maintain health[1]. These forms in foods can be quantified by microbiological

and chromatographic methods. Microbiological assay gives the total folate values which is inclusive of all forms of folate whereas chromatographic quantifies the individual forms of folate.

Chromatography is a versatile method used for the segregation of mixtures. In general, foods exhibit different matrices and therefore selectivity is an important step. In the past 10 years of work on folate shows that HPLC used fluorescence (LC-FD)[2], electrochemical detection (ED)[3] and mass spectrometry (LC-MS) detectors to identify folate derivatives. HPLC with FD and ED detector lacks specificity and are subjected to sample interference. HPLC (LC–MS/MS) combined with mass spectrometry effectively, identifies folate forms. It is also used widely for quantitative determination in blood, serum and food. It possesses inherent selectivity and specificity making it suitable to analyse many coenzyme species. The development of labeled analogues as an internal standard in stable isotope dilution assays (SIDAs) using LC-MS detection is a rapid method to quantify folate vitamers[4]. It was developed in 1980 to study the bioavailability of folate. It is considered to be one of the most accurate methods because the use of radiolabelled folates [5,6] or deuterium[7] acts as internal standards to correct for folate losses during preparation and analysis [8]. Simultaneously, the advent of ultra high-performance liquid chromatography (UHPLC) shortens the analysis time, allows for higher flow rate and rapid gradient curves when compared to HPLC [9]. The analysis by LCMS is very expensive therefore, the present study utilised the HPLC method to estimate vitamers of selected foods. The quantified vitamers data is needed for intervention trials, and there is no data existing on cooked foods, therefore, foods mostly commonly consumed by the population were selected for the study.

Methodology

Selection of foods

To foods chosen for analysis was identified through, a survey conducted among women population in the age group of 18-45 years residing at Coimbatore district, Tamil Nadu, India. The subjects comprised of two groups namely South Indian and North Indian women. The Northern Indian women refers to

population migrated to southern parts of India. Their dietary pattern differs, between the population hence, the group. A food frequency questionnaire (FFQ) was administered to elicit information about the frequency of food consumption on daily, weekly, monthly, occasionally and rarely basis. A score was assigned to the selected foods (FFQ) for quantification. The foods scoring more than 90% were selected for HPLC analysis.

Cooking of foods

The selected foods were standardised based on the standard method of preparation. Sensory evaluation (taste, texture, appearance, flavour and overall acceptability) was carried out for the prepared foods using thirty semi-trained panel members. The results were compiled and foods were taken for the analysis. The foods were prepared in batches and stored under dark conditions as folate is sensitive to heat, light and temperatures. The selected foods were homogenised in a blender and about 1g was taken for extraction. The samples were taken in triplicates. Quality control samples were carried out along with samples.

Method of Cooking followed by the population

Cereal based preparation

Rice : pressure cooking

Roti : roasting

Cereal-pulse based preparation

Pulses : Boiling and pressure cooking

Vegetable based preparation

Vegetables : Boiling

Chemicals and reagents

α-amylase (20mg/ml) from aspergillus oryzae, Sigma-Aldrich (St.Louis, MO, USA)
b. Protease (2mg/ml)from streptomysus griesus, Sigma-Aldrich (St.Louis, MO, USA)
c. Deconjugase(kidney acetone powder, Sigma-Aldrich, St.Louis, MO, USA)(5mg/ml).
All the enzymes were prepared in milli-Q water.

Standards

1. 10-formyl folic acid (catalogue no:16.215)
2. Tetrahydrofolic acid (catalogue no:16.207)
3. 5-formyl -5,6,7,8 Tetrahydrofolic acid, calcium salt (catalogue no:16.221)
4. 5-methyl-5,6,7,8 Tetrahydrofolic acid, calcium salt (catalogue no:16.235)
5. Folic acid (Sigma, F7876)

Standard preparation (200ng/µl) was purchased from Schircks Laboratories, Switzerland. The standards were prepared by dissolving in buffer (pH 6.0).

Sample Extraction

According to Arcot and Shreshta (2005) extraction conditions varies depending on the nature and type of food chosen for analysis. Therefore, in the present study, food samples were subjected to modified trienzyme extraction with protease, amylase and conjugase. The enzyme treated food extracts were purified using strong anion exchange and SPE purification, before being injected into the column.

Sample Purification

Sample purification is an important step in HPLC analysis because interference in the sample may damage the column. Interference overlaps the analyte peak in separation and can affect assay results. Solid Phase Extraction (SPE) is an efficient separation process, which makes it easier to obtain a higher recovery of the analyte. SPE was carried out before injecting the sample into the column, and it provides a complete removal of interference from the analyte fraction. Large particles are trapped by the SPE cartridge. In the present study, the cartridge is conditioned with 3 ml of hexane, methanol and water, and then equilibrated with 3 ml of buffer (pH 7.0). Then 2 ml of Standard was passed through the cartridge followed by 3 ml of conditioning buffer with pH 7.5. The compounds were collected by passing 1ml of elution buffer. Therefore, all samples were purified using SPE-Cartridge 500 mg, 6ml (Bond Elut, Agilent Technologies). The eluent were then filtered through PVDF filter (Agilent make) and injected into the column for separation. Positive pressure manifold system was used to provide flow control for each of the cartridge.[11]

HPLC Instrumentation

The HPLC system used in the study was Schimadzu make HPLC, C18 reversed phase column (Eclipse plus 5 micron, 4.6x250mm, Agilent technologies). Hundred microliters of the sample solutions were analysed using gradient elution with phosphate buffer pH 2.2 (Solvent A) and acetonitrile (Solvent B) at a flow rate of 1.0ml/min. An initial 0.01 min 2% hold of acetonitrile was ramped to 1-min hold of 5% acetonitrile. Then, a 5-min run of 10% acetonitrile and 5.50 min of 5% acetonitrile over 9-min was brought to 2% acetonitrile to re–equilibrate the column. Photo Diode Array (PDA) and Fluorescent (FD) detector was used. In FD detector, emission was set at 290 nm and excitation at 355 nm. In PDA detector, wavelength was set at 280 nm. The temperature was maintained at 40˚C [6,12].

Data Calculation

Recovery tubes were carried along with the samples to test for quality. The data was calculated based on the formula given below:

$$\frac{makeup\ vol}{samp\ wt} \times \frac{makeup\ vol}{vol\ taken} \times \frac{makeup\ vol}{vol\ taken} \times \frac{samp\ area}{inj\ vol(ml)} \times \frac{std\ conc}{std\ area} \times \frac{1}{1000} \times 100$$

Results and discussion

Folate and its vitamers of selected samples

Water soluble vitamins are a highly diverse group of compounds with differing functions. A single vitamin consists of several vitamers and they exhibit different biological functions *in vivo*. Vitamin compounds are non-volatile, and their hydrophilic nature makes them suitable for reverse phase HPLC analysis. HPLC provides increased speed, precision, accuracy, specificity and potential for simultaneous quantification of vitamers [13]. Folate is a water soluble vitamin, and quantification is difficult due to its characteristics and instability. As stated earlier, microbiological assay gives the picture of total folate whereas individual forms can be quantified by the HPLC method. It is less time-consuming and allows determination of folate forms such as folic acid, tetrahydrofolic acid, 5-

methyltetrahydrofolic acid, 5-formyltetrahydrofolic acid [14]. The present study determined the folate forms in selected food samples.

Table -1 **Folate vitamers content of Selected South and North Indian preparations**

S.No	**Food preparations**	**Folate vitamers (µg/100g)**				
		THF	**MTHF**	**10,FFA**	**5,FTHF**	**FA**
South Indian preparations						
1.	Cooked rice	BDL	BDL	BDL	19	BDL
2.	Idli	BDL	BDL	6	7	27.5
3.	Dosa	BDL	BDL	5	11	25.8
4.	Semolina upma	BDL	12	BDL	4	BDL
5.	Sambhar	72	BDL	BDL	22	55.8
6.	Rasam	195	423	BDL	792	77.8
7.	Potato poriyal	132	44	BDL	19	BDL
8.	Carrot poriyal	277	304	BDL	10	35.6
9.	Broad beans kootu	56	BDL	BDL	62	401
10.	Ladies finger poriyal	68	BDL	BDL	13	365
North Indian preparation						
1.	Roti	BDL	BDL	BDL	BDL	BDL
2.	Paratha	BDL	BDL	BDL	BDL	BDL
3.	Kadhi	21	BDL	BDL	16	545
4.	Kichidi	305	202	BDL	13	482
5.	Bajra roti	75	300	BDL	21	696
6.	Mixed vegetable subji	1752	46	BDL	1411	70
7.	Rice flakes upma	BDL	BDL	BDL	BDL	47.8
8.	Dhal fry	BDL	BDL	BDL	396	BDL
9.	Ghatta	BDL	58	BDL	BDL	BDL
10.	Vegetable salad	233	BDL	BDL	9	621

THF - tetrahydrofolic acid, MTHF- 5, methyltetrahydrofolic acid

10,FFA- 10, formylfolic acid, FA- folic acid,

5,FTHF – 5, formyltetrahydrofolic acid, BDL-Below detection limit

Folate is a crucial vitamin because it plays various metabolic roles in the body; mammals cannot synthesise folate and is dependent on external sources. It exists in several oxidative states and each of is essential to the role of cofactor in metabolism [15]. Tetrahydrofolate functions as coenzyme in the utilization of single carbon units. The metabolism of serine, glycine, methionine and histidine are dependent on tetrahydrofolate. It is capable of binding single carbon units including methyl, methylene and formyl species [15]. The present study shows that carrot poriyal (277 µg/100g) and mixed vegetable subji (1752 µg/100g) had higher amount of tetrahydrofolic acid.

Cereals based preparation

The rice and wheat are the staple food of South and North Indian population. The rice is cooked by boiling method whereas after harvesting, wheat is processed as flour to use in variety of foods. The study revealed that rice had formyl compounds alone and wheat had levels much below the detection levels. The study is an accordance with Blancquaert *et al* (2015) and Islam *et al*., 2020 they opine that rice and wheat contains total folate in low levels. Cereals crops takes long duration to grow hence they are stored. The storage and milling process decreases the folate content. The folate is lost by 23% when rice is stored for a year and boiling contributes to 48 % of loss. The Genetically modified rice brought a 50 % loss after four months off storage [18]. In wheat, 26% of loss was seen after 8 months of storage and processing like boiling, steaming and baking of flour leads to 11-16% of folate loss[19].

The 5-formyl and 5-methyl species of tetrahydrofolate functions as regulators and it exerts a role in the level of intracellular 5,10-methylene tetrahydrofolate. In turn, it takes part in methionine cycle, purine and thymidylate synthesis [20]. The 5-formyl THF is the stable natural derivate found in seeds. According to Pfeiffer, Rogers and Gregory (1997) cereals are good sources of 5-formyltetrahydrofolate, 5-methyltetrahydrofolate and folic acid whereas in the present study, presence of formyl compound was

observed in rice alone. Among cereals, bajra roti had the highest amount of 21µg/100g of 5-formyltetrahydrofolic acid and the least was seen in upma (4 µg/100g).

Roti and paratha are wheat based preparations. Wheat is the staple food of North Indian population. Folates are found in germ layer of wheat kernels, processing removes the folate present in wheat. Roti and paratha had 78 and 33 µg/100g of total folate when analysed through microbiological assay however, HPLC method could not produce results. It can be attributed to the fact that there are nine forms of folate existing today. The HPLC can quantity only five vitamers and LCMS/MS alone can quantify nine forms of the vitamers. The word 'total folate' means it includes all folate forms that exist. Therefore, in the present study HPLC could not quantity the vitamers that were present in roti and paratha.

Pulse based preparation

Legumes are considered as a good source of folate. The naturally occurring folate derivative in pulses are 5- MTHF, 10-FTHF, THF. A 100g serving of pulses provides 50 - 73 % of folate RDA for adults. Han and Tyler (2003) found that environment and soil influences the folate content of pulses. Pulses are consumed globally by different methods of processing like soaking, boiling, pressure cooking, germination, fermentation, canned legumes. All these treatment influence the folate content present in pulses. Fermentation process is widely used in daily cooking and it influences the folate content of processed pulses significantly [22, 23]. Delchier *et al* (2014a) opines folate vitamers content were altered after thermal treatment. Soaking increases the folate content by 40-60 % and germination increases by 2.4 fold[25]. Folate retention is higher by non - thermal processing whereas boiling decreases the folate content of legumes by 10-64%. Delchier *et al* (2012) found that folate is lost by leaching in legumes. Heat treatments like deep-frying does not cause folate loss in pulses[26]. Blanching is a common process widely practised in cooking, it reduces the folate content in faba beans and chick peas by 10 and 20 percent respectively [27]. Retort packing resulted in folate loss of 30-40% in chick peas[28]. The folate retention in household cooking depends on the methods of cooking. When folate is lost through

leaching consumption of water will help to regain folate. In the present study, sambar and dhal fry are a pulse based preparation of South and North Indian preparations. They are prepared by boiling and pressure cooking method. In the present study, pulses were found to have high folic acid when compared to cereal based preparation. Dhal fry had higher amount of formyl tetrahydrofolic acid (396 µg/100g). Hefni and Witthoft (2014) reported that autoclaving of pulses significantly reduced the folate content in chick pea by 40%. The HPLC method could not quantify vitamers present in cooked pulse based preparation it can be attributed to the fact that application of heat treatment could have contributed to loss of folate vitamers in pulse based Indian foods.

Vegetable based preparation

The fruits and vegetables are good source of tetrahydrofolate and 5-methyl tetrahydrofolate (Pfeiffer, Rogers and Gregory, 1997). Vegetables are consumed after cooking process. It involves cooking methods like blanching, boiling, juicing, microwave cooking and steaming. It is stored by application of various technique like freezing, canning and all this process brings out folate loss. Bureau *et al* (2015) found that folate is lost by 65% and 50% in spinach after canning and boiling method. Boiling and pressure cooking involves 60 and 54 % of folate lost by this method. Munyaka *et al* (2009) observed in Broccoli that High temperature short time cooking had better folate retention than low temperature long time cooking. Fajardo-Matin *et al* (2012), found that ready-to-eat vegetable foods leads to loss of folate. High pressure processing leads to folate loss in vegetables (Nguyen *et al*., 2003). Juicing of fruits and vegetables brings folate loss, it is seen in vegetables that carrots had 10 % and tomatoes had 50 % of loss. Folate is degraded in the presence of oxygen Delchier *et al* (2014a) studied the degradation of beans under anaerobic condition and found no loss in the folate content. Folate is stable in the presence of antioxidants, vegetables are source of antioxidant. Ascorbic acid prevents degradation of folates in foods (Ng et al., 2008). Interconversion also takes place in food either during analysis or spontaneously. The presence of carbohydrates especially fructose significantly causes degradation of methyl forms of folate in vegetables (Verlinde et al., 2010). Leaching is common among fruits and vegetables when in contact with water. The pH levels also causes degradation. Fruits and

vegetables have lower pH, and are easily degraded. The water content in vegetables corresponds to folate loss[36].

In vegetable based preparation, mixed vegetable subji (46 µg/100g) had the least and carrot poriyal (304 µg/100g) had the highest amount of 5-methyltetrahydrofolic acid. The present study data goes in agreement with the above mentioned study by Pfeiffer, Rogers and Gregory (1997).

According to Leichter (1980) cooking deactivates the endogenous folate. The endogenous folate present in fruits and vegetables cleaves polyglutamte to monoglutamate during preparation leading to lower amount of polyglutamate in cooked foods. The difference in folate levels in foods is influenced by region, season of harvest, climate and cooking conditions (Soongsongkiat *et al.*, 2010). The same was observed in the present study.

Folic acid is the synthetic form of folate and used in supplements and fortified foods. It is easily absorbable and highly bioavailable. Broad beans kootu (401 µg/100g) and vegetable salad (621 µg/100g) had the highest amount of folic acid and least was noted in carrot poriyal (35.6 µg/100g).

Conclusion

The developed countries had mandated folic acid fortification to prevent deficiency diseases. Folic acid is added synthetically to foods. Excess consumption of synthetic folic acid raise concern in the population. Folate is an unstable compound and many studies found that folate loss occurs through cooking methods. The study did not focus on the different methods of cooking to estimate folate loss. The data composition table list the folate vitamers content of raw foods alone. Thus, the study made an attempt to estimate the folate vitamers in selected foods most frequently consumed by the population. Although the data generated gave an insight about the folate vitamers present in foods. The HPLC method carried out for the present study could not identify vitamers further studies are needed to draw conclusion.

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Conflict of Interest

The authors have no conflict of interest

Reference

1. Scott J, Rebeille F and Fletcher J. (2000). Folic acid and Folates: The Feasibility for Nutritional Enhancement in Plant Foods. *Journal of the Science of Food and Agriculture* 80:795–824.
2. Mueller H. (1993). Determination of the Folic acid Content of Grain, Cereal Products, Bakery Products and Legumes by means of HPLC. *Food Chem* 197:573-577.
3. Bagley PJ and Selhub J. (2000). Analysis of Folate form Distribution by Affinity Followed by Reversed-Phase Chromatography with Electrochemical Detection. *Clinical Chemistry* 46(3):404–411.
4. Rychlik M, Englert K, Kapfer S, Kirchhoff E. (2007). Folate Contents of Legumes Determined by Optimized Enzyme Treatment and Stable Isotope Dilution Assays. *J Food Compos Anal* 20:411-419.
5. Chandra-Hioe MV, Bucknall MP, Arcot J. (2011). Folate Analysis in Foods by UPLC-MS/MS:Development and Validation of a Novel, High throughput Quantitative Assay; Folate Levels Determined in Australian Fortified Breads. *Anal Bioanal Chem* 401:1035-1042.
6. Brouwer V, Storozhenko S, VanDeSteene JC, Wille SMR, Stove CP, VanDerStraeten D, Lambert WE. (2008). Optimisation and Validation of a Liquid Chromatography-Tandem Mass Spectrometry Method for Folates in Rice. *J Chromatogr A* 1215(1-2):125-132.
7. Freisleben A, Schieberle P and Rychlik M. (2003). Specific and Sensitive Quantification of Folate Vitamers in Foods by Stable Isotope Dilution Assays using High Performance Liquid Chromatography-Tandem Mass Spectrometry. *Anal Bioanal Chem 376*:*149–56*.

8. Wieling J. (2002). LC-MS-MS Experiences with Internal Standards. *Chromatographia* 55:S107-S113.
9. Stefano VD, Avellone G, Bongiorno D, Cunsolo V, Muccilli V, Sforza S, Dossena A, Drahos L, Vékey K. (2012). Applications of Liquid Chromatography–Mass Spectrometry for Food Analysis. *Journal of Chromatography A* 1259:74–85.
10. Arcot J and Shrestha A. (2005). Folate:Methods of Analysis. Trends in Food Science and Technology 16: 253–266.
11. Snyder LR, Kirkland JJ, Dolan JW. (2011). Introduction to Modern Liquid Chromatography, 3rd edition, New Jersey: John Wiley and Sons, 757-780.
12. Rader JI, Weaver CM and Angyal G. (1998). Use of a Microbiological Assay with Trienzyme Extraction for Measurement of Pre-fortification Levels of Folates in Enriched Cereal-Grain Products. *Food Chemistry* 62 (4):451-465.
13. Russell LF. (2000). Quantitative determination of water soluble vitamins, In: Nollet LML, ed. 2nd edition, Food Analysis by HPLC, USA:Marcel Dekker, 403-450.
14. Jastrebova J, Witthoft C, Grahn A, Svensson U, Jagerstad M. (2003). HPLC Determination of Folates in Raw and Processed Beetroots. *Food Chemistry* 80:579–588.
15. Donnelly JG. (2001). Folic Acid. *Critical Reviews in Clinical Laboratory Sciences*. 38(3):183-223.
16. Blancquaert, D., Van Daele, J., Strobbe, S., Kiekens, F., Storozhenko, S., De Steur, H., Gellynck, X., Lambert, W., Stove, C., Van Der Straeten, D., (2015). Improving folate (vitamin B-9) stability in biofortified rice through metabolic engineering. Nat. Biotechnol. 33 (10), 1076–1078.
17. Islam M S., Liu J, Jiang L, Zhang C, Liang Q (2021), Folate Content in Fresh Corn: Effects of Harvest Time, Storage and Cooking Methods, Journal of Food Composition and Analysis 103, 104123.
18. Dong W, Cheng Z, Wang X, Wang B, Zhang H, Su N, Yamamaro C, Lei C, Wang J, Wang J, Zhang X, Guo X, Wu F, Zhai H & Wan J (2011). Determination of folate content in rice

germplasm (*Oryza sativa* L.) using tri-enzyme extraction and microbiological assays. International Journal of Food Sciences and Nutrition, 62:5, 537-543.

19. Liang Q, Wang K, Shariful I, Ye X, Zhang C, (2020) Folate content and retention in wheat grains and wheat-based foods: Effects of storage, processing, and cooking methods. Food Chemistry, 333, 127459,
20. Donnelly JG. (2001). Folic Acid. Critical Reviews in Clinical Laboratory Sciences. 38(3):183-223.
21. Pffeifer CM, Rogers LM, and Gregory JF. (1997). Determination of Folate in Cereal-grain Food Products using Tri-enzyme Extraction and Combined Affinity and Reversed Phase Liquid Chromatography. *Journal of Agricultural and Food Chemistry* 45:407-413.
22. Masuda, M., Ide, M., Utsumi, H., Niiro, T., Shimamura, Y., & Murata, M. (2012). Production potency of folate, vitamin B12, and thiamine by lactic acid bacteria iso- lated from Japanese pickles. Bioscience Biotechnology and Biochemistry, 76(11), 2061–2067.
23. Saubade, F., Hemery, Y. M., Guyot, J. P., & Humblot, C. (2017). Lactic acid fermentation as a tool for increasing the folate content of foods. Critical Reviews in Food Science And Nutrition, 57(18), 3894–3910.
24. Delchier, N., Ringling, C., Maingonnat, J. F., Rychlik, M., & Renard, C. M. G. C. (2014). Mechanisms of folate losses during processing: Diffusion vs. heat degradation. Food Chemistry, 157, 439–447.
25. Delchier N, Ringling C, Maingonnat J-F, Rychlik M, Renard CMGC. 2014a. Mechanisms of folate losses during processing: Diffusion vs. heat degradation. Food Chem 157:439-47.
26. Hefni, M., & Witthoft, C. M. (2014). Folate content in processed legume foods commonly consumed in Egypt. Lwt-Food Science and Technology, 57(1), 337–343.
27. Munyaka AW, Oey I, Verlinde P, Van Loey A, Hendrickx M. 2009. Acidification, crushing and thermal treatments can influence the profile and stability of folate poly-gamma- glutamates in broccoli (Brassica oleracea L. var. italica). Food Chem 117(3):568-75.

28. Fajardo-Martin V, Alonso-Aperte E, Varela-Moreiras G. 2012. Lack of data on folate in convenience foods: Should ready-to-eat products be considered relevant for folate intake? The European challenge. J Food Comp Anal 28(2):155-63.
29. Nguyen MT, Indrawati, Hendrickx M. 2003. Model studies on the stability of folic acid and 5-methyltetrahydrofolic acid degradation during thermal treatment in combination with high hydrostatic pressure. J Agric Food Chem 51(11):3352-7.
30. Ng X, Lucock M, Veysey M. 2008. Physicochemical effect of pH and antioxidants on mono and triglutamate forms of 5-methyltetrahydrofolate, and evaluation of vitamin stability in human gastric juice: Implications for folate bioavailability. Food Chem 106(1):200-10.
31. Verlinde P, Oey I, Lemmens L, Deborggraeve WM, Hendrickx ME, Van Loey AM. 2010. Influence of reducing carbohydrates on (6S)-5-Methyltetrahydrofolic acid degradation during thermal treatments. J Agric Food Chem 58(10):6190-9.
32. Wusigale and Liang, (2020), Folates: Stability and interaction with biological molecules, Journal of Agriculture and Food Research, Volume 2, 100039.
33. Leichter J. (1980). Folate Content in the Solid and Liquid Portions of Canned Vegetables. *Can Inst Food Sci Technol* 13:33–4.
34. Soongsongkiat M, Puwastien P, Jittinandana S, Dee-Uam A, Sungpuag P. (2010). Testing of Folate Conjugase from Chicken Pancreas vs Commercial Enzyme and Studying the Effect of Cooking on Folate Retention in Thai Foods. *Journal of Food Composition and Analysis* 23:681-688.
35. Kapil U and Bhadoria AS, (2014) Prevalence of Folate, Ferritin and Cobalamin Deficiencies amongst Adolescent in India, J Family Med Prim Care, Jul-Sep; 3(3): 247–249.