



MICROBIOLOGICAL ASSAY OF FOLATE CONTENT IN COMMONLY CONSUMED FOODS BY URBAN WOMEN OF PRAYAGRAJ, INDIA

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Abstract

The deficiency of folic acid increases the risk of chronic diseases that affect human health, particularly among women. This study aimed to examine the most commonly consumed food items within the chosen population which contribute to dietary intake of folate. A cross-sectional study was conducted to assess the folate content in commonly consumed food in the diet of urban women of Prayagraj. Foods consumed on daily basis or within 2-3 days were selected for further analysis. The folic acid in the selected food was estimated by a microbiological assay using *Lactobacillus casei* (ATCC 7469) as test organism with trienzyme extraction of protease, α -amylase and conjugase (from human plasma). The total folate content of 22 food items was analyzed and value ranged from 9.7 $\mu\text{g}/100\text{ g}$ to 257.7 $\mu\text{g}/100\text{ g}$. The highest folate content was recorded in gram flour (257.7 μg) followed by oats (174.14 μg), white bread (159.92 μg), spinach (129 μg), pulses (green gram dhal -116.36 & red gram dhal -79.02 μg), paneer (118.14 μg) and ladies finger (78.14 μg) for every 100 g food sample, respectively. The least folate content was recorded in milk (9.7 $\mu\text{g}/100\text{ g}$) and apple (11.48 $\mu\text{g}/100\text{ g}$). Commercial breakfast cereals showed major contributions in daily recommended intake of folate. This study concluded that some of these commonly consumed food items are fine sources of folate and may possibly fulfil the recommended dietary intake of total folate.

Key words: Microbiological assay, Trienzyme extraction, Folate, Folic acid deficiency.

Introduction

Folate is one of the most nutritionally significant water-soluble vitamins. Public concern has been escalated in folate in the past few years because of its functioning in health maintenance and disease prevention. It is a vitamin B group member and has got much consideration in the field of human nutrition, as it plays a crucial part in the vast area of biochemical alleys. Foliates exist in different forms of tetrahydrofolate (THF) and serve as substrates, coenzymes for growth, transport, for metabolism of nucleic and amino acids where they are involved in enzymatic process of one carbon atom transfer (Cook *et al.*, 2001). Folate dearth in a human body reduces DNA biosynthesis (Scott *et al.*, 2000; Hanson and Roje, 2001) and hence many cellular activities get affected. It has been reported that mothers with inadequate folic acid status are more likely to have neural tube defects among children or other types of birth defects (Botto *et al.*, 1999). There are a variety of disorders that can be caused by the inadequacy of folate, including major

illnesses like megaloblastic anemia, neural tube defect and minor illness such as Alzheimer's disease, vascular disease and depression (Singh and Yadav, 2020). There is solid confirmation of a relation between deficient folic acid status and increased homocysteine levels in the human body that causes the risk of cardiovascular condition. Since human cells can't incorporate folate *de novo*, hence, they should get the folate from their diet's items only. Whether the folate intake is adequate in the general population as well as for the vulnerable group remains unanswered as this evidence is based on literature, which has been excoriated to underestimate the folate content. Data on folic acid content of commonly consumed food in India are very scanty, till date no study was done to analyze the folate content of commonly consumed food among adult women in India where the infants, maternal mortality rates and anemia are more prevalent that directly link to folate deficiency. In a report given by an Indian Council of Medical Research group of expert, mentioned the need for more research on reliable data related to folate content in food consumed

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by Indians (ICMR, 2010).

This study was aimed to reveal the folate content in the diet of adult women by identifying and analyzing the maximum frequently consumed food items that contribute to folate intake in this population.

Materials and Methods

Study Location: This study was conducted from March to July 2018 and data on food folate consumed in northern region particularly in Prayagraj, Uttar Pradesh were collected. Eatables were selected based on the accessibility throughout the year and frequency of consumption. This study was ethically approved by the Institutional Ethical Review Board (IERB), University of Allahabad, (IERB ID: 2018-111).

Selection of Food Items: The food items were collected in raw form from different food groups. Food frequency table was used to calculate the consumption of food items recorded in the questionnaire by the respondents and the food items which were consumed daily or within 2-3 days were selected. A total of 22 food items were estimated for folic acid content per 100 gram by Microbiological Assay- Tri enzyme extraction method (Rahman. *et al.*, 2015; Tamura *et al.*, 1997).

Sample extraction: Solid food samples were washed with distilled water to remove dirt and dust particles (except egg, whole wheat flour, semolina, milk and packed commercial food items), dried and grind to fine powder whereas fruits and vegetables were made to a fine paste. Approx one gram of the sample was extracted in dim light where the sample was mixed with 100 ml of a freshly made working buffer prepared by mixing of stock buffer A (Sodium Phos. monobasic dehydrate) and buffer B (Sodium Phos. Dibasic dodecahydrate) and placed in the shaker to obtain a homogenized mixture with adjusted pH of 6.1 (by NaOH of 0.1 N or 0.05 N HCl), autoclave the extraction mixture at 120°C and 15 lbs pressure for few minutes.

Preparation of Folic acid Standard: Stock standard of folate (100µg/ml) prepared in dim light by dissolving 25 mg of USP Folic acid in ethyl alcohol of 25 percent and changing the pH of this solution to 7.0 by 0.1N NaOH. This stock standard can be stored in dark for 3 months at -80°C. The working standard of folate of 1ng/ml was prepared freshly before use by dilution of the stock standard with phosphate buffer and pH adjusted to 7 by 0.1 N NaOH or 0.05 N HCl.

Inoculum preparation: The lyophilized (ATCC 7469) *Lactobacillus casei subspecies Rhamnosus* preserved in sterile glass vials was obtained from the

National Collection of Dairy Cultures (NCDC), Karnal. The inoculums were prepared according to Rahman *et al.*, (2015); Chew *et al.*, (2012).

Media preparation: Folic acid casei medium purchased from Himedia Chem Co. (M543-100G) prepared by dissolving 9.4 gm in 100 ml of distilled water and boiled for 1-2 mins.

Trienzyme treatment: The cooled extraction samples were treated with protease, α-amylase and conjugase. Protease (2mg/ml) from *Streptomyces griseus* purchased from Sigma Chemical Co (P5147-100MG), added 1 ml in a food sample and incubated for 12 hours at 37°C, inactivated for few minutes in a water bath at 100°C. α- Amylase (20mg/ml) from *Aspergillus oryzae* purchased from Sigma Chemical Co (10065-10G) added 1 ml (1 mg dissolved in 50 ml distilled water in 100 ml beaker and blended firmly for 5 mins) then incubated at 37°C for 3 hours, inactivated at 100°C for 5 minutes in a water bath. After treatment with α- amylase the sample pH was changed by NaOH to 7.2. Conjugase from human plasma (Vishnumohan *et al.*, 2017; Gregory, 1989; Arcot and Shrestha, 2005) obtained from the blood bank, Prayagraj, added 50 µL to the α- amylase treated food extract and incubated for 3 hours at 37°C and heated at 100°C for 5 minutes in a water bath. Centrifugation of the sample was done at 4000 rpm for 15 mins. The supernatant was collected and used for folate determination and can be stored for future use at -80°C.

After enzyme treatment: To the cooled trienzyme treated sample with adjusted pH of 6.2, working buffer and media were added, autoclaved at a pressure of 15 lbs, at 121°C for 10 minutes. Once each test tube was cooled, 15 µL (*L.casei*) inoculum prepared from culture was added, each test tubes was vortexed for few minutes and incubated at room temperature 37°C for a time period of 18-24 hours, deactivated to discontinue the growth of bacteria by boiling at 100°C in a water bath. Absorbance was taken at 540 nm using UV-visible spectrophotometer.

Control: About. 100ml of working buffer with pH adjusted to 6.1 was taken for control and autoclaved, accompanied by enzyme treatment, inactivation, dilution and filtration measures as done in case of sample.

Construction of Std. Graph for Evaluation of Folate Content in tested food samples: A std. graph is obtained by turbidity values of UV- visible spectro because of *L. casei growth* in the media having (0.2, 0.4, 0.6, 0.8, 1) nanogram per tube of the replicate set of standard solutions. Absorbance was recorded at a wavelength of 540 nm. The optical density of all the above 5 sets of std. tubes were passed down to form the graph

to obtain a calibration curve having an R-square value of 0.994.

The folic acid level in Food Sample: The folic acid was measured using the AOAC method in provided food items after trienzymes extraction of folate in food form and diluted with media, the growth, or the turbidity of the *L. casei* was compared with the standard folate solutions. The concentration of control at 540 nm is 3.023 that is important to determine the control of folic acid after dilution. Concentrations of folic acid from separate food items were observed following dilution which was later used to determine the concentration of folic acid from std. graph and calculated by using the following formula:

$$\text{Folate in food } \mu\text{g}/100\text{g} = \frac{\text{Concentration (ng / tube)}}{\text{Vol. of extract per tube}} \times \frac{\text{Dilution factor}}{1000} - \text{O.D. of control} \times 100$$

Statistical analysis: The obtained data were entered

Table 1: Folate content of commonly consumed food items in the present study compared with Reference values of IFCT, 2017.

Food Samples	Experimental value ($\mu\text{g}/100\text{g}$)	IFCT, 2017 ($\mu\text{g}/100\text{g}$)	P-value
Spinach	129.69 \pm 1.41	142 \pm 10.30	0.204
Potato	17.69 \pm 1.15	15.69 \pm 1.93	0.074
Tomato	26.58 \pm 4.07	19.46 \pm 2.99	0.199
Carrot	35.48 \pm 4.07	23.67 \pm 3.25	0.115
Onion	40.81 \pm 4.07	29.68 \pm 1.97	0.094
Ladies Finger	78.14 \pm 5.55	63.68 \pm 10.75	0.051
Apple	11.48 \pm 4.07	3.52 \pm 0.36	0.099
Banana	28.36 \pm 2.66	10.90 \pm 3.45	0.021*
Green Gram Dhal	116.36 \pm 2.66	92.11 \pm 5.11	0.030*
Red Gram Dhal	79.02 \pm 0.05	108 \pm 8.69	0.031*
Gram Flour	257.7 \pm 7.05	NA	-
Milk	9.7 \pm 1.09	8.57 \pm 0.44	0.057
Paneer	118.14 \pm 4.07	93.3 \pm 14.35	0.054
Egg	58.59 \pm 6.71	49.32 \pm 2.34	0.231
Whole Wheat Flour	33.69 \pm 1.15	29.22 \pm 1.92	0.013*
Dalia	97.69 \pm 26.68	26.3 \pm 3.60	0.033*
Rice Flakes	22.14 \pm 5.55	8.46 \pm 0.92	0.053
Rice	14.14 \pm 4.07	9.32 \pm 1.93	0.265
Semolina	37.25 \pm 5.55	25.68 \pm 3.64	0.156
Oats	174.14 \pm 4.07	NA	-
Cornflakes	51.48 \pm 6.71	NA	-
Bread	159.92 \pm 55.51	NA	-

Note: NA not available by IFCT, 2017

*Sig (p<0.05)

in MS-Office 2007 excel worksheet and a standard curve was made. Student unpaired t-test was applied to compare the experimental data with reference values of Indian Food Composition Table (Longvah *et al.*, 2017). Data were analyzed using STATA version 13.

Results

Overall 22 commonly consumed food items were analyzed out of which 19 food items were representative of all food groups and three were commercial breakfast products of cereals (oats, cornflakes and white bread). Each sample was analyzed in triplicate and mean value was calculated with standard deviation. The value of folate content among 22 food items depicted in the table.1, ranged from 9.7 $\mu\text{g}/100\text{g}$ to 257.7 $\mu\text{g}/100\text{g}$. Highest folate content was found in gram flour (257.7 $\mu\text{g}/100\text{g}$) followed by oats (174.14 $\mu\text{g}/100\text{g}$), white bread (159.92 $\mu\text{g}/100\text{g}$), spinach (129 $\mu\text{g}/100\text{g}$), pulses (green gram dhal -116.36 $\mu\text{g}/100\text{g}$ & red gram dhal - 79.02 $\mu\text{g}/100\text{g}$), paneer (118.14 $\mu\text{g}/100\text{g}$) and ladies finger (78.14 $\mu\text{g}/100\text{g}$) respectively, whereas lowest folate content was estimated in milk (9.7 $\mu\text{g}/100\text{g}$) and apple (11.48 $\mu\text{g}/100\text{g}$). Experimental values were mostly higher when compared with IFCT, 2017 values (Longvah *et al.*, 2017) except in case of banana, spinach and red gram.

As illustrated in Fig. 1 folate content ($\mu\text{g}/100\text{g}$) of food analyzed in the present study, when compared with the ICMR RDA, 2010 for adult, pregnant and, lactating women showed that pulses, vegetables and commercial breakfast cereals product contribute more to percentage RDA.

Discussion

Folic acid occurs in many different forms in nature and it is more sensitive to the environment as compared to other micronutrient, hence its estimation becomes a more challenging task. Aside from that, a deliberate election of type of buffer, conjugate form and incubation condition is a must since all of these processes are probably going to alter the ultimate yield of the folate. The microbiological assay method is more reliable and most tedious so not adopted by most of the studies.

The folate content is given by IFCT, 2017 (Longvah *et al.*, 2017) and our experimental values show some variations which might be due to the difference in the method chosen for analysis. In both cases, trienzyme treatment was given, however in IFCT, 2017 they followed U-HPLC-UV or fluorescence detector used and in our study UV-visible spectrophotometer is used that might show different ranges for detection of food folate. Similar results were also reported by Rahman *et*

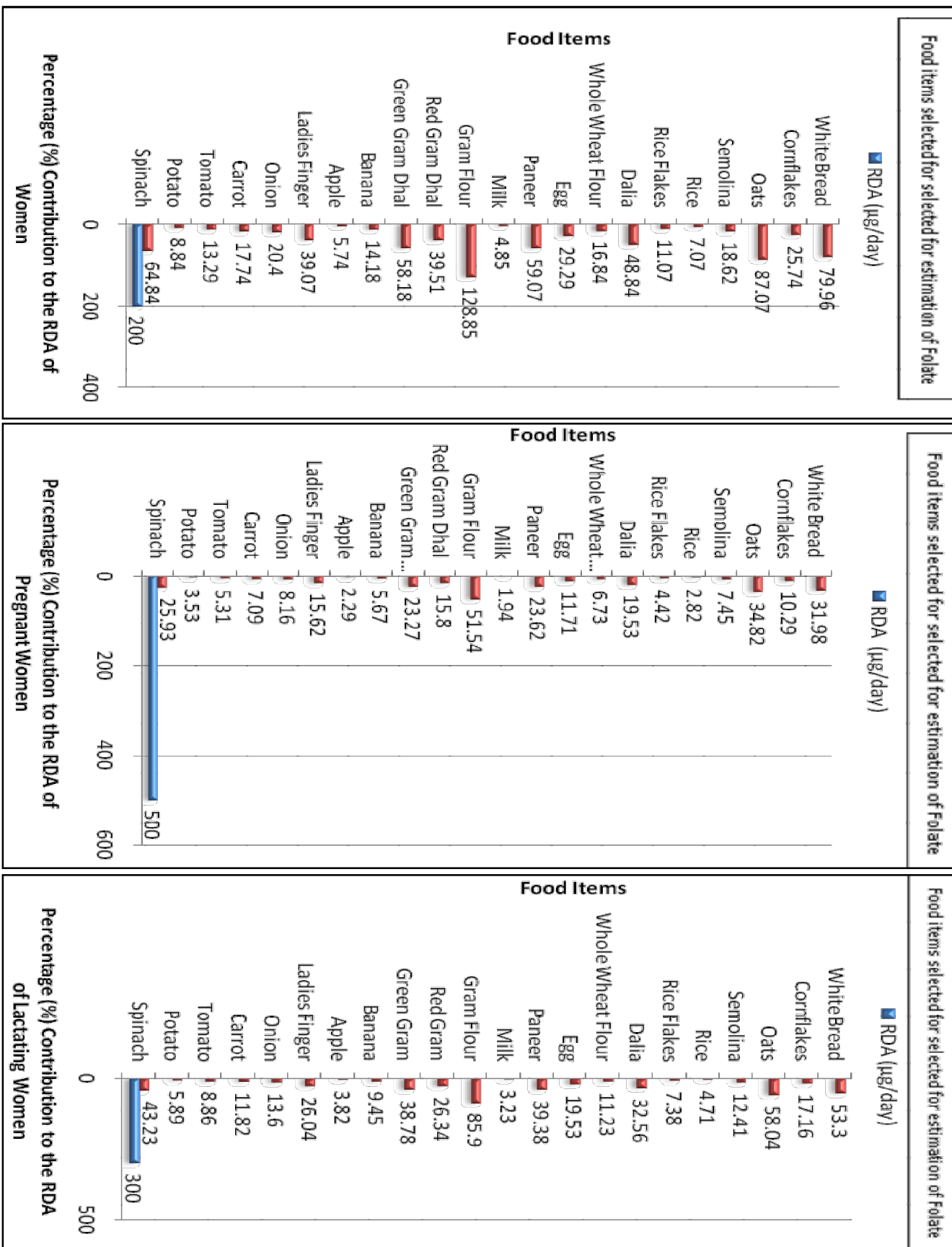


Fig. 1: Percentage contribution of estimated folate content in selected foods to recommended dietary allowances (A)-200 µg/day for sedentary women; (B) -500 µg/day for pregnant women; (C) -300 µg/day for lactating women.

et al., (2015), the difference observed in values may also be due to the agricultural practices, season, climate, geography, soil composition, geology, the maturity of the plant, variety/species, the difference in the extraction procedure used and the chemical stability of folate during the processing conditions (Iwitani *et al.*, 2002; Schakel *et al.*, 1997; Rahman *et al.*, 2015; Shohag *et al.*, 2012; Vishnumohan *et al.*, 2009).

Increased value of folate in white bread may be due to the process of fermentation where microorganisms showed a positive impact on folate content of food and enhance B vitamins synthesis (Srilakshmi, B. 2010; Vishnumohan *et al.*, 2009).

Folic acid values of breakfast cereals could not be compared as data was not reported by IFCT, 2017 (Longvah *et al.*, 2017), however in present study these food items were analyzed due to higher frequency of consumption and also higher contribution of these food towards daily recommended folate intake in selected population, possibly due to the processing techniques used in their preparation that may further enhance folate values. Fig. 1 Also depicts that among selected food analyzed for folate content, breakfast cereals and pulses (viz oats, white bread, gram flour and spinach among leafy vegetables contribute more than 1/3rd of daily RDA of sedentary women. Previous studies in the country also encouraged the consumption of breakfast cereals and vegetables for folate intake (Vishnumohan *et al.*, 2009). A study was done by Shohag *et al.*, (2012) also reported that the consumption of vegetables contributes more to % RDA among women.

Although microbiological assay is the prevailing approach of folic acid estimation is time-inefficient, requires extreme responsibility and cannot distinguish the specific folic acid like free or conjugated form. HPLC is an advanced detailed approach but includes a complex extraction and purification method immunoassay approaches are fast, basic & inexpensive but not advisable for evaluation of folic acid in diet products (Rahman *et al.*, 2015). It comes to be as none of the folic acid interpretive approaches is perfect. The prime of a specific approach is greatly decided by the aim of the study for example food content and least value by the number of resources in hand, assessment time & expenditure and scrutiny itself. A more comprehensive data on various folate food sources and its bioavailability needs to be generated; this will help in mapping the deficiency and sufficiency of dietary folic acid among population.

Conclusion

Among selected food items, gram flour, oats, bread,

paneer and spinach are rich sources of folic acid and may be recommended in appreciable amounts or advocated to increase folate content in the diet. Percentage RDA value from the present study can also be used as a guideline for promoting folate-rich sources where consumption of breakfast cereals, vegetables and pulses are recommended among adult women. This study stated that the diet of majority of respondents in the selected population may be adequate in folate intake, so efficiently meet the requirement. However, the study is on a lesser number of food items, therefore further studies should be conducted to ensure the total folate content of most of the food items. The effect of various cooking methods on the folate content in foods and recipes prepared by using these food items should also be considered for future research.

Acknowledgments

Authors would like to acknowledge the Food and Analysis Research Laboratory (FARL), Centre of Food Technology, University of Allahabad, Prayagraj-211002, U.P., India for the support of this work to be successfully done.

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