October 2002 Issue 5

**FOLATE:**
**From Food to Functionality and Optimal Health**

# Folate Func Health

## Introduction

Welcome to the 5th issue of our Newsletter. In this issue, we have reports on folate bioaccessibility and bioavailability from dairy foods, a novel hydroponics system for preparing intrinsically labelled spinach for bioavailability studies and details of a new combined liquid chromatography-microbiological assay for quantifying individual folates in foods and biological samples. If you would like any further information, please contact either **paul.finglas@bbsrc.ac.uk** or **dawn.wright@bbsrc.ac.uk**, or visit our web site at **www.ifr.bbsrc.ac.uk/folate.**

**Paul Finglas**
*Scientific Coordinator*

# Folate Bioaccessibility from dairy Foods using TIM

On average, milk and dairy products provide 10-15% of the daily folate intake in many Western countries, especially among the younger population. Although the folate concentration in milk is only approximately 5µg/100g, milk is of interest due to the high consumption and the presence of a folate binding protein (FBP). The exact function of FBP is still unknown. The conditions during passage of dairy products through the gastro-intestinal tract can influence the FBP stability and binding capacity. FBP may play an important role in folate stabilisation and may also have an impact on the absorption in the gastro-intestinal tract and bioavailability of dietary folate. Dairy products can therefore be considered as potential foods for folate fortification.

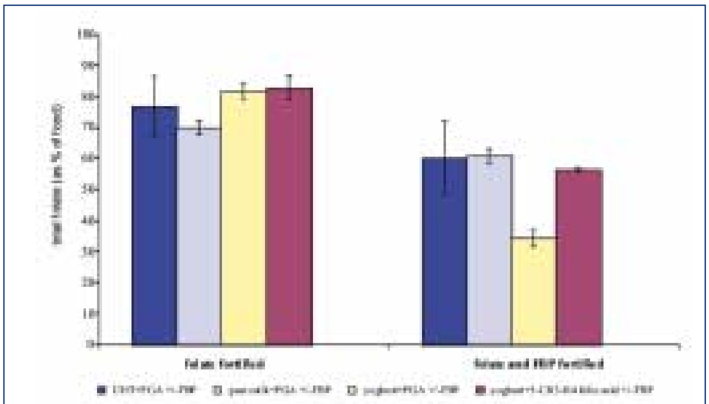
Folate bioaccessibility from various dairy products has been studied in an *in vitro* dynamic computer-controlled gastro-intestinal model (TIM) by TNO in collaboration with the Swedish University of Agricultural Sciences. The TIM system was described in issue 3 (June 2001) of this newsletter. The aims of the study were:

- What is the folate bioaccessibility from dairy products?
- Is there a difference in folate bioaccessibility between PGA- and 5-$CH_3$-$H_4$ folic acid-enriched dairy products?
- Does FBP have an impact on folate bioaccessibility?

Ten dairy products fortified with two different folate forms (40µg/100g), with and without FBP (molar folate:FBP ratio of 1:1) were produced (Table 1). All products were applied into TIM as test portions of 300g under the same digestion conditions. Total dialysate was collected during 0-1, 1-2, 2-3 and 3-5 hours from the jejunal and ileal compartments to study the kinetics of folate bioaccessibility. From the ileal delivery fraction a pooled sample was collected. The dialysate fractions contained the potentially "absorbable" (bioaccessible) fraction of folate, while the ileal delivery samples represented the "non-absorbable" fraction. Folate was quantified by radio protein-binding assay (RPBA) and HPLC. For FBP quantification an ELISA method was used.

*Table 1. Characteristics of milk products tested in the in vitro gastro-intestinal model.*

| Number | Product | Folate fortification form | FBP fortification |
|---|---|---|---|
| 1 | pasteurised milk | PGA | - |
| 2 | pasteurised milk | PGA | + |
| 3 | pasteurised milk | 5-$CH_3$-$H_4$ folic acid | - |
| 4 | pasteurised milk | 5-$CH_3$-$H_4$ folic acid | + |
| 5 | UHT milk | PGA | - |
| 6 | UHT milk | PGA | + |
| 7 | yoghurt plain | PGA | - |
| 8 | yoghurt plain | PGA | + |
| 9 | yoghurt plain | 5-$CH_3$-$H_4$ folic acid | - |
| 10 | yoghurt plain | 5-$CH_3$-$H_4$ folic acid | + |



The stability (recovery) of PGA and 5-$CH_3$-$H_4$ folic acid during TIM passage was 80-90%. No difference in stability was observed between the two folate forms. Folate bioaccessibility data from folate-fortified dairy products showed that 70-75% and 85% of the initial folate content in the dairy products (milk and yoghurt, respectively) was absorbed from the jejunal and ileal compartments (Figure 1). In the FBP-enriched milk products, the absorbable PGA fraction (jejunum+ileum) was lowered from 70-75% to 60%. In the FBP enriched yoghurt the absorbable PGA fraction was lowered from 85% to 35%, while the 5-$CH_3$-H4 folic acid fraction was lowered from 85% to 55%. This indicates FBP binding activity during gastro-intestinal passage, but differently for the type of folate and the food matrix.

Further research will be done to investigate the role of FBP on folate transport across the intestinal wall. TIM results will be compared with results from a human intervention study (FOLMELK, also in this issue) and a human ileostomy study, in which some of these products were studied.

For further information please contact **Miriam Verwei** or **Robert Havenaar** (TNO)(Verwei@voeding.tno.nl), or **Karin Arkbåge** (SLU)(Karin.Arkbage@lmv.slu.se).

*Figure 1. Average (+/- range; n=2) folate bioaccessibility from PGA and 5-$CH_3$-$H_4$ folic acid fortified dairy products without (left) and with FBP (right). The bars represent total 'absorbable folate' analysed by RPBA in jejunal+ileal dialysate, expressed as percentage of intake with the fortified dairy products.*

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# Folic acid added to milk increases folate status and lowers plasma homocysteine in healthy volunteers

A low folate status has been associated with various health problems. Although it is generally accepted that folate intake on population level should increase; the best means to increase intake are still under debate, especially in European countries where folic acid fortification is not permitted. Since the bioavailability from natural sources such as fruits and vegetables might not be optimal, enriched food products might be a future alternative. For this purpose milk was thought as a promising food, at least for the Netherlands. Milk is commonly consumed in relatively constant and predictable amounts, providing 10-15% of the daily folate intake. Bioavailability of folate from the milk matrix is considered to be high. In addition, the presence of milk in the diet seems to enhance folate bioavailability compared to diets without milk. The reason for this might be the folate binding proteins (FBPs), present in excess compared to the amount of folate in milk.

The objectives of the present study were to determine absorption and homocysteine-lowering capacity of folates from fortified milk, with or without its endogenous FBP. Folate in milk is essentially bound to FBPs. However, ultra-high temperature (UHT) processing of milk appears to destroy FBP and consequently its folate-binding capacity. Therefore, we investigated bioavailability of folic acid added to pasteurized and UHT processed milks.

An intervention study was performed that started with a run-in period of 4 weeks in which the volunteers were instructed to limit their folate intake. Subsequently, the 72 volunteers were divided over 4 treatment groups, each receiving the same fully controlled diet, together with 0.5 L of one of the 4 types of milk studied (see Figure 1). At the start and end of the intervention, serum and red blood cell folate (5-MTHF), and plasma total homocysteine were measured.

Only minor differences in results were observed between the results of the two non-fortified milk groups, and between the results of the two fortified milk groups. Therefore, average results are presented for the non-fortified milk groups and the fortified milk groups (see Table 1). Serum folate concentrations of the fortified milk groups were 89% higher (p<0.001) than of the non-fortified milk groups; mean red blood cell folate concentrations were 16% higher (p<0.001), and total homocysteine concentrations were 14% lower (p<0.001) in these groups.

This study shows that milk is a suitable source for folic acid fortification, showing beneficial effects within four weeks. Within this period folate status increased substantially and plasma homocysteine concentration decreased. Further exploration of the data (not presented here) indicated that FBP had no significant effect on folate bioavailability.

**For further information, please contact Trinette van Vliet (T.vanVliet@voeding.tno.nl)**

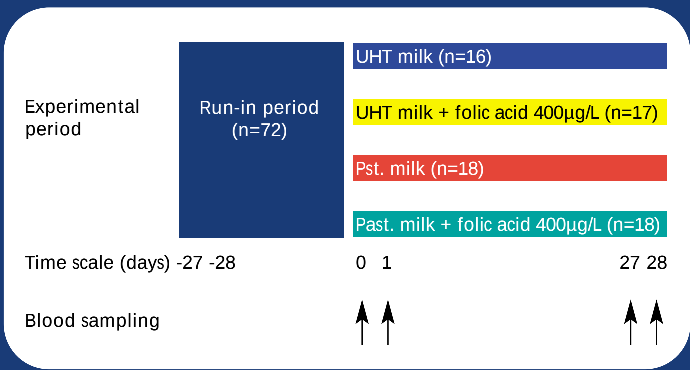


*Figure 1. Schedule of the nutrition intervention study.*

*Table 1. Serum folate (5-MTHF), red blood cell folate (5-MTHF) and total plasma homocysteine concentrations at start and end of dietary intervention.*

| Parameter | | Unfortified milk (n=34) | Fortified milk (n=35) |
|---|---|---|---|
| Serum folate (nmol/L) [1] | Start<br>End | 9.0 ± 2.6<br>7.4 ± 2.0 | 8.9 ± 2.9<br>14.0 ± 4.6 |
| Red blood cell folate (nmol/L) [1] | Start<br>End | 337 ± 89<br>318 ± 84 | 357 ± 118<br>369 ± 103 |
| Plasma total homocysteine (µmol/L) [2] | Start<br>End | 8.5 (7.9 – 9.2)<br>9.0 (8.4 – 9.6) | 8.6 (8.1 – 9.2)<br>8.0 (7.5 – 8.6) |

[1] Mean ± SD, [2] Geometric mean (95% confidence interval)

# HPLC Coupled with a Microbiological Assay (HPLC- MA): A Powerful Analytical Alternative for Food Folate Analysis

Although improvements in the determination of folates in foodstuff over the last years have positively impacted on the quality of data, new validated methods for the analysis of individual folate metabolites in foodstuff are still of high importance for nutritional studies. Improved methods may lead to a better understanding of the folate bioavailability from diets and folate metabolism in man, and thus provide us with more reliable advice for an optimal folate supply for disease prevention.

For that reason, an alternative analytical method for folate analysis in combination with a very simple sample preparation technique was developed and successfully applied to the determination of folate metabolites in various food samples of animal and plant origin.
This analytical method directly combines the effective separation of folate monoglutamates with an isocratic ion-pair-HPLC system and the highly selective and sensitive detection using a microbiological assay employing a chloramphenicol resistant strain of Lactobacillus casei [Fig. 1].

Our initial results show, that satisfactory qualitative and reproducible quantitative data are obtained without the use of an extensive sample clean-up (e.g. SPE or affinity chromatography). Due to the high selectivity of the microbiological detection system, even food samples with a complex folate pattern or crude food matrices, such as cereals, can be analysed without

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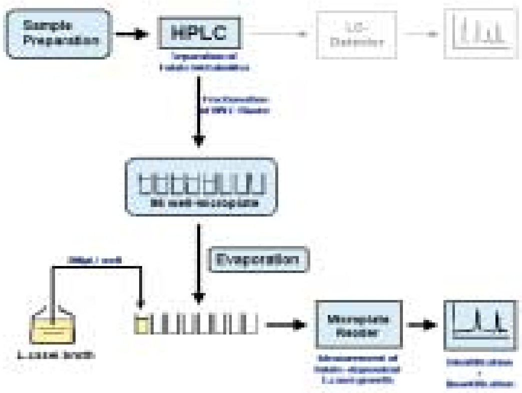
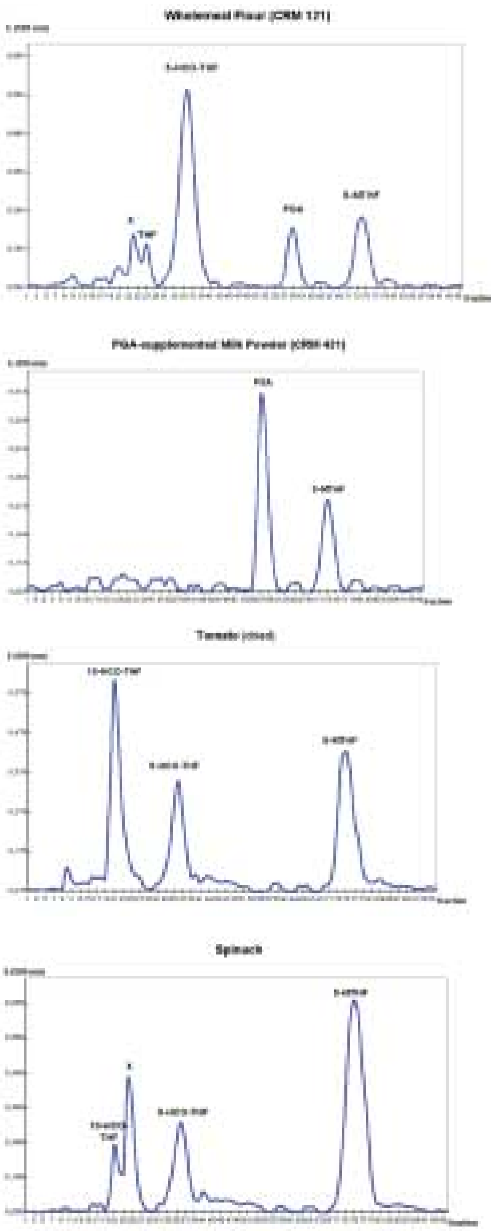



significant problems with interferences. The presented HPLC-MA methodology offers a powerful analytical alternative to conventional chromatographic or microbiological methods, as it is a combination of both allowing the ultra-sensitive detection of the biological active folate metabolites. The method is currently being used to provide data on the folate distribution patterns in model foods used in processing and human studies. Results are presented in Figure 2 for two certified reference materials (wholemeal flour, CRM 121 & milk poweder, CRM 421) and two model foods (dried tomatoes and spinach).




For further information on this technique, please contact **Ulla Kehlenbach [ukehlen@gmx.de],** or **Prof Heinz Nau [Heinz.Nau@tiho-hannover.de] at the University of Hannover.**

## Project News

There are two plenary meetings remaining in the project: Rome (16-18 January, 2003) and Warsaw (either July or September 2003 – dates to be confirmed). It is hoped that the final meeting will be organised as a conference – further details on the project web site and a call for papers will be made shortly.

## Hydroponics

Problem: **to produce a batch of spinach containing a stable isotope for use in food intervention studies.**

Solution: **Hydroponics.**

We have developed a method to grow spinach, labelled with the stable isotope $^{15}N$, suitable for feeding to volunteers in food studies to study the absorption and metabolism of folates. Spinach seeds (of the variety *Ballet* ) were sown in 9cm diameter plant pots, filled with a mixture of *Vermiculite* and *Pearlite* as a support. The pots were then placed in trays of fifty, and were watered in with an un-labelled nutrient solution.

Once the seedlings had established, weak plants were removed, and nutrient was provided by means of a nutrient flow system, where the medium flowed from a header tank to a reservoir tank through the trays in which the pots stood. A pump was used to pump the medium back up to the header tank at a rate of ½L per min (fig 1).

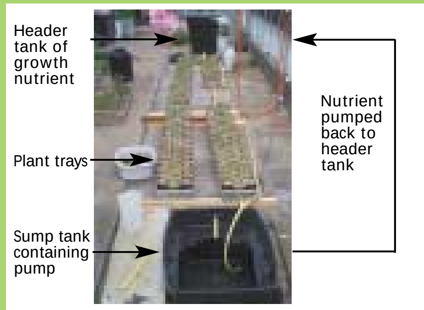



*Figure 1: Hydroponics equipment for growing spinach.*

Plants were fed with a nutrient solution based on a formulation of Bentley *et al* (1959), with around 73% nitrogen coming from nitrates and 27% from ammonium sulphate. 50% of the nitrogen was provided as $^{15}N$-labelled ammonium sulphate, potassium nitrate and sodium nitrate.

The pH of the nutrient medium was determined daily and adjusted if necessary to maintain levels between 5.5 and 6.5 Analyses for magnesium (performed by atomic absorption) and potassium (by atomic emission) were performed regularly with the frequency rising as the leaf mass increased. The nitrate levels were also monitored by means of a test-kit, involving a colorimetric method using the sulphonylic acid and chromotropic acid reaction.

Plants were harvested after 10 weeks growth, with an average mass of 21.8g per plant. The majority of the stalks were removed, the leaves washed in lightly salted water, then clean water, weighed into 250g portions, cooked for 2 minutes in a microwave (800 watt) and frozen. When ready for use, they were liquidised and turned into soup to be fed to volunteers.

Incorporation of $^{15}N$ stable isotope into 5-methyl tetrahydrofolate (5-MeTHF) present within the leaf matter was determined by negative-ion mass spectrometry, and was estimated at 41% of N as $^{15}N$.

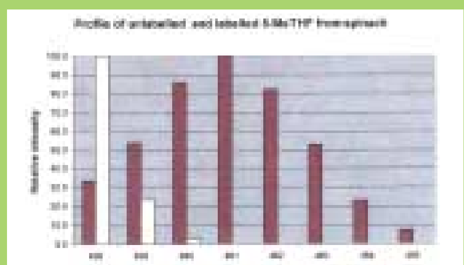


Figure 2 shows the profile of labelled 5-MeTHF compared with that of unlabelled from the spinach. At natural abundance, 5-MeTHF containing 2 extra mass units (M+2) accounted for 2.6% of the total, with M+3 not detected. However, using the hydroponics system to grow the spinach yielded 5-MeTHF with an isotope distribution with a maxima at M+3, and detectable to M+7.


***References:***

*Bentley. M (1959) 'Commercial Hydroponics – Facts and Figures' (p604). Bendon Books, Johannesburg.*

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