October 2003 Issue 6

FOLATE:
From Food to Functionality and Optimal Health



Folate Func Health

Introduction

Welcome to the 6th issue of our Newsletter. In this issue, we have details of the new work by the two NAS partners who have joined the project this year, some results and findings from various parts of the project and details of the conference **"EUROFOODFOLATE2004."** 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.ac.uk/folate**

Paul Finglas
Scientific Coordinator

Old Town, Warsaw

EUROFOODFOLATE 2004

The final project meeting will be combined with the "**First International Conference on Folates – Analysis, Bioavailability and Health"**, **11-14 February 2004, Warsaw, Poland,** which is being supported under EC QoL Food Nutrition and Health (Key Action 1; QLAM-2001-00475). This innovative conference will encompass the exciting advances in our understanding of folate nutrition and function, interactions with other B-group vitamins and homocysteine metabolism and its significant contribution to human health and well-being. The conference will offer a unique opportunity to share experiences, exchange ideas and encourage discussions between leading food and plant scientists, nutritionists, molecular biologists and consumer scientists, and between academia and industry.

The topics will include:

- **Analytical methods**
- **Folate synthesis and enhancement in food**
- **Food processing and biotechnology**
- **Bioavailability and metabolism**
- **Studies on the intake, biological effects and dose-response data**
- **Optimal folate requirements including nutrient-gene interactions**
- **Recommendations for optimal health**
- **Fortification strategies**
- **Effective communication strategies for health professionals and consumers**

The provisional list of speakers include:
Prof Jess Gregory
University of Florida, USA
Dr Christine Pfeiffer
Centers for Disease Control and Prevention, Atlanta, USA
Prof Anne Molloy
Trinity College Dublin, Ireland
Dr Fabrice Rebeille
University of Joseph Fourier, Grenoble, France
Dr Hilary Powers
(University of Sheffield, UK)
and various members of the project.

There are a number of bursaries available to assist scientists and representatives of SMEs from NAS countries, students and young researchers from European countries with expenses to the meeting.

The deadline for the submission of abstracts has been extended to 31 October 2003.

For full details visit **www.ifr.ac.uk/folate** or contact **Dawn Wright** (EUROFOODFOLATE 2004 Conference Secretariat, **Tel: +44 (0) 1603 255394, fax: +44 (0) 1603 255168, Email: dawn.wright@bbsrc.ac.uk).**

Please register early as places at the conference will be limited!

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Folates and fermented milk products

Cow's milk is recognized as an important dietary source of folates, supplying up to 10-15% of RDA. Folates are essential cofactors in bacterial metabolism and, as such, will be present in most bacteria. Many bacteria are able to synthesize this cofactor by themselves, but some, including many lactic acid bacteria, have strict growth requirement for folate. Because of this the content of folates in fermented milk products is dependent on both the type of fermentation starter and fermentation conditions.

The aim of the current work in Prague is to determine if, by careful selection of fermentation bacteria, fermented milk products with naturally increased folate content can be produced. The low concentration of folates occurring in foods, together with their instability, makes analysis of folate very demanding. The validation of the method used is necessary in order to obtain reliable data. Thus, the first step of the work included validation of HPLC method for folate determination by the analysis of a reference material and by comparing sample analyses with results from Partner 5.

The content of 5-methyltetrahydrofolate (5-MTHF), the main folate form found in milk, was measured in 15 fermented milk products bought in retail stores. The 5-MTHF content was between 0.40 and 4.01 µg in 100g of sample. In comparison, an average content 24 ± 1.5 µg/100g of 5-MTHF was found in analysis of several samples of defatted dried milks produced over a one year period. Even taking dry matter into account, fermented milk products with much higher folate concentrations are available to the consumer.

In cooperation with Milcom Ltd. (a Czech producer of fermentation starters for the dairy industry), the model fermented samples were prepared. Several strains of lactic acid bacteria (*Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium adolescentis* and *Streptococcus salivarius* subsp. *Thermophilus)* from Milcom's microbial collection were used in these experiments. Almost 1 order differences in 5-MTHF concentration were found between the model samples. Fig.1 shows the maximal value found within the individual species. In all samples the decline after 12 hours of fermentation at 37°C was observed. The highest folate production was observed in samples fermented with *Streptococcus salivarius* subsp. *thermophilus,* and *Bifidobacterium longum* followed.

Further planned experiments will evaluate other microbial species and strains in terms of folate production, possible influences of growth stimulants and effects of co-cultivation. Sensory evaluation of the products will also be undertaken.

For further information, please contact
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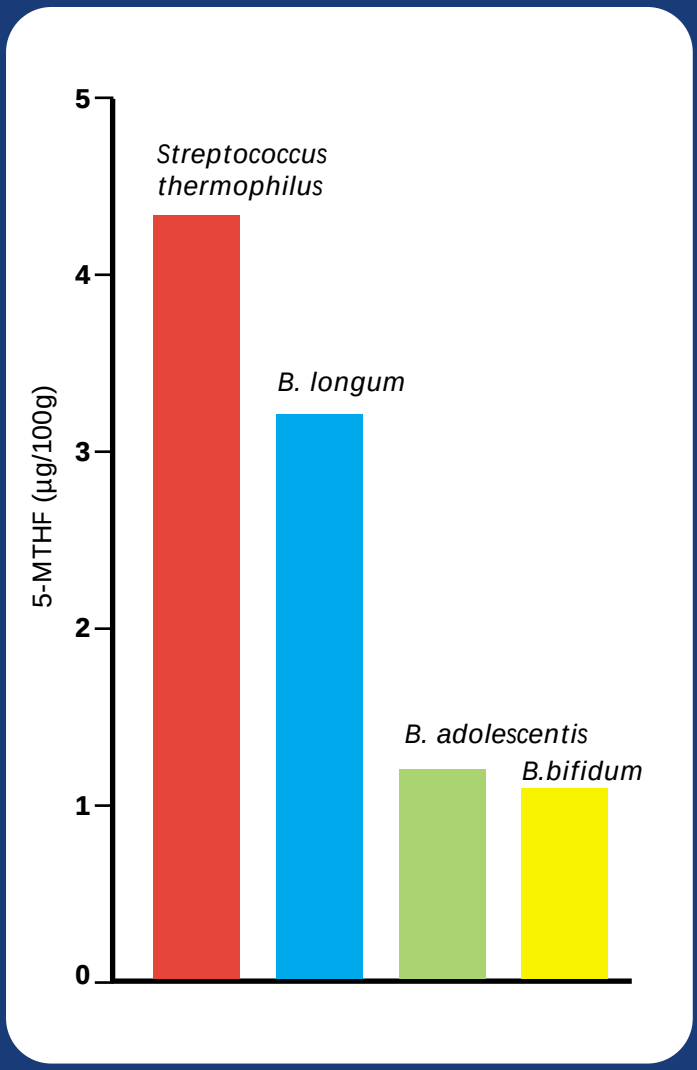

Fig.1: 5-MTHF concentration in model samples after 12 hours fermentation

Plasma Homocysteine Lowering in the Elderly

An intervention study investigating different strategies for lowering plasma homocysteine level is being conducted by Prof Anna Brzozowska and her team at the Department of Human Nutrition, Warsaw Agricultural University (SGGW). Healthy free-living volunteers (50-65 years old) will receive either 400 µg of folic acid or 5-methytetrahydrofolate, both in the form of capsules, or 400 µg of folic acid in white bread and apple juice, for a period of 4 weeks. The fortified bread for the study was prepared by the Research Institute of Agricultural and Food Biotechnology (Department of Grain Processing and Bakery) in Warsaw, and the fortified juice was produced by Alima-Gerber S.A. in Rzeszow.

In addition to the human study, SGGW will collaborate with Prof Prof Hanna Kunachowicz at the National Institute of Food and Nutrition in Warsaw to investigate the accuracy and reliability of recently published Polish data on folates in food products, and compare them with data from other European countries. On the bases of food composition tables and food product intakes, collected through the Main Statistical Bureau, the intake of folate in the households in Poland will be calculated to extend data gathered by other partners in the project.

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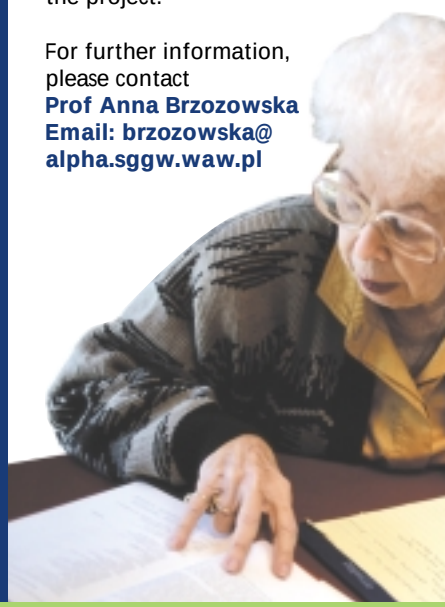

A project funded by the European Union under Key Action 1: Food, Nutrition and Health QLK1-1999-00576

EFFECTS OF PROCESSING ON FOLATES IN RYE BREADS

The effect of baking and sourdough fermentation on folates in rye and wheat breads has been investigated by the University of Helsinki. Baker's yeast was able to compensate for folate losses (typically 25%) during baking by its high folate content but also by synthesising natural folates. Changes in folate content during baking were accompanied by changes in the individual folate vitamer distribution. Factors affecting folate content of rye breads included differences in the amyloytic and microbial activity of the rye flour, type of Baker's yeast, fermentation time and baking temperature**.** It was concluded that sourdough fermentation affects sensory and microbiological quality of the bread but also the nutritional quality. The results suggest that by screening and selecting appropriate yeast and lactic acid bacteria, it is possible to enhance natural folate content in rye breads.

Total folates (µg/100g)[a] during sourdough fermentation of rye

Baking Step	Fermentation 1 (*S. cerevisiae*)	Fermentation 2 (*S. cerevisiae*)	Fermentation 3 (endogenous *C. milleri*)
Flour	66	62	62
Sourdough Start	77	71	58
Sourdough End	119	162	61
Dough	94	106	56
Proofed dough	93	111	54
Bread	93	79	40

[a] determined by microbiological assay; values on dry weight basis

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TIM Studies using Dairy Foods

Folate bioaccessibility from UHT and pasteurised milk was investigated using the TNO TIM *in vitro* model. Results show that folate is easily released from these matrices and is readily available for absorption (60-70%). Thus, both these milks would make suitable carriers for fortification. A small but significant difference was found in the bioaccessibility for folic acid (60%) compared to 5-methyltetrahydrofolic acid in milks. In addition, it was found that folic acid remains partly bound to folate binding protein during passage through the small intestine, which inhibits folic acid bioaccessibility from milk in this model. Thus, FBP does not appear beneficial for the enhancement of folate bioavailability. The TIM results are being compared to results obtained from the human study and folate bioaccessibility of other model foods (e.g. rye bread, beer, gazpacho and other vegetables) are also being determined.

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Developments in Folate Techniques

API 3000 triple quadruple tandem mass spectrometer (Applied Biosciences)

As part of the human study being carried out at the Amsterdam Free University Hospital, a state-of-the -art liquid chromatography tandem mass spectrometry method (LC/MS/MS) has been developed and validated for the quantification of *in vivo* folate enrichment measurements. This is the first technique of this type to be applied to folate studies in humans. Measuring 5-methyltetrahydrofolate enrichments in plasma gives the potential for further *in vivo* uptake and metabolism studies in humans.

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