February 2002 Issue 4

FOLATE:
From Food to Functionality and Optimal Health

Folate Func Health

Introduction

Welcome to the 4th issue of our Newsletter. In this issue, we have two articles on varietal and processing effects on folates in vegetables, and an article on the preliminary results from a human study in Rome investigating the effect of different dietary folate strategies on the reduction of plasma homocysteine concentrations. 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

Project News

Dr Henk van den Berg has left TNO (Partner 8) to join the Dutch Nutrition Foundation and his role in the project has been replaced by Dr Trinette van Vliet, supported by Dr Rob Havenaar (both TNO). Rosemarijn de Jong also replaces Dr Jacqueline Castenmiller at Wageningen Agricultural University.
There is also a new Scientific Officer for the project in Brussels, Alkmini Katsada, and she has replaced Dr Torbjörn Ingemansson.
Dr Ingemansson will continue to work for the Life Sciences Directorate but now with the responsibility for exploitation of results in the biotechnology area (Cell Factory Key Action).
Discussions are underway to extend this project to include two additional partners from Poland and the Czech Republic under the Commission's call for "Newly Associated States" (QoL-2002-NAS). Further information will be available in the next issue.

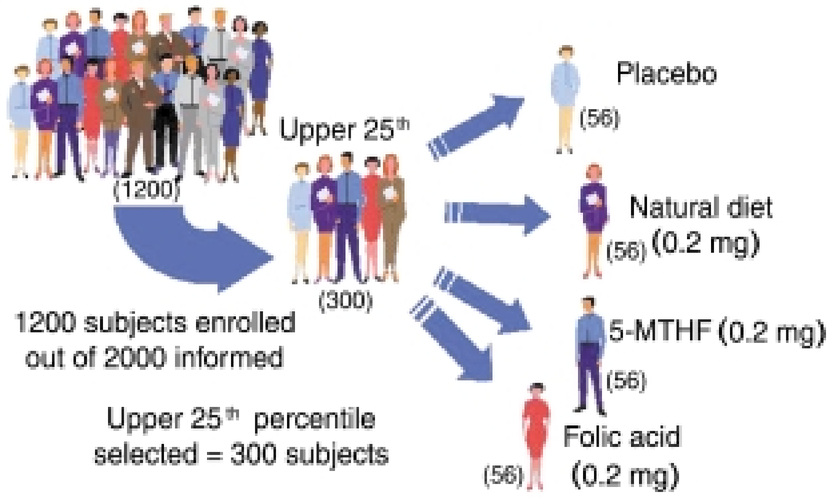
Homocysteine Lowering Study in Rome

Mild hyperhomocysteinemia is a recognised risk factor for cardiovascular and cerebrovascular diseases, and recurrent thrombosis, resulting from both genetic (mainly methylenetetrahydrofolate reductase and cystathionine synthase mutations) and nutritional causes involving sub-optimal deficiencies of folate, and vitamins B_{12} and B_6. The aim of the study is to evaluate the efficacy of different homocysteine lowering strategies by using different dietary folate interventions in a group of 2500 healthy volunteers from a Mediterranean region, and taking into account the influence of different MTHFR genotypes. The participants in the study were recruited from the local university, military academy and the National Institute of Nutrition.
The volunteers are screened for total plasma homocysteine (tHcy) in two consecutive phases, identical for season, duration and number of participants. Volunteers whose homocysteine falls in the upper 25th centile, were subsequently enrolled in the intervention trial and randomly assigned to one of the following four groups (see figure).

For phase 1, a total of 1220 healthy volunteers (757 women & 463 men; aged 18-59y) with no history of hypertension, diabetes, coronary heart disease or cerebrovascular disease, were evaluated for tHcy. Plasma tHcy showed a median of 7.8 µmol/L and 75th percentile of 10.0 µmol/L. The MTHFR polymorphism was evaluated in the group of volunteers with high tHcy (>75th percentile).

The median tHcy was markedly higher in TT subjects but no difference were found between CT and CC individuals. Men (TT) had higher median tHcy values than women with TT (18.2 vs 12.3 µmol/L), suggesting an interaction between sex and TT, not explained by smoking.

As previously planned, the randomized intervention study commenced in April 2001 and lasted for 12 weeks.



Human Study : Phase A

Plasma tHcy was higher in men (median 9.3 =µmol/L) than in women (median 7.0 µmol/L), was unaffected by age or body mass index but increased with smoking (smokers: 8.1 µmol/L vs non-smokers: 6 µmol). The effect of smoking was more evident in women, and with low intake of fresh fruits and vegetables.
The frequency of MTHFR TT polymorphism in volunteers with high plasma tHcy (30 women & 52 men) was ca. 33% for both sexes.

The second phase commenced in September 2001.
Results from this study will be compared with results from two other shorter-term intervention studies with specific folate-rich foods and translated into nutritional advice for optimal plasma tHcy reduction in the general population using dietary means.
For further information, please contact Dr Bruno Zappacosta (Catholic University of Sacro Cuore; **b.zappacosta@uniserv.ccr.rm.cnr.it.**

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Folates in fermented vegetables

Vegetables are good sources of folates, with concentrations 50-250 µg/100g. During traditional thermal processing of vegetables, e.g. boiling and canning, substantial losses of folates usually occur, mostly by leakage and/or oxidation. There are also, however, food process techniques with a potential to enhance folate concentrations. In particular, many fermented dairy products, e.g. yoghurt and dessert cheese, contain 2-3 fold higher amounts of folate compared to the original milk, which they are manufactured from. The increase in folates is explained by folate synthesis from starter cultures (1). Except for fermented white cabbage (Sauerkraut), little work has been reported in the literature on fermentation as a tool to increase folates in vegetables.

The present work aims to evaluate the possibility that fermentation, on an industrial scale, can retain or even increase folate concentrations in vegetables making these foods superior to similar foods produced by the more common preservation techniques. Pilot studies have started in collaboration with a food company (Orkla Foods A.S./Procordia Food AB, Sweden). An HPLC method has been developed and validated for determination of three folate forms, which are mainly present in food: tetrahydrofolate (H_4PteGlu), 5-methyltetrahydrofolate (5-CH_3H_4PteGlu) and 5-formyltetrahydrofolate (5-HCOH_4PteGlu) (2). Some preliminary and new data on folates are presented in white cabbage, red beets and turnips subjected to grating, blanching, canning or fermentation.

Fermented white cabbage (Sauerkraut)

In a screening of some commercial Sauerkraut products and one sample from a local small-scale producer, where the Sauerkraut was stored cooled for 11 months, folate concentrations varied between 5-20µg/100g of 5-CH_3H_4PteGlu. No H_4PteGlu and 5-HCO-H_4PteGlu forms were detected. The juice and the drained fermented white cabbage contained similar amounts of 5-CH_3H_4PteGlu, indicating either leakage or extracellular production of folates during fermentation. Two raw white cabbage samples were found to contain only 10-11µg of total folate (mainly as 5-CH3H_4PteGlu), which is much lower than indicated in the national food data tables, often based in the microbiological assay (Table 1). This difference between folate values obtained by HPLC and microbiological assay depends upon the fact that microbiological assay determines all folate forms present in food samples, whereas our HPLC methods determines only three major folate forms.

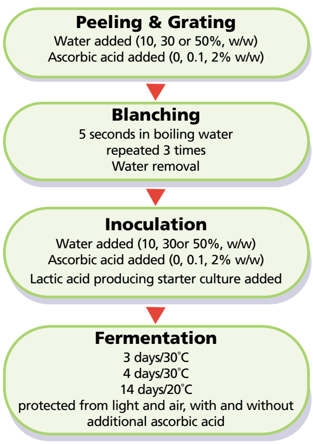

Figure 1. Fermentation in industrial pilot scale (Orkla FOODS/Procordia Foods AB)

Processing of root vegetables

Typical root vegetables, e.g. red beets and turnips, were evaluated as candidates for fermented vegetables. According to our HPLC analyses, 5-CH_3H_4PteGlu is the major folate form in these vegetables. Folate content of raw red beets was found to be 72-95 µg/100g, whereas folate content of raw turnips was found to be lower, 40µg/100g. These values are in agreement with national food tables (Table 1).

The manufacture of fermented root vegetables, is outlined in Figure 1 along with parameters varied in different experiments. It was found that in spite of adding ascorbic acid to protect the labile folates during grating and blanching, these steps resulted in folate losses between 20-60%. Since grating and blanching included addition, as well as, removal of process water, some leakage of folates might have occurred, enhanced by the markedly increased surface area as a result of grating. A further decrease of folate content was observed after the fermentation step, corresponding to folate losses in the range of 10-25%. Different starter cultures, mainly from lactic acid producing bacteria and in one case, from a propionic acid producing bacteria, were not able to produce any significant amounts of folates. In comparison, preservation of red beets, which is often processed by sterilisation of sliced and pickled red beets in diluted acetic acid, resulted in folate concentrations between 32-46 µg/100g drained red beets. These figures indicate an average loss of 50-60%. The work on fermentation of vegetables to increase folate concentrations for health benefits is challenging and offers potential for the production of foods with enhanced folate content.

For further information please contact Prof Margaretha Jägerstad **(mailto:Margaretha.Jagerstad@lmv.slu.se)** at the University of Uppsala.

References

1. Forssén, K., Jägerstad, M., Wigertz, K., Witthöft, C. Folates and dairy products: A critical update. J Am. Coll. Nutr., **19**(2) 100S-110S, 2000.

2. Jastrebova, J., Witthöft, C., Grahn, A., Svensson,U.,Jägerstad, M. HPLC determination of folates in raw and processed red beets (to be submitted to Food Chemistry).

Vegetables	Raw and fresh (µg/100g fresh weight)		Preserved (pickled) (µg/100g fresh weight)		Fermented (µg/100g fresh weight)	
	Analysed	*Food tables[1]*	*Analysed*	*Food tables[1]*	*Analysed*	*Food tables[1]*
White Cabbage	**10**	**50-75**	**-**	**-**	**5-22**	**5-22**
Red beets	**72-95**	**68-150**	**32-46**	**2-53**	**15-20[2]**	**-**
Turnips	**40**	**27-90**	**-**	**-**	**24**	**-**

Key:
"-" no data available
1 Swedish, Danish, English and American Food Tables
2 a mixture (50:50) of red beets and turnips

Table 1. Total folates in selected raw and processed vegetables based on HPLC analyses and according to national food tables.

Root vegetables such a Red Beets are a rich source of

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Kariluoto, S. *et al*. (2001). Intercomparison of current methods for folate determinations in food. 4th International Food Data conference, 24-26 August, Bratislava, Slovak Republic.

Witthöft C. *et al*. Determination of folate bioavailability from food by a human ileostomy model. International Congress of Nutrition 2001, Vienna, Austria, 27-31 August 2001. Annals of Nutrition and Metabolism, 45 (S1), 62.

Finglas PM. *et al*. Folic acid bioavailability from fortified cereal foods determined by using a dual-label stable isotope protocol and GC-MS. 17th International Congress of Nutrition, 27-31 Aug 2001, Vienna, Austria. Annals of Nutrition and Metabolism, 45 (S1), 355.

Meer, de K. & Jakobs, C. (2001). *In vivo* stable isotope measurements of methyl

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folates

metabolism: applications in pathphysiology and interventions involving folate, vitamin B-6 and B-12. 17th International Congress of Nutrition, 27-31 Aug 2001, Vienna, Austria. Annals of Nutrition and Metabolism, 45 (S1), 355.

Publications

Highlighted Recent Publications

Forssén KM, Jägerstad MI *et al* (2000). Folates and dairy products: a critical update. *JACN*, 19 (2), 100S-110S.

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Garbis, S.D., Melse-Boonstra, A., West, C.E. & van Breemen, R.B. (2001). Determination of folates in human plasma using hydrophilic interaction chromatography-tandem mass spectrometry. *Anal Chem*, **73**, 5358-5364.

Finglas, P.M. *et al*. (2002). The use of an oral/i.v. dual-label stable isotope protocol in the determination of folic acid bioavailability, in women, from fortified cereal grain foods. *J. Nutrition*, In Press.

Variety, Ripening and Processing Effects on Folates in Vegetables

Plant development, maturity, as well as cultivar or variety, have a marked influence on folate content in vegetables and are especially intense during post-harvest storage, and in the early stages of processing before enzyme inactivation. Folate retention in peas and spinach is drastically reduced after storage at ambient temperature for 5 days (50% loss), and freezing is better than chilled storage. Also, due to endogenous enzyme activity, that remains after harvesting, folate polyglutamate are converted into monoglutamate forms, which may have reduced stability. It may be possible to modify processing conditions that enhance natural folate stability and give products with improved folate content.

Little information is available on the folate content and individual forms of the current varieties of peas, tomato for Southern European countries such as Spain. Our preliminary results for peas using HPLC analysis of current varieties have found that cultivar is an important folate factor.

Pea Variety	Stage	5-CH_3H_4Pteglu Content (µg/100g)
Dinos	Refrigerated	10 (0.8)
Globo	Refrigerated	6 (0.4)
Dinos	Frozen	23 (4)
Globo	Frozen	14 (5)

The predominant folate identified was 5-methyltetrahydrofolate (5-CH_3H_4Pteglu). The mean (+/- SD) folate content of two varieties of peas, at two stages during the production chain are shown in the table above.

Frozen peas appear to have about twice folate content following freezing, and this may be due to better extraction of folate from the matrix following processing. Further tests will be carried out in order to optimise conditions for maximum folate content. It should also be noted that that pea varieties for canning or freezing constantly change from to season to season due to genetic improvements by the seed producers and newer varieties will also be tested for folate.

Tomato is important because it is the main ingredient (0.5Kg/L) of Gazpacho , a cold Spanish vegetable soup, which is of increasing interest in the market because of its sensory quality, natural ingredients composition and `healthy image.' Other minor vegetables in Gazpacho, such as green pepper and onion, also provide rich sources of folate.

The predominant forms found in tomatoes as identified by HPLC were 5-CH_3H_4Pteglu and tetrahydrofolate (H_4Pteglu), although other forms have also been found using a more sensitive and specific combined liquid chromatography-microbiological procedure, which is currently being run in collaboration with the University of Hannover.

Tomato Size	Variety	5-CH_3H_4Pteglu (µg/100g)	H_4Pteglu (µg/100g)
Small Cherry	Pera	93 (11)	ND*
Large wrinkled skin	Rugoso	51 (1)	4 (0.5)
Large soft skin	Ronaldo	15 (2)	1.3 (0.2)
	Tina	11 (0.7)	4 (0.1)

*ND = not detected

Based on our preliminary HPLC results, there are significant differences on the folate content in tomatoes, depending on both variety and size. The tomatoes studied were classified into small cherries, large wrinkled skin (for salads) and large soft skin (for salads and processing).
The mean (+/- SD) folate results are shown in the table opposite.

Over recent years, there has appeared a wide range of new Gazpacho formulations (pasteurised, sterilized and frozen) in the market and we have also started to investigate the folate content in these products. Initial results have indicated that folate content is relatively low in all these products (<5µg/100g) and we have identified that the particle size of the ingredients used, and homogenisation time during processing, as the key parameters affecting folate content. The folate content of two types of Gazpacho [homogenisation time = 1.5 min (`thick'), and 1 min (`thin') and the residue by-product are shown in the table above.

Product & Residue	5-CH_3H_4Pteglu(µg/100g)
Gazpacho	
• Thin	3.1
• Thick	7.2
Residue	
• Thin	3.6
• Thick	12.2

Further studies in collaboration with two local industries (Hero and Tropicana-Alvalle) will be carried out in order to optimise folate content in processed peas and tomato products, such as Gazpacho and tomato sauces, focusing on variety selection, critical processing steps and other potential ingredients.

For further information, please contact
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