Folate content in fermented milk products and possibility for its enhancement

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AIMS and BACKGROUND

The aim of this work was the evaluation of possibilities for folate level increase in fermented milk products by selection of microorganism, fermentation conditions and fruit component.

Folates represent an essential nutrition component involved in many metabolic pathways, mainly in one carbon transfer reactions such as purine and pyrimidine biosynthesis and amino acid interconversion. Natural folates exist primarily as reduced, one-carbon-substituted forms of pteroyl-glutamates, with up to seven glutamyl residues attached to the p-aminobenzoic group. The daily recommended intake for an adult varies between 200 and 400 μg. Folate deficiency has been associated with incidence of neural tube defects during embryo development. Low plasma folate concentration correlates with elevated level of homocystein that is recognized as risk factor in coronary heart disease. Furthermore there seems to be relation between low folate status and incidence of certain forms of cancer.

Milk is well known source of folates. Food composition tables and review papers based on microbiological assay report folate values for cow's milk in range 5 - 7 μg/100g. Most HPLC studies indicate 5-methyltetrahydrofolate (5-MTHF) as the major form of folates in milk. Fermented milk products are reported to contain higher amounts of folate. This high level is result of the production of additional folates by the bacteria. However many bacteria synthesize this cofactor by themselves from a simple precursors, but some auxotrophic bacteria, including many lactic acid bacteria, have a strict growth requirement for folic acid. The production and consumption of folates by applied microorganisms will be probably the most important factor determining the folate level in fermented milk products. In general, *Lactobacillus* strains did not produce folates with exception of *Lactobacillus plantarum*. *Streptococcus thermophilus* is reported to produce folates, however large differences were observed in production ability of individual strains. Some other lactic acid bacteria – *Lactococcus lactis, Leuconostoc lactis*, *Bifidobacterium longum* were recognized as a folate producers. Some strains of *Propionibacteria*, well known vitamin B12 producer, are able to increase folate level as well.

Contents of folates in fruit and berries range from a few μg to approx. 100 μg/100g. The highest concentration of about 50-100 mg/100g are to be found in frozen concentrated orange and grapefruit juices and strawberries.

METHODOLOGY

Model fermentation

Substrate: UHT milk, fat content 1.5 %, sterilized 114°C/20 min
Raw milk, fat content 1.5 %, pasteurized 85°C/7 min
Inoculation: 2% *Bifidobacterium longum*
1% *Streptococcus thermophilus*
5% *Propionibacterium freudenreichii* ssp. *shermanii*
0.3% butter starter
Fermentation temperature: 37°C, 30°C
Fermentation time: 12 and 18 hrs (final pH < 4.5)
Sample treatment: extraction and hydrolysis after end of fermentation, stored at 18°C until purification and quantification

Fruit components

Samples of fruit components (pineapple, sour cherry, kiwi, apricot, peach, apple, strawberry, blueberry and raspberry) were obtained from Frujo Tvrdonice, Czech Republic. Fruit content: 27-32 %, refractometric dry matter: 62-63°Brix

Commercial fermented products

30 samples of fermented milk products from 10 producers bought in retail store

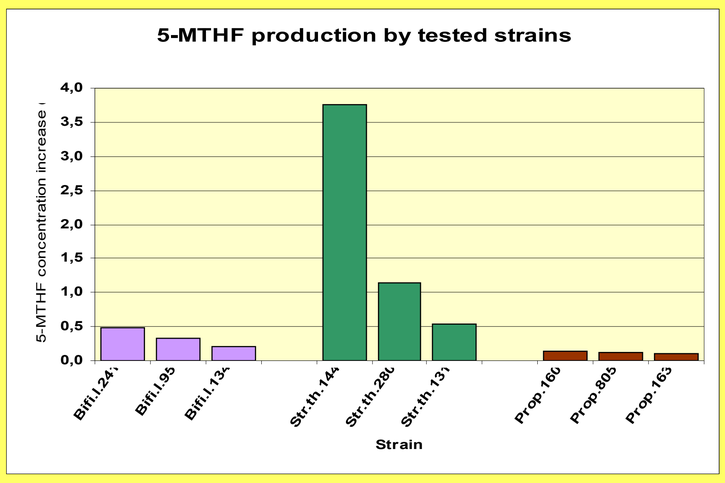
Analytical procedure

Extraction:
0.075 M phosphate buffer pH 6.0 with mercaptoethanol and ascorbic acid, 10 min/100°C, centrifugation 15 min/15000 rpm, 2°C
Deconjugation:
at pH 4.9 using in house conjugase from hog kidney, incubation for 2 hrs at 37°C, enzyme inactivation 5 min/100°C, centrifugation 10 min/3000 rpm, 2°C
Purification:
SPE with SAX cartridges (AccuBond SAX), washing with water, elution by 0.1 M sodium acetate containing 10% NaCl and ascorbic acid
HPLC determination:
reversed phase (Lichrospher 100RP18, 5 μm, 250 x 4 mm), mobile phase - 8% ACN in phosphate buffer pH 2.3, gradient elution, flow – 1 ml/min, sample loop - 100μl, fluorescence detector at 280/360 nm
Identification and quantification: external standard method

RESULTS

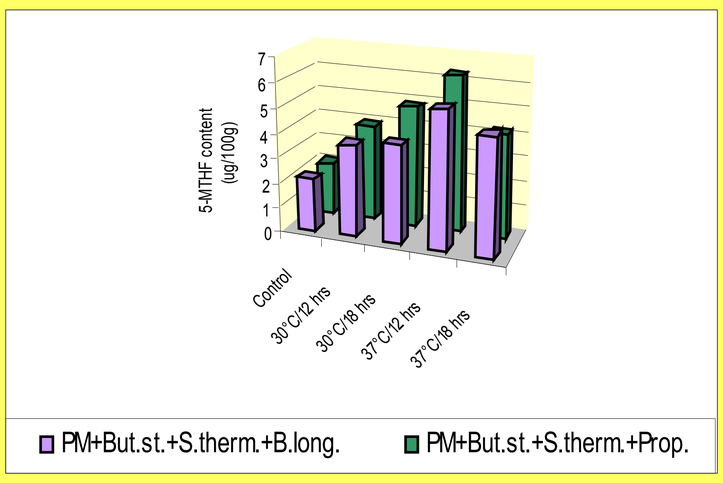
Str. thermophilus and Prop.freudenreichii ssp.shermanii

Substrate – UHT milk sterilized 114°C/20 min
Fermentation 37°C, 12 hrs



B.longum 241, Str. thermophilus144 and Propionibacter. freudenreichii ssp.shermanii 160

Substrate – raw milk pasteurized 85°C/7 min
Fermentation 30, 37°C; 12, 18 hrs

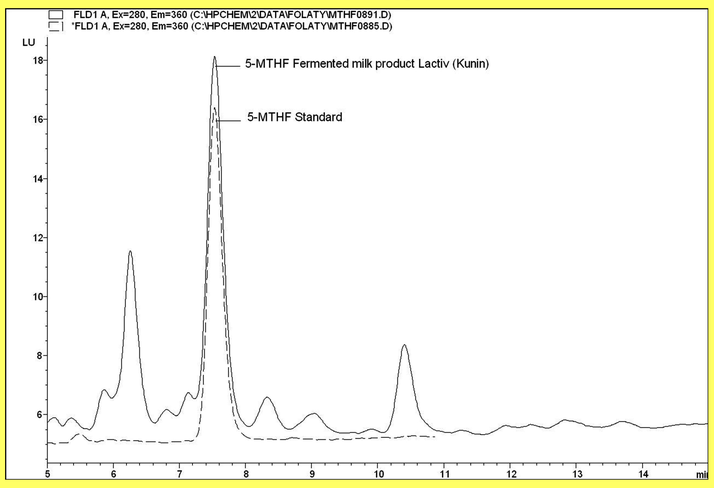


5-MTHF increase during cofermentation of strains B.longum 241, Str. thermophilus144 and Prop.freudenreichii ssp.shermanii 160

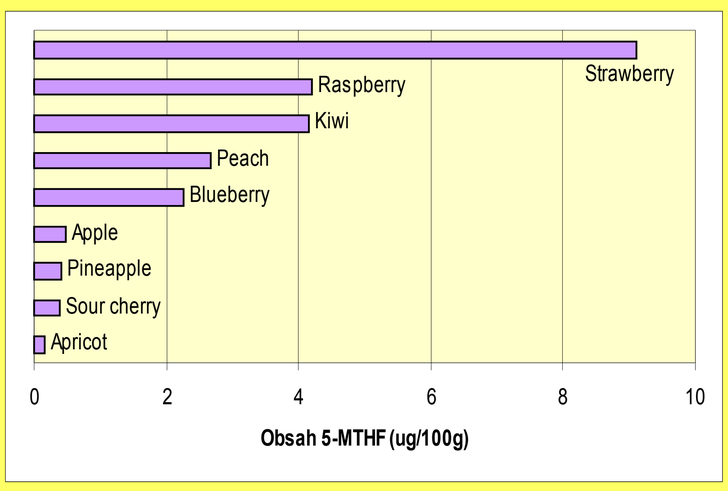
Substrate – raw milk pasteurized 85°C/7 min
Fermentation 30, 37°C; 12, 18 hrs

Condition of fermentation	5-MTHF increase (μg/100g)	
	PM+But.st.+ S.therm.+B.long	PM+But.st.+ S.therm.+Prop.
30°C/12 hrs	1.59	1.84
30°C/18 hrs	1.83	2.80
37°C/12 hrs	3.39	4.23
37°C/18 hrs	2.59	2.11

Chromatogram of 5-MTHF analysis in commercial product

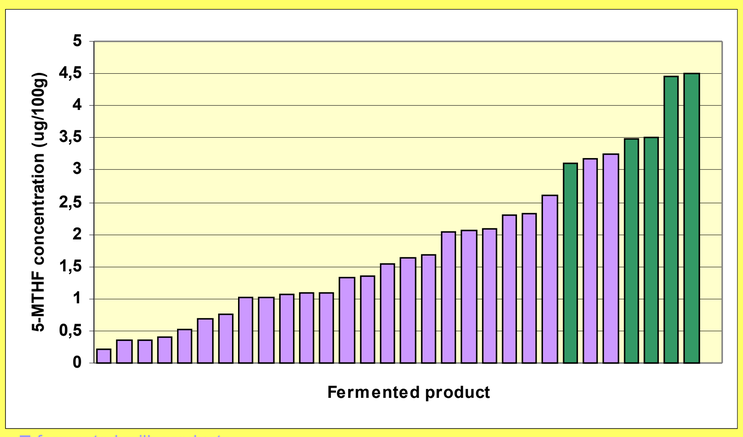


5-MTHF content in fruit component



5-MTHF determination in fermented milk samples from retail store

(30 fermented milk products from 10 producers)



CONCLUSION

- **5-MTHF production of tested individual microbial species in fermented milk differs. The most productive strain was *Streptococcus thermophilus* 144. *Propionibacterium freudenreichii* ssp. *shermanii and Bifidobacterium longum were* recognized as a mild folate producers.**
- **The highest increase of folate level (4.23 μg/100g) was observed in cofermentation of selected strain *Streptococcus thermophilus* 144 with *Propionibacterium freudenreichii* ssp. *shermanii* 160 and butter starter after 12 hrs fermentation at 37 °C.**
- **Fruit components used in flavouring can donate folate into product. Among tested components the strawberry one represented the most rich folate source (9.11μg/100g).**
- **5-MTHF content between 0.21 - 4.55 μg/100g (median 1.49 μg/100g) was found in 30 analyzed fermented milk products from retail store.**
- **By inoculation of pasteurized milk by butter starter and *Streptococcus thermophilus* No.144 in combination with *Propionibacterium freudenreichii* subsp. *shermanii* No.160 and butter starter, fermented at 37°C for 12 hrs and flavoured by 17 % of strawberry component the product with folate content 7.17 μg/100g was reached. The content is by more than 50% higher than the content found in the most rich commercial product. Fermentation and fruit component addition caused thus 5-MTHF increase 4.8 μg/100g in comparison with pasteurized milk. Of this 69 % originated from fermentation and 31 % from fruit component addition.**

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