

AIMS and BACKGROUND

▪The aim of the work was to evaluate the ability of selected bacterial species and strains to produce folate and to determine if cofermentation of these micro-organisms can lead to fermented milk products with increased folate level and acceptable sensorial properties. Folates represent the essential component in human diet. They participate in one-carbon transfer reactions required in many metabolic pathways, especially purine and pyrimidine biosynthesis and amino acid interconversion. Folate deficiency has been associated with incidence of neural tubes defects during embryo development. Low plasma folate concentration correlates with elevated level of plasma homocysteine, a risk factor for 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 with 5-methyl tetrahydrofolate (5-MTHF) as a predominating form. It contains between 2-5 ug folates/100g and thus contributes significantly to the daily requirement. Fermented milk products are reported to contain even higher amounts of folates. Reported value vary widely, ranging from 2 ug to 10 ug/100g. This elevated level is result of bacterial production of additional folate during fermentation. However many bacteria are able to 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.

METHODOLOGY

Model fermentation

Substrate: Raw milk, fat content 1.5 %, pasteurized 85°C/7 min
 Inoculation: 2% *Bifidobacterium longum* 241
 1% *Streptococcus thermophilus* 144
 5% *Propionibacterium freudenreichii* ssp. *shermanii* 160
 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

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/13000 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, external standard method
 ▪Identification and quantification: external standard
 ▪Method validation: analysis of CRM - milk powder 421, recovery of standard addition

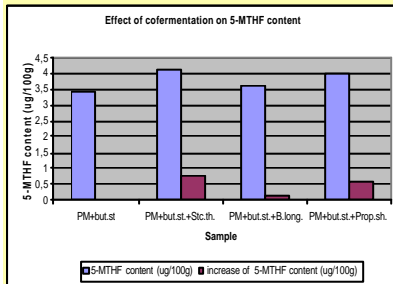
Commercial fermented products

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

RESULTS

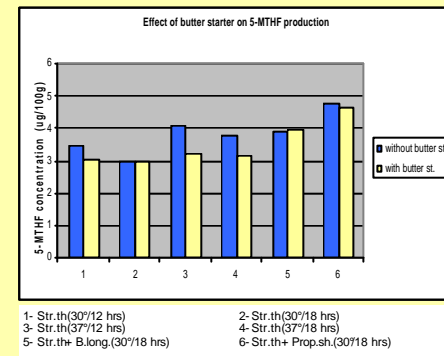
5-MTHF production by *B.longum*, *Str. thermophilus* and *Prop.freudenreichii* ssp.*shermanii*

Substrate – pasteurized milk
 Fermentation at 30°C, 18 hrs

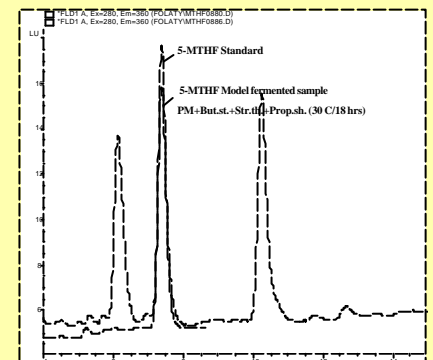


Influence of butter starter on 5-MTHF production

Substrate - pasteurized milk, butter starter

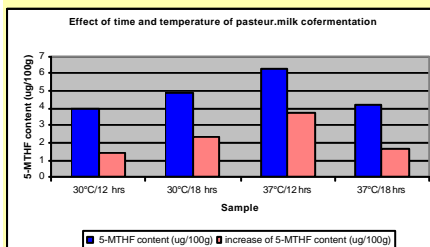


Chromatogram of 5-MTHF analysis in model sample



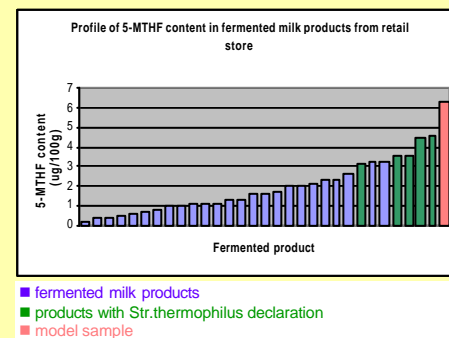
Effect of cofermentation on 5-MTHF level

Substrate - pasteurized milk
 Inoculation – *Str.thermophilus* 144 with *Prop.freudenreichii* ssp.*s hermanii* 160, butter starter
 Fermentation - 30°C, 37°C/12 hrs, 18 hrs

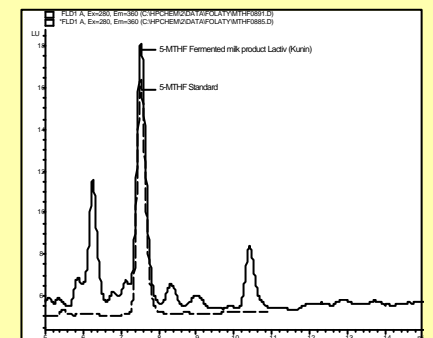


5-MTHF determination in fermented milk samples from retail store

(30 fermented milk products from 10 producers)



Chromatogram of 5-MTHF analysis in commercial product



CONCLUSION

- 5-MTHF production of tested individual microbial species in fermented pasteurized milk differs. The most productive strains were *Streptococcus thermophilus* 144 (5-MTHF increase 0.73 ug/100g) and *Propionibacterium freudenreichii* ssp. *shermanii* 160 (5-MTHF increase 0.57 ug/100g). *Bifidobacterium longum* 241 was recognized as a mild folate producer (5-MTHF increase 0.15 ug/100g).
- Application of butter starter caused in average 10% decrease of 5-MTHF content (80 - 101%)
- By cofermentation of selected strains *Streptococcus thermophilus* 144 and *Propionibacterium freudenreichii* ssp. *shermanii* 160 with butter starter (37°C, 12 hrs) 5-MTHF content 6.34 ug/100g was reached.
- 5-MTHF content between 0.21 - 4.55 ug/100g was found in 30 analyzed fermented milk products from retail store.
- By selection and combination of micro-organisms and by optimizing of conditions used in milk fermentation the model sample with 5-MTHF content exceeding this in analyzed commercial milk products was reached. Related to min. and max. 5-MTHF contents in commercial fermented milk products it represented increase 30 times and by 40%, resp.