Possibility of Increasing Natural Folate Content in Fermented Milk Products by Fermentation and Fruit Component Addition

Marie HOLASOVÁ[1], Vlasta FIEDLEROVÁ[1], Petr ROUBAL[2] and Martina PECHAČOVÁ[2]

[1]Department of Nutritive Substances, Food Research Institute Prague, Prague, Czech Republic; [2]Dairy Research Institute, MILCOM, Corp., Prague, Czech Republic

Abstract

Holasová M., Fiedlerová V., Roubal P., Pechačová M. (2005): **Possibility of increasing natural folate content in fermented milk products by fermentation and fruit component addition**. Czech J. Food Sci., **23**: 196–201.

The possibility of the increase of the natural folate content in fermented milk products by the fermentation process and by the addition of fruit component was evaluated. 5-methyltetrahydrofolate (5-MTHF) content was followed after the inoculation of pasteurised milk with the butter starter and the selected strains of *Streptococcus thermophilus* in combination with *Bifidobacterium longum* or *Propionibacterium freudenreichii* subsp. s*hermanii* followed by fermentation at 30°C and 37°C for 12 and 18 h. 5-MTHF was determined by HPLC method after deconjugation with hog kidney conjugase and SPE SAX purification. The highest 5-MTHF content increase of 4.03 ± 0.44 μg/100 g was found at cofermentation with *Streptococcus thermophilus* No. 144 and *Propionibacterium freudenreichii* subsp. s*hermanii* No. 160 at 37°C after 12 h of fermentation. 9 commercial fruit components used in dairy industry contained between 0.17–9.11 μg 5-MTHF/100 g. The components produced with pineapple, sour cherry, apricot, and apple contained low amounts of 5-MTHF – less than 1 μg/100 g. Among the rest of the components tested, the strawberry component proved to be the best source of folate with the content of 9.11 μg 5-MTHF/100 g. The values in 7 varieties of fresh fully ripe strawberries fluctuated between 25.5 and 54.0 μg/100 g fresh sample, i.e. 272 and 554 μg/100 g dry matter with the highest content found in the varieties Elsanta and Honeyoe. Fully ripe berries of the variety Senga Sengana contained by 63% more 5-MTHF in comparison with unripe berries. In order to reach maximum folate content in the fruit component, fully ripe strawberries of the cultivars Elsanta and Honeyoe are recommended for the processing. The folate content in the fermented milk product may be increased in this way by 4.8 μg/100 g, with 69% originating from the fermentation and 31% from the fruit component addition.

Keywords: folate; fermentation; fruit; fermented milk products

Folates belong to the vitamin B group and are represented by compounds resembling in their chemical and nutritional properties pteroyl-L-glutamic acid (folic acid). Natural folates exist primarily as reduced, one-carbon-substituted forms of pteroyl-glutamates, with up to seven glutamyl

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QD0179, and European Commission, Project No. QLRT-2002-2740.

residues attached to the p-aminobenzoic group (Ball 1994). Deficiency of the folate intake proved to increase the risk of neural tube defects during pregnancy (Daly *et al.* 1995; Shaw *et al.* 1995). It was found that the increased consumption of folic acid lowers the levels of plasma homocysteine which is recognised as a risk factor for the coronary heart disease (Morrison *et al.* 1995; Brattstrom 1996). Folate status is possibly also linked to the incidence of certain forms of cancer (Ames 1999).

Polyglutamyl folate is an essential biochemical constituent of living cells, and most foods contribute to the folate intake. Yeast, liver, wheat bran, beetroot, green leafy vegetables, chick peas, and nuts are rich natural sources of folate. Other useful sources include oatmeal, whole grain cereals, potatoes, eggs, cheese, kidney, muscle meats, fish, and fruit (Ball 1994).

The contents of folate in fruit and berries range from a few µg to approx. 100 µg/100 g. The highest concentrations of about 50–100 µg/100 g are found in frozen concentrated orange and grapefruit juices and in strawberries (Witthöft *et al.* 1999).

The folate content in milk is rather low but in respect to its share in the diet, milk represents an important dietary source of folates. The annual consumption of milk and milk products in Czech Republic (CR) in 2003 was estimated at 223.4 kg/person. After a decrease in the consumption between the years 1990 and 1997, a slight permanent increase was recorded (CZSO 2004). The consumption of yoghurts and other fermented milk products is estimated at 12.8 kg/person/year. In 2003, the production of yoghurts and other fermented milk products in CR was 86 672 tons and 24 031 tons, resp. with the annual increase of 15.1% in the production of other fermented milk products category. Another 19 190 tons of yoghurts and other fermented milk products were imported (Křivánek 2004).

The food composition tables and review papers based on microbiological assay report the folate values in cow's milk in the range of 5–7 µg/100 g. Most HPLC studies indicate 5-methyltetrahydrofolate as the major form of folates in milk (Forssén *et al.* 2000). Heat treatment such as pasteurisation and UHT processing are known to reduce the folate content (Wigertz *et al.* 1996). However, milk processing can lead to a positive effect when milk fermentation is applied. The fermented milk products are reported to contain higher amounts of folate. The values reported vary widely. The folate contents in yoghurts commercially available in the Netherlands varied between less than 2 and more than 10 µg/100 g (Smid *et al.* 2001). In 22 low-fat commercial yoghurts from US market, the folate contents of 6.5 ± 1.7 µg/100 g were found by microbiological assay after the conjugase treatment (Johnston *et al.* 2002).

The objective of this study was to evaluate the possibility of the increase of the natural folate content in fermented milk products by the fermentation process and by the addition of the fruit component. Combinations of recognised folate microbial producers and fermentation conditions were tested. 9 commercially available fruit components were evaluated for 5-MTHF content and the influence of the fruit sort.

MATERIAL AND METHODS

Organisms and fermentation conditions. The most dominant folate producers selected from the Collection of dairy micro-organisms Lactoflora MILCOM (Holasová *et al.* 2004) were applied, i.e.: *Streptococcus thermophilus* strain No. 144, *Bifidobacterium longum* strain No. 241, and *Propionibacterium freudenreichii* subsp. *shermanii* strain No. 160. For the improvement of the sensorial properties, the butter starter Sl_{30} (But. st.) was simultaneously used. All strains were stored in freeze-dried or deep frozen forms and except *Propionibacterium* strains, they were propagated in sterilised milk. *Propionibacterium* strains were grown in broth containing whey, yeast extract, and calcium lactate. After microscopic examination, the cultures or their mixtures were used for inoculation. For inoculation, 1%, 2%, 1% and 0.3% of inoculum were applied with *Streptococcus thermophilus, Bifidobacterium*, *Propionibacterium*, and the butter starter, resp. Pasteurised milk (85–88°C/7 min) with 4.1 ± 0.5% fat content represented the substrate (PM). CPM/1 ml of raw milk was between 2.0×10^3 and 2.0×10^5 and decreased after pasteurisation to 7.0×10^1–1.4×10^2. The substrate without inoculation was used as the control sample. Fermentation was conducted at 37°C and 30°C without agitation. Samples were taken after 12 and 18 h of fermentation, subjected to extraction and deconjugation, stored at –18°C until purification and quantification. Folate concentrations were calculated from two parallel determinations. The production abilities of the strain mixtures tested were evaluated in relation to 5-MTHF content in the substrate without inoculation found at time 0.

Fruit components and fruits. Samples of fruit components (pineapple, sour cherry, kiwi, apricot, peach, apple, strawberry, blueberry, and raspberry) were obtained from Frujo Tvrdonice, Czech Republic. They contained 27–32% of frozen or sterilised fruits and were characterised by refractometric dry matter 62–63 Brix. Fully ripe strawberries cv. Elsanta I, Honeyoe, Dukat, Dita, and Elista were harvested in June 2004 and were collected from the strawberry farm J. Sixta, Sedlčanky, in central Bohemia. Strawberries cv. Elsanta II, Senga Sengana I and II and Carmen were obtained from small growers. Strawberries Senga Sengana were harvested at three stages-unripe, ripe and fully ripe. The berries were placed in plastic bags, sealed under vacuum and stored at –40°C until analysis.

5-MTHF determination. 5-MTHF concentrations were determined using HPLC method including the extraction of folate in phosphate buffer, pH 6, with antioxidant addition, deconjugation by conjugase from hog kidney, purification by SPE on SAX columns, and HPLC determination on reversed phase with fluorimetric detection (Holasová *et al*. 2004).

RESULTS AND DISCUSSION

Fermentation

In the recent study (Holasová *et al*. 2004), we tested 5 strains of *Bifidobacterium,* 3 strains of *Streptococcus thermophilus* and 3 strains of *Propionibacterium freudenreichii* subsp. *shermanii* for the 5-MTHF production ability during fermentation of UHT milk. The dominant producers among the individual species were the strains *Bifidobacterium longum* No. 241, *Streptococcus thermophilus* No. 144, and *Propionibacterium freudenreichii* subsp. *shermanii* No. 160. *Streptococcus thermophilus* No. 144 was recognised as the most productive one, producing 3.7 μg 5-MTHF/100 g during 12 h fermentation at 37°C. The increase of 5-MTHF content caused by *Bifidobacterium longum* No. 241, and *Propionibacterium freudenreichii* subsp. *shermanii* No. 160 was lower, 0.5 μg/100 g and 0.12 μg/100 g, resp.

In the present study, the effect of cocultivation was evaluated. Model samples were prepared combining the inoculation of pasteurised milk with (*i)* 1% of *Streptococcus thermophilus* No. 144, 2% of *Bifidobacterium longum* No. 241, and 0.3% of the butter starter, and (*ii*) 1% of *Streptococcus thermophilus* No. 144, 1% of *Propionibacterium freudenreichii* subsp. *shermanii* No. 160, and 0.3% of the butter starter, fermented for 12 h or 18 h at 37°C or 30°C. The 5-MTHF content found after 12 h and 18 h of fermentation is demonstrated in Figure 1.

In both samples the fermentation at 37°C was more effective in view of the 5-MTHF production in comparison with the fermentation at 30°C. Whereas at the temperature of 30°C the increase in the folate concentration was found between 12h and 18 h of fermentation, at 37°C the folate concentration declined during this time interval. With the exception of the sample fermented for 18 h at 37°C, the production in cofermentation (*ii*) (1% of *Streptococcus thermophilus* No. 144, 1% of *Propionibacterium freudenreichii* subsp. *shermanii* No. 160, and 0.3% of the butter starter)

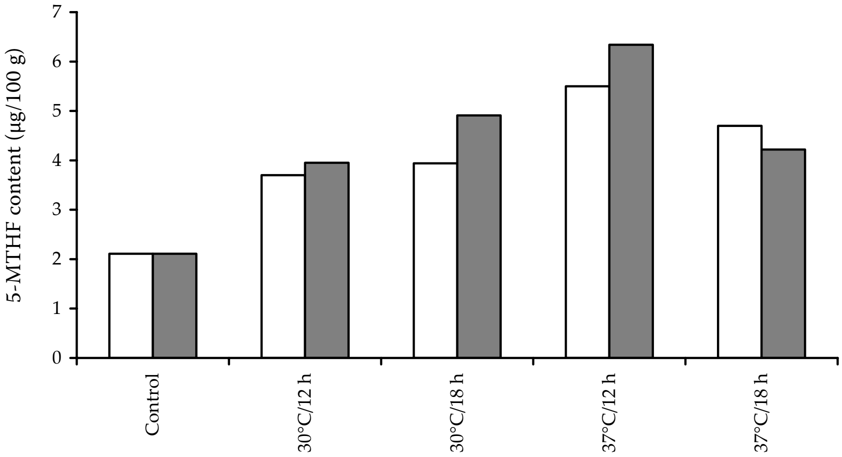

Figure 1. 5-MTHF production in cofermented samples

was superior to that obtained in cofermentation (*i*) (1% of *Streptococcus thermophilus* No. 144, 2% of *Bifidobacterium longum* No. 241, and 0.3% of the butter starter). In Table 1 are summarised the values of 5-MTHF increase in µg/100 g related to 5-MTHF content in pasteurised milk. Pasteurised milk used as a control sample contained 2.11 µg 5-MTHF/100 g. In 7 samples of commercial pasteurised milk the values between 1.95–4.65 µg 5-MTHF/100 g were found (unpublished).

It shows that under the conditions applied the highest value was found with cofermentation of *Streptococcus thermophilus* No. 144 and *Propionibacterium freudenreichii* subsp. *shermanii* No. 160 at 37°C after 12 h of fermentation. In 4 repeated experiments an average increase of 4.03 ± 0.44 µg 5-MTHF/100 g was found. The value even exceeded the expected sum of the individual contributions, thus indicating a possible synergistic effect of this combination. However, the differences between the residual microflora in UHT milk used in the individual strains testing and pasteurised milk used in the present study might be of importance as well.

Testing of 30 commercial yoghurts and other fermented milk products obtained from Czech retail store (unpublished) showed the 5-MTHF content between 0.21–4.55 µg/100 g, median 1.49 µg 5-MTHF/100 g. Laboratory fermentation under the conditions described above resulted in the 5-MTHF content increase by 325% related to the median content in the commercial products analysed, indicating thus that the selection of microorganisms and the conditions used in fermentation may lead to an increase in the folate content.

Fruit component

Fermented milk products are often flavoured by fruit components. The folate content in fruits reaches the values from a few µg up to 100 µg in 100 g of fresh fruits with 5-MTHF being the dominant form (Witthöft *et al.* 1999; Stralsjö *et al.* 2003); thus the fruit components used in the fermented milk production may lead to their enrichment. In the present study the 5-MTHF content was determined in 9 commercial fruit components used in the dairy industry. The results shown in Table 2 demonstrate that the 5-MTHF content in the samples tested differs in the dependence on the fruit sort. The components produced with pineapple, sour cherry, apricot and apple contain low amounts of 5-MTHF – less than 1 µg/100 g. Among the rest of the components tested the strawberry component proved to be the best source of folate with the content of 9.11 µg/100 g. The figures represent the average of two independent determinations in each sample.

Table 1. 5-MTHF content increase during cofermentation

Condition of fermentation	5-MTHF increase (µg/100 g)	
	pasteurised milk + butter starter + *Streptococcus thermophilus* + *Bifidobacterium longum*	pasteurised milk + butter starter + *Streptococcus thermophilus* + *Propionibacterium*
30°C/12 h	1.59	1.84
30°C/18 h	1.83	2.80
37°C/12 h	3.39	4.23
37°C/18 h	2.59	2.11

Table 2. 5-MTHF content in fruit components

Sort	5-MTHF content (µg/100 g)	Sort	5-MTHF content (µg/100 g)
Pineapple 2515/A	0.41	Apple 2520/A	0.47
Sour cherry 2516/A	0.39	Strawberry 2521/A	9.11
Kiwi 2517/A	4.16	Blueberry 2522/A	2.27
Apricot 2518/A	0.17	Raspberry 2523/A	4.21
Peach 2519/A	2.66		

The 5-MTHF content in the fruit components will be influenced by the applied technology and by its original content in the processed fruit. In 7 strawberry cultivars harvested in the Czech Republic the 5-MTHF content was determined. The results (Table 3) demonstrated that differences exist in the 5-MTHF content between the cultivars tested. The values in fully ripe strawberries fluctuated between 25.5 and 54.0 μg/100 g fresh sample, i.e. 272 and 554 μg/100 g dry matter. The highest content was found in the varieties Elsanta and Honeyoe, the lowest one in the cultivar Senga Sengana. These results correspond well to the literature data. Stralsjö *et al.* (2003) published the results of the RPBA determination in 13 strawberry varieties grown in southern Sweden giving the values between 36–69 μg/100 g. They proved that apart from the cultivar, the year of the harvest is a significant factor affecting the folate content in strawberries. However, her results also recognised the varieties Elsanta and Honeyoe as relatively very rich.

The effect of ripeness was checked by analysis of unripe, ripe, and fully ripe berries of the variety Senga Sengana, that is recognised as an important variety for industrial processing (Table 4). It was found that fully ripe berries contain by 63% more 5-MTHF in comparison with unripe berries. Literature data are not consistent in findings concerning the influence of ripeness. In the experiments carried out in three successive years, Stralsjö *et al.* (2003) found the increase in the folate content of 25% and 13%, but the decrease of 21% as well.

The results of the fruit components and strawberries analysis show that the fruit component addition to the fermented milk products may increase the natural folate content, and that strawberry flavouring is the most effective one. According to the producer's recommendation, 17% is added into the fermented product. Thus the strawberry component under our experimental conditions may donate 1.5 μg of folate to 100 g of the fermented milk product. In order to reach maximum folate content in the fruit component, fully ripe berries of the cultivars Elsanta and Honeyoe are recommended for the processing.

The results of this study indicate that, by the selection of the productive microorganisms and

Table 3. 5-MTHF content in 7 strawberry cultivars

Cultivar	Dry matter (g/100 g)	5-MTHF content	
		μg/100 g FW	μg/100 g DM
Elsanta I	9.74	54.0 ± 7.2	554 ± 74
Elsanta II	8.81	43.2 ± 3.9	490 ± 44
Honeyoe	9.99	54.4 ± 2.2	545 ± 22
Dukat	8.65	35.6 ± 1.7	412 ± 20
Dita	9.12	33.1 ± 2.0	363 ± 22
Elista	9.31	29.2 ± 2.8	314 ± 30
Senga Sengana I	8.21	25.5 ± 0.4	311 ± 50
Senga Sengana II	10.60	28.8 ± 3.5	272 ± 33
Carmen	9.35	36.6 ± 5.5	391 ± 59

Table 4. 5-MTHF content in strawberries (cv. Senga Sengana) of different ripeness

Ripness	Dry matter (g/100 g)	5-MTHF content	
		μg/100 g FW	μg/100 g DM
Unripe	8.07	13.2 ± 2.4	164 ± 30
Ripe	9.63	19.8 ± 1.0	206 ± 10
Fully ripe	10.15	27.2 ± 1.3	268 ± 13

optimised conditions in milk fermentation and by flavouring with the selected fruit component, the increase of the natural folate content can be reached. The folate content in the fermented milk product prepared by inoculation of pasteurised milk by the butter starter and *Streptococcus thermophilus* No. 144 in combination with *Propionibacterium freudenreichii* subsp. *shermanii* No. 160, fermented at 37°C for 12 h and flavoured with the strawberry component, was in this way increased by 4.8 μg/100 g, with 69% originating from fermentation and 31% from the fruit component addition. The folate losses after 15 days of storage in the refrigerator did not exceed 8%.

The folate intake may be increased, besides food fortification, by including sources rich in natural folate into the diet. The fermented milk products produced by appropriate fermentation and flavoured with an appropriate fruit component represent one of the ways to increase the natural folate content in foods offered to the consumers.

References

Ames B.N. (1999): Micronutrient deficiencies cause DNA damage and cancer. Food Science and Agricultural Chemistry, **1**: 1–15.

Ball G.F.M. (1994): Water-soluble vitamin assays in human nutrition. Chapman & Hall, London: 60.

Brattstrom L. (1996): Vitamins as homocysteine-lowering agents. The Journal of Nutrition, **126**: 1276S–1280S.

Czech Statistical Office (2004): No. 1618/2004-3330, Code 3004-04.

Daly L.E., Kirke P.N., Molloy A., Weir D.G., Scott J.M. (1995): Folate levels and neural tube defects. Journal of the American Medical Association, **247**: 1698–1702.

Forssén K.M., Jägerstad M.I., Wigertz K., Witthöft C.M. (2000): Folates and dairy products: A critical update. Journal of the American College of Nutrition, **19**: 100S–110S.

Holasová M., Fiedlerová V., Roubal P., Pechačová M. (2004): Biosynthesis of folates by lactic acid bacteria and propionibacteria in fermented milk. Czech Journal of Food Sciences, **22**: 175–181.

Johnston K.E., Dirienzo D.B., Tamura T. (2002): Folate content of dairy products measured by microbiological assay with trienzyme treatment. Journal of Food Science, **67**: 817–820.

Křivánek M. (2004): Výroba mlékárenských výrobků v roce 2003. Mlékařské listy, Zpravodaj, **82**: 9–12.

Morrison H.I., Schaubel D., Desmeules M., Wigle D.T. (1995): Serum folate and risk of fatal coronary heart disease. Journal of the American Medical Association, **275**: 1893–1896.

Shaw G.M., Schaffer D., Velie E.M., Morland K., Harris J.A. (1995): Periconceptional vitamin use, dietary folate and the occurrence of neural tube defects. Epidemiology, **6**: 212–226.

Smid E.J., Starrenburg M.J.C., Mieraul I., Sybesma W., Hugenholz J. (2001): Increase of folate level in fermented foods. Innovation in Food Technology, February/March: 13–15.

Stralsjö L.M., Witthöft C.M., Sjöhalm I.M., Jägerstad M.I. (2003): Folate content in strawberries: effect of cultivar, ripeness, year of harvest, storage and commercial processing. Journal of Agricultural and Food Chemistry, **51**: 128–133.

Wigertz K., Hansen I., Hoier-Madsen M., Holm J., Jägerstad M. (1996): Effect of milk processing on the concentration of folate-binding protein, folate-binding capacity and retention of 5-methyltetrahydrofolate. International Journal of Food Sciences and Nutrition, **47**: 315–322.

Witthöft C.M., Forseén K., Johannesson L., Jägerstad M. (1999): Folates – food sources, analyses, retention and bioavailability. Scandinavian Journal of Nutrition, **43**: 138–146.

Received for publication March 3, 2005
Accepted after corrections April 12, 2005

Corresponding author:

Ing. Marie Holasová, Výzkumný ústav potravinářský Praha, Oddělení výživových látek, Radiová 7, 102 31 Praha 10, Česká republika
tel.: + 420 296 792 271, fax: + 420 272 701 983, e-mail: m.holasova@vupp.cz