LWT - Food Science and Technology 54 (2013) 1–5

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Development of a high folate concentration yogurt naturally bio-enriched using selected lactic acid bacteria

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ARTICLE INFO

Article history:
Received 15 February 2013
Received in revised form
13 May 2013
Accepted 18 May 2013

Keywords:
Folates
Vitamin enrichment
Yogurt
Lactobacillus bulgaricus
Streptococcus thermophilus

ABSTRACT

Folate concentrations in yogurts vary widely and are normally considered low due to an inadequate selection of starter cultures and fermentation conditions. In this study, folate producing *Lactobacillus delbrueckii* subsp. *bulgaricus* (3 strains) and *Streptococcus thermophilus* (2 strains) were combined and used to elaborate 15 different yogurts. Samples were taken at different time points during the fermentation process and folate concentrations were determined. The yogurt elaborated with strains CRL871 + CRL803 + CRL415 and incubated at 42 °C had significantly higher folate concentrations (reaching 180 ± 10 μg/L) which implies almost a 250% increase in respect to non-fermented milk, and about 125% compared to commercial yogurts. No variations in folate concentration were observed during 28 days of storage at 4 °C. This is the first report of a yogurt naturally bio-enriched in folate using native folate producing starter cultures.

1. Introduction

Tetrahydrofolate (THF) and its derivates (generally grouped under the denomination folates or vitamin B_9) are important co-factors of metabolic enzymes that are involved in one-carbon transfer reactions participating in vital pathways such as DNA replication, repair and methylation, biosynthesis of nucleic acids, some amino acids, pantothenate and other vitamins (Hanson & Roje, 2001).

This vitamin belongs to the water-soluble B-group vitamins and is present in a wide variety of foods. However, deficiencies in folates are very frequently in many parts of the world, not only in developing countries, but also in some industrialized countries such as Germany (Konings et al., 2001). In some population groups there is an increased risk of vitamin deficiency, especially in the elderly, because their food intake is lower, and in children, who sometimes consume a low variety of foods (Allen, 2003; Brachet et al., 2004). Folate deficiencies may contribute to the etiology of many important diseases such as Alzheimer, cancer, cardiovascular disease, and neural tube defects (NTDs) (Basset, Quinlivan, Gregory Iii, & Hanson, 2005; Luchsinger, Tang, Miller, Green, & Mayeux, 2007). Therefore, fortification has been proposed by many governments (Canada, USA and Argentina amongst others) in order to increase folate intakes of the general population. However, a number of studies have shown that high intakes of folic acid, the chemically synthesized form of folate, can cause adverse health effects such as the masking of the early hematological manifestations of vitamin B_{12} deficiency, alteration in the activity of the hepatic dihydrofolate reductase enzyme (Bailey & Ayling, 2009) or promote cancer (Baggott, Oster, & Tamura, 2012; Melnyk et al., 1999; Ulrich & Potter, 2006). Natural folates, by contrast, such as those found in foods or produced by certain microorganisms (Kariluoto et al., 2006; Lin & Young, 2000; Santos, Wegkamp, de Vos, Smid, & Hugenholtz, 2008), do not cause such adverse health effects in individuals (Wright, Dainty, & Finglas, 2007). Certain lactic acid bacteria (LAB) strains have the capability to synthesize folates being this property strongly dependent on species, strain, growth time, and cultivation conditions (Crittenden, Martinez, & Playne, 2003; Lin & Young, 2000; Sybesma, Starrenburg, Tijsseling, Hoefnagel, & Hugenholtz, 2003), whereas other LAB consume it.

Milk is not considered a rich source of dietary folate. However, many dairy products are processed using microbial fermentations in which folate could be synthesized. In this regard it was

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http://dx.doi.org/10.1016/j.lwt.2013.05.035

previously described that certain yogurts contain a three-fold increase in folate concentrations compared to non-fermented milk (Wouters, Ayad, Hugenholtz, & Smit, 2002). The FAO/WHO Codex Alimentarius Commission defines yogurt as "a coagulated milk product obtained by lactic acid fermentation through the action of *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* (*S. thermophilus*) from milk (pasteurized or concentrated milk) with or without additions (milk powder, skim milk powder, etc.). The microorganisms in the final product must be viable and abundant". In the production of yogurt, there is a symbiosis between these two microbial species; i) *L. bulgaricus*, unlike *S. thermophilus*, possesses extracellular cell-wall bound protease and therefore can supply *S. thermophilus* with peptides and amino acids, ii) *S. thermophilus* can stimulate the growth of *L. bulgaricus* through formic acid formation (a limiting step in the purine biosynthesis), excretion of CO_2 by degrading milk urea (CO_2 concentration are low in milk after heat treatment affecting lactobacilli growth) and producing folates. This latter example largely depends on the strains used as starter cultures since it was recently shown that certain strains of *L. delbrueckii* subsp. *bulgaricus* are capable to produce folate in large amounts when grow in folate-free culture medium and milk (Laiño, LeBlanc, & Savoy de Giori, 2012). Taking into account that the optimization of fermentation process using folate-producing strains constitutes a promising approach, the objective of this study was to select the best combination of folic acid-producing lactic acid bacteria in order to obtain a yogurt bio-enriched in folate and to evaluate the vitamin content during its storage.

2. Materials and methods

2.1. Microorganisms and growth conditions

L. bulgaricus CRL863, CRL871 and CRL872 and *S. thermophilus* CRL803 and CRL415 belonging to the culture collection of the Centro de Referencia para Lactobacilos (CERELA-CONICET, Tucumán, Argentina) (CRL) were used in this study. These strains were previously selected for their folate-producing capacities (Laiño et al., 2012). Lactobacilli were grown without agitation in De Man–Rogosa–Sharpe (MRS) broth culture media (De Man, Rogosa, & Sharpe, 1960) at 37 °C for 16 h, whereas streptococci were grown for 16 h at 42 °C without agitation in LAPTg broth culture media containing (w/v) 1.5% peptone, 1% tryptone, 1% yeast extract, 1% glucose and 0.1 %Tween 80.

2.2. Elaboration of fermented milks and yogurts

Cultures were transferred twice in 200 mL reconstituted non-fat powdered milk (Svelty Calcio Plus, Nestle, Argentina) prior to experimental use; 16 h old cultures (2% v/v) were used as inocula combined (Table 1) and incubated at 37 and 42 °C for 24 h.

For the elaboration of yogurts, five combinations (G, L, M, N and O) were selected to be used as starter cultures for yogurt manufacture where the resulting products were named as Y1, Y2, Y3, Y4, and Y5, respectively.

Reconstituted non-fat powdered milk (250 mL) (Svelty Calcio Plus, Nestle, Argentina) was heat treated at 87 °C for 30 min, cooled to 37 °C, and inoculated with 2% of previously selected yogurt starter cultures. Folate producing mixtures, Y1, Y2 and Y3 (cocci:-bacilli ratio = 2:1), and Y4 and Y5 (1:1 ratio) were incubated at 42 °C for 6 h. Fermentation was stopped by rapidly cooling the yogurt in an iced bath, then chilled in water bath and stored at 4 °C for 28 days. Folate concentration, viable cell count and pH were determined after 0, 3, 7, 14, 21 and 28 days of storage.

Table 1
Folate production by different combinations of folate producing strains in milk at 37 °C.

Strains & fermented milks	Hours of fermentation							
	0		6		8		24	
	Folates[a]	SD	Folates[a]	SD	Folates[a]	SD	Folates[a]	SD
CRL 863-803 (A)	47.2	1.4	80.7	0.1	75.4	0.4	64.5	1.8
CRL 863-415 (B)	52.4	0.7	53.4	0.9	54.3	0.9	43.9	0.1
CRL 871-803 (C)	51.9	1.1	60.7	0.2	78.2	1.5	60.2	0.2
CRL 871-415 (D)	51.2	0.3	57.6	0.6	70.1	2.6	56.5	0.9
CRL 872-803 (E)	51.8	0.2	51	0.8	58.1	0.2	57.2	0.8
CRL 872-415 (F)	49.5	0.2	54.2	0.2	45.7	0.5	60.5	0.5
CRL 863-803-415 (G)	58	0.9	69.3	1.1	76.7	0.6	65.2	0.9
CRL 863-871-803 (H)	49.6	1.4	99.3*	0.8	88.4**	2.6	107.3‡	0.1
CRL 863-871-415 (I)	49.3	2.9	43.5	1.5	34.6	1.7	32.4	0.7
CRL 863-872-803 (J)	51.7	2.3	63.9	1.8	93.9†	1.7	95.5†	0.9
CRL 863-872-415 (K)	54.7	2.7	18.4	0.5	22.5	0.5	26.9	0.9
CRL 871-803-415 (L)	51.8	0.6	84.1	0.8	54.3	1.1	61.4	0.8
CRL 872-803-415 (M)	54.3	0.2	82.2	0.2	61.6	0.1	69.3	0.2
CRL 863-871-803-415 (N)	53.7	1.3	90.3†	2.9	28	0.9	102.1††	1.3
CRL 863-872-803-415 (O)	49.9	1.6	55.1	0.6	104.9††	0.6	95	0.5
Milk (P)	47.4	0.4	45	0.3	42	0.5	48	0.4

[a] Folate levels are expressed as the mean (μg/L) ± standard deviation (SD). *,**,†,††,‡ Means with different symbols differ significantly ($p < 0.05$).

Viable *L. bulgaricus* were plate counted in LBS agar (Difco, Becton, Dickinson, and Co., Sparks, Maryland) after incubation during 48 h at 37 °C and *S. thermophilus* on M17 agar (Biokar Diagnostics, Beauvais, France) after incubation of 48 h at 42 °C. Both LBS agar and M17 agar allowed a successfully isolation and separation of both bacteria species.

2.3. Sample preparation for folate determination

Samples of milk/yogurt (500 μL) were aseptically withdrawn at different times and mixed with 500 μL of protection buffer (0.1 mol/L phosphate buffer, pH 6.8, containing 1.5% (w/v) ascorbic acid to prevent vitamin oxidation and degradation) to evaluate folates production. The resulting mixture (1 mL) was boiled (100 °C) for 5 min to precipitate proteins and release folate from binding proteins present in milk/yogurt and was then centrifuged (10,000 *g* for 6 min). The supernatant was collected and stored at −70 °C for total folates determination.

In every case, non-inoculated samples, used as control, were analyzed simultaneously.

2.4. Folate determination

Folate determination was performed using a modified microbiological assay (Horne & Patterson, 1988) using *Lactobacillus casei* subsp. *rhamnosus* (*L. rhamnosus*) NCIMB 10463 as the indicator strain (O'Brien et al., 2001), which is naturally resistant to chloramphenicol (up to 500 μg/mL). The strain, stored at −70 °C, was inoculated in fresh MRS and incubated 24 h at 37 °C before use. After growth, an aliquot of 1 mL was taken and washed 3 times with saline solution, resuspended in the original volume and used to inoculate at 2% v/v fresh FACM and incubated 24 h at 37 °C. This last step was repeated and the second culture was used to perform the folate determination (this procedure was performed to deplete folate reserves in the indicator strain).

All frozen samples were thawed at room temperature (21 °C) in the absence of light and processed in light reduced conditions. The samples were diluted with protection buffer and each sample (100 μL) was placed into one well of a 96 well sterile microplate (Deltalab, Argentina). The folate concentration of each sample was

determined in triplicate. The reference strain (*L. rhamnosus* NCIMB 10463) grown in FACM as described above, was inoculated at 4% v/v in 10 mL of 2× FACM containing 20 μg/mL chloramphenicol (to decrease the potential of microbial contaminants), and a fraction (100 μL) was added to each well and mixed. Sterile plate covers were placed on the microtiter plates that were then incubated 48 h statically at 37 °C. After this optimized incubation period, the optical density (OD) was read at 580 nm using a microplate reader (VERSAmax tuneable microplate reader, Molecular Devices, USA).

In each microplate, a standard curve was realized using HPLC grade folic acid (Fluka BioChemica, Sigma–Aldrich, Switzerland) instead of samples at different concentrations (between 0 and 1.0 μg/L) diluted in the protection buffer. Samples were diluted (normally in a 1/80 relation in protection buffer), in order to obtain values within the range of the standard curve. To obtain the final folate concentrations, the values obtained from the standard curve were multiplied by the dilution factor and expressed as μg/L.

Folate determination were performed after applying a trienzymatic treatment as described previously by Iyer, Tomar, Singh, and Sharma (2009), α-amylase from *Aspergillus oryzae*, and protease from *Streptomyces griseus* (Sigma Chemical, St. Louis, MO, USA) were dissolved in distilled-deionized water at concentrations of 4 mg/mL and filter-sterilized (0.22 μm). Although the protease did not contain any measurable folate by microbiological assay, α-amylase contained approximately 1.54 ng of folate per mg of solid enzyme. Rat plasma, obtained from rats provided by Instituto de Estudios Biológicos (INSIBIO-CONICET-Universidad Nacional de Tucumán, Tucumán, Argentina), was used as a source of folate deconjugase enzyme as described previously containing 1.2 μg/L (Aiso & Tamura, 1998). The endogenous folate in each enzyme was determined by the microbiological assay described above after folate conjugase treatment and subtracted for the final calculation of food folate content.

2.5. Statistical analysis

The results were obtained from three independent experiments and each data point was measured in triplicate ($n = 9$). All values were expressed as means ± standard deviations (SD). Statistical analyses were performed with the software package SigmaPlot for Windows Version 12.0 (Systat Software Inc., Chicago IL, USA) using ANOVA GLM followed by a Tukey's posthoc test, and differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Influence of the temperature and time of fermentation over the folate production

Folate concentration of the different mixtures of yogurt starter cultures, incubated at 37 and 42 °C, are shown in Tables 1 and 2. From a total of 15 mixtures of yogurt starter cultures incubated at 37 °C, four of them (H, J, N y O) showed the highest folate levels (90.3–104.9 μg/L) between 6 and 8 h of fermentation (hf) (Table 1) being significantly higher as compared with others mixtures (18.4–78.2 μg/L). Some cultures (I and K) consumed folate instead of increasing it (Table 1), demonstrating that vitamin production was a strain-dependent trait and that the food matrix can affect folate production since all of the strains were able to increase this vitamin in a folate-free medium (Laiño et al., 2012). At 42 °C, the highest folate concentrations (107.3–110.6 μg/L) were found in the mixture L (CRL871 + CRL803 + CRL415) at 6 hf and did not change during the incubation period up to 24 hf, whereas the others combinations produced different folate concentrations but all above 90 μg/L (Table 2).

Table 2
Folate production by different combinations of folate producing strains in milk at 42 °C.

Strains & fermented milks	Hours of fermentation							
	0		6		8		24	
	Folates[a]	SD	Folates[a]	SD	Folates[a]	SD	Folates[a]	SD
CRL 863-803 (A)	23.2	1.4	66	0.1	62.7	0.3	78.1	1.8
CRL 863-415 (B)	40.3	0.7	39.4	0.9	49.2	0.4	60.3	0.1
CRL 871-803 (C)	55.4	1.1	74.6	0.7	89.3	0.9	79.9	0.1
CRL 871-415 (D)	44.5	0.7	38.2	0.7	49.1	0.2	41.6	0.6
CRL 872-803 (E)	44.8	1.1	74.4	0.7	78.3	0.7	63.1	0.5
CRL 872-415 (F)	45.1	0.7	46.9	0.3	61.8	2.1	42.4	0.3
CRL 863-803-415 (G)	33.8	0.9	64.6	1.1	61.7	0.3	76.7	0.9
CRL 863-871-803 (H)	49.6	1.3	93.8*	0.8	101.2**	0.6	101.2**	0.1
CRL 863-871-415 (I)	49.3	2.9	30.9	1.5	63.8	2.6	23.2	0.7
CRL 863-872-803 (J)	51.7	2.3	35.4	1.8	25.4	1.7	83.3	0.9
CRL 863-872-415 (K)	54.7	2.7	14.5	0.5	16.3	1.7	26.4	0.9
CRL 871-803-415 (L)	50.9	0.7	107.3†	0.6	108.3†	0.5	110.6†	0.4
CRL 872-803-415 (M)	46.8	0.5	40.1	0.1	50.7	0.1	39.3	0.4
CRL 863-871-803-415 (N)	53.7	1.3	99.9**	2.9	93.4*	0.4	96.7**	1.3
CRL 863-872-803-415 (O)	49.9	1.6	34.6	0.6	52.9	0.9	63.5	0.5
Milk (P)	50.1	2.4	48.7	1.2	45.7	0.6	47.2	0.9

[a] Folate levels are expressed as the mean (μg/L) ± standard deviation (SD). *,**,† Means with different symbols differ significantly ($p < 0.05$).

From these results, the best conditions to increase folate concentration during milk fermentation were 6 h of incubation at 42 °C.

3.2. Folate levels in yogurt manufactured using folate producing starter cultures. Stability of vitamin during storage

Folate level was highest in yogurt Y2 with respect to other yogurts at the end of the production of yogurt (Fig. 1). After the previous fermentation, folate concentration remained stable during 28 days of refrigerated storage (Fig. 1). Total viable cell count did not show any significant difference during all storage period at 4 °C until 28 d, showing that both bacteria remain viable through storage. Only *L. bulgaricus* showed a slight decrease after 14 d of storage (Fig. 2A). In contrast, *S. thermophilus* showed a constant value of viable cell until 28 d (Fig. 2B).

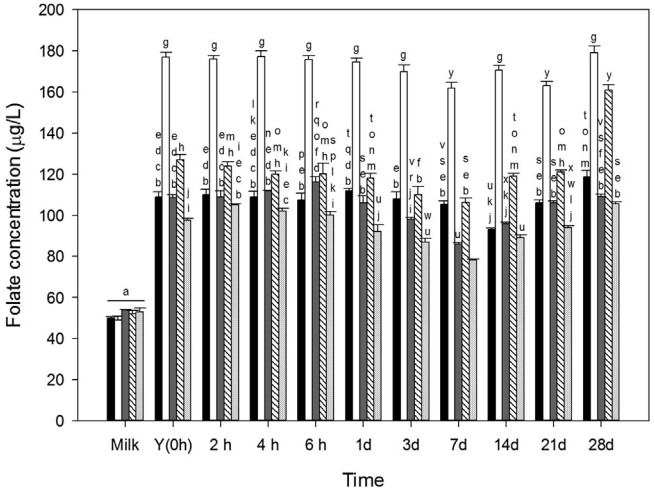

Fig. 1. Folate concentration in yogurt during shelf-life. Time is expressed as hour (h) and day (d) after fermentation. Y (0 h) represents the end of yogurt elaboration. Values are expressed as the mean ± standard deviation (SD). [a–w]Means with different letter differ significantly ($p < 0.05$). Black bars represent Y1 (CRL863 + CRL803 + CRL415), empty bars represent Y2 (CRL871 + CRL803 + CRL415), dark gray bars represent Y3 (CRL872 + CRL803 + CRL415), diagonally stripped bars represent Y4 (CRL863 + CRL871 + CRL803 + CRL415), and dotted bars represent Y5 (CRL863 + CRL872 + CRL803 + CRL415).

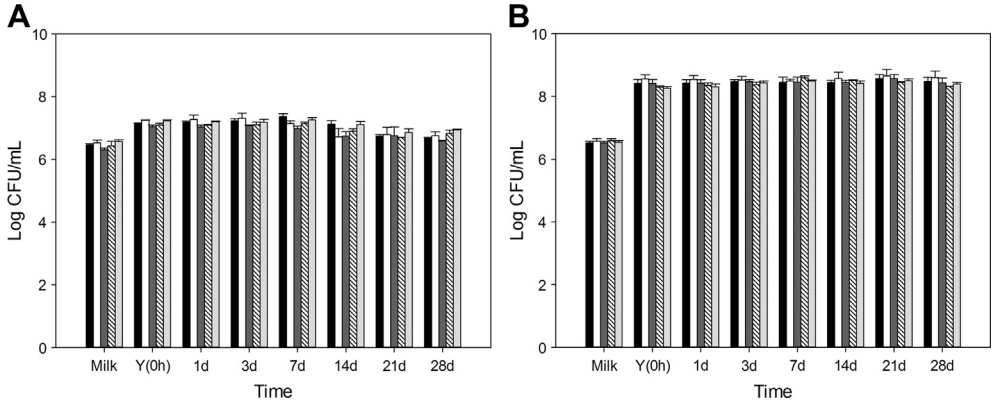

Fig. 2. Viable cell count of *L. bulgaricus* and *S. thermophilus* in yogurt during storage at 4 °C. A. Viable cell count of *L. bulgaricus* in LBS agar at 37 °C for 48–72 h. B. Viable cell count of *S. thermophilus* in M17 agar at 42 °C for 48 h. Values are expressed as the mean ± standard deviation (SD). Black bars represent Y1 (CRL863 + CRL803 + CRL415), empty bars represent Y2 (CRL871 + CRL803 + CRL415), dark gray bars represent Y3 (CRL872 + CRL803 + CRL415), diagonally stripped bars represent Y4 (CRL863 + CRL871 + CRL803 + CRL415), and dotted bars represent Y5 (CRL863 + CRL872 + CRL803 + CRL415).

4. Discussion

Although folates are present in milk (20–50 μg/L), their concentrations are not considered important. In order to increase folate concentrations in milk, microbiological fermentation in which folates could be synthesized *in situ* is a promising alternative. Previous studies demonstrated that the ability to synthetize folate by yogurt starter cultures (*L. bulgaricus* and *S. thermophilus*) is very variable and is strain-dependant (Alm, 1982; Crittenden et al., 2003; Kneifel, Holub, & Wirthmann, 1989; Kneifel, Kaufmann, Fleischer, & Ulberth, 1992). Crittenden et al. (2003) and other authors (Kneifel, Erhard, & Jaros, 1991; Rao, Reddy, Pulusani, & Cornwell, 1984) reported that *L. bulgaricus* are a folate consumers. However, it was recently shown that there are certain strains of *L. bulgaricus* that are able to produce folate when grown in a folate-free culture medium and in milk (Laiño et al., 2012). Based on this recent discovery and the fact that there are no commercial yogurts or any other fermented dairy product that are naturally enriched in folate, folate production by different combinations of folate producing strains and the influence of fermentation conditions over folate production in yogurts were evaluated. Also the stability of vitamin during storage at 4 °C (yogurt shelf life) was studied. The vitamin production showed a wide variation in its levels, due not only to incubation temperatures and times, but principally due to the combination of starter cultures used to produce the yogurts.

When results at both temperatures were compared, it was observed that at 42 °C the maximum folate values were reached quicker (6 hf) than at 37 °C (8 hf and 24 hf). CRL871 + CRL803 + CRL415 (mixture L) showed the highest values of folate at 6 hf. As those folate levels were obtained in a short time of fermentation (6 hf) and they were kept until 24 hf, it allowed the elaboration of different kinds of dairy products. Although, there are previous reports about folate production by bacteria in milk and other fermented products (Jägerstad, Jastrebova, & Svensson, 2004; Kariluoto et al., 2006; Murdock & Fields, 1984), none of these evaluated folate production in function of temperature and incubation time.

On the basis of these results, the best conditions of fermentation were selected and 5 yogurts were elaborated using folate producing strains. Shelf life of yogurts at 4 °C for 28 d was studied. Starter cultures viability did not show significant modifications through storage. Folate concentrations kept constant in function of the storage period. These results suggest that in fact no decrease in folate concentration is observed during storage of yogurts at 4 °C during one month. An explanation could be that the increase in the length of glutamic tail of folate, would allow folates to remain inside the starter cultures and could in fact be beneficial to maintain and protect folates during long storage times or in extreme conditions.

Yogurt CRL871 + CRL803 + CRL415 showed the highest folate concentration at the end of its elaboration, and those values remained constant during storage. These results conflict with what was reported by Reddy, Shahani, and Kulkarni (1976) where vitamin levels decreased about 54% when yogurt was stored at 5 °C for 16 d.

The World Health Organization recommends the daily intake of 400 μg folate per day by adults and 200 μg folate per day by children (FAO/WHO, 2002). It has also been stated that a food is considered a good source of folate when it contributes to at least 10%–20% of daily recommended intake (Ohio State University, 2005) and by this definition, traditional or commercial yogurts are not considered a good source of folate (Ohio State University, 2005). In this study, one portion (225 mL) of the yogurt prepared with strains CRL871 + CRL803 + CRL415 would contribute to 10% RDA for adults and 20% RDA for children. Other authors reported that it is possible to develop fermented products enriched in folates, including yogurts, but none have done so by adequately selecting the starter cultures, normally they simple add other food products to increase vitamin concentrations (Holasova, Fiedlerova, Roubal, & Pechacova, 2005).

5. Conclusion

To the best of our knowledge, this is the first study where a significant and important concentration of folate was obtained in a yogurt using selected starter cultures. These novel bio-enriched yogurts could be able to satisfy the growing demands of consumers for more natural products and provide novel tools to prevent folate deficiencies without having to recur to fortification with chemicals such as folic acid. The production costs of folate bio-enriched products would be the same as traditional ones, but the latter would have an increased economic value because they would provide consumers an additional benefit.

Disclosure statement

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank Mrs. Carole Johnston and Dr. Helga Refsum of the Department of Pharmacology, University of

Oxford, for their kindly donation of the folate reference strain *L. rhamnosus* NCIMB 10463.

Also, the authors would like to thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), and the Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT) for their financial support.

References

Aiso, K., & Tamura, T. (1998). Trienzyme treatment for food folate analysis: optimal pH and incubation time for alpha-amylase and protease treatment. *Journal of Nutritional Science and Vitaminology, 44*(3), 361–370.

Allen, L. H. (2003). B vitamins: proposed fortification levels for complementary foods for young children. *Journal of Nutrition, 133*(9), 3000S–3007S.

Alm, L. (1982). Effect of fermentation on B-vitamin content of milk in Sweden. *Journal of Dairy Science, 65*(3), 353–359.

Baggott, J. E., Oster, R. A., & Tamura, T. (2012). Meta-analysis of cancer risk in folic acid supplementation trials. *Cancer Epidemiology, 36*(1), 78–81. doi:S1877-7821(11)00084-1 [pii] 10.1016/j.canep.2011.05.003.

Bailey, S. W., & Ayling, J. E. (2009). The extremely slow and variable activity of dihydrofolate reductase in human liver and its implications for high folic acid intake. *Proceedings of the National Academy of Sciences of the United States of America, 106*(36), 15424–15429. doi:0902072106 [pii] 10.1073/pnas.0902072106.

Basset, G. J. C., Quinlivan, E. P., Gregory Iii, J. F., & Hanson, A. D. (2005). Folate synthesis and metabolism in plants and prospects for biofortification. *Crop Science, 45*(2), 449–453.

Brachet, P., Chanson, A., Demigne, C., Batifoulier, F., Alexandre-Gouabau, M. C., & Tyssandier, V. (2004). Age-associated B vitamin deficiency as a determinant of chronic diseases. *Nutrition Research Reviews, 17*(1), 55–68. doi:S0954422404000058 [pii] 10.1079/NRR200478.

Crittenden, R. G., Martinez, N. R., & Playne, M. J. (2003). Synthesis and utilisation of folate by yoghurt starter cultures and probiotic bacteria. *International Journal of Food Microbiology, 80*(3), 217–222. doi:S0168160502001708 [pii].

De Man, J. D., Rogosa, M., & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology, 23*, 130–135.

FAO/WHO. (2002). Human vitamin and mineral requirements. In FAO/WHO (Ed.), *Human vitamin and mineral requirements*. Bangkok, Thailand.

Hanson, A. D., & Roje, S. (2001). One-carbon metabolism in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology, 52*, 119–137. doi:10.1146/annurev.arplant.52.1.119 52/1/119 [pii].

Holasova, M., Fiedlerova, V., Roubal, P., & Pechacova, M. (2005). Possibility of increasing natural folate content in fermented milk products by fermentation and fruit component addition. *Czech Journal of Food Sciences, 23*(5), 196–201.

Horne, D. W., & Patterson, D. (1988). *Lactobacillus casei* microbiological assay of folic acid derivatives in 96-well microtiter plates. *Clinical Chemistry, 34*(11), 2357–2359.

Iyer, R., Tomar, S. K., Singh, R., & Sharma, R. (2009). Estimation of folate in milk by microbiological assay using tri-enzyme extraction method. *Milchwissenschaft, 64*(2), 125–127.

Jägerstad, M., Jastrebova, J., & Svensson, U. (2004). Folates in fermented vegetables—a pilot study. *LWT – Food Science and Technology, 37*(6), 603–611.

Kariluoto, S., Aittamaa, M., Korhola, M., Salovaara, H., Vahteristo, L., & Piironen, V. (2006). Effects of yeasts and bacteria on the levels of folates in rye sourdoughs. *International Journal of Food Microbiology, 106*(2), 137–143. doi:S0168-1605(05)00422-8 [pii] 10.1016/j.ijfoodmicro.2005.06.013.

Kneifel, W., Erhard, F., & Jaros, D. (1991). Production and utilisation of some water soluble vitamins by yogurt and yogurt-related starter cultures. *Milchwissenschaft, 46*, 685–690.

Kneifel, W., Holub, S., & Wirthmann, M. (1989). Monitoring of B-complex vitamins in yogurt during fermentation. *Journal of Dairy Research, 56*(04), 651–656. http://dx.doi.org/10.1017/S0022029900029174.

Kneifel, W., Kaufmann, M., Fleischer, A., & Ulberth, F. (1992). Screening of commercially available mesophilic dairy starter cultures: biochemical, sensory, and microbiological properties. *Journal of Dairy Science, 75*(11), 3158–3166.

Konings, E. J., Roomans, H. H., Dorant, E., Goldbohm, R. A., Saris, W. H., & van den Brandt, P. A. (2001). Folate intake of the Dutch population according to newly established liquid chromatography data for foods. *The American Journal of Clinical Nutrition, 73*(4), 765–776.

Laiño, J. E., LeBlanc, J. G., & Savoy de Giori, G. (2012). Production of natural folates by lactic acid bacteria starter cultures isolated from artisanal Argentinean yogurts. *Canadian Journal of Microbiology, 58*(5), 581–588. http://dx.doi.org/10.1139/w2012-026.

Lin, M. Y., & Young, C. M. (2000). Folate levels in cultures of lactic acid bacteria. *International Dairy Journal, 10*(5–6), 409–413.

Luchsinger, J. A., Tang, M. X., Miller, J., Green, R., & Mayeux, R. (2007). Relation of higher folate intake to lower risk of Alzheimer disease in the elderly. *Archives of Neurology, 64*(1), 86–92. doi:64/1/86 [pii] 10.1001/archneur.64.1.86.

Melnyk, S., Pogribna, M., Miller, B. J., Basnakian, A. G., Pogribny, I. P., & James, S. J. (1999). Uracil misincorporation, DNA strand breaks, and gene amplification are associated with tumorigenic cell transformation in folate deficient/repleted Chinese hamster ovary cells. *Cancer Letters, 146*(1), 35–44. doi:S030438359900213X [pii].

Murdock, F. A., & Fields, M. L. (1984). B-vitamin content of natural lactic acid fermented cornmeal. *Journal of Food Science, 49*(2), 373–375. http://dx.doi.org/10.1111/j.1365-2621.1984.tb12425.x.

O'Brien, M. M., Kiely, M., Harrington, K. E., Robson, P. J., Strain, J. J., & Flynn, A. (2001). The North/South Ireland food consumption survey: vitamin intakes in 18-64-year-old adults. *Public Health Nutrition, 4*(5A), 1069–1079.

Ohio State University. (2005). Extension fact sheet – Folate (folacin, folic acid). In *Ohio State University extension fact sheet – Folate (folacin, folic acid), HYG 5553-05* (pp. 1–3).

Rao, D. R., Reddy, A. V., Pulusani, S. R., & Cornwell, P. E. (1984). Biosynthesis and utilization of folic acid and vitamin B12 by lactic cultures in skim milk. *Journal of Dairy Science, 67*, 1169–1174.

Reddy, K. P., Shahani, K. M., & Kulkarni, S. M. (1976). B-complex vitamins in cultured and acidified yogurt. *Journal of Dairy Science, 59*(2), 191–195. doi:S0022-0302(76)84182-3 [pii] 10.3168/jds.S0022-0302(76)84182-3.

Santos, F., Wegkamp, A., de Vos, W. M., Smid, E. J., & Hugenholtz, J. (2008). High-Level folate production in fermented foods by the B12 producer *Lactobacillus reuteri* JCM1112. *Applied and Environmental Microbiology, 74*(10), 3291–3294. doi:AEM.02719-07 [pii] 10.1128/AEM.02719-07.

Sybesma, W., Starrenburg, M., Tijsseling, L., Hoefnagel, M. H., & Hugenholtz, J. (2003). Effects of cultivation conditions on folate production by lactic acid bacteria. *Applied and Environmental Microbiology, 69*(8), 4542–4548.

Ulrich, C. M., & Potter, J. D. (2006). Folate supplementation: too much of a good thing? *Cancer Epidemiology, Biomarkers & Prevention, 15*(2), 189–193. doi:15/2/189 [pii] 10.1158/1055-9965.EPI-152CO.

Wouters, J. T. M., Ayad, E. H. E., Hugenholtz, J., & Smit, G. (2002). Microbes from raw milk for fermented dairy products. *International Dairy Journal, 12*(2–3), 91–109.

Wright, A. J., Dainty, J. R., & Finglas, P. M. (2007). Folic acid metabolism in human subjects revisited: potential implications for proposed mandatory folic acid fortification in the UK. *British Journal of Nutrition, 98*(4), 667–675. doi:S0007114507777140 [pii] 10.1017/S0007114507777140.