Folate bio-fortification of yoghurt and fermented milk: a review

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Abstract Folate, an essential vitamin, plays an important role in human life for the synthesis of nucleotides, vitamins and some amino acid. This vitamin cannot be synthesized by humans and must be obtained exogenously to prevent folate deficiency, neural tube defects (NTDs) and other related diseases. Folic acid, the chemically synthesized form of folate, is used for fortification and supplementation, but it can cause adverse effects such as vitamin B₁₂ deficiency in high intake. Therefore, novel methods to increase concentrations of naturally occurring folate in foods have grabbed the interest of researchers. The application of bacterial cultures in food fermentation is a novel strategy to increase “natural” folate levels. Dairy products, especially yoghurt, are an appropriate choice for bio-fortification of folate as they contain folate-binding protein which improves folate bioavailability. This study will review the folate production by probiotic bacteria in fermented milk based on various strains and culture conditions.

Keywords Probiotic · Folate · Folic acid · Bio-fortification · Yoghurt

1 Introduction

Folate belongs to the water-soluble B-group of vitamins. It is also referred to as vitamin B₉, vitamin B_c (or folacin), and pteroyl-L-glutamate. This vitamin is found in a wide variety of foods. However, folate deficiencies are a very frequent phenomenon in many

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countries. There is an increased risk of vitamin deficiency in some population groups, especially in the elderly, because their food intake is lower, in children, who sometimes consume a limited variety of foods, and for pregnant women because of the critical role played by folate in foetus neural tube development. Low folate intake increases the risk of birth defects in newborn babies.

Probiotic bacteria confer beneficial effects to their hosts and are known for the production of folate in high amounts (Hugenschmidt et al. 2010, 2011; Iyer et al. 2010; LeBlanc et al. 2007).

Different types of products have been proposed as carrier foods for probiotic microorganisms by which consumers can take in large amounts of probiotic cells for therapeutic effects. Yoghurt has long been recognized as a product with many desirable effects for consumers, and it is also important that most consumers consider yoghurt to be healthy.

Strains exhibiting high vitamin production capacities have high application potential for enriching fermented products with natural vitamins (Lourens-Hattingh and Viljoen 2001).

This study follows a brief presentation of folate in terms of chemistry, nutritional aspects, biosynthesis, bioavailability and analysis. After that, fermented dairy products with emphasis on yoghurt are reviewed for their folate content. Special emphasis is placed on the probiotic strains that have significant capacity to produce folate in different fermented milks with various culture conditions.

2 Folate

2.1 Chemical structure of folate

The term “folate” refers to all groups of folic acid derivatives, containing the natural folate form present in foods and synthetic folic acid form used for food fortification and vitamin supplements. Folic acid (pteroyl-L-glutamic acid; Fig. 1a) (PGA) is composed of para-aminobenzoic acid (pABA), pteridine ring and L-glutamic acid (Fig. 1a). The natural folate forms exist primarily as reduced, with different reduction levels of the pteroyl group, the number of substituents and that of glutamyl residues attached to the pteroyl group (Fig. 1b).

The various folate derivatives include 5-methyltetrahydrofolate (5-MTHF), 5-formyltetrahydrofolate (5-formyl-THF), 10-formyltetrahydrofolate (10-formyl-THF), 5,10-methylenetetrahydrofolate (5,10-methylene-THF), 5,10-methenyltetrahydrofolate (5,10-methenyl-THF), 5-formiminotetrahydrofolate (5-formimino-THF), 5,6,7,8-tetrahydrofolate (THF) and dihydrofolate (DHF). Over 100 distinct forms of this vitamin may exist naturally as a result of this chemical diversity, although fewer than 50 principal forms of folate exist in most animal and plant tissues (Gregory 1989).

Most naturally occurring dietary folates have two to seven glutamate units attached on peptide linkages. The intracellular folates are mostly pteroylpentaglutamates, while the pteroylmonoglutamates are dominant forms of extracellular folate. Naturally, pteroylpolyglutamates with a maximum of 11 glutamic acid residues exist (Forssén et al. 2000; Holasova et al. 2005; LeBlanc et al. 2007; Sybesma et al. 2003; Sybesma 2003).

Fig. 1 Structure and name of some folate derivatives with fully oxidized (folic acid) (a) and fully reduced pteridine ring (natural folate) (b). *N* The number of extra glutamate units

2.2 Nutritional and health aspects

Folate is not synthesized in mammalian cells; hence, it must be supplied exogenously. This vitamin plays important roles in various metabolic pathways, acting as cofactor for one carbon transferring reactions in methylation cycle, DNA replication as well as biosynthesis of nucleic acids, some amino acids and vitamins (Hanson and Roje 2001; Laiño et al. 2013). In addition to the mentioned functions, folate has an antioxidant property which protects the genome against free radical damage (Duthie et al. 2002; LeBlanc et al. 2007).

The approved daily recommended intake of folate in the European Union is $400 \mu\text{g}\cdot\text{day}^{-1}$ for adults (FAO and WHO 2005). Except pregnant and lactating women, the minimum folate requirement for adults has been estimated at $50 \mu\text{g}\cdot\text{day}^{-1}$ (Crittenden et al. 2003). In addition, dietary intake of $400\text{--}600 \mu\text{g}\cdot\text{day}^{-1}$ is commonly recommended for pregnant women (Allen 2003; Brachet et al. 2004; Crittenden et al. 2003; Laiño et al. 2013).

Folate is mainly found in yeast, liver, wheat bran, beetroot, green leafy vegetables, chick peas, nuts, legumes, citrus and some fruits. Other sources include oatmeal, whole grain cereals, potatoes, eggs, kidney, muscle meats, fish and fermented dairy products (Eitenmiller et al. 2007; Holasova et al. 2005; LeBlanc et al. 2007). It has been stated that a food is considered a good source of folate when it contributes to at least 10–20% of daily recommended intake (FAO and WHO 2005).

Despite the wide range of dietary sources for folate, folate deficiencies still occur frequently. Such deficiency has been reported in a wide variety of disorders. A noteworthy disease related to folate deficiency is neural tube defect (NTD) (Geisel 2003; Storozhenko et al. 2005). Homocysteine, a metabolite of folate deficiency, is raised in blood samples and is called homocysteinuria. Hyper homocysteinuria is a predictor of cardiovascular disease and a risk factor for dementia and Alzheimer's disease (LeBlanc et al. 2007; Luchsinger et al. 2007; Storozhenko et al. 2005). In addition, coronary heart disease, osteoporosis, breast and colorectal cancer (Rossi et al. 2011), pancreatic, cervical cancer and leukaemia (Díaz de la Garza et al. 2007; Storozhenko et al. 2005), and hearing loss (Laiño et al. 2012) are related to folate deficiency.

Insufficient folate intakes have been reported among various population groups such as pregnancy-age women in many parts of the world, including developing countries, and some industrialized countries (LeBlanc et al. 2007; Morris and Tangney 2007). The high prevalence of NTD, a result of folate deficiency in pregnant women, obligates the governing bodies of many countries to fortify foods with folic acid. On the other hand, the potential side effects of folic acid fortification are a reason for not adopting folic acid fortification in some countries (Sherwood et al. 2006). For example, over consumption of folic acid (more than 1 mg/day) is not toxic but may cover the appearance of vitamin B₁₂ deficiency (Ray et al. 2003; Storozhenko et al. 2005). The other adverse effects of high intake of chemically synthesized form of folate include change in activity of the hepatic dihydrofolatereductase enzyme (Bailey and Ayling 2009) or promotion of cancer (Baggott et al. 2012; Laiño et al. 2013; Ulrich and Potter 2006). Recently, it has been reported that foetus exposure to excess folic acid results in methylenetetrahydrofolate polymorphism associated with a range of debilitating illnesses (Sweeney et al. 2007). In contrast, natural folate found in foods and produced by

microorganisms does not have the mentioned adverse health effects (Laiño et al. 2013; Wright et al. 2007).

2.3 Biosynthesis and metabolism

Biosynthesis of folate pathway in microorganisms is composed of several parts. The three main structural parts of folate are pteridine, para-aminobenzoic acid (pABA) and glutamate. The pteridine moiety of folate is made from guanosine triphosphate (GTP), which is synthesized in purine biosynthesis pathway (Fig. 2). The reaction of GTP to dihydroneopterin is catalyzed by GTP cyclohydrolase. Then phosphatase removes a phosphate residue. After that, dihydroneopterin aldolase acts on the product to give glycolaldehyde and 6-hydroxymethyl-7,8-dihydropterin, which is converted to 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPPP) by 6-hydroxymethyl-dihydropteridine pyrophosphokinase. Dihydropteroate synthase pairs DHPPP to para-aminobenzoic acid. pABA originates from chorismate in the same pathways involved in aromatic amino acid, glycolysis, pentose phosphate pathway and shikimate pathway. The joining of C-N bond of DHPPP to pABA forms dihydropteroate (DHP). DHP is glutamylated with glutamate taken up from the medium and is then reduced by DHF reductase to the biologically active cofactor tetrahydrofolate (THF). Finally, multiple glutamate moieties are added by polyglutamate folate synthetase to yield THF-polyglutamate (Rossi et al. 2011; Sybesma 2003).

The metabolism of folate happens in several enzymatic pathways; the reductase enzyme reduces the pterin ring in kidney and liver, reaction of polyglutamyl side chain by polyglutamatesynthetase, and adding one-carbon units to certain positions of the pterin ring. The metabolically active forms of folate include 5-methyl-THF, 5-formyl-THF, 10-formyl-THF, 5,10-methylene-THF, 5,10-methenyl-THF and 5-formimino-THF (Mahan et al. 2012).

2.4 Bioavailability

Bioavailability is defined as a fraction of ingested nutrient which is available for metabolic process or storage in the body. While folate is found in foods as monoglutamate and polyglutamate, the polyglutamate form has 60–80% bioavailability compared to monoglutamate. Therefore, the attachment of polyglutamate chain to folate may decrease bioavailability of folate (LeBlanc et al. 2007). However, the γ -glutamyl hydrolase or human conjugase in brush border of the small intestine removes the polyglutamate chain, after which the monoglutamate form can be absorbed (Melse-Boonstra et al. 2004; Sybesma 2003). Compared to synthetic folic acid, naturally occurring dietary folate is only 80% bioavailable (Mahan et al. 2012). The folate-binding protein in milk may increase folate absorption by protecting dietary folate uptake by gut bacteria (Eitenmiller et al. 2007; Forssén et al. 2000; LeBlanc et al. 2007). Furthermore, dietary factors such as alcoholism, chelation and certain drugs may affect intestinal pH and thus folate absorption (LeBlanc et al. 2007). The nutritional status of folate depends on intake from food and supplements with various bioavailabilities. Therefore, the level of folate bioavailability as well as the level of folate polyglutamylation determines the effective intake of folate (Clifford et al. 1991;

Fig. 2 Pathway of folate biosynthesis in lactic acid bacteria (LAB). *GTP* guanosine triphosphate

Gregory III 1989; Melse-Boonstra et al. 2004; Sybesma 2003). It is believed that predominant natural conjugase inhibitors such as anionic species of organic acids (citrate, malate and ascorbate) in food inhibit the action of conjugase and decrease efficient folate uptake in the gastrointestinal tract leading to a decline in folate

bioavailability as opposed to the chemically synthesized folic acid in supplements and fortified foods (Gregory 2001; Sybesma 2003).

2.5 Folate analysis methods

Folate is sensitive to light, oxidation, heat and acids, so an analytical method with high sensitivity is needed. Some analytical techniques used for the determination of folate content are biological, such as microbiological and competitive protein-binding procedures. In spite of sensitivity, these methods lack the specificity to separate different folate forms. Other methods are chemical ones, such as high-performance liquid chromatography (HPLC) method (LeBlanc et al. 2007; Storozhenko et al. 2005; Vahteristo and Finglas 2000).

In the competitive protein-binding assay, folate content is determined through developed colour measurement in microtitration plate which results from competition between sample folate and radio-labelled folate for attaching to folate-binding protein. Despite the speed and convenience of this assay, this method has varied affinity for different forms of folate and is suitable only for foods with the same form of folate. It is less useful for analyzing foods with several types of folate forms and more complex matrices (Arcot and Shrestha 2005; LeBlanc et al. 2007).

As the traditional method of folate analysis, microbiological assay is based on the nutritional requirements of microorganisms, especially *Lactobacillus rhamnosus* (Horne and Patterson 1988), and is used for the analysis of total folate in foods and responds to most native folate. In this assay, the growth of microorganisms is dependent on the amount of folate in the medium; therefore, the rate of microorganism growth is measured by change in solution turbidity, presents the amount of folate. The microbiological method has been considered to be one of the most versatile methods for determining food folate, but it has some limitations such as providing inadequate information on the forms of folate, as well as the fact that it needs experts, and it can also be a tedious procedure (Arcot and Shrestha 2005).

The HPLC method, which separates and detects the different forms based on their chemical structure, is more interesting. The advantages of HPLC are high specificity and reliability, and minimal interference from food enzymes. In the HPLC method, one needs to use all known forms of folate to quantify total folate. Some of them are hard and expensive to detect, which constitutes a potential major limitation. In addition, HPLC is not able to separate folates based on their structure and size at the same time (Arcot and Shrestha 2005; LeBlanc et al. 2007; Lin and Young 2000). However, HPLC has been used to measure folate in several foods including dairy products (Gregory 3rd et al. 1984; Holt et al. 1988).

The known analytical methods to be used for a particular biological material depend on the sample matrix and purpose of the assay. Basically, three main parts of folate analysis in plants and foods include extraction, deconjugation and detection/quantification. Depending on the sample matrix and on the chemical structure of the target compounds, the extraction process can vary considerably. The sample preparation and extraction methods largely affect the amount of extracted folate content, which is then evaluated by a chosen method (Arcot and Shrestha 2005; Vahteristo and Finglas 2000).

3 Natural synthesis of folate by probiotic lactic and propionic acid bacteria

The consumption of live microorganisms called probiotics that provide health benefits for human physiology has become a common practice. According to the common definition accepted by FAO/WHO, probiotics are defined as live microbes which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO 2006). For a strain to be determined as probiotic, it should preferably be of human origin, have a status generally recognized as safe (GRAS), be able to survive through the gastrointestinal tract, adhere to the human intestinal cells, be antagonistic towards potential pathogenic microorganisms and have several health benefit effects on the host (Collins et al. 1998; Iyer et al. 2010). Probiotic bacteria have a positive effect on the immune system and intestinal microbiotic composition and functions and have been claimed to be therapeutic or preventive against infections and several autoimmune and atopic diseases (Collins et al. 1998; Homayouni Rad et al. 2013a, 2013b; Rossi et al. 2011; Schrezenmeir and de Vrese 2001; Singh et al. 2011).

Probiotic microorganisms have been recognized within different bacteria and yeast phylum. Besides *Lactobacillus* and *Bifidobacterium*, species belonging to the genera *Lactococcus*, *Enterococcus*, *Streptococcus* and *Propionibacterium* are currently known to be probiotic microorganisms, conferring a number of health benefits such as vitamin production (Rad AH et al. 2012; Iyer et al. 2010; Rossi et al. 2011; Sanders and Huis 1999).

Recent reports have shown that some probiotic microorganisms have the potential ability to synthesize folate (Table 1). It is reported by Sybesma et al. that *Lactococcus lactis* has a great potential to produce high amounts of folate (Sybesma et al. 2003; Sybesma 2003). Other researchers have also reported that many strains of *Bifidobacterium* were able to produce folate. Several folate-producing *Bifidobacterium* strains have been investigated, but the produced folate does not seem to be widespread within the genus strains of *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *Bifidobacterium longum*, and *Bifidobacterium adolescentis* cultured in a folate-free semisynthetic medium. Most of bifidobacteria belonging to the species *B. adolescentis* and *Bifidobacterium pseudocatenulatum* have a high potential to produce folate, but other strains produce less folate (Pompei et al. 2007; Rossi et al. 2011). In another study, *B. longum* strains cultured in reconstituted skim milk (RSM) had higher levels of folate than other tested species as shown in Table 1 (Lin and Young 2000).

Among the *Lactobacillus* species, only *Lactobacillus plantarum* can produce folate, whereas other species were shown to consume folate during growth (Sybesma et al. 2003). However, several studies have reported that different kinds of *Lactobacillus* can be included on the list of folate-producing bacteria (Hugenschmidt et al. 2010). These properties depend on many factors such as the strain of bacteria, culturing media, incubation time and others (Crittenden et al. 2003; Laiño et al. 2012; Lin and Young 2000; Nor et al. 2010; Sybesma et al. 2003). Propionibacteria, other probiotic bacteria, have been demonstrated as folate-producing bacteria, able to produce vitamin B₁₂ and stimulate growth of *L. plantarum*. Their other properties include inhibition of undesirable microflora in fermented foods via releasing of organic acids and bacteriocins. They were shown to modulate the colon flora both in animals and humans beneficially, by enhancing the indigenous bifidobacteria population (Bougle et al. 1999; Chaia et al. 1999; Hugenholtz et al. 2002). Hugenschmidt et al. showed that optimized culture

Table 1 Folate production by lactic and propionic acid bacteria in different culture conditions

Microbial species	Folate ($\mu\text{g}\cdot\text{L}^{-1}$)	Folate derivatives	Analytical method	Culture media	Incubation condition	Ref
<i>Lactobacillus</i> species						
<i>L. acidophilus</i> *	1 ^a	–	MA	MRS medium	37 °C	(W. Sybesma et al. 2003)
<i>L. brevis</i> *	31–43 ^{ac}	THF 5-MeTHF 5-FmTHF	HPLC	Fermented milk	37 °C, 6 h	(Lin and Young 2000)
<i>L. bulgaricus</i>	54–131 ^b	–	MA	SWP	30 °C, 1–2 days anaerobic	(Hugenschmidt et al. 2010)
	54 ^a	–	MA	MRS medium	37 °C	(W. Sybesma et al. 2003)
	40–46 ^{ac}	THF 5-MeTHF 5-FmTHF	HPLC	Fermented milk	37 °C, 6 h	(Lin and Young 2000)
	70–75 ^b	–	MA	Fermented milk	37 °C, 16 h	(Laiño et al. 2012)
<i>L. casei</i> *	–13 ^a	–	MA	MRS medium	37 °C	(W. Sybesma et al. 2003)
<i>L. fermentum</i> *	2–10 ^a	–	MA	Fermented milk	37 °C, 24–48 h	(Crittenden et al. 2003)
	84 ± 32 ^b	–	MA	SWP	37 °C, 1–2 days anaerobic	(Hugenschmidt et al. 2010)
<i>L. helveticus</i> *	2–89 ^a	–	MA	MRS medium	37 °C	(W. Sybesma et al. 2003)
<i>L. johnsonii</i> *	4–10 ^a	–	MA	Fermented milk	37 °C, 24–48 h	(Crittenden et al. 2003)
<i>L. plantarum</i> *	45 ^{ac}	–	MA	MRS medium	37 °C	(W. Sybesma et al. 2003)
	397 ± 60 ^b	–	MA	SWP	30 °C, 1–2 days anaerobic	(Hugenschmidt et al. 2010)
	8399 ± 784 ^b	–	MA-HPLC	SWP ^d	33 °C, 3 days anaerobic/4 days aerobic ^h	(Hugenschmidt, et al. 2011)
	4413 ± 248 ^{bc}	5-FmTHF	MA-HPLC	SWP ^d	33 °C, 3 days anaerobic/4 days aerobic	(Hugenschmidt et al. 2011)
<i>L. reuteri</i> *	0–10 ^a	–	MA	Fermented milk	37 °C, 24–48 h	(Crittenden et al. 2003)
	125 ± 28 ^b	–	MA	SWP	37 °C, 1–2 days anaerobic	(Hugenschmidt et al. 2010)
<i>L. rhamnosus</i>	–63 ^{ac}	–	MA	MRS medium	37 °C	(W. Sybesma et al. 2003)
<i>Lactococcus lactis</i> subspecies						
<i>L. lactis</i> subsp. <i>cremoris</i> *	92–116 ^{ac}	5,10-methenyl THF 10-FmTHF	MA-HPLC	M17 medium	30 °C	(W. Sybesma et al. 2003)
<i>L. lactis</i> subsp. <i>lactis</i> *	57–291 ^{ac}	5,10-methenyl THF 10-FmTHF	MA-HPLC	M17 medium	30 °C	(W. Sybesma et al. 2003)

Table 1 (continued)

Microbial species	Folate ($\mu\text{g.L}^{-1}$)	Folate derivatives	Analytical method	Culture media	Incubation condition	Ref
<i>Lc. lactis</i> subsp. <i>lactis</i> biovar diacetylactis*	79–100 ^{ac}	5,10-methenyl THF 10-FmTHF	MA-HPLC	M17 medium	30 °C	(W. Sybesma et al. 2003)
<i>Enterococcus</i> species						
<i>E. faecium</i> *	18–28 ^a	–	MA	Fermented milk	37 °C, 24–48 h	(Crittenden et al. 2003)
<i>Streptococcus</i> species						
<i>S. thermophilus</i>	29–202 ^{ac}	5,10-methenyl THF 5-FmTHF	MA-HPLC	M17 medium	37 °C	(W. Sybesma et al. 2003)
	536 ^{ac}	–	MA	Modified M17 ^e	37 °C ^f	(W. Sybesma et al. 2003)
	24–36 ^c	THF 5-MeTHF 5-FmTHF	HPLC	Fermented milk	37 °C, 6 h	(Lin and Young 2000)
	37 ^c	5-MeTHF	HPLC	Fermented milk	37 °C, 12 h	(Holasova et al. 2005)
<i>Leuconostoc</i> species						
<i>Leuconostoc lactis</i>	45 ^{ac}	–	MA	MRS medium	37 °C	(W. Sybesma et al. 2003)
<i>Leuconostoc paramesenteroides</i>	44 ^{ac}	–	MA	MRS medium	37 °C	(W. Sybesma et al. 2003)
<i>Propionibacterium</i> species						
<i>P. thoenii</i> *	36 ^a	–	MA	–	–	(Hugenholtz et al. 2002)
<i>P. acidipropionici</i> *	36 ^a	–	MA	–	–	(Hugenholtz et al. 2002)
<i>P. jensenii</i> *	40 ^a	–	MA	–	–	(Hugenholtz et al. 2002)
<i>P. freudenreichii</i> ssp. <i>Shermanii</i> *	17–78 ^a	–	MA	–	–	(Hugenholtz et al. 2002)
<i>Propionibacterium</i> sp.*	9–29 ^a	–	MA	–	–	(Hugenholtz et al. 2002)
<i>Bifidus</i> species						
<i>B. adulescentis</i> *	1–65 ^b	–	MA	SM ^g	37 °C, 48 h	(Crittenden et al. 2003)
<i>B. animalis</i> *	26 ^b	–	MA	SM	37 °C, 48 h	(Crittenden et al. 2003)
	15–25 ^a	–	MA	Fermented milk	37 °C, 24–48 h	(Crittenden et al. 2003)
<i>B. bifidum</i> *	0.6 ^b	–	MA	SM	37 °C, 48 h	(Crittenden et al. 2003)
<i>B. breve</i> *	1–3 ^b	–	MA	SM	37 °C, 48 h	(Crittenden et al. 2003)

Table 1 (continued)

Microbial species	Folate ($\mu\text{g.L}^{-1}$)	Folate derivatives	Analytical method	Culture media	Incubation condition	Ref
<i>B. dentium</i> [*]	40–55 ^a	–	MA	Fermented milk	37 °C, 24–48 h	(Crittenden et al. 2003)
<i>B. infantis</i> [*]	29 ^b	–	MA	SM	37 °C, 48 h	(Crittenden et al. 2003)
<i>B. infantis</i> [*]	27 ^b	–	MA	SM	37 °C, 48 h	(Crittenden et al. 2003)
<i>B. infantis</i> [*]	25–45 ^a	–	MA	Fermented milk	37 °C, 24–48 h	(Crittenden et al. 2003)
<i>B. longum</i> [*]	69–103 ^{ac}	THF 5-MeTHF 5-FmTHF	HPLC	Fermented milk	37 °C, 6 h	(Lin & Young 2000)
	9–12 ^c	5-MeTHF	HPLC	Fermented milk		(Holasoova et al. 2005)
<i>B. lactis</i> [*]	20–30 ^a	–	MA	Fermented milk	37 °C, 24–48 h	(Crittenden et al. 2003)
<i>B. pseudocatenulatum</i> [*]	12–82 ^b	–	MA	SM	37 °C, 48 h	(Pompei et al. 2007)

MA microbiological assay, HPLC high-performance liquid chromatography, THF tetrahydrofolate, 5-MeTHF 5-methyltetrahydrofolate, 5-FmTHF 5-formyltetrahydrofolate, SWP supplemented whey permeate

^a Total folate
^b Extracellular folate
^c HPLC analysis
^d Whey permeate medium supplemented with yeast extract, with 5 mg.L⁻¹ cobalt chloride, 15 mg.L⁻¹ dimethylbenzimidazole (DMBI) and 10 mg.L⁻¹ para-aminobenzoic acid (pABA)
^e Potassium phosphate instead of β -glycerophosphate
^f pH6.5, adding of 0.5% lactose
^g Folate-free synthetic medium (SM)
^h Co-culturing with *Propionibacterium freudenreichii*
–, not reported
^{*}, probiotic strains

media accompanied with *Propionibacterium freudenreichii* stimulated *L. plantarum* and led to ten times higher increases in folate levels than maximum values reported for natural fermentations (Hugenschmidt et al. 2011).

4 Folate production in milk and yoghurt

Milk is not a rich source of dietary folate, but fermented dairy products such as yoghurt processed by starter culture are regarded as important dietary sources of folate (Kneifel et al. 1989; LeBlanc et al. 2007). The ability of yoghurt starter cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) to produce folate is very variable and depends on strains (Alm 1982; Crittenden et al. 2003; Kneifel et al. 1989, 1992; Laiño et al. 2013). It is reported that *L. bulgaricus* are folate consumers (Kneifel et al. 1991; Laiño et al. 2012, 2013; Rao et al. 1984). However, certain strains of *L. bulgaricus* are able to produce folate when grown in a folate-free medium and in milk (Laiño et al. 2012, 2013). Against various data regarding the synthesis of this vitamin by *Lactobacillus* species, it was demonstrated that *L. plantarum* has the ability to produce high levels of folate, when grown in an optimized medium, as shown in Table 1 (Hugenschmidt et al. 2011). Most previous and recent studies based on HPLC method have indicated that 5-methyl-tetrahydrofolate (5-methyl-THF) is the major form of folate in milk and yoghurt. Based on the three main folate analysis methods, HPLC, radio protein binding and microbiological assays, the same ranges of folate have been detected in cow's milk (50–100 $\mu\text{g}\cdot\text{L}^{-1}$), while its concentration may be increased to more than 200 $\mu\text{g}\cdot\text{L}^{-1}$ depending on applied starter cultures and storage conditions (Hugenschmidt et al. 2011; Laiño et al. 2014).

In this line, optimal combination of *S. thermophilus* and *L. bulgaricus* strains results in organoleptically desirable yoghurt with increased folate levels. Therefore, using different starters may be an explanation for folate content and variations in its form (Forssén et al. 2000; Holasova et al. 2005).

It is reported that the use of folate-producing probiotic bacteria in combination with *S. thermophilus* and/or *L. bulgaricus* provides the largest increase in folate during the fermentation process of probiotic yoghurt compared to original milk and conventional fermented milk (Crittenden et al. 2003; Jägerstad et al. 2005; Rossi et al. 2011).

5 Conclusions

This review has concluded that bio-fortification of folate in yoghurts and fermented milks is possible through proper selection of the microbial strains of probiotics and cultivation conditions. It is believed that bio-fortified fermented milks containing elevated levels of natural folate with improved stability and bioavailability would be a good source of folate. Based on the fact that traditional or commercial yoghurts cannot meet at least 10–20% of daily recommended intake as well as there are no commercial yoghurts or any other fermented dairy products naturally fortified with folate, there is an opportunity for food industry settings to take the next step and use this information for selecting folate-producing strains as part of their starter cultures in order to produce fermented products with elevated levels of this essential vitamin. This

attempt is a cost-effective move by food manufacturers since increased “natural” folate concentrations would be an important value-added effect without increasing production costs. The proper selection of probiotic folate-producing strains provides a strategy for the development of novel and functional foods with increased nutritional values.

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