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Abstract

Folate is an essential micronutrient involved in numerous vital biological reactions. The dietary consumption of naturally occurring vitamin B9 is often inadequate in many countries, and supplementation or fortification programs (using synthetic folic acid) are implemented to alleviate folate deficiency. Other food-based alternatives are possible, such as the use of lactic acid bacteria (LAB) to synthesize folate during fermentation. Many studies have been conducted on this topic, and promising results were reported for some fermented dairy products. However, in other studies, folate consumption by LAB or rather low folate production were observed, resulting in fermented foods that may not significantly contribute to the recommended B9 intake. In addition, the optimum conditions for folate biosynthesis by LAB are still not clear. The aim of this review was thus to (i) clarify the ability of LAB to produce folate in food products, (ii) check if the production of folate by LAB in various fermented foods is sufficient to meet human vitamin B9 requirements and (iii) suggest ways to optimize folate production by LAB in fermented food products.

1- Introduction

Folate, or the B9 vitamin family, is involved in essential functions such as amino acid metabolism and DNA replication and repair, and is thus essential for cell division (Ball, 2005). For example, some vitamin B9 forms (called vitamers) are involved in one-carbon transfer reactions that lead to the synthesis of adenine, guanine and thymine, three essential constituents of DNA. As humans are unable to synthesize folate de novo, too low intake of these vitamins leads to folate deficiency. In humans, among the diseases caused by folate deficiency, the most studied are megaloblastic anaemia, as well as congenital malformations, including neural tube defects (NTD) (FAO/WHO, 2005; Metz, 1963). Folate deficiency remains a health problem worldwide, and has been recorded in many countries on all continents, and in different nutritional contexts, both in developing and developed countries, and in different age groups (McLean et al., 2008; Viñas et al., 2011; Youngblood et al., 2013).

Folate (Figure 1) is composed of a pteridine moiety joined by a methylene bridge to para-aminobenzoic acid and linked to one or more molecules of L-glutamic acid (Ball, 2005). There are many forms of vitamin B9, called vitamers. Folic acid, the synthetic form of B9 vitamin, has only one glutamate residue, whereas the naturally occurring forms generally comprise a polyglutamate chain. Moreover, folic acid exhibits a fully oxidized pteridine ring, while the other vitamers are generally either partially reduced (at the 7,8-position) in the case of dihydrofolate forms, or fully reduced (at the 5,6,7,8-position) in the case of tetrahydrofolate compounds. The vitamers can also be substituted, either at the N5 position by formyl, methyl or formimino groups, or at the N10 position by a formyl group (Blakley, 1987). The N5 and N10 positions can also be bridged by methylene or methenyl groups.

The main strategies used to address the problem of vitamin deficiencies are (i) supplementation, (ii) food fortification, and (iii) dietary diversification (Bhutta et al., 2013). Daily folic acid supplementation in pregnant women is recommended to reduce the risk of low birth weight, maternal anemia and NTD (WHO, 2012). In fortification programs, folic acid is added to wheat and/or maize flour at the industrial level to increase the folate intake of the whole population of a country or region to prevent folate deficiency. In 2015, flour fortification with folic acid was mandatory in 63 countries and authorized in four countries (Food Fortification Initiative, 2015). It has been estimated that folic acid consumption in appropriate amounts in the fortified products could prevent up to 75% of the most common NTD: spina bifida and anencephaly (Bell and Oakley, 2009). However, food fortification programs are not always effective as Youngblood et al. (2013) estimated that currently, less than 25.5% of the folic acid preventable NTD are actually prevented. In addition, it has been shown that the absorption of high amounts of folic acid can mask the symptoms of vitamin B12 deficiency, which may result in the progression of neuropathy to an irreversible point (FAO/WHO, 2005).

Dietary diversification, by promoting the consumption of folate-rich foods such as liver and green leafy vegetables, is a possible sustainable way of preventing folate deficiency (FAO/WHO, 2005; Souci et al., 2000; USDA, 2015).. However, folate-rich foods are not always available, depending on the season, and on the geographic, agro-ecological and socio-economic context. In these cases, populations at risk, e.g. young children and pregnant women, may not be able to meet their folate requirements through their daily food intake (FAO/WHO, 2005). Folate deficiency thus still occurs frequently in many countries and sustainable solutions are needed.

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In this context, the use of folate producing microorganisms to increase the B9 vitamin content of fermented foods could be an interesting approach. Different studies suggest that the folate content of food products could be increased by *in situ* fortification through fermentation (LeBlanc et al., 2008, 2011; Moslehi-Jenabian et al., 2010). Fermented foods are consumed in many countries worldwide (Campbell-Platt, 1994). Numerous microorganisms are involved in the traditional fermentation processes, including lactic acid bacteria (LAB) and yeast (Beuchat, 1997). Yeasts are well known for their ability to produce folate during food fermentation (Moslehi-Jenabian et al., 2010), but the folate production capacity of LAB is not yet clear, as both folate production and folate consumption have been observed in culture media and in fermented foods (LeBlanc et al., 2008). Nevertheless, several studies have shown that the folate content of different food products was increased after fermentation by LAB selected for their folate biosynthesis capacities (Gangadharan and Nampoothiri, 2011; Holasova et al., 2004; Jägerstad et al., 2004; Kariluoto et al., 2006a). However, most of the published studies focused on the lactic acid fermentation of dairy products, and only a few of them investigated if the observed increases in vitamin B9 significantly contribute to the satisfaction of folate requirements.

In this review, we discuss the occurrence of folate in food products, the stability of folate vitamers during food processing, and their bioavailability. Next, we focus on the production of folate by LAB and (i) draw up a list of LAB strains able to produce folate in culture media and/or fermented foods, (ii) estimate the possible nutritional benefits of the consumption of fermented foods enriched in folate by LAB, in terms of contribution to the satisfaction of folate

requirements, and (iii) suggest which parameters could be adjusted to improve folate production by LAB in fermented foods.

2- Folate: essential but sensitive molecules

2-1- Folate requirements

Recommendations for folate intakes are given in Table 1, based on the folate requirements of different age groups. Two values can be used for each age group, the Estimated Average Requirement (EAR) and the Recommended Nutrient Intake (RNI). EAR corresponds to the daily intake necessary to cover the needs of 50% of the people in the age group concerned. RNI corresponds to the daily intake necessary to cover the needs of 97.5% of the people in the age group concerned. An intake of 400 µg of folate per day would satisfy the folate requirements of most of the population. However, 400 µg is not enough to cover the needs of pregnant women, who represent one of the main populations at risk. Yet, adequate folate intake is particularly important during pregnancy, as folate deficiency has a high negative impact during the fetal development stage, when the cell multiplication is high (FAO/WHO, 2005). Therefore, the value of 600 µg/day, which is the RNI for pregnant women, is used in the rest of this review as a reference for the calculation of folate requirements.

To our knowledge, no global data are available on the prevalence of folate deficiency worldwide, although the deficiency has been detected in countries as different as Burkina Faso, Guatemala, Iran, Ireland, Thailand and United States (Arsenault et al., 2014; Assantachai and Lekhakula, 2007; Brunst et al., 2014; Harrington et al., 2008; Rosenthal et al., 2015; Sedehi, 2013). Folate deficiency affects different age groups depending on the country (McLean, 2008). In rural Burkina Faso, a study recorded the food consumption of 36-59 month-old children and their

mothers using the quantitative 24 h recall method, and estimated that the probability of folate adequacy in these populations varied with the season. The median probability of adequate folate intake was zero for women in both the lean and post-harvest seasons, and for children during the lean season, but the median probability of adequate folate intake rose to 0.98 in children in the post-harvest season (Arsenault et al., 2014). Folate intake in men and women in Ireland was assessed through a food frequency questionnaire and showed that 40% of the Irish population is below the recommended dietary allowance for folate (Harrington et al., 2008). In an urban US population, Brunst et al. (2014) estimated that 16% of pregnant women have inadequate folate intake. Analyses of the serum level of folate in women of childbearing age showed that 5.1% of women in Guatemala and around 30% of women in Iran had folate deficiency (Rosenthal et al., 2015; Sedehi, 2013). In Thailand, Assantachai and Lekhakula (2007) evaluated the serum level of folate of subjects aged 60 years and over, and detected folate deficiency in 39% of this population group. All these studies underline the fact that folate deficiency is still a global public health problem that affects different age categories.

2-2- Dietary sources of folate

It is generally considered that the following foods are the best dietary sources of folate: liver (136-963 µg/100 g FM), green leafy vegetables (88-187 µg/100 g FM), other green vegetables such as asparagus (108 µg/100 g FM), Brussels sprouts (101 µg/100 g FM) and broccoli (114 µg/100 g FM), legumes and pulses (140-540 µg/100 g non-processed FM), nuts (39-169 µg/100 g FM), cereal-based foods (26-170 µg/100 g FM for wholegrain products), and some fruits including oranges (27-42 µg/100 g FM), strawberries (36-65 µg/100 g FM) and cherries (52-75 µg/100 g FM) (FAO/WHO, 2005; Souci et al., 2000; USDA, 2015). In the case of cereals, folate

is located in the outer layers and in the germ, which explains why refined flours have a much lower concentration (10-17 µg/100 g FM) than wholegrain products (Pomeranz, 1988; Souci et al., 2000). However, food products with low folate content but that are frequently consumed may be better dietary sources of folate than some folate-rich foods that are rarely consumed. Consequently to assess whether a food is a good dietary source of folate, not only its folate content but also its consumption frequency should be taken into account. Table 2 presents theoretical values of folate intake (µg/capita/day); these were calculated taking into account the folate content of various food products (µg/100 g FM) and their average supply worldwide in the period 2001-2011 (g/capita/day).

The food supply data in Table 2 suggest that cereals are the most frequently consumed food group, which can be explained by the high consumption of wheat and rice worldwide. Vegetables are the second most available food category, followed by dairy products, fruits, starchy roots and tubers, and meat. The highest folate content is found in offal, especially in liver, the organ in which folate is stored. With the exception of offal, the folate content of meat products is generally less than 20 µg/100 g FM. Pulses and green leafy vegetables have a high folate content, generally between 80 and 400 µg/100 g FM. In the other food categories, folate content is generally between 1 and 100 µg/100 g FM. Table 2 shows that the food categories with the highest folate content are not those that contribute the most to theoretical folate intake. For example, even if offal and pulses have a high folate content, their theoretical folate intake values are similar to those of other foods, such as rice and maize products, whose folate content is lower. This is explained by the more frequent consumption of cereal products than of pulses and offal worldwide.

According to the U.S. Food and Drug Administration, a food can be considered as a good source of folate if it provides at least 10% of the RDA, which is equivalent to the RNI (FAO/WHO, 2005; FDA, 2016). In this review, we use the value 60 µg/100 g FM as a reference, which corresponds to 10% of the folate requirements of pregnant women. According to theoretical folate intake values, only some wheat products, vegetables and dairy products supply more than 60 µg of folate/capita/day. However, for wheat products, if we exclude wholegrain products (87 µg of folate/100 g FM), the maximum theoretical folate intake is only 38 µg of folate/capita/day. LeBlanc et al. (2008) already showed that the consumption of some fermented dairy products could cover more than 10% of the folate requirements of pregnant women. Although green leafy vegetables are considered as one of the best sources of folate, the folate intake from this food category cannot be calculated because of the lack of consumption data.

Even if global data help understand the contribution of different food products to folate consumption, they may not reflect reality, because diets differ considerably from one region to another. The nutritional context needs to be taken into account to determine the main dietary sources of folate in any given area. Figure 2 shows the availability of the most consumed food categories in Europe and Africa for the period 2001-2011 (FAOSTAT, 2015). It shows that, in Africa, cereal-based foods (409g/capita/day) are 1 to 9 fold more available than the other food categories, so cereal products may be the main source of folate in Africa. In contrast, in Europe, dairy products (593g/capita/day) are 1 to 3 fold more available than other food categories, and so could greatly contribute to folate intakes, at least in some countries.

A large proportion of dairy products and cereal-based foods are consumed as fermented foods (Blandino et al., 2003; Campbell-Platt, 1994; Guyot, 2012; Nout, 2009; Oyewole, 1997). For

example, in some European countries, fermented dairy products (cheese, yoghurt and other fermented milk products) can represent up to 43% of total dairy consumption (160 to 478 g/day) for adults (Hjartaker et al., 2002). Some studies showed that, the consumption of a portion (225 mL) of some dairy products fermented by LAB could contribute to 10-15% of the RDA for adults (Laiño et al., 2013, 2014). However, depending on the LAB involved in the fermentation, some fermented dairy products could have lower folate content and consequently contribute to less than 10% of the RDA for adults (Laiño et al., 2012). Some fermented dairy products could contribute to only 2% of the recommended dietary intake for women of child-bearing age (Crittenden et al., 2003). In Burkina Faso, it has been estimated that 31% of the population consumes pearl-millet based fermented porridge every day (Mouquet-Rivier et al., 2008). According to Kariluoto (2008), cereal products, mostly rye bread contribute more than one third of daily folate intake in Finland. Many studies suggest that fermented foods contain more folate than the original raw material (Ekıncı, 2005; Forssén et al., 2000; Murdock and Fields, 1984). For that reason, fermented dairy products could be interesting sources of folate in Europe, and cereal based fermented foods in Africa. However, other processing steps can cause folate losses, due to folate instability, or to the removal of the outer layers of cereal grains.

2-3- Folate stability and the influence of food processing

Different factors can cause interconversion and/or degradation of folate, which can lead to irreversible loss of vitamin activity. This is the case of temperature, exposure to oxidizing agents, unfavorable pH, exposure to UV and light, and interaction with other food components such as metal cations. Both oxidized and reduced folate forms are sensitive to degradation by UV (Strandler et al., 2015). Figure 3 summarizes the effects of these factors on folate vitamers.

These effects can occur during food processing and food storage. In non-fortified foods, folic acid is a degradation product of H_4folate, but it is the most stable vitamer with 10-HCO-folic acid (Ball, 2005; Strandler et al., 2015). Reduced one-carbon-substituted folate vitamers are relatively stable, especially, folate vitamers substituted at the N5 position (Strandler et al., 2015). This could be due to "steric hindrance in restricting the access of oxygen or other oxidants to the pteridine ring" (Strandler et al., 2015). On the contrary, reduced unsubstituted folate, whose pteridine ring is not protected, is more easily oxidized. Many folate vitamers are pH-sensitive. For example, the lowest stability of reduced unsubstituted vitamers is between pH 4 and 6, whereas folic acid is stable under neutral and alkaline conditions but less stable at pH below 5 (Gregory, 1989). At acid pH, 5-CHO-H_4folate and 10-CHO-H_4folate are cyclized to form 5,10-CH-H_4folate (Tabor and Wyngarden, 1959).

As folate vitamers are not all equally stable, folate losses may differ in two food products exposed to the same factors, depending on the composition of the folate vitamers. In fact, the composition of folate vitamers differs from one food category to another (Table 3). In all food categories, the most common vitamer is generally 5-CH_3-H_4folate, and to a lesser extent 5-CHO-H_4folate, 10-CHO-folic acid and H_4folate. For example, H_4folate is unstable when exposed to high temperatures (Wilson and Horne, 1983). As H_4folate is the main vitamer in some pulses and in liver, major losses of folate may occur during cooking.

Table 4 lists some examples of changes in folate content during different processing steps. When heating is involved, non-negligible folate losses can occur especially in the case of hydrothermal cooking, where vitamins are leached into the cooking water (Eitenmiller and Laden, 1999). The degree of fragmentation of the food products is also important: the larger the ratio of surface area

to volume, the greater the folate loss due to leaching (Czarnowska and Gujska, 2012). Oxidation can also occur when food products are exposed to oxygen and oxidizing agents, for example during storage over a period of several months (Sotiriadis and Hoskins, 1982). To prevent oxidation, folate can be protected by antioxidants that are either naturally present in some food products or added during processing, such as ascorbic acid. For example, Sotiriadis and Hoskins (1982) showed that the addition of ascorbic acid to canned processed foods significantly limited folate loss during a six-month storage period. Exposure to light can also occur during most processing steps. It has been shown that the loss of folic acid in juices stored in the light was significantly higher than in those stored in the dark (Frommherz et al., 2014). However, some compounds naturally present in foods, such as β-lactoglobulin, bovine serum albumin and α-lactalbumin can delay the photodecomposition of folate (Liang et al., 2013). The food matrix can also play a positive role by entrapping the vitamins and protecting them from exposure to oxidizing agents and light. Conversely, the fractionation of food products, such as cereal grains, may lead to a higher exposition of folate to oxidizing agents and light and to an increase in folate loss in the following processing steps. Moreover, the folate-rich fractions (bran and germ) may be removed during cereal processing, thereby reducing folate content (Monks et al., 2013), whereas other forms of processing make it possible to obtain the folate-rich fractions from wheat bran (Hemery et al., 2011).

Other processing steps can positively influence the folate content of food products. Folate is produced by plant cells during the germination of cereals and pulses (Hefni and Witthöft, 2011; Hefni et al., 2015). A modification in the composition of vitamers may also occur during germination, even though $5\text{-}CH_3\text{-}H_4\text{folate}$ is usually predominant (Koehler et al., 2007; Rychlik

and Adam, 2008). During the fermentation of food products (Table 4), the production of vitamins by yeasts and some bacteria can multiply the folate content up to 7 fold, but folate is consumed by other bacteria (LeBlanc et al., 2008; Moslehi-Jenabian et al., 2010). However, fermented foods have an acid pH, and most folate vitamers are less stable at acid pH, especially during heating or in presence of metal cations. As a result, the folate produced by microorganisms - especially reduced unsubstituted folate - might be degraded in a cooking step following fermentation.

2-4- Bioavailability in foods

Folate bioaccessibility is defined as "the fraction that is released from food matrix and is available for intestinal absorption", and folate bioavailability as "the fraction of the ingested nutrient that is available for utilization in normal physiologic functions and for storage" (Gregory III et al., 2005). Folate is absorbed by intestinal epithelial cells, mainly in the jejunum. Naturally occurring folate forms are generally folylpolyglutamyl folate, which has to be deconjugated into monoglutamyl forms before absorption (Ball, 2005). The deconjugation of folylpolyglutamate is controlled by the intestinal brush-border enzyme glutamate carboxypeptidase II (GCPII; EC 3.4.17.21), which is stable at pH 6.5 (Chandler et al., 1986; KEGG, 2014). Folate is mainly absorbed by transporters, even if in the case of folic acid, diffusion is also possible when the transporters are saturated (Kelly et al., 1997; Zhao et al., 2011). According to Reisenauer and Halsted (1987), human intestinal brush-border conjugase is generally present in a sufficient amount and is therefore not a limiting factor for folate absorption. Folate could also be synthesized by some bacteria present in the large intestine, mainly in the form of monoglutamyl folate, and then absorbed in the colon and metabolized (Rong et al., 1991).

Eighty-five percent of folic acid is considered to be bioavailable in a food matrix (FAO/WHO, 2005). Previous studies have reported that the bioavailability rates of naturally occurring vitamers are 30% to 98% that of folic acid (used as reference) (Brouwer et al., 1999; Hannon-Fletcher et al., 2004). However, Gregory (2012) and McNulty and Pentieva (2004) emphasized that the bioavailability of folate is a complex topic, and that the variability previously reported for the different vitamers may be due to (i) the use of animal bioassays on rodents, which may not suffice for the prediction of dietary folate bioavailability in humans due to the differences in the intestinal deglutamylation mechanism and in the enzymes involved, and (ii) the properties of the food matrices, as listed below.

The entrapment of folylpolyglutamates in the food matrix can protect folate against degradation, but also limit folate bioaccessibility, as shown in the study of Van het Hof et al. (1999), who demonstrated that the consumption of minced vegetables significantly increased the plasma response to folate, in comparison to non-minced vegetables. The consumption of acid food products could thus reduce absorption of folate, due to incomplete intestinal deconjugation, by lowering the pH of the jejunum content below the optimum pH for the GCPII enzyme. For example, Tamura et al. (1976) observed that the bioavailability of folylpolyglutamates was lower than that of folic acid in orange juice at pH 3.7, or in aqueous solutions with citric acid at the same pH. Conversely, when the orange juice was neutralized to pH 6.4, the bioavailability of folylpolyglutamates did not significantly differ from that of folic acid. However, some naturally occurring folate vitamers are unstable at low pH (Figure 3), and degradation of these vitamers could also explain the lower bioavailability observed at pH 3.7. Many studies have shown that folate bioavailability can also be reduced or increased by some food components, such as metal

cations (Ghishan et al., 1986). For example, at approaching neutral (pH 6.4) and mildly acid (pH 3.5) conditions, which can occur under normal physiological conditions, folate can form a complex with zinc (Lucock et al., 1994). As reviewed by Ball (2005), many food compounds are suspected to exert a negative influence on folate bioavailability by inhibiting conjugase activity. However, the conjugase activity in many raw foods of both plant and animal origin, such as asparagus and chicken offal, can positively influence folate bioavailability by allowing the conversion of folylpolyglutamates into monoglutamates within the food matrix (Leichter et al., 1979; Saini and Rosenberg, 1974).

3- Folate production by lactic acid bacteria

In the previous section, we showed that the dietary supply of folate is not always adequate. Dairy and cereal products appear to be good sources of folate in Europe and Africa, respectively. These food products are often fermented by LAB alone or in combination with other microorganisms before consumption. In the following section, we discuss the potential of LAB to increase folate content of fermented food products. In this review, Bifidobacteria are considered as LAB.

3-1- Biosynthetic pathway

The general biosynthetic pathway of folate with the indication of the enzymes present in *Lactobacillus plantarum* WCFS1 is depicted in Figure 4. This pathway is similar to that of plants and other microorganisms including yeasts. According to other data from the KEGG (2014) database, almost all LABs are unable to produce folate because some genes coding for enzymes involved in folate biosynthesis are lacking.

There are 17 enzymatic reactions involved in the common folate *de novo* biosynthesis pathway, or 45 if the synthesis of folate vitamers from dihydrofolate is included (KEGG, 2014). From this

pathway, three molecules that can be found in food products are generally considered as precursors: para-aminobenzoic acid (PABA), guanosine triphosphate (GTP) and glutamate. The folate biosynthetic pathway corresponds to three steps: (i) the conversion of the D-erythrose-4-phosphate or the phosphoenolpyruvate into PABA, (ii) the conversion of GTP into 2-amino-4-hydroxy-6hydroxymethyl-7,8-dihydropteridine-P2 (pteridine moiety) and (iii) the linkage of the pteridine moiety, PABA and glutamate units.

According to Figure 4, *L. plantarum* WCFS1, a well-known folate producing strain, does not have all the genes involved in the folate biosynthesis pathway. In the first step leading to the synthesis of PABA, this strain and other LAB from different species generally have all the genes involved in the synthesis of PABA, except two genes coding for enzymes involved in the synthesis of 4-amino-4-deoxychorismate and PABA from chorismate (KEGG, 2014). These two genes have been described in *Bifidobacterium adolescentis* ATCC 15703: *pabA* (EC 2.6.1.85) and *pabC* (EC 4.1.3.38). Two other LAB (*L. fermentum* IFO 3956 and *L. reuteri* JCM 1112) able to produce folate *de novo* possess neither these two genes nor orthologs of them, suggesting that an alternative pathway for PABA biosynthesis exists in these strains (Kuratsu et al., 2010; Satoh et al., 2014). Moreover, in *L. plantarum* WCFS1 and other LAB genera, at least one gene coding for an enzyme involved in the second step is missing (i.e. for the synthesis of dihydroneopteridine phosphate and dihydroneopteridine from 7,8- dihydroneopteridine 3'-triphosphate). These reactions may be catalyzed by non-specific phosphatases that can be synthesized by LAB, and especially by *L. plantarum* (Zamudio et al., 2001). The genes involved in the third step are generally found in all LAB (KEGG, 2014). In addition, it has been shown that the two proteins FolK (EC 2.7.6.3) and FolP (2.5.1.15) can be used as signature proteins for

folate synthesis, since they were detected in 13 LAB able to produce *de novo* folate, but not in 18 LAB dependent on folate salvage (de Crecy-Lagard et al., 2007).

Considering these genetic data, it is likely that if precursors are available in the culture medium or in the food, many LAB could produce folate. These results thus suggest that the potential of LAB to produce folate has probably been underestimated.

3-2- Lactic acid bacteria able to produce folate in culture medium

Interest in the potential use of LAB to produce folate in food products significantly increased after 2000 (Holasova et al., 2004; Lin and Young, 2000a, 2000b). In culture media, the strains belonging to the genus *Lactobacillus* and *Bifidobacterium* were the most investigated. The focus on these genus may be due (i) to the occurrence of Lactobacilli in a wide variety of foods (Hugenschmidt et al., 2010), and (ii) to the fact that Bifidobacteria are often considered as probiotics and may be able to produce folate in the colon (Pompei et al., 2007). Table 5 lists all the LAB species whose ability to produce folate in a culture medium has been tested. They were isolated from different sources including dairy products, vegetables and cereals.

It is sometimes impossible to compare data on folate production by LAB in different studies due to the differences in methods used and units of expression of the results. In some studies, folate production was measured in the cell biomass but in others in the supernatant of the culture medium (D'Aimmo et al., 2012, 2014; Kariluoto et al., 2006a). As the quantity of biomass is not given in these studies, it is impossible to estimate the production of folate in the medium, and these results cannot be compared with the data presented in Table 5. However, these studies give interesting results that can be compared to the production of folate by yeasts in culture media. For instance, folate production by 10 *Bifidobacterium* strains in a folate-free medium ranged

from 500 to 9,500 µg/100 g dry matter (DM) of cell biomass (D'Aimmo et al., 2012). The production of folate by 25 *Saccharomyces cerevisiae* strains ranged from 4,000 to 14,500 µg/100 g DM of cell biomass in a synthetic medium (Hjortmo et al., 2005). Therefore, in some cases, the capacity of some LAB to produce folate is similar to that of some yeasts.

Table 5 shows that folate may also be consumed or produced in different culture media, and that this is highly variable and strain dependent. *L. plantarum* JA71 produced the highest concentration of folate in MRS medium (9,030 ng/mL) (Park et al., 2014) and *L. plantarum* SM39 produced 4,413 ng/mL in a supplemented whey permeate medium (Hugenschmidt et al., 2011). Folate production also depends on growth kinetics and on the culture conditions. According to numerous studies, folate is synthesized during the exponential growth phase or at the beginning of the stationary phase, and is then consumed (D'Aimmo et al., 2012; Iyer et al., 2010; Laiño et al., 2012, 2014; Padalino et al., 2012). In addition, it has been proved that folate production depends on the composition of the medium (Nor et al., 2010). A high rate of production of folate by *L. plantarum* SM39 was obtained in a medium supplemented with 10 mg/L of PABA (Hugenschmidt et al., 2011) which is generally recognized as the limiting precursor (Sybesma et al., 2003). Moreover, LAB can produce large amounts of folate even when folate is already available in the medium (Lin and Young, 2000a; Padalino et al., 2012; Sybesma et al., 2003). Therefore, to select folate-producing strains, it is important to take all these elements into account.

Studying the synthesis of folate by LAB in culture media is useful to understand the influence of different parameters, but may not be suitable to select LAB strains with the aim of increasing the folate content of a food product. Many studies have shown that the composition of the medium

has an impact on folate synthesis by LAB. Therefore, for the selection of strains, it would be better to investigate folate production by LAB in the food matrices concerned.

3-3- Folate production in fermented food

Table 6 summarizes the studies on inoculation of different food matrices with LAB previously selected for their ability to synthesize folate in culture medium. In most cases, an increase in folate was reported. Cereal, vegetable and milk-based food products were investigated, although most of the studies focused on dairy products. Dairy foods are not representative of the wide variety of fermented food products in the world, especially as fermented dairy products are mostly consumed in developed countries, whereas cereal-based fermented foods are far more widely consumed in African countries. The production of folate in dairy products by pure culture of LAB strains has already been reviewed (LeBlanc et al., 2008).

Only a few studies have been conducted on the production of folate in cereal based fermented foods. Most studied the effect of yeasts on the folate content of this food category (Hjortmo et al., 2008a, 2008b), but two studies showed that the folate content of fermented rye and oat sourdoughs was increased by LAB (Kariluoto et al., 2006a, 2014). LAB can produce from 2 to 16 µg/100g FM in food matrices (Table 6). In comparison, yeasts alone can produce from 9 to 17 µg/100g FM in various cereal-based matrices (Kariluoto et al., 2006a; Korhola et al., 2014). LAB can thus produce similar amounts of folate as some yeasts in food products. However, given the small number of studies dedicated to this topic, it is difficult to generalize and further studies are needed on the production of folate by LAB alone or in co-culture with yeasts.

To assess if the enriched food products listed in Table 6 can be considered as good dietary sources of folate, and to determine if the increase in folate content in these food products by

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LAB is high enough to satisfy nutritional requirements, the portion of each food needed to cover 10% of the needs of a pregnant woman (based on the RNI: 600 µg/day) was calculated. Most of the values obtained are not realistic, for example, more than 1 kg of some fermented milk would need to be consumed per day. Therefore, even if LAB can significantly increase the folate content in food products, this would still not be enough to cover people's folate needs. Other solutions should be investigated to further increase the folate content of food products fermented by LAB.

4- Ways to preserve and improve folate content in fermented food

The previous parts of this review showed that folate synthesis by LAB depends on the strain, the incubation time and the composition of the culture medium. In this section, we discuss these parameters in detail and suggest ways to optimize folate synthesis by LAB in food matrices.

4-1- Folate production and selection of strains

In addition to the selection of high folate producing strains, the use of a combination of different LAB may be more efficient than the use of single cultures (Table 5 and 6). For example, in a reconstituted skim milk, a co-culture of two *B. animalis* and *L. acidophilus* strains produced at least 30% more folate than the single cultures (Crittenden et al., 2003). Similarly, in a supplemented wheat permeate medium, two co-cultivated strains of *L. plantarum* and *P. freudenreichii* produced at least 35% more extracellular folate than *L. plantarum* and *P. freudenreichii* alone (Hugenschmidt et al., 2011). In another study, among different combinations, the best was the mix of one *L. delbruecki* subsp. *bulgaricus* strain with two *S. thermophilus* strains in milk (Laiño et al., 2013). Similarly, among various combinations, the

combination of strains of *Lactococcus lactis* subsp. lactis and *Lc. lactis* subsp. *cremoris* gave the best results in Domiati cheese (Ayad, 2009).

Combinations of LAB with other folate producing microorganisms such as yeasts could also be used to increase the folate content of fermented food products. A few studies have evaluated the impact of LAB and yeast co-cultures on the folate content of fermented food products. However, in almost all cases, yeasts alone or the natural endogenous microbiota were more efficient than the selected strains. Indeed, a combination of *L. rhamnosus* LC-705 and *Saccharomyces cerevisiae* ABM5131 (in 3.5% oat bran solution with 2% added glucose) produced 6 fold more folate than the LAB alone and a similar amount of folate to yeast alone (Korhola et al., 2014). In another study, the highest folate concentrations in sourdoughs made from rye flour was reached with the non-sterile control containing naturally occurring bacteria and yeasts or with some of the monocultures of yeasts, whereas folate concentrations were significantly lower with LAB monocultures (Kariluoto et al., 2006a). Considering the limited number of attempts reported until now, it would be interesting to further investigate this hypothesis.

As reviewed previously, metabolic engineering can be used to obtain overproducing strains of LAB and, as a consequence, food products with higher amounts of folate (Capozzi et al., 2012; LeBlanc et al., 2011). Consumption of food fermented with the overproducing LAB strains was even able to improve the folate status of deficient rats (LeBlanc et al., 2010). However, the use of genetically modified microorganisms may be limited by ethical and regulatory considerations. LAB have co-evolved with their habitat and are the result of a combination of extensive gene loss and key gene acquisition via horizontal gene transfer (Makarova et al., 2006). Therefore, isolating strains from a different niche could be a way to find naturally overproducing bacteria.

For example, the two genes *folK* (EC 2.7.6.3) and *folP* (EC 2.5.1.15) were detected in 96 LAB strains isolated from African starchy foods (Turpin et al., 2011). This work suggests that many different bacteria would be able to produce folate. Exerting selection pressure by isolating LAB on culture medium without folate would also be a way to find naturally producing bacteria. For example, among eight *L. acidophilus* strains, only two were able to grow and to produce folate after seven passages in a folate free medium at 37 °C for 16 h (Laiño et al., 2014).

Another promising way to increase the folate content of foods, would be to promote the production of folate by LAB strains mainly able to produce the most stable vitamers. Indeed, it was shown in section 2.3 that many processing steps can reduce the folate content, due to oxidation and other reactions, especially when the food product is fragmented before fermentation, and cooked and/or stored after fermentation. According to the few data available, the production of vitamers appears to be a strain dependent trait (D'Aimmo et al., 2012). 5-methyl-THF appears to be the main vitamer produced by LAB, but THF can also be produced at higher levels by some LAB (D'Aimmo et al., 2014; Lin and Young, 2000a). However, Figure 3 shows that THF, among other vitamers, is oxidized into other folate forms at low pH, which may explain its low detection rate. Therefore, the lowering of the pH in the food matrix due to fermentation is likely to induce degradation and/or interconversion of folate vitamers. As 5-methyl-THF is more stable than THF, in some cases it would be advisable to use strains that mainly produce this vitamer than other strains able to produce more folate but mainly THF.

4-2- Kinetics of folate production

The choice of the strain is very important to produce large amounts of folate, but as mentioned above, folate production by LAB also depends on the period of incubation. For example, with a

L. plantarum strain, the highest folate content was reached at the beginning of the stationary phase in a folate free medium and similar results were obtained with a mixed culture of a *L. plantarum* strain with a *Propionibacterium* strain in supplemented whey permeate medium (Hugenschmidt et al., 2011; Masuda et al., 2012). However, with *Bifidobacterium* strains in single culture in a folate free medium or co-cultivated with *S. thermophilus* in reconstituted non-fat dry milk, the highest folate contents were obtained during the exponential growth phase (D'Aimmo et al., 2012; Lin and Young, 2000a). Moreover, the distribution of folate vitamers produced by yeasts depends on the length of the fermentation period (Kariluoto et al., 2014). These results indicate that the duration of the fermentation should be optimized for each fermented food.

4-3- Precursors of folate biosynthesis

Three main precursors are required for folate synthesis: para-aminobenzoic acid (PABA), guanosine triphosphate (GTP) and glutamate (KEGG, 2014). According to the KEGG (2014) database, many LAB are unable to synthesize at least PABA. For now, only the effect of PABA and glutamate on the production of folate by LABs has been evaluated (Gangadharan and Nampoothiri, 2011; Hugenschmidt et al., 2011; Nor et al., 2010; Pompei et al., 2007; Santos et al., 2008; Sybesma et al., 2003). GTP is an essential molecule synthesized by all the LAB and should not be a limiting factor.

Conversely, PABA can be a limiting precursor for folate biosynthesis. Even if many LABs are able to produce PABA without the genes *pabA* (EC 2.6.1.85), and *pabC* (EC 4.1.3.38), many LAB need PABA in the medium to produce folate or larger amounts of folate. For example, in their study, Sybesma et al. (2003) showed that most folate was produced by a *Lc. lactis* strain in

a chemically defined medium containing 14 mg/L of PABA. In another study, the optimal PABA concentration in a modified MRS medium was around 1 mg/L for a *L. plantarum* strain (Nor et al., 2010). These two studies reported very different optimum concentrations with different explanations: all strains do not have the same PABA requirements. For example, PABA concentrations required for folate production varied between 0.04 mg/L and 1.37 mg/L for different strains of *Bifidobacterium* in a folate-free semisynthetic medium (Pompei et al., 2007). In addition, rich media such as MRS or food matrices may already contain PABA, thus limiting the quantity of precursor that needs to be added to increase folate production.

In food matrices for example, 10 mg/L was the best concentration of added PABA for a *Lc. lactis* strain in skim milk (Gangadharan and Nampoothiri, 2011). Similarly, with a wild strain and a mutant strain of *L. reuteri,* the addition of 10 mg/L of PABA in a cucumber juice led to a two-fold and a fifteen-fold increase, respectively, in folate content in comparison with the same matrix without PABA (Santos et al., 2008). However, it should be kept in mind that a high concentration of PABA can inhibit folate biosynthesis (Pompei et al., 2007). It would thus be worth measuring the PABA content of a food product before trying to increase its folate content using LAB.

Finally, the addition of glutamate could also increase folate production by LAB. For example, 10 mg/L of glutamate in skim milk led to an increase in folate production by a *Lc. lactis* subsp. *cremoris* (Gangadharan and Nampoothiri, 2011). However the ratio of PABA to glutamate appears to be critical. A tenfold increase in folate production by a combination of *L. plantarum* and *P. freudenreichii* was obtained in whey permeate supplemented with 10 mg/L of PABA, but

production was a little lower when 10 mg/L of glutamate was added (Hugenschmidt et al., 2011). The PABA:glutamate ratio thus deserves more attention in future studies.

Understanding the physiology of the strains with respect to the need for a precursor is of importance in basic research, but the question remains whether it is desirable to add these precursors to food products: changing the composition of food may lead to undesirable effects. For example, as reviewed by Ende (2015), the consumption of high doses of glutamate could increase impulsivity and anxiety.

4-4- Modulating folate production by other food components

A few studies attempted to better understand folate production in culture medium by investigating other parameters. For example, it has been shown that the growth rate is inversely correlated with folate production, so the use of growth inhibitory compounds such as NaCl could improve folate production by LAB (Sybesma et al., 2003). However, the use of prebiotics such as sorbitol and mannitol could also improve folate production by LAB (Gangadharan and Nampoothiri, 2011). Carbon and nitrogen sources could also play a role in folate production. Nor et al. (2010) showed that lactose was a better carbon source for folate production for a *L. plantarum* strain in a modified MRS medium than glucose and maltose. However, the best lactose concentration for folate production appears to depend on the strain (Iyer et al., 2010; Tomar et al., 2009). Similarly, meat extract was a better nitrogen source than yeast extract for a *L. plantarum* strain (Nor et al., 2010). The impact of growth inhibitory molecules and nutrient sources on folate production also deserves more attention.

5- Conclusion

In many countries, dietary folate consumption is not sufficient to prevent folate deficiencies, leading to the implementation of fortification programs using synthetic folic acid. Another alternative to fortification worth considering is *in situ* folate biofortification of fermented foods by LAB. Fermentation is an ancestral, sustainable and low cost way of preserving food products, and most fermented foods have higher concentrations of folate than non-fermented foods. Moreover, fermented foods are frequently consumed in many countries worldwide. For all these reasons, numerous studies have been conducted to assess the potential of LAB to produce folate during fermentation of various food products.

This review highlighted the fact that folate can be degraded during food processing due to physical-chemical factors. The activation of biological pathways during germination and fermentation can lead to a significant increase in folate content. Food products are complex media, in which oxidizing and reducing agents are naturally present and can induce and/or influence reactions. This review also evidenced that even if numerous LAB strains have been shown to consume folate, others are able to increase the folate content of cereal, vegetable, fruit or milk-based fermented foods. In addition, some LABs are able to produce similar amounts of folate as yeasts in both culture media and food matrices. However, the folate content of fermented foods enriched by LAB is generally still too low to significantly contribute to the satisfaction of folate requirements.

Future research should thus focus on the following points: (i) selecting strains able to produce high amounts of folate and if possible, mainly folate vitamers that are resistant to oxidation, acid pH, and heat treatments, (ii) testing co-cultures of folate-producing microorganisms, (iii)

estimating the effect of different parameters *in situ* (kinetics, precursors, growth inhibitors, etc), (iv) limiting folate losses during the different processing steps. The use of LAB to naturally fortify food can be an advantage if bacteria are consumed alive, since folate could be produced in the gut. This aspect also deserves further investigation. The combination of LAB with different ability to improve the nutritional quality of food could also be considered to cumulate beneficial properties.

Acknowledgments

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Table 1: Estimated average requirements (EARs) and recommended nutrient intakes (RNIs) of folic acid expressed as dietary folate equivalents, according to age group, FAO/WHO (2005)

Group	EAR (µg/day)	RNI (µg/day)
Infants and children		
0-12 months [a]	65	80
1-3 years	120	150
4-6 years	160	200
7-9 years	250	300
Adolescents, 10-18 years	330	400
Adults		
19-65 + years	320	400
Pregnancy	520	600
Lactation	450	500

[a] Based on a human milk intake of 0.75 l/day

Table 2: Theoretical contribution of different food products to folate consumption worldwide

Food category	Food product	Average food supply[a] (g/capita/day)	Folate content [b] (µg/100 g FM)	Theoretical folate intake (µg/capita/day)
Cereals and products	**Total (excluding beer)**	**402**	ND	NC
	Wheat and products	182	10-87	18-159
	Rice (Milled Equivalent)	146	10-16	15-23
	Maize and products	46	10-26	5-12
	Sorghum and products	10	30-38	3-4
	Millet and products	9	8-50	0.8-5
Pulses	**Total**	**17**	ND	NC
	Beans	6	140-360	9-23
	Peas	2	151-340	3-7
Vegetables	**Total (excepted starchy foods and tubers)**	**343**	11-187	38-641
	Green leafy vegetables	ND	88-187	NC
Fruits	**Total (Excluding Wine)**	**187**	ND	NC
	Oranges, Mandarins	33	7-29	2-10

	Bananas	28	13-17	4-6
	Apples and products	24	3-12	0.8-3
Starchy roots and tubers	**Total**	**169**	ND	NC
	Potatoes and products	90	22-40	20-36
	Cassava and products	37	16-56	6-21
	Sweet potatoes	26	7-43	2-11
Nuts and products	**Total**	**5**	39-169	2-9
Meat products	**Total**	**109**	ND	NC
	Pig meat	41	3-10	1-4
	Poultry Meat	35	4-16	1-6
	Bovine Meat	26	1-10	0.3-3
	Offal*	6	4-592	0.2-36
Fish and Sea products	**Total**	**48**	1-37	0.5-18
Dairy products	**Total (Milk, Yoghurt, Cheese and Whey)**	**219**	1-66	2-145
	Raw milk	**ND**	0.8-7	NC
	Cheese	**ND**	10-65	NC

[a]The food supply data were obtained from FAOSTAT database for the 2001-2011 period (FAOSTAT, 2015).

[b]Folate contents of food products were obtained mainly from Souci et al., (2000) and when data were missing, other databases were used (Stadlmayr et al., 2012; USDA, 2015).

ND: Not Determined (no supply data were available in the databases). NC: Not Calculated

*offal includes folate-rich products such as liver and kidney, as well as products with a much lower folate content such as heart and brain.

Table 3: Distribution of folate vitamers in different food categories

Food categories	5-CH$_3$-H^{4}folate	5-CHO-H$_4$folate	10-CHO-folic acid	H$_4$folate	folic acid	5,10-CH$^+$-H$_4$folate	10-HCO-H$_2$folate	Total folate content (µg/100 g FM)	References
Cereal and products	4-47%	5-48%	9-41%	0-15%	0-17%	1-27%	0-70%	17 to 73	(Edelmann et al., 2012, 2013; Gujska et al., 2009; Kariluoto et al., 2004; Konings, 1999; Ringling and Rychlik, 2013)
Pulses	6-85%	0-75%	0-9%	0-67%	0-	0-1%	0-1%	50 to	(Ringling

					10 %			340	and Rychlik, 2013; Rychlik et al., 2007; Shohag et al., 2012)
Leafy vegetables	20-74%	6-49%	1-58%	0-24%	0-1%	0-15%	0-34%	54 to 227	(O'Hare et al., 2012; Phillips et al., 2014; Ringling and Rychlik, 2013; Shohag et al., 2011, 2012; Wang et al., 2013)

Other vegetables	17-85%	4-53%	0-14%	7-27%	0-23%	0-2%	1-3%	16 to 103	(Phillips et al., 2006, 2014; Ringling and Rychlik, 2013; Rychlik and Adam, 2008; Rychlik et al., 2007; Shohag et al., 2012; Wang et al., 2013)
Mushrooms	0-90%	10-60%	0-40%	NA	NA	NA	NA	1 to 70	(Phillips et al., 2011)

Liver	18-81%	0-10%	NA	10-81%	0-1%	NA	ND	375 to 1500	(Konings, 1999; Vahteristo et al., 1996)
Dairy products	6-100%	0-93%	16%	0-33%	3%	0.3%	6%	4 to 104	(Forssén et al., 2000; Ringling and Rychlik, 2013)

NA: Not Analyzed; ND: Not Detectable

5-CH$_3$-H$_4$folate: 5-methyltetrahydrofolate, 5-HCO-H$_4$folate: 5-formyltetrahydrofolate, 10-HCO-folic acid: 10-formylfolic acid, H$_4$folate: tetrahydrofolate, 5,10-CH$^+$=H$_4$folate: 5,10-methenyltetrahydrofolate, and 10-HCO-H$_2$folate: 10-formyldihydrofolate.

Table 4: Influence of food processing on folate content: examples of common processing steps

Processing steps	Food product	Conditions	Influence on B9	References
Cooking or heating	Rye and wheat bread	200 °C/60 min	-25%	Kariluoto et al. (2004)
	Soybeans	Blanched (85 °C/5 min)	-26%	Arcot et al. (2002)
	Broccoli	25-140 °C/30 min	-22 to -49%	Munyaka et al. (2010)
	Milk	Pasteurization (62.5 °C, 30 min), microwaving (720 watts, 30 s), or heating (40 °C, 10 min)	0 to -16%	Donnelly-Vanderloo et al. (1994)
Hydrothermal cooking	Rice	Boiling/duration not given	-16 to -39%	Wieringa et al. (2014)
	Navy beans	100 °C; 10-40 min, in distilled water/water-oil mixture (seed to water ratio 1:80 g/mL)	-11 to -85%	Xue et al. (2011)
Fractionation	Rice	Degree of polishing: 10%	-88%	Monks et al. (2013)
	Wheat	Electrostatic separation of bran fractions	-37% to	Hemery et al.

			+23%	(2011)
Soaking	Navy beans	0-12 h; seed to water ratio at 1:3 or 1:7	-1 to -45%	Xue et al. (2011)
	Soybeans	24 h/30 °C in tap water without added LAB or 16 h/20 °C in tap water	-27 to -59%	Mo et al. (2013)
Storage	Cowpeas and okra	Canned and stored for 3 to 24 weeks	-39 to -76%	Sotiriadis and Hoskins (1982)
	Brussels sprouts	Fresh sprouts stored at -21 °C for 31 to 188 days	-6 to -48%	Malin (1977)
	Various vegetables	3 to 12 months/<-18 °C	0 to -99%	Czarnowska and Gujska (2012)
	Whole milk powder	60-70 °C/1 to 8 weeks	-53 to -100%	Ford et al. (1983)
Germination	Wheat grains	20-35 °C; 48 h	+200 to +336%	Hefni and Witthöft (2011)
	Rye grains	5 to 25 °C; 6 days	+70 to +280%	Kariluoto et al. (2006b)
Ferment	Fermented	37 °C/14 to 40 h; single bacteria cultures	-83 to	Crittenden et

ation	skim milk		+267%	al. (2003)
	Togwa (maize based porridge)	30 °C/5 to 46 h; single yeast cultures	0 to +700%	Hjortmo et al. (2008b)

Table 5: Lactic acid bacteria species able to produce folate in a culture medium or in food.

LAB species	Medium (number of strains studied)	Folate production (ng/mL)	References
B. adolescentis	MRS (n=1)	-50 to 150	(Padalino et al., 2012)
	SM7 (n=10)	1 to 65	(Pompei et al., 2007)
B. animalis	SM7 (n=7)	26	(Pompei et al., 2007)
B. bifidum	SM7 (n=6)	1	(Pompei et al., 2007)
B. breve	SM7 (n=15)	1 to 3	(Pompei et al., 2007)
B. catenulatum	MRS (n=1)	0 to 25	(Padalino et al., 2012)
	SM7 (n=1)	3	(Pompei et al., 2007)
B. dentium	SM7 (n=1)	29	(Pompei et al., 2007)
B. longum	MRS (n=2)	10 to 30	(Lin and Young, 2000a)
	SM7 (n=17)	2	(Pompei et al., 2007)
B. pseudocatenatulum	SM7 (n=3)	12 to 82	(Pompei et al., 2007)
L. acidophilus	FFM (n=8)	0 to 38	(Laiño et al., 2014)
	MRS (n=3)	1 to 20	(Lin and Young, 2000a; Sybesma et al., 2003)
	SWP (n=5)	0*	(Hugenschmidt et al., 2010)
L. amylovorus	FFM (n=1)	75 to 87	(Laino et al. 2014)
L. brevis	SWP (n=9)	0 to 150*	(Hugenschmidt et al., 2010)

L. buchneri	SWP (n=1)	0*	(Hugenschmidt et al., 2010)
L. casei	FFM (n=4)	0 to 2	(Laino et al. 2014)
	MRS (n=2)	-63 to -13	(Sybesma et al., 2003)
	SWP (n=19)	0 to 20*	(Hugenschmidt et al., 2010)
L. coryniformis	FFM (n=2)	80 to 100	(Masuda et al., 2012)
L. curvatus	SWP (n=6)	0 to 20*	(Hugenschmidt et al., 2010)
L. delbrueckii	MRS (n=4)	-50 to 200	(Lin and Young, 2000a; Padalino et al., 2012; Sybesma et al., 2003)
L. fermentum	FFM (n=15)	0 to 148	(Cárdenas et al., 2015; Laiño et al., 2014; Masuda et al., 2012)
	SWP (n=24)	-10 to 116*	(Hugenschmidt et al., 2010)
L. fructivorans	SWP (n=1)	0 to 20*	(Hugenschmidt et al., 2010)
L. helveticus	MRS (n=2)	2 to 89	(Sybesma et al., 2003)
	SWP (n=3)	0*	(Hugenschmidt et al., 2010)
L. johnsonii	MRS (n=1)	28	(Nor et al., 2010)
L. paracasei	FFM (n=12)	0 to 40	(Laino et al., 2014)
	SWP (n=18)	0*	(Hugenschmidt et al., 2010)
L. pentosus	FFM (n=2)	0 to 4	(Masuda et al., 2012)
L. plantarum	CDM (n=1)	36 to 60	(Nor et al., 2010)
	FFM (n=30)	0 to 108	(Laiño et al., 2014; Masuda et

			al., 2012)
	MRS (n=2)	-10 to 9030	(Hugenschmidt et al., 2011; Padalino et al., 2012; Park et al., 2014)
	SWP (n=28)	0 to 4413	(Hugenschmidt et al., 2010, 2011)
L. reuteri	CDM (n=1)	59	(Santos et al., 2008)
	SWP (n=3)	0 to 125*	(Hugenschmidt et al., 2010)
L. rhamnosus	SWP (n=1)	-10 to 0*	(Hugenschmidt et al., 2010)
	YPD (n=3)	<0*	(Herranen et al., 2010)
L. sakei	FFM (n=2)	101 to 107	(Masuda et al., 2012)
Lc. lactis	M17 (n=16)	57 to 291	(Sybesma et al., 2003)
	YPD (n=2)	<0*	(Herranen et al., 2010)
Leuconostoc lactis	MRS (n=1)	45	(Sybesma et al., 2003)
Ln. paramesenteroides	MRS (n=1)	44	(Sybesma et al., 2003)
Pediococcus. parvulus	FFM (n=10)	40 to 60	(Masuda et al., 2012)
P. pentosaceus	FFM (n=3)	0 to 40	(Masuda et al., 2012)
S. thermophilus	FFM (n=51)	0 to 170	(Laiño et al., 2012)
	MRS (n=1)	0 to 200	(Padalino et al., 2012)

	M17 (n=2)	4 to 202	(Lin and Young, 2000a; Sybesma et al., 2003)
	SWP (n=33)	0 to 50*	(Hugenschmidt et al., 2010)
	YPD (n=1)	100 to 190	(Herranen et al., 2010)
Weissella confusa	FFM (n=1)	0 to20	(Masuda et al., 2012)

MRS: de Man Rogosa and Sharp; YPD: Yeast extract, peptone, dextrose; SWP: supplemented

Whey permeate, CDM: Chemically defined medium; TPY: tripticase, phytone, yeast extract;

FFM: folate free medium. *: extracellular production only.

Table 6: Folate measurement after fermentation of different food inoculated with LAB only

ND: not determined. * Folate content values are expressed in fresh weight basis

Raw Material		Fermented food		Portion needed to cover 10% of RNI of a pregnant woman	Producing microorganisms	Reference
Type	Folate content*	Name	Folate content*			
Cereal	ND	Fermented oat sourdough	15-17 µg/100 g	353-400 g	*L. rhamnosus*	(Kariluoto et al., 2014)
Cereal	4-6 µg/100 g	Fermented rye sourdough	8-13 µg/100 g	462-750 g	*S. thermophilus*	(Kariluoto et al., 2006a)
Vegetable	15-16 µg/100 g	Fermented Beetroot mix	10-17 µg/100 g	353-600 g	*L. plantarum, Lc. lactis, Ln sp.*	(Jägerstad et al., 2004)
Vegetable	1±0.0 µg/100 mL	Fermented cucumber	5-7 µg/100 mL	857-1200 mL	*Lc. lactis*	(Gangadharan and Nampoothiri, 2011)

Fruit	1-2 µg/100 mL	Fermented watermelon juice	2-3 µg/100 mL	2000-3000 mL	*Lc. lactis*	(Gangadharan and Nampoothiri, 2011)
Milk	1-2 µg/100 g	Fermented milk	6-9 µg/100 g	667-1000 g	*B. animalis* and *S. thermophilus*	(Crittenden et al., 2003)
Milk	2-3 µg/100 g	Fermented milk	3-6 µg/100 g	1000-2000 g	*B. longum, S. thermophilus*	(Holasova et al., 2005)
Milk	2-3 µg/100 mL	Fermented milk	5-10 µg/100 mL	600-1200 mL	*B. longum*	(Lin and Young, 2000a)
Milk	3-5 µg/100 mL	Fermented milk	25-28 µg/100 mL	214-240 mL	*L. amylovorus, S. thermophilus and L. bulgaricus*	(Laiño et al., 2014)
Milk	ND	Fermented milk	1-7 µg/100 mL	857-6000 mL	*L. delbrueckii*	(Lin and Young, 2000b)
Milk	4-6 µg/100 mL	Fermented milk	8-18 µg/100 mL	333-750 mL	*L. delbrueckii and S. thermophilus*	(Laiño et al., 2013)
Milk	Not	Fermented	6-7	857-1000	*L. lactis*	(Sanna et al.,

	detectable	milk	µg/100 g	g		2005)
Milk	ND	Fermented milk	0.2-7 µg/100 mL	857-30000 mL	*L. sp.*	(Dana et al., 2010)
Milk	11-12 µg/100 g	Fermented milk	12-13 µg/100 g	462-500 g	*Lc. lactis*	(Ayad, 2009)
Milk	0.2-0.4 µg/100 mL	Fermented milk	0.2-2 µg/100 mL	3000-30000 mL	*Lc. lactis*	(Gangadharan and Nampoothiri, 2011)
Milk	0.5-1 µg/100 g	Fermented milk	0.5-5 µg/100 g	1200-12000 g	*S. thermophilus*	(Holasova et al., 2004)
Milk	ND	Fermented milk	2-5 µg/100 mL	1200-3000 mL	*S. thermophilus*	(Iyer et al., 2011)
Milk	1-2 µg/100 g	Fermented milk	10-11 µg/100 g	545-600 g	*S. thermophilus*	(Wouters et al., 2002)
Milk	4-5 µg/100 g	Fermented milk	5-20 µg/100 g	300-1200 g	*S. thermophilus*	(Padalino et al., 2012)
Milk	2-3 µg/100	Fermented milk	7-8 µg/100	750-857 mL	*S. thermophilus*	(Laiño et al., 2012)

	mL		mL			
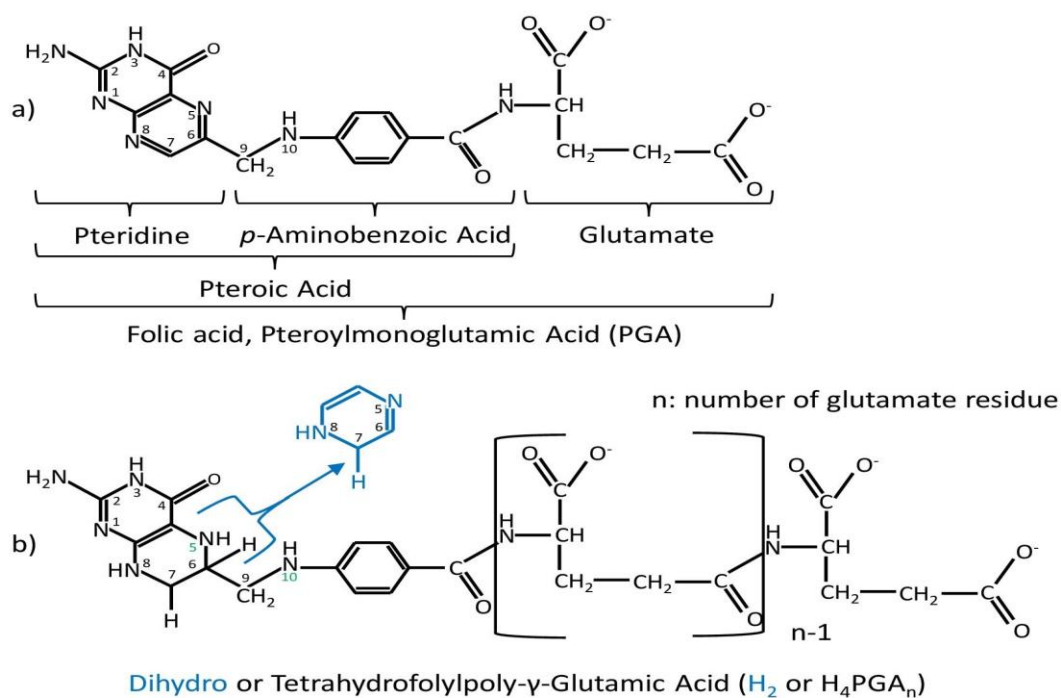
One Carbon Substituent		Position	Oxidation State	Vitamers
Methyl	—CH$_3$	N-5	Methanol	5-CH$_3$-H$_4$folate 5-CH$_3$-H$_2$folate
Methylene	—CH$_2$—	N-5, N-10	Formaldehyde	5,10-CH$_2$-H$_4$folate
Methenyl	—CH=	N-5, N-10	Formate	5,10-CH$^+$-H$_4$folate
Formyl	—CHO	N-5 or N-10	Formate	5-HCO-H$_4$folate 10-HCO-H$_4$folate 10-HCO-H$_2$folate 10-HCO-PGA
Formimino	HN=CH—	N-5	Formate	5-HN=CH-H$_4$folate

Figure 1: Structure of a) folic acid, and b) naturally occurring folate

5-CH$_3$-H$_4$folate: 5-methyltetrahydrofolate; 5-CH$_3$-H$_2$folate: 5-methyltedihydrofolate; 5,10-CH$_2$-H$_4$folate: 5,10-methylenetetrahydrofolate; 5,10-CH$^+$-H$_4$folate: 5,10-methenyltetrahydrofolate; 5-HCO-H$_4$folate: 5-formyltetrahydrofolate; 10-HCO-H$_4$folate: 10-formyltetrahydrofolate; 10-HCO-H$_2$folate: 10-formyldihydrofolate; 10-HCO-PGA: 10-formylfolic acid; 5-HN=CH-H$_4$folate: 5-formiminotetrahydrofolate
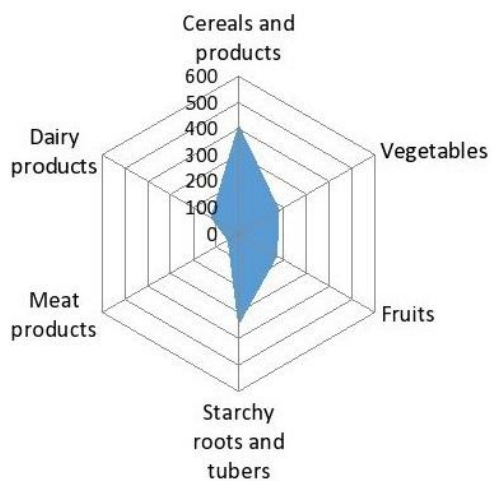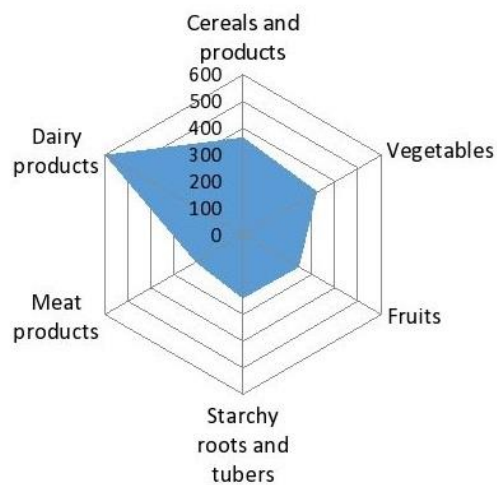
Figure 2: Consumption (in g/capita/day) of different food categories in A) Africa and B) Europe,
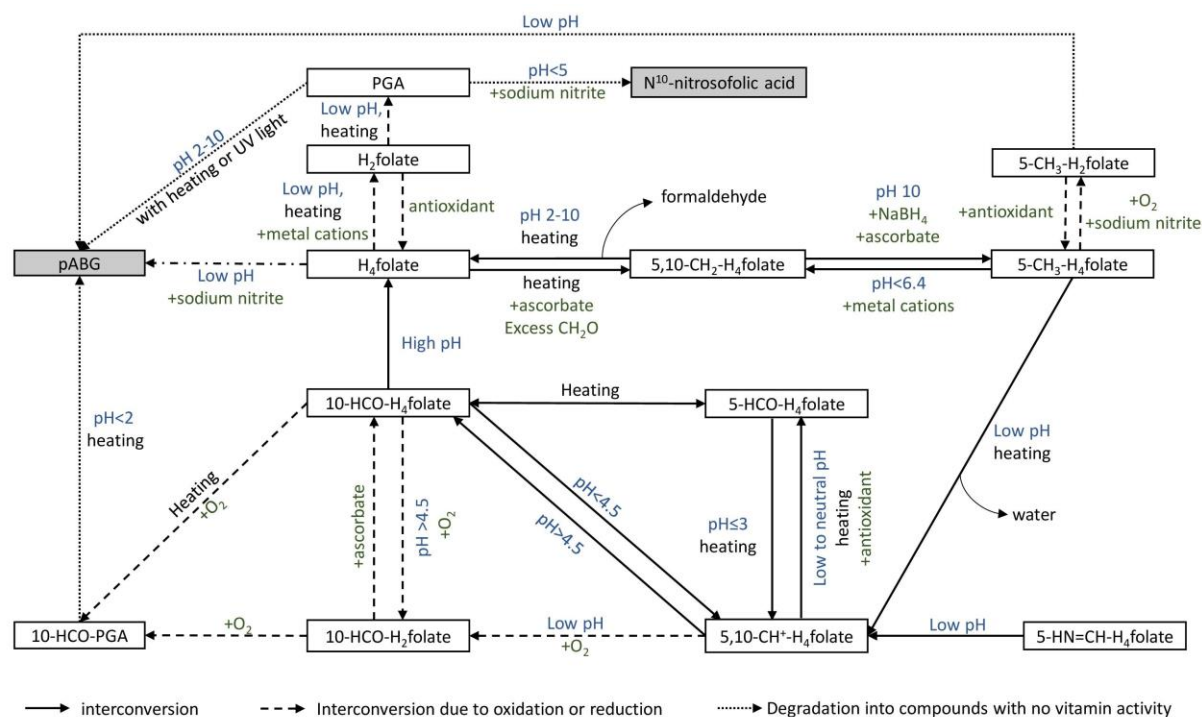
in 2011 (FAOSTAT, 2015)

Figure 3: Degradation and interconversion of folate vitamers under different conditions. This figure was adapted from that of Edelmann (2014) and is based on data in different publications (Akhtar et al., 1999, 2003; De Brouwer et al., 2007; Jägerstad and Jastrebova, 2013; Lucock et al., 1994; Mnkeni and Beveridge, 1982; Paine-Wilson and Chen, 1979; Quinlivan et al., 2006; Strandler et al., 2015; Tyagi et al., 2009; Wilson and Horne, 1983).

H_4folate: tetrahydrofolate, 5-CH$_3$-H$_4$folate: 5-methyltetrahydrofolate, 5,10-CH+=H$_4$folate: 5,10-methenyltetrahydrofolate, 5,10-CH$_2$-H$_4$folate: 5,10-methylenetetrahydrofolate, 5-HCO-H$_4$folate: 5-formyltetrahydrofolate, 10-HCO-H$_2$folate: 10-formyldihydrofolate, 10-HCO-folic acid: 10-formylfolic acid and pABG: para-aminobenzoylglutamate.
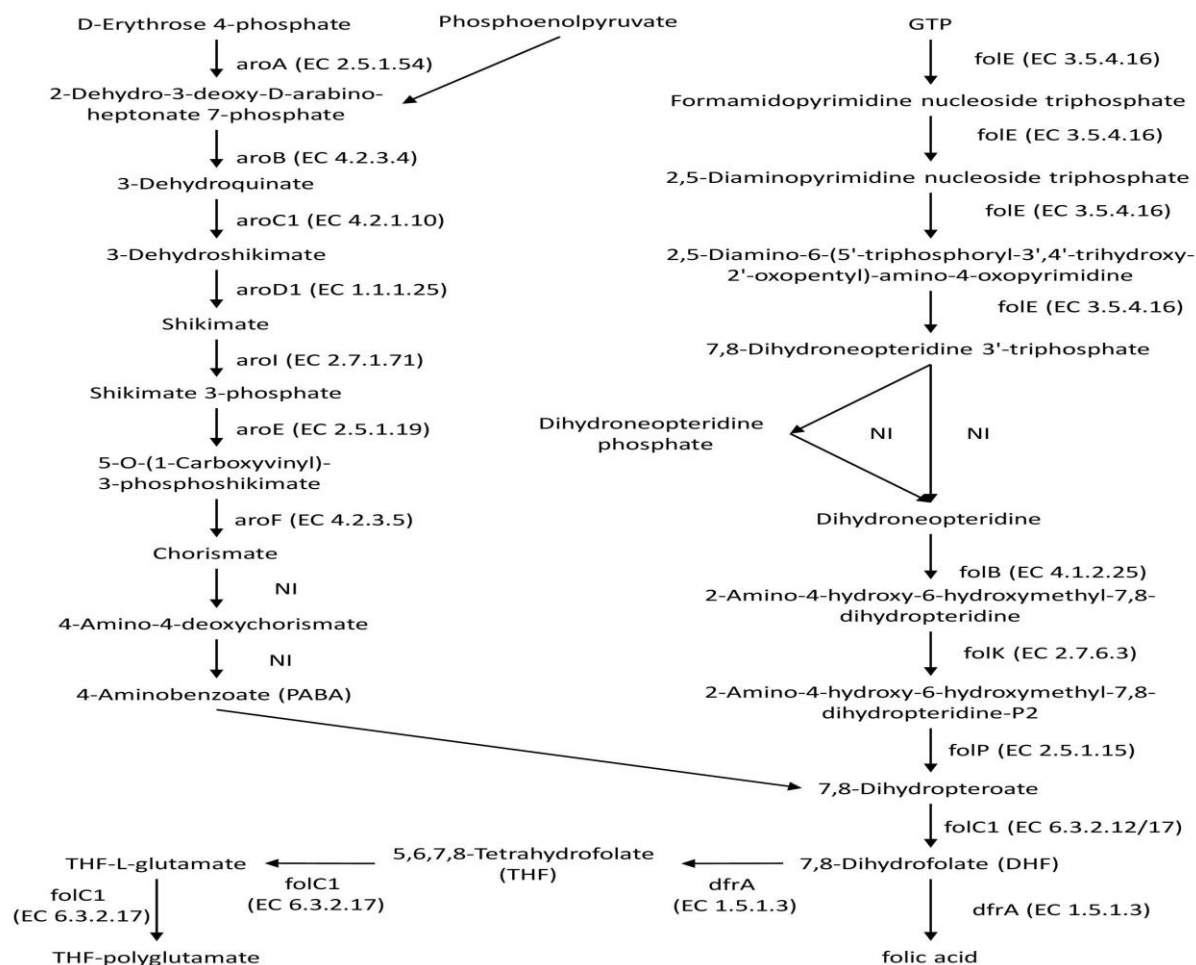
Grey boxes: compounds with no vitamin activity

Figure 4: General biosynthetic pathway of folate with indication of the enzyme present in *L. plantarum* WCFS1 according to KEGG (2014). NI: Not identified (No information on the gene coding for this enzyme in *L. plantarum* WCFS1)