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Folate Biofortification of Fermented Foods by Using Lactic Acid Bacteria

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Abstract

Folate, an essential vitamin and an important factor takes part in human life for the synthesis of nucleotides, vitamins and some amino acids. This vitamin cannot be synthesized in humans' metabolism and must be taken exogenously to prevent folate deficiency, neural tube defects (NTDs) and other related diseases. The chemically synthesized form of folate is folic acid used for fortification and supplementation of foods, but it can cause some problems such as vitamin B-12 deficiency in high intake. Milk and fermented dairy products represent a good source of natural folate and folate binding proteins, which increase the bioavailability and stability of folate.

Many plants, fungi and bacteria synthesize and produce folate. However, folate concentrations in fermented dairy products are higher than in non-fermented dairy products. Some bacteria and yeasts are used as co-culture for natural folate bio-enrichment of fermented milk in the last years.

The selection and use of folate-producing microorganisms is an interesting approach to supplement "natural" folate levels in foods. The certain bacteria can contribute to the folate intake directly with foods such as fermented dairy products, or in the intestine as probiotic microorganisms or part of the natural microbiota.

Keywords: Lactic Acid Bacteria; Microorganisms; Fortification; Fermented foods

Introduction

Food fortification (FF) is explained as the addition of essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting that showed deficiency of one or more nutrients in the population or specific groups. Fortification generally uses for main foods as vehicles to transfer micronutrients generally deficient or not contained in adequate amounts in the diet of a population. Fortification has been applied for some specific health conditions such as iodine deficiency through the iodization of salt, anemia through the fortification of cereals with vitamins and iron, and neural tube defects through the fortification of wheat flour with folic acid since 1930's. Food fortification contains biofortification, microbial biofortification, commercial and industrial fortification, and home fortification. The several types of FF are defined because different techniques and approaches are used to fortify the foods. Biofortification involves creating micronutrient-dense staple crops using traditional breeding techniques and/or biotechnology.

Using biotechnology (genetic engineering) to biofortify staple crops is more modern and has gained much attention in recent years. Microbial biofortification involves using probiotic bacteria (mostly lactic acid bacteria), which ferment to produce β-carotene either in the foods we eat or directly in the human intestine [1].

This vitamin is defined as a water-soluble B-group vitamins and it is present in many kind of foods. However, deficiencies in folates are very often in many areas of the world, not only in developing countries, but also in some developed countries such as Germany. In some population groups vitamin deficiency is an increased risk, generally in the elderly, because their food consume is lower, and in children, who sometimes take a low kind of foods (Folate deficiencies may show to the etiology of many kind of diseases such as Alzheimer, cancer, (e.g. bladder, colon and breast) cardiovascular disease, megaloblastic anaemia and neural tube defects (NTDs) [2-8].

The recommended daily intake of folate, by for instance the Nordic Nutrition Recommendations are 400 μg for women, 300 μg for men and 500 μg for lactating or pregnant women. But these levels not easy to take. Foods rich in folate are, for example, beans and fruits such as oranges, green leafy vegetables like spinach and broccoli, liver and yeast. In taking folate with such products in the diet depends on availability, economy and eating habits. The U.S. Dept. of Agriculture (USDA) suggests the consumption of minimum 3 servings of milk products as a part of a healthy daily diet. According to this recommendation and considering that a serving consists of 240 mL fermented milk products could show significantly to the reference daily intake of folates (up to 23% of RDI). Besides fermented dairy products, microorganisms are capable of increasing folate content in much kind of other nondairy foods as rye, sourdough bread, beer, wine and fermented vegetables. Generally folate is present in a normal human diet but folate deficiencies still occur frequently in developed countries [6,9].

However, a number of researches are shown that high level intakes of folic acid (the chemically synthesized form of folate) can cause adverse health effects such as the late determination of the early hematological problems causing vitamin B12 deficiency, alteration in the activity of the hepatic dihydrofolate reductase enzyme or promote cancer. In addition, a recent study has showed that folic acid supplementation during pregnancy is related with an increased risk of respiratory infections in newborns. Natural folates, such as those found in foods or produced by some microorganisms, do not cause such adverse health effects in individuals [5,10].

Since natural folates (for example; 5-methyltetrahydrofolate) that are generally found in foods and produced by microorganisms are not masked B12 deficiency, this folate form can be a more efficient and safe alternative than addition folic acid to foods. Furthermore, it was shown that synthetic folic acid is absorbed and transported to the liver where it is reduced and a portion is methylated. However, natural folates (such as 5-MTHF) are reduced and methylated before being absorbed and natural folate become more bioavailable than folic acid at these conditions [11].

Fermentation and Folate Production in Foods

Fermented milk products are known to contain 2–4 fold higher amounts of folate when compared with natural milk because folate production of lactic acid bacteria (LAB). Daily consumption of these fermented milk products (150–175 g) will provide 15–20% of minimum required folate intake for an adult. Therefore, lactic acid bacteria producing important amount of folate as well as capable of surviving in the gastro intestinal system can be used as an effective probiotic to combat folate [12-14].

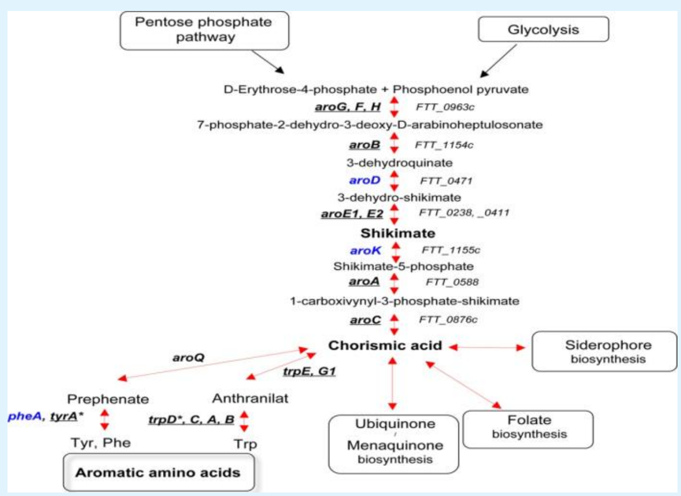

Figure 1: Folate biosynthesis [15].

LAB can produce from 2 to 16 μg/100g folate in food matrices. Also, the addition of glutamate could 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* [16].

Lb. amylovorus CRL887 is shown to be effective as a co-culture together with the yoghurt starter cultures and increases the folate concentrations of fermented foods. Such products, with increased natural folate concentrations, can be applied as an alternative to current fortification protocols that use the chemical form (folic acid) of this vitamin [17].

Certain strains of *Streptococcus thermophilus*, an important dairy starter, are been reported to produce larger amounts of folate compared with other bacteria, most of which is excreted into milk during the fermentation process. Yoghurts contain higher folate amounts than non-fermented milk because of folate production by S. *thermophilus*; however, it is shown that some *Lactobacillus delbrueckii* subsp. *bulgaricus* strains may able to increase the initial folate concentrations in milk by almost 190%, it can be said that these species can produce natural folates [3,18]. However, *S. thermophilus* may also reduce folates during the fermentation process. Therefore, the major problem when using these organisms for bio-fortification of folate, is the possibility that the folate present in the fermented milk product may be reduced by co-cultures when used as a starter. The among of these food grade bacteria and dairy starters, *S. thermophilus* is known as a good folate producer. These microorganisms produce folate extracellularly in the milk products during fermentation, unlike *Lb. lactis* and *Leuconostoc* which accumulate folate intracellularly, and therefore, the folate is not excreted into the milk. *Streptococcus salivarius* subsp. *thermophilus* is a gram-positive bacterium and homofermentative facultative anaerobe which does not form endospores [19]. The species of bacteria generally are used in fermented milk products can positively change the folate content that milk or dairy products may supply to consumers. However, the addition of prebiotics (fructooligosaccharides-FOS or galactooligosaccharides-GOS) does not meet the aim of increasing bacterial folate production, which is reached only in specific conditions depending on the bacteria strains choosen. More specifically, FOS increased folate production only in *L. plantarum*, whereas FOS reduced it in the other bacteria determined. In the case of the other prebiotic tested, only *S. thermophilus* exhibited high folate production rate when this bacterium is grown in GOS-supplemented trypticase-soy- yeast extract (TSYE) broth [10].

Twenty bacteria isolated from three commercial oat bran products are researched for their folate production ability. The bacteria as well as some reference microorganisms are grown until stationary phase on a rich medium (YPD), and the amount of total folate in the different cell mass and the culture medium (supernatant) is tested by microbiological procedure. The producers are determined as *Bacillus subtilis* ON4, *Chryseobacterium* sp. NR7, *Janthinobacterium* sp. RB4, *Pantoea agglomerans* ON2, and *Pseudomonas* sp ON8. The amount of folate produced in culture medium is the highest for *B. subtilis* ON5, *Chryseobacterium* sp. NR7, *Curtobacterium* sp. ON7, *Enterococcus durans* ON9, *Janthinobacterium* sp. RB4, *Paenibacillus* sp. ON10, *Propionibacterium* sp. RB9, and *Staphylococcus kloosii* RB7. Intracellular folate amount is higher when the bacteria are grown at 28 °C than at 18 °C or 37 °C and also higher at the conditions of pH 7 than at pH 5.5 [20].

It is proved in a depletion-repletion research that folate produced by metabolic engineering of *Lactococcus lactis* is exactly bioavailable and uses folate deficiency. Fermentation of cereal substrates makes an economical way of improving nutritional value, functional and sensory properties, and shelf life. Traditional fermented products, cereal-based foods include a wide variety of products are not only used by lactic acid fermentation bacteria but also other endogenous bacteria, yeasts and moulds. It is shown that endogenous bacteria may produce folate, and that traditional sourdough fermentation increases the folate content in rye bread. Biofortification and particularly fermentation fortification are researched in many studies at recent years [21,22].

It is determined that folate content and composition of food are dynamics and depend on medium components, physiological state and specific strain. If these factors are properly applied, high amounts of folate per unit biomass can be obtained and hence expected to make an important difference either in foods, such as dairy products, or in the intestine as folate-trophic probiotics or natural microbiota [6].

Folate bio-enriched fermented milk can be regarded a viable, economic, useful, and efficient alternative to folic acid fortification with no side effects. Consumers also can be improved from this type of product because they could increase their folate levels while consuming foods together with their normal diets [3].

Food products are complex media, in which include oxidizing and reducing components are naturally present and can stimulate and/or influence reactions. It is stated that many LAB strains are shown to consume folate; others are able to increase the folate content of cereal, vegetable, fruit or milk-based fermented foods [16].

Product	Microorganisms Used	Results	References
Yoghurt	*Lactobacillus delbrueckii* subsp. *bulgaricus* (3 strains) *Streptococcus thermophilus* (2 strains)	Folate concentrations are significantly higher (180 ± 10 μg/L, 250% increase) than non-fermented milk.	[5]
Folate free culture medium	55 strains of *Lactobacillus* species	*Lactobacillus amylovorous* can be successfully used as co-culture for natural folate bioenrichment of fermented milk.	[11]
Sourdough	*Saccharomyces cerevisiae, Torulaspora delbrueckii , Candida mulleri*	Folate contents of sterilized rye flour-water mixtures increased to about 3-folds.	[23,24]
Kefir	Russian kefir granules	Showed high folate production capacity.	[25]
Snythetic folate free media	19 strains of *Bifidobacteria* species	The highest folate content was found in *Bifidobacterium catenulatum* ATCC 27539 and the lowest in *Bf. animalis* ssp. *animalis* ATCC 25527 mediums.	[6]
Fermented milk	*Lb.bulgaricus* CRL871, *Str.thermophilus* CRL803, *Str.macedonicus* CRL415	Using folate producing starter cultures was found effective in improving folate content and also prevent folate deficiency.	[3]
Synthetic growth medium	*Saccharomyces cerevisiae*	By changing cultivation procedure increasing folate concentration caused Baker's yeast mass increasing also caused increasing folate concentrations in yeast fermented foods.	[7]
Fermented maize based porridge (Togwa)	*Candida glabrata* *Pichia anomala* *Issatchenkia orientails* *Saccharomyces cerevisiae* *Kluyveromyces marxianus*	The highest folate concentration was found fermentation with *C. glabrata* TY26 (23 fold increasing compared with nonfermented togwa.	[26]
Oat and barley based models	*Saccharomyces cerevisiae* ALK0743 *Candida Milleri* ABM4949 *Pseudomonas* spp. ON8 *Janthinobacterium* spp. RB4	Folate producing capacity of these microorganisms will enable the development of products rich in folate.	[21]
Fermented milk	*Str. thermophilus*	Folate-rich fermented milks have the potential to significantly increase the hemoglobin level of blood.	[27]
Indian fermented milk products	*Str. thermophilus*	Application of this microorganism as a probiotic starter is possible.	[28]

Skim milk, cucumber and water melon juices	*Lactococcus lactis* ssp. *cremoris*	*L. lactis* proved to be a good source for the enrichment of the folate content in these drinks.	[29]
Idli batter (wet grounded rice and black gram)	*Lc. lactis* N8, *S. boulardii* SAA655	Folate levels were increased with 40-90% compared with controls.	[30]
In milk and complex media	*Bifidobacterium catelunatum, Bf. adolescentis, Lb. plantarum , Lb. delbrueckii ssp. bulgaricus, Str. thermophilus*	*Bf. catelunatum* and *Str. thermophilus* produced the highest level of folate in complex media and milk.	[10]
Goat's milk yoghurt with water soluble soybean extract	*B. lactis*	Folate contents in yoghurts increased with the addition of probiotic culture and soybean extract.	[31]
Reduced fat soft cheese	*Str. thermophilus* culture	The content of folates by up to 67% compared to control cheeses obtained using a commercial starter culture for soft cheeses.	[32]
Traditional cereal based African fermented food	*Pichia kudriavzevii*	*P. kudriavzevii* is a suitable starter for fermented cereal-based products. It can survive the conditions of the human GIT, to adhere to intestinal cell lines, and to produce folate and phytases.	[33]
Cassava	*Lb. rhamnosus, Lb. plantarum, Lb. acidophilus, Lb. reuteri, Lb. fermentum, Lb. brevis, Lb. salivarus*	LAB can be produced natural folate.	[34]

Table 1: Some researches about folate biofortification with microorganisms.

Conclusion

In next researches, development of folate producing LAB and yeast strains and combinations of them for food applications should be supported. More needs to be learnt about folate forms produced and the relations between food matrix and folate producing microorganisms. Folate production ability of microorganism strains used in the production of fermented milk starter cultures should be tested; formulations can be optimized for natural fortification of the folate content in food products. The increase in folate levels in yogurts and fermented milks is possible through effective selection of the microbial species through isolation with native strains of folate-producing microorganisms from different traditional (ethnic foods, fruits and vegetables, vegetation, and so on) and cultivation medium. Such products would supply economic advantageous to food producers since increased "natural" folate amounts would be an important value-added effect without increasing production costs.

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