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Enhancing folate levels in different fermented milks through biofortification and content evaluation during storage using HPLC-MS/MS analysis

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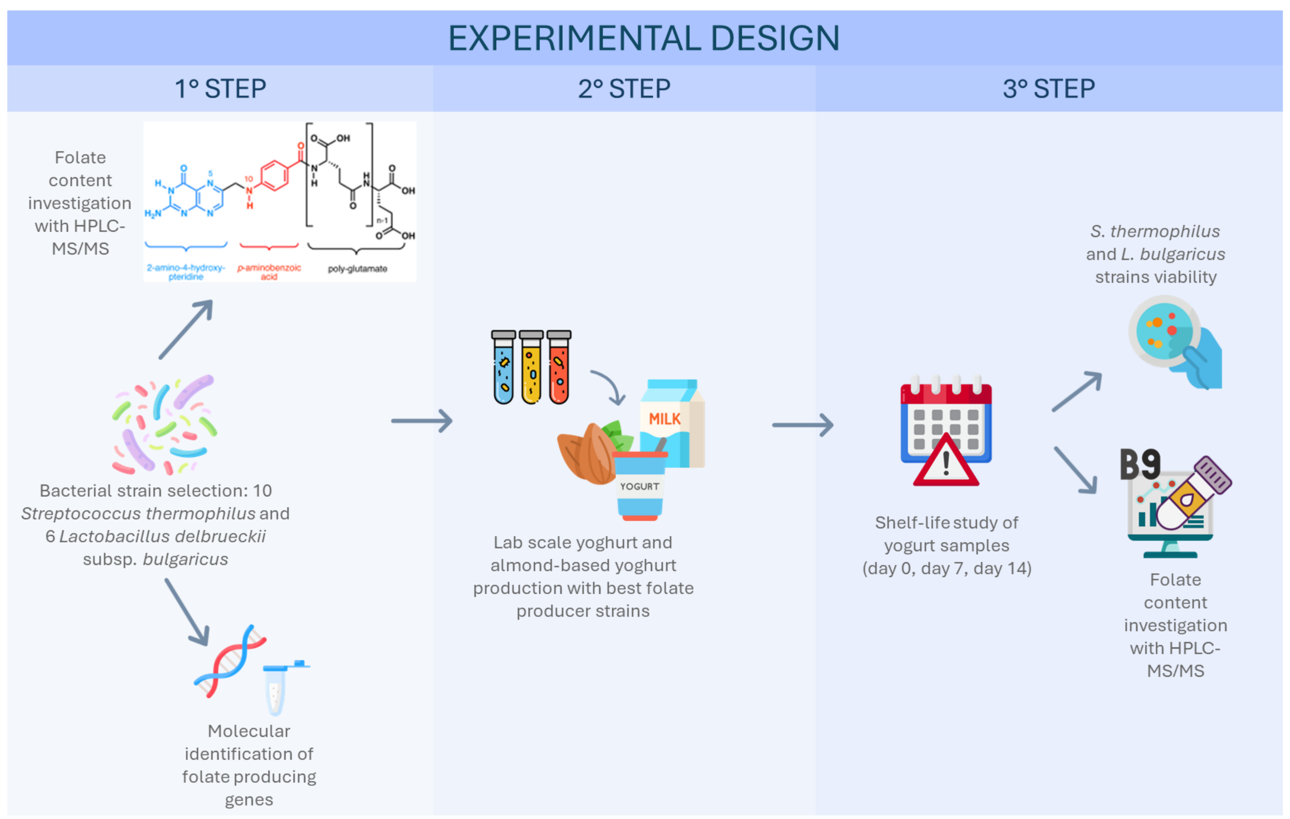
INTRODUCTION

Folate, the natural form of vitamin B9, plays a role in several biological processes, including DNA synthesis, repair and methylation, as well as erythropoiesis and critical growth phases such as pregnancy and infancy. This vitamin is naturally present in many foods and can not be synthesised by animal cells, making dietary intake crucial for maintaining adequate levels. Folate deficiencies are common and can lead to different health problems such as neural tube defects and megaloblastic anaemia.

In recent years, there has been a growing focus on the development of functional foods with enhanced nutritional value. Biofortification has emerged as a promising strategy to increase the natural folate content of foods, particularly when applied to fermented foods.

The objective of this study was to enhance folate levels in yoghurts produced from cow's milk and almond milk through biofortification.



RESULTS

1. Strain selection

In this study, 16 strains of *Streptococcus thermophilus* and *Lactobacillus delbruckii* subsp. *bulgaricus* were tested for their ability to produce folate. PCR analysis revealed the presence of four genes involved in folate biosynthesis in *Lactobacillus* species in all the tested strains: *folA* (dihydrofolate reductase), *folC* (folate synthetase/folyl polyglutamate synthetase), *folK* (2-amino-4-hydroxy-6-hydroxymethyldihydropterideine pyrophosphokinase) and *folP* (dihydropteroate synthase).

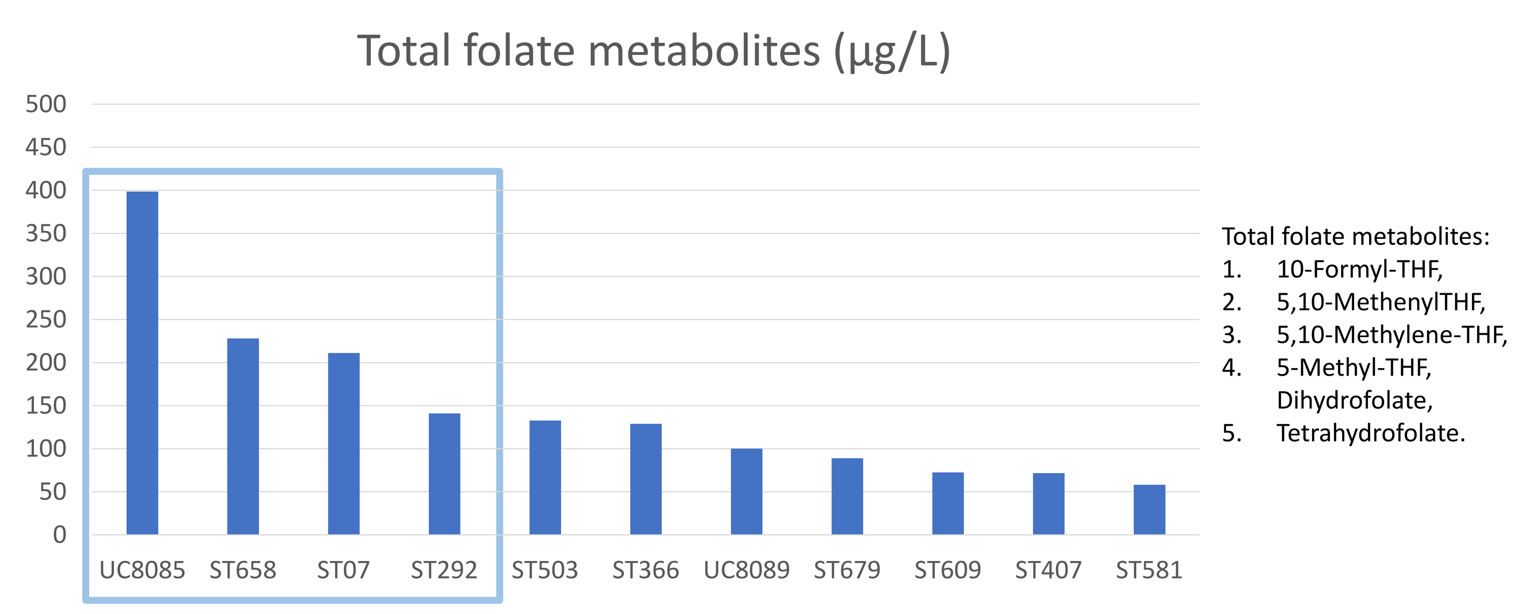


Fig. 1 – Semi-quantification of six folate metabolites produced by S. thermophilus and L. bulgaricus strains in milk samples.

UHPLC-MS/MS was used to analyze folate production, shifting from traditional microbiological methods. Six key folate metabolites were identified and grouped as total folic acid metabolites. (Fig. 1). Semi-quantification was performed using the 5-MTHF calibration curve.

2. Yoghurt preparation

Product source	Name	*S. thermophilus*	*L. bulgaricus*	Ratio	% inoculated
Cow milk	YCB1	ST 07	UC 8085	2:1	8%:4%
	YCB2	ST 292	UC 8085	2:1	8%:4%
	YCB3	ST 658	UC 8085	2:1	8%:4%
Almond milk	YMB1	ST 07	UC 8085	1:1	4%:4%
	YMB2	ST 292	UC 8085	1:1	4%:4%
	YMB3	ST 658	UC 8085	1:1	4%:4%

Tab. 1 - Three distinct blends of the most effective strains of S. thermophilus and L. bulgaricus used to create the different cow and almond yoghurts.

Three blends (Tab. 1) were inoculated into cow's and almond milks, with an initial bacterial count of 8 log CFU/ml. Over time, bacterial levels in cow yoghurts stayed constant or slightly increased, while those in almond yoghurts dropped by 1-2 log CFU/ml. The highest bacterial concentration in cow yoghurt was in blend YCB3, while in almond yoghurt it was in blend YMB2.

3. Folates quantification in yoghurt

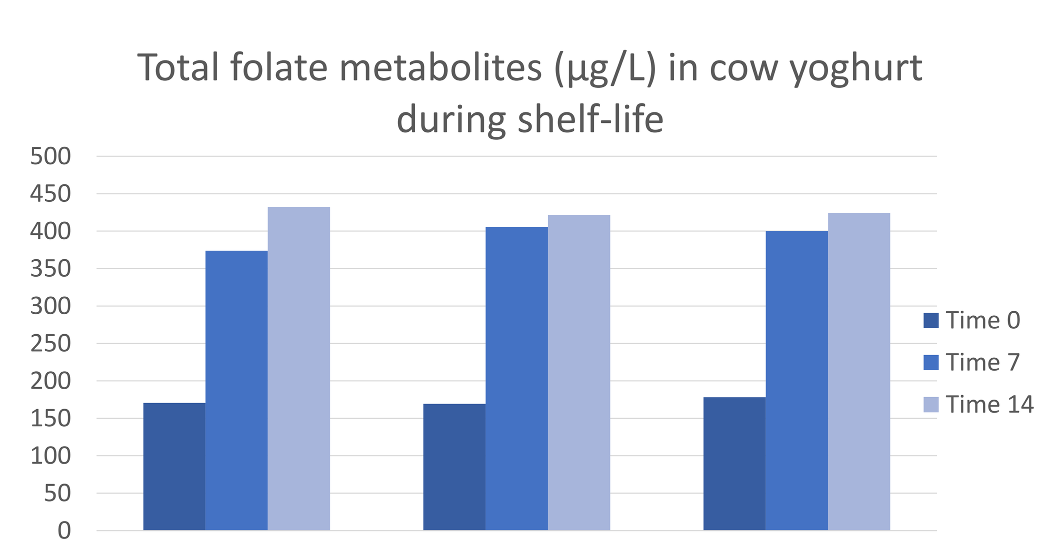


Fig. 2 - Folates content in cow yoghurt during shelf-life.

The folate levels in cow yoghurt samples increased over the shelf-life period (Fig. 2). The blend YCB1, representing the optimal combination of bacterial species, showed the highest folate content at 432.08 µg/L.

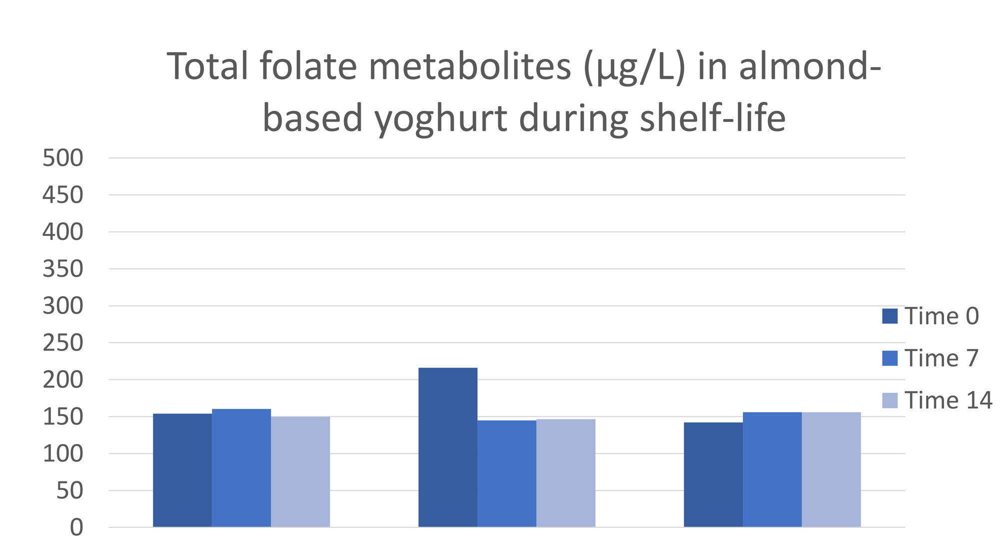


Fig. 3 - Folates content in almond-based yoghurt during shelf-life.

Levels of folate metabolites in almond-based yoghurt fluctuate during its estimated shelf-life, with levels after 14 days significantly lower than in cow's yogurt, showing a fourfold reduction (Fig. 3). In this case, the optimal solution was the YMB3 mixture, with values of 155.81 µg/L.

CONCLUSIONS

- ✓ *S. thermophilus* strains ST 07, ST 292 and ST 658, and *L. bulgaricus* UC 8085 were identified as the most efficient producers of folate and were selected as starter cultures for yoghurt and almond alternatives.
- ✓ Final level of total folate metabolites in the cow yoghurt resulted to be much higher with respect to those of almond-based alternative.
- ✓ A portion of biofortified yoghurt (125ml) contains a folate content that is below the daily requirement for the general population, as defined by EFSA (330 µg/day).
- ✓ The study has demonstrated that the folate content of yoghurt can be enhanced through the biofortification of lactic acid bacteria. Further investigations are recommended to optimise this potential.

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