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# Utilization of beneficial micro-organisms in mango pulp variety: Panchavarnam and its impact on folic acid content

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**Abstract**
Folic acid is one of the vital micronutrients that help in the regular growth and development of the fetus. Folic acid deficiency leads to neural tube deficiency during pregnancy. Fruits are sources of micronutrients. Mango is also a good source of folic acid. Several studies proved that some of the beneficial microorganisms synthesize folate. Mainly, these strains were used in dairy and bakery products, but less in fruit-based products. In this study, four microorganisms were used: *Lactobacillus acidophilus, Leuconostoc mesenteroides, Streptococcus thermophilus, and Saccharomyces cerevisiae.* Initially, mango pulp concentration was 0.231 mg/kg. After the fermentation, *Saccharomyces cerevisiae* synthesized a higher concentration compared to the remaining three bacterial strains. There are three different concentrations of mango pulp used for folate synthesis. The yeast synthesize the 1.46, 1.36, and 1.26 mg/kg concentrations of folates in 10%, 20%, and 30% of mango pulp, respectively. *Lactobacillus acidophilus* also synthesizes folates of about 0.381, 0.499, and 0.462 mg/kg in mango pulp concentrations of 10%, 20%, and 30%, respectively. The remaining *Leuconostoc mesenteroides* and *Streptococcus thermophilus* also synthesized folate with lower concentrations as compared to the other two.

**Keywords:** Micro-organisms, pulp variety, Panchavarnam, folic acid

## 1. Introduction
Fruits are rich sources of micronutrients compared to macronutrients. Vitamins and minerals are considered micronutrients and are present in fruits and vegetables. The body's growth and development is greatly impacted by vitamins. The human body requires vitamins in very small amounts compared to macronutrients. Even though a smaller amount of vitamins leads to different disorders, resupplying the vitamins helps overcome the deficiency (Maqbool Muhammad Amir *et al*. 2017) [11]. Vitamin B9 or folic acid or folates, or folacin are the water-soluble vitamins. Folic acid is a crucial vitamin that plays an important role during pregnancy. This is an important nutrient that helps prevent neural tube deficiency. NTD forms different conditions in newborn babies; 1. Spina bifida: a condition when spinal cord does not properly develop and causes paralysis of the limbs, as well as effects urinary and faecal. 2. Anencephaly: this is the severe condition of folic acid deficiency that leads to incomplete formation of the skull and improper development of the brain (Sutton, Daly, and Kirke 2008) [12]. In 1965, folic acid deficiency was connected with neural tube defects. (Crider, Bailey, and Berry 2011) [5]. The previous history of affected pregnant women should consume 4000µg of folic acid daily starting at the planning stage of a pregnancy, which was recommended by the Centers for Disease Control and Prevention during 1992 (Centers for Disease Control and Prevention 1991) [3]. In addition, to prevent neural tube deficiency, the United States Public Health Service advised all women of childbearing age to ingest 400g of folic acid daily through supplements and fortification in 1992. (Centers for Disease Control and Prevention (CDC) 1993) [4]. The Institute of Medicine recommends that all pregnant women consume 400µg of folic acid through diet or supplements, or fortification to decrease the occurrence of deficiency (Institute of Medicine. Folate 1998) [9]. Further, the World Health Organization and Agricultural Organizations of the United States made guidelines for the fortification of flour with folic acid (Allen *et al*. 2006) [1] and several countries started to implement the fortification of folic acid to decrease the prevalence of deficiency (Crider, Bailey, and Berry 2011) [5].

In the human body, folate is present in the form of 5-methyltetrahydrofolate. Naturally occurring folate is in the form of tetrahydrofolate with glutamate residues, which are present in foods. Folic acid is the fully man-made synthesis, which is the completely oxidized form of monoglutamate used in the fortification of foods (Bailey, Lynn B. 2012) [2]. Naturally, folates have different forms, but they only differ in their carbon rings. Foods rich in folate includes fruits and vegetable, mainly spinach, asparagus, but high concentrations are found in yeasts, wheat germ and some pulses and cereals (Wusigale and Liang 2020; Delchier *et al*. 2016) [14, 6]. Several studies were carried out to synthesize the folic acid in that by using beneficial microorganisms that produce folate via fermentation. In this study, four beneficial microorganisms were used, namely: *Streptococcus thermophilus and Saccharomyces cerevisiae Lactobacillus acidophilus, Leuconostoc mesenteroides are involved* in the production of folic acid in mango pulp.

## 2. Materials and Methods

### 2.1 Sample collections

Panchavarnam mangoes were received from the Government Horticulture Marungulam Farm, Marungulam, Thanjavur, India. The media required for microbial growth were received from Hi-media.

### 2.2 Sample preparation

Fresh, pulpy mango was washed, peeled and removed seed. The pulp was homogenized for fermentation purposes.

### 2.3 Microbial culture preparation

Firstly, broth was prepared and autoclaved. After being autoclaved, loops of culture were taken and added to the respective broth. The Lactic acid Bacteria strains of *Lactobacillus acidophilus* and *Leuconostoc mesenteroides* were inoculated in LAB MRS Broth and incubated period was 18-24hour at 35–37 °C. Then *Streptococcus thermophilus* was grown in nutrient broth at 35–37 °C for 18–48 hours, whereas, *Saccharomyces cerevisiae* were inoculated in Potato Dextrose Broth and incubated period was 24-48hrs at 25-30 ℃. Further broth was centrifuged and washed off cells with buffer phosphate solution to get pellets. 10 ml of sterile water added to the pellet.

### 2.4 Pulp preparation for fermentation

For fermentation different concentration of pulp were used. The concentration of pulp was 10%, 20% and 30%. The *Lactobacillus acidophilus and Leuconostoc mesenteroides* and *Streptococcus thermophilus* of 10ml culture added to pulp and kept in incubated conditions for 24 hours at 37 °C, whereas *Saccharomyces cerevisiae* was added and kept in incubated conditions for 24hrs at 25 °C. Pulp was incubated in incubator shaker and maintained 120 rpm.

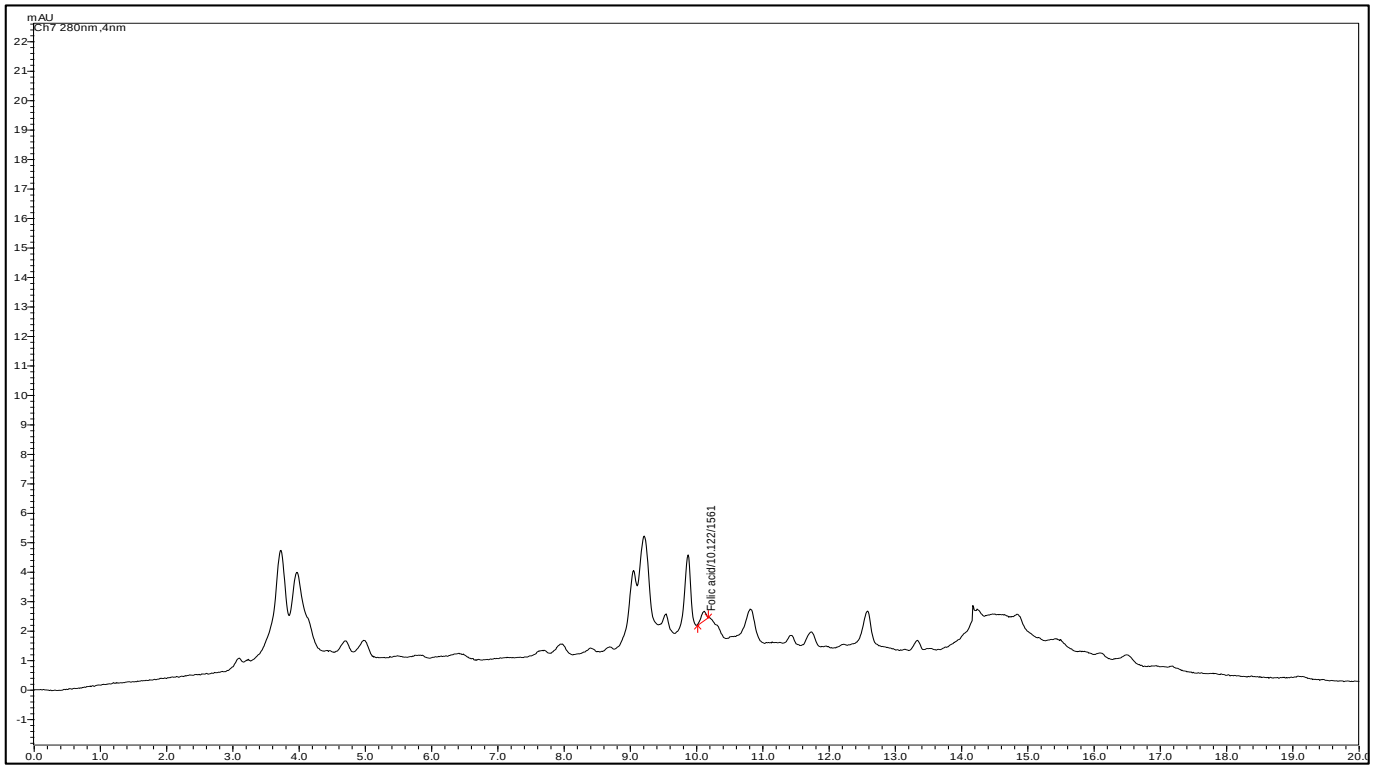
### 2.5 Sample preparation for HPLC analysis

The mango fermented pulp were prepared by direct solvent extraction. Firstly mango fermented pulp samples were diluted and then it analysed. Added accurately weighed 10 g of homogenised sample into a 50-mL centrifuge tube and added 25 mL of buffer extraction (0.1 M phosphate buffer pH 7.0) and kept for ultrasonic extraction for 15 min. Then added the extraction solution to make up the volume of 50 ml. Then the samples were centrifuged at 4,500 rpm, the supernatant was filtered through a 0.22 µm filter, and the samples were injected into the HPLC system.

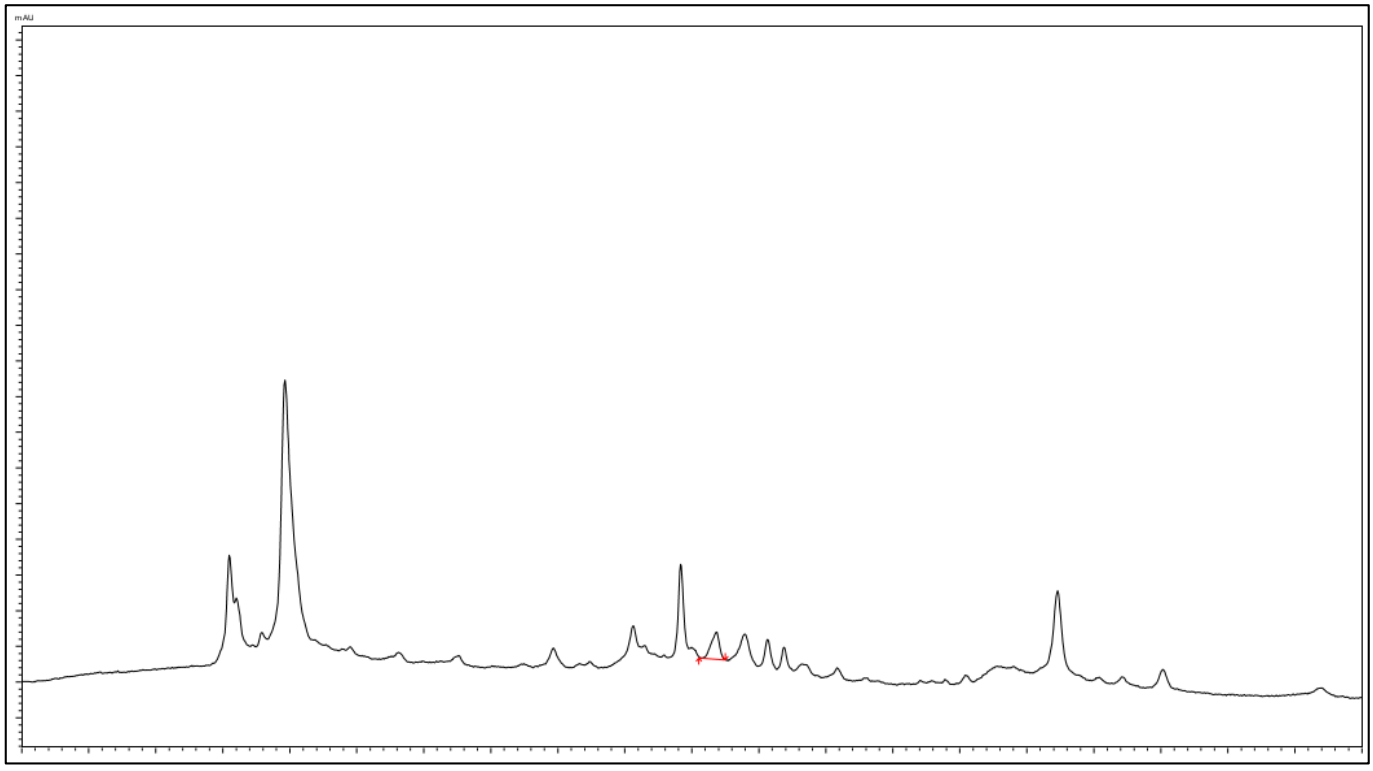
## 3. Results and Discussion

The HPLC results are tabulated in Table No.1. The initial mango pulp folic acid concentration was 0.231 mg/kg. The four beneficial microorganisms were added to mango pulp and kept in a shaker incubator. The fermentation process plays an important role in synthesizing the folic acid in mango pulp. The HPLC chromatogram results are represented in Figure 1. *Saccharomyces cerevisiae* synthesized folic acid at a higher concentration as compared to *Lactobacillus acidophilus and Leuconostoc mesenteroides and Streptococcus thermophilus.* The different concentrations of mango pulp fermented yeast synthesized 1.46, 1.36 and 1.26mg/kg of folic acid in 10%, 20% and 30% of the fruit pulp, respectively. Several studies proved that Baker's yeast has the ability to synthesise folic acid. *Saccharomyces cerevisiae* used in bread formulation for enrichment of folates by the biofortification method and to enhance folates in wine by the bioengineering method were studied (Walkey *et al*. 2015; Hjortmo *et al*. 2008) [13, 8]. In *Lactobacillus acidophilus* 20% fermented mango pulp synthesis, the folic acid has a higher concentration of 0.499 mg/kg compared to 10% and 30% of mano pulp. In milk and milk products, *Lactobacillus acidophilus* synthesises folic acid. Many different Lab strains were used for the production of folic acid in different milk products. The remaining two LAB cultures were also synthesised, but at a lower concentration as compared to *Saccharomyces cerevisiae and Lactobacillus acidophilus.* The least concentration was observed in 30% of mango pulp cultures with *Leuconostoc mesenteroides.* The probiotic strain *Streptococcus thermophilus* also synthesised folic acid, but in a lower concentration relative to the three different mango pulp concentrations. The LAB strains of *Lactobacillus acidophilus, Leuconostoc mesenteroides and Streptococcus thermophilus* synthesised a good amount of folate in all milk and milk products (Lin and Young 2000; De Giori and LeBlanc 2018) [10, 7]. The LAB strains were also used for the synthesis of folates in bakery products, but it revealed that compared to synthesis, they consumed the folates in sourdough products (De Giori and LeBlanc 2018) [7].

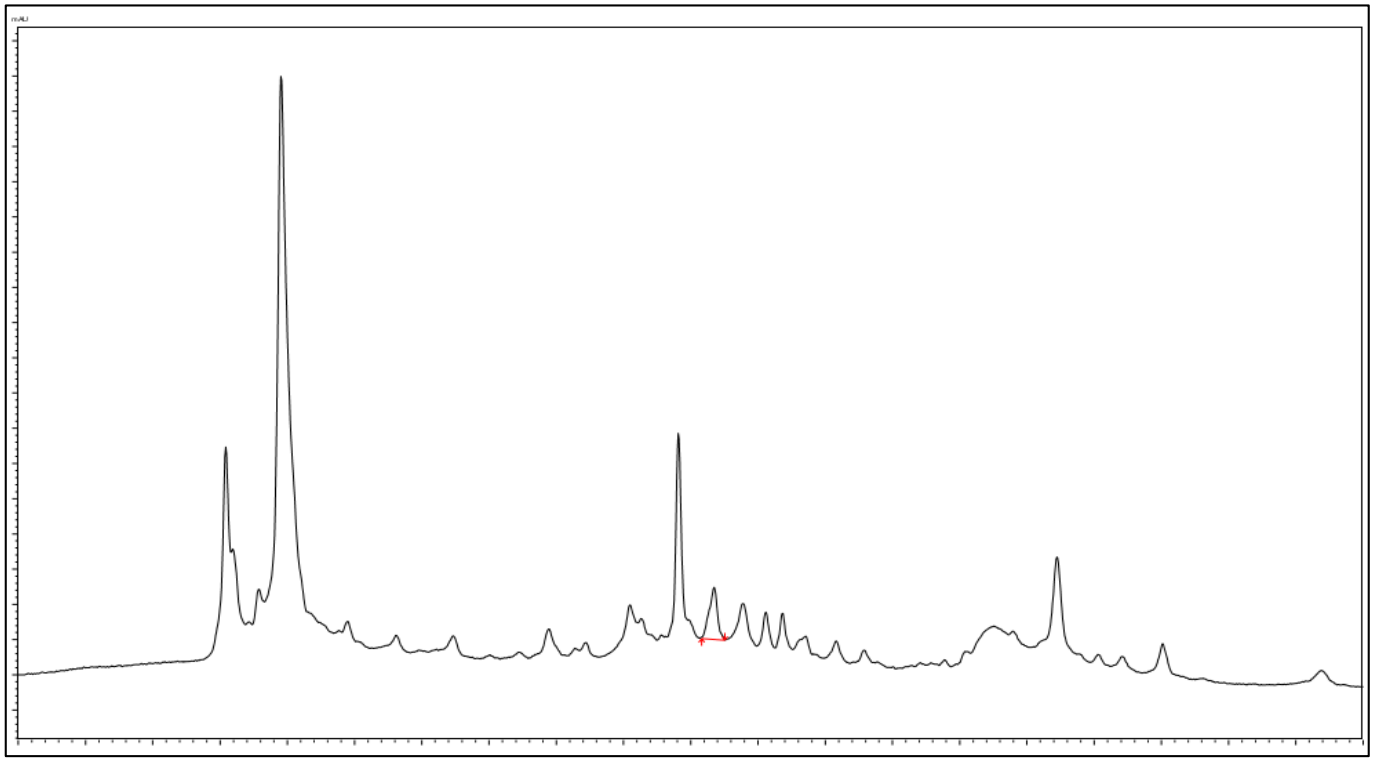
Mango pulp control




10% mango with *S. cerevisiae*



20% mango with *S. cerevisiae*



30% mango with *S. cerevisiae*

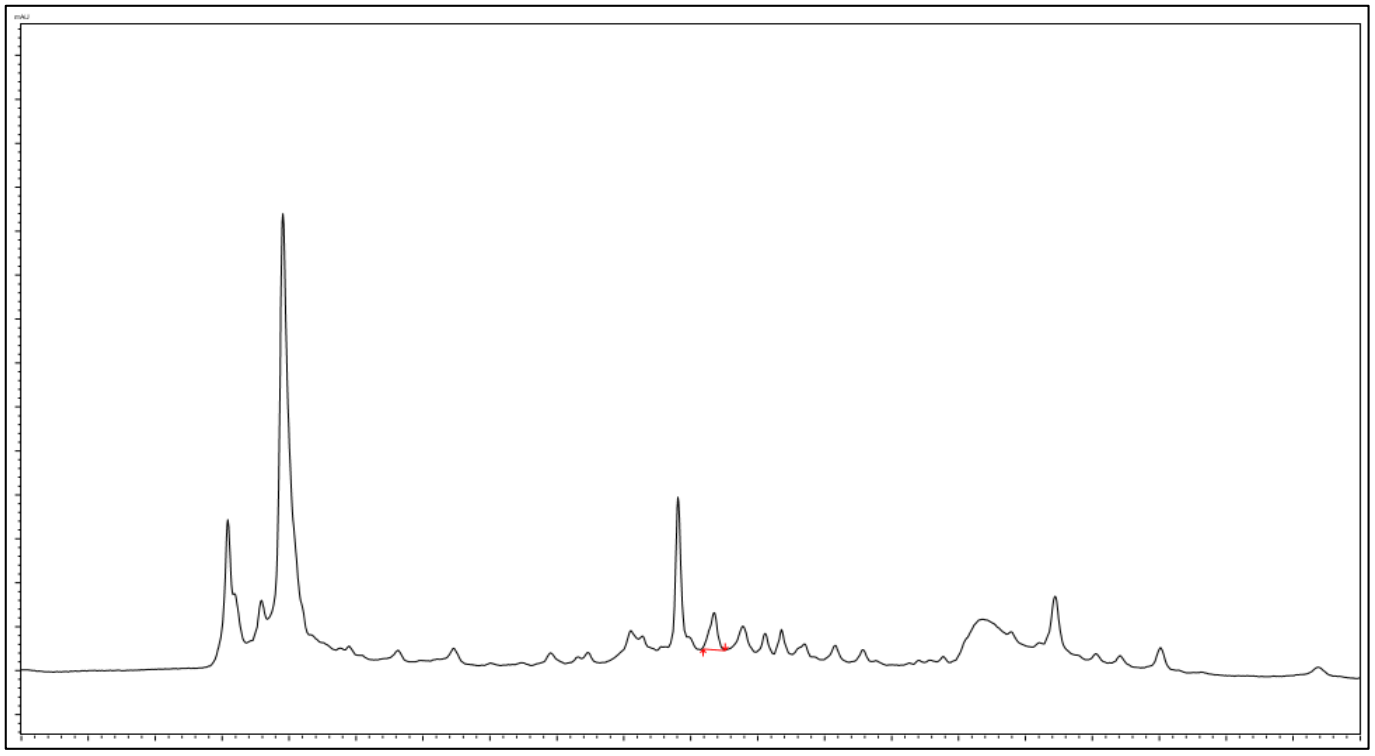
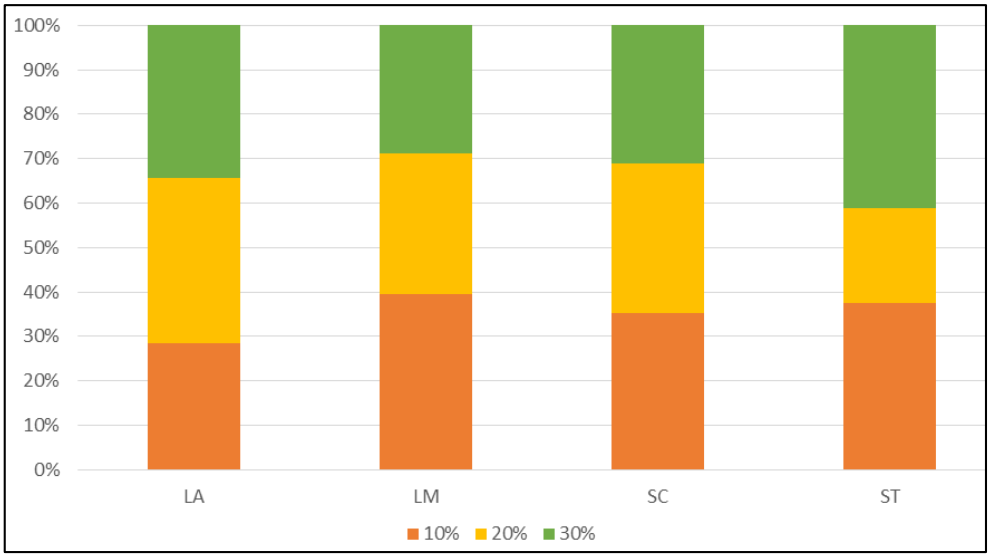


**Fig 1:** HPLC result of folic acid in mango pulp

**Table 1:** The concentration of folic acid in different concentration of mango pulp

| Micro-organisms | Concentration of mango | | |
|---|---|---|---|
| | 10% | 20% | 30% |
| *Lactobacillus acidophilus* | 0.381 | 0.499 | 0.462 |
| *Leuconostoc mesenteroides* | 0.179 | 0.143 | 0.13 |
| *Saccharomyces cerevisiae* | 1.42 | 1.36 | 1.26 |
| *Streptococcus thermophilus* | 0.4 | 0.23 | 0.44 |




Folate content of different concentration of mango pulp

## 4. Conclusion

This result supports the idea that the strains used for this study have the ability to synthesise folates and can be used for further studies. The majority of folate enrichment studies considered dairy based products. This will help with upcoming research on fruit-enriched folate products. This will give ideas for the development of new products based on fruits.

## 5. Acknowledgement

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