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Article

Mango (*Mangifera indica* L) var Banganapalli: Impact of in-situ intervention on folic acid concentration and its changes in physicochemical property

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Abstract: Fruits are a source of vitamins. Mango is one of the abundantly nutritional fruits. Vitamin B9, or folic acid, is one of the important vital amines due to its role in preventing neural deficiency. Several beneficial micro-organisms are used for the synthesis of folic acid. In this study, *Lactobacillus acidophilus*, *Leuconostoc mesenteroides*, *Streptococcus thermophilus*, and *Saccharomyces cerevisiae* were used. *Saccharomyces cerevisiae* synthesized folic acid as compared to other organisms. There were five different concentrations of mango pulp that were analyzed for folic acid synthesis (5%, 10%, 15%, 20%, and 30%). The initial concentration of pulp was 133.37 mg kg^{-1}, but after fermentation with four micro-organisms it got reduced. As compared to the other three organisms, *Saccharomyces cerevisiae* synthesizes 17.15 mg kg^{-1}, 30.14 mg kg^{-1}, 28.62 mg kg^{-1}, 21.70 mg kg^{-1}, and 21.78 mg kg^{-1}, respectively, at different pulp concentrations of 5%, 10%, 15, 20%, and 30%. Vitamin C increased to 320 mg as compared to the control, and there was no significant difference between the four micro-organisms. Antioxidants also showed positive results at different concentrations of pulp. There was an increase in titratable acidity and a decrease in pH recorded for the 24 h fermentation period. In this variety, the color of mango pulp slightly changes to yellow shades due to the breakdown of pigments, so this effects the **b* value in between the pulp concentrations. Data supports the enrichment of folic acid, which will further support the utilization of beneficial micro-organisms in food beverages.

Keywords: *Saccharomyces cerevisiae*; HPLC; vitamin C; Lactic acid bacteria and pH

1. Introduction

India is one of the leading countries in the production of fruits and vegetables. It accounts for 50% of world production and holds the rank of first [1,2]. China, Mexico, Thailand, Pakistan, Brazil, Egypt, Nigeria, the Philippines, and Indonesia are the other major mango producing countries. Mango is the king of fruit in India, and regarding economic importance, it is the third largest agricultural produce. The large number of cultivars and wild varieties leads to huge diversification in mango production. Its source is abundant in nutrition, adaptability, variety, and excellent flavour and taste. This is the most tropical dessert worldwide, compared to other tropical fruits. In India, Andra Pradesh is the leading producer of mango, followed by Bihar, Gujarath, and

Karnataka. Banganaplli (*Mangifera indica* L) is a variety of mango cultivated in and around Banganapalle, Andra Pradesh, India [3]. This is also one of the commercial varieties in Tamil Nadu and Andra Pradesh, banganapalli is also known as safeda, chapta, chapai, and baneshan [2,4]. Mango is a rich source of macronutrients and micronutrients; sucrose is the principal sugar component present in ripened mango, and it also contributes 15% of total sugars [5]. The ripened mango tastes sweet due to the presence of malic acid and the blending of reducing sugars. Suar to acid ratio in mango represents the ripeness of mango. In some of the mango variety it ranges from 4% to 11% in immature stage while in ripened 29.7% to 43% this observed by Rastegar and Rahimzadeh [6]. It's a rich source of β-carotene (vitamin A) and the antioxidant vitamin C. In mango, pulp is the major consumable, functional, nutritional, and application in various food beverages as a flavoring substance. According to the USDA data base, mango pulp contains the major four vitamins: vitamin A, C, E, and K. Even though vitamin A and C contribute a higher concentration compared to the other two, it depends on the ripening stage of mango. Mango pulp is also a source of macro- and micro-minerals, especially calcium, copper, sodium, phosphorus, iron, magnesium, manganese, zinc, selenium, and boron. The protein and fatty acid concentrations are lower, and the presence of essential fatty acids represents the maturation stage of mango [7]. The potential antioxidant types present in natural fruits, which have more biological effects on the human body, were studied by Rabie et al. [8].

According to the National Institute of Nutrition (NIN), the banganapalli (*Mangifera indica* L) variety contributes 82.05 ± 7.90 of total folates. Vitamin B9, or folic acid or folate is one of the crucial water-soluble nutrients. The key role that folates play in our bodies is in preventing neural tube defects. It also interacts in conjunction with cobalamin to help repair, synthesize, and methylate DNA, as well as act as a cofactor in a wide range of biological processes. Megaloblastic anaemia is caused by folic acid deficiency, which also causes neural tube abnormalities in infants [9] and an increased risk of cardiovascular diseases such as Alzheimer's disease and colorectal cancers [10]. The stability of water-soluble vitamins does not last long during the processing of food. It is one of the important nutrients that cannot be synthesized by body. The dietary intake of folate rich foods contributes to the daily intake requirement for the body. Due to the fact that a lack of dietary intake of folic acid leads to deficiency during pregnancy, fortification of folic acid in several foods has been made mandatory by the Food and Drug Administration (FDA) [11]. The drawback of synthetic forms of folic acid is that they will mask the cobalamin or vitamin B12 deficiency. Several fermentation foods, green vegetables, and leguminous plants can be sources of natural folates. It's been reported that several micro-organisms have the ability to produce folate due to its strict requirements for growth. The beneficial micro-organisms synthesize the folate through *De novo*. Numerous researchers reported that some of the strains of Lactic Acid Bacteria (LAB) [12–14] for industrial importance starter such as *Leuconostoc*, *S. thermophilus*, *Lactococcus lactis*, *Propionibacterium*, and *Bifidobacteria* species, have the ability to synthesize folic acid. These micro-organisms are the nature of probiotics and, in the same way, have a positive impact on folic acid production during milk fermentation. Not only bacteria, but even brewer yeast is one of the important strains for folate

production. There is a larger difference in folate content among the different strains of *Saccharomyces cerevisiae,* so it is very important to choose the proper culture while optimizing folate content in yeast-fermented food. The traditional method of fermentation in cereal-based products like oats barely reported higher folate content by utilizing selected lactic acid bacteria and yeasts. It was reported that yeast isolates from bran increased folate production in oat fermentation [15,16].

In this study, three lactic acid-producing organisms and one yeast strain were used, namely: *Lactobacillus acidophilus*, *Leuconostoc mesenteroides*, *Streptococcus thermophilus*, and *Saccharomyces cerevisiae*. Folates are naturally present in fruits, but their utilization is less. The four beneficial microbes were used in milk and milk products, wines, and wheat-based products but fruits-based products were less studied. The main objective of the study was to investigate the folic acid synthesis by the selected beneficial microbes and its impact on the physiochemical properties of fruit pulp. This result supports further product development.

2. Material and method

2.1. Sample collections

Mango samples were collected from the local farmer Bangalore Karnataka. The mango was directly plucked from the tree in the morning and roughly temperature was 22 ℃. The mangoes were received in immature stage and laterally ripened the mango by placing in between paddy straw for 3–4 days in dark condition. The media required for cultures were procured from Hi-Media. The folic acid standards and HPLC-grade solvents were procured from Sigma-Aldrich.

2.2. Sample preparation

The ripened *Mangifera indica* L banganapalli was washed, peeled, and the seeds removed. Pulping of fruits was done by using a colloidal ball mill. The pulped fruits were sterilized and kept for further study.

2.3. Microbial growth conditions

The LAB strains, mainly *Lactobacillus acidophilus* MTCC 10307 and *Leuconostoc mesenteroides* MTCC 867, were obtained from the Microbial Type Culture Collection. IMTECH, Chandigarh, India, and *Saccharomyces cerevisiae* NCIM-3059 received from the National Collection of Industrial Microorganisms, Pune, India, and *Streptococcus thermophilus* NCDC 459 obtained from the National Collection of Dairy Cultures, Dairy Microbiology Division, National Dairy Research Institute, Karnal, Haryana, India. The LAB culture was inoculated in Lactobacillus MRS agar and incubated at 35–37 ℃ for 18–24 h, whereas *Saccharomyces cerevisiae* inoculated in Chloramphenicol Yeast Glucose Agar were incubated at 22–25 ℃ for 2–5 days. The *Streptococcus thermophilus* were inoculated in *Streptococcus thermophilus* isolation agar and incubated at 35–37 ℃ for 48–72 h. After the incubation period, the cultures were stored under refrigeration for further use.

2.4. Selections of culture for folate production

The different broths were used for four different organisms, which were prepared according to the technical data given by Hi-Media. The prepared broth was autoclaved and cooled down for inoculation. The loopful of *Lactobacillus acidophilus* and *Leuconostoc mesenteroides* were grown in LAB MRS broth at 35–37 ℃ for 18–24 h, then in potato dextrose broth at 35–37 ℃ for 18–24 h, whereas *Streptococcus thermophilus* was grown in nutrient broth at 35–37 ℃ for 18–48 h. Thereafter, activated broth cultures were centrifuged and washed three times with buffer phosphate solution. The pellet cells were used for folate production in fruit pulp.

2.5. Pulp preparation for fermentation

The mango pulp concentrations of 5 g, 10 g, 15 g, 20 g, and 30 g were used for folate production. For this, 10 mL of each culture is added, bringing the total volume to 100%. Each 10 mL of culture contains 10^8–10^{10} cfu/mL. The cultures of *Lactobacillus acidophilus, Leuconostoc mesenteroides* and *Streptococcus thermophilus* were added to fruit pulp and incubated at 37 ℃ for 24 h, whereas yeast was incubated at 25 ℃ for 24 h. Pulp was incubated in a shaker incubator at 120 rpm. After the incubation period, samples were analyzed through HPLC for folic acid content in pulp.

2.6. Chromatography parameter

The determination of folic acid was performed by using high-performance liquid chromatography (Shimadzu, Japan). The system is equipped with a quaternary pump (LC-30AD), a degasser (DGU-20A3), an autosampler (SIL-30AC), a column, computer software (LabSolution), and an ultraviolet detector. The chromatographic parameters were: column Shim-pack 4.6 × 150 mm, 3 μm C18, column oven temperature maintained at 35 ℃, data acquisition time 25 min, injection volume 20 μL, flow rate 0.4 mL/min, and solvent used for mobile phase A: 25 mM hydrogen dipotassium phosphate (HK2PO4) pH-7.0, mobile phase B: acetonitrile. The gradient elution with hydrogen dipotassium phosphate and acetonitrile solution started at 1% (v/v); this was for the initial time and for 5 min. Gradually, for 15 min and 20 min, it was 30% (v/v), and finally, for 20 min, 10 min, and 25 min, the gradient elution was 1% (v/v). The post-run time was maintained for 5 min. The ultraviolet detectors are used to detect folic acid concentration in fruit pulp at a wavelength of 280 nm [17].

2.7. Sample preparation for HPLC

The samples were prepared by direct solvent extraction. Firstly, mango pulp samples were diluted and then it analyzed. Added accurately weighed 10 g of homogenized sample into a 50-mL centrifuge tube and added 25 mL of extraction buffer (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 4500 rpm, the supernatant was filtered through a 0.22 μm filter, and the samples were injected into the HPLC system [18,19].

2.8. pH

pH was recorded by using Mettler Toledo pH and conductivity meter. pH was recorded for every 6 h till 24 h of fermentation.

2.9. Titratable acidity

Titratable acidities were carried out as per method describe by Ragnanna [20] and FSSAI manual [21]. Ten mL of fermented pulp were taken in conical flask. Then added two-three drops of phenolphthalein indicator and shaken vigorously. Then titrated against 0.1 N NaOH, till appeared of permanent pale pink colour. Finally Recorded the titer value which represent volume of NaOH required for Titration. The titratable acidity was calculated by using the formula:

$$\%Titratble\ acidity = \left[\frac{T \times N \times V1 \times E}{V2 \times W \times 1000}\right] \times 100$$

whereas, *T*: titer value, *N*: normality of NaOH, *V*1: volume made up to, *V*2: volume extracted, *E*: equivalent weight of the acid *W*: sample weight.

2.10. Color measurement

Hunter colour flex model has been used to measure colour. It operates on the concept of absorbing light and measuring energy from reflected samples across the full visible spectrum. Prior to beginning the sample, the equipment used to assess colour was standardized using standards like white and black. The L*a* and b* of the samples were calculated, where L stands for lightness and darkness, a* for red (+a) and green (−a), and b* for blue (+b) and yellow (−b).

2.11. Vitamin C

The vitamin C or ascorbic acid was determined by the volumetric method [22]. Dye solution was prepared. It includes 42 mg sodium bicarbonate into a small volume of distilled water and added 52 mg of 2.6-dichrolo phenol indophenol and make up to 200 mL with distilled water. Standard solution:100 mg of ascorbic acid was dissolved in 100 mL of 4% oxalic acid solution in a volumetric flask. It represents 1 mg/1 mL, from this 10 mL of working standard solution was prepared by dissolving in 100 mL of oxalic acid. The working standard concentration was 100 µg/mL. From the working standard 5 mL pipette out into a 100 mL conical flask then added 10 mL of 4% oxalic acid and titrated against dye solution. The end point was appearance of pink colour which stable only few minutes. The sample weighed about 5 g and added known volume of 4% oxalic acid into centrifuge tubes then samples were centrifuge for 10 min at 4500 rpm. Then 5 mL of supernatant pipette out into a 50 mL conical flask and added 10 mL of 4% oxalic acid and titrated against dye.

$$Ascorbic\ acid\ \frac{mg}{100g} = \frac{0.5mg \times V_2ml \times 100ml}{V_1ml \times 5ml \times weight\ of\ smaple} \times 100$$

where,

V_1 = titer value of standard,

V_2 = titer value of sample.

2.12. Anti-oxidants

The antioxidant percentage present in pulp and juice was determined as mentioned by Brand-Williams et al. [23]. The sample weighed about 100 mg and contained 100 mL of methanol. then shook it overnight and filtered it. 10 mL of the filtrated sample was taken and diluted with 100 mL of distilled water. The stock standard was prepared by diluting 100 mg in 100 mL of methanol. Then 10 mL of stock solutions were diluted with 100 mL of methanol to give a working standard. Mainly DPPH (2,2-diphenyl-1-piccrlhydrazyl hydrate) was prepared by diluting 4 mg of DPPH in 100 mL of methanol and keeping it in dark conditions. From the standard and sample, 100 µL was taken, then volume was made up to 1 mL with methanol and 4 mL of DPPH was added. It was incubated for 30 min in dark conditions. Further samples and a standard solution were used for absorbance readings at 517 nm by using UV spectrometry.

$$Antioxidant\ \% = \frac{OD\ of\ control - OD\ of\ test}{OD\ of\ control} \times 100$$

where: *OD*: optical density.

2.13. Total phenols

The total phenols content present in fruit pulp and juice was determine as mentioned by Chandra et al. [24]. The fruit sample and pulp sample 50 mg were taken and added 5 mL of methanol. This solution was sonicated for 45 min at 40 ℃, followed by centrifugation at 1000 rpm for 10 min. The supernatant was collected, from this 200 µL was taken and added 600 µL of water with that 200 µL of FC reagents were added. After 5 min 1 mL of 8% saturated sodium carbonate solution was added. At that point volume was made up to 3 mL with distilled water and kept the sample in dark conditions for 30 min. The absorbance reading was recorded at 765 nm. The results were expressed by mg/g of gallic acid.

$$Y = mx + C$$

2.14. Statistics

All the sample analysis were done in triplicate. All the raw data obtained were inserted in Minitab software to determine the significant differences in the treatments given to SWPP using ANOVA and grouping information was provided by the Tukey's range test.

3. Results and discussion

3.1. Folic acid production of different strains in different concentrations of fruit pulp

The initial concentration of folic acid in mango pulp was 133 mg kg^{-1}. The HPLC results of folic acid in different concentration of fruit pulp mentioned in **Figure 1**, calibration of HPLC system **Figure 2**. The effects of *Lactobacillus acidophilus*, *Leuconostoc mesenteroides*, *Streptococcus thermophilus* and *Saccharomyces cerevisiae* on folic acid content in different concentration of fermented pulp (5%, 10%, 15%, 20% and 30%) has mentioned in **Table 1**. After the 24 h fermentation folic acid

content in control pulp gradually decreased, even though *Saccharomyces cerevisiae* synthesis the folic acid as compared to remaining three micro-organisms. In 5% mango pulp concentration only *Saccharomyces cerevisiae* synthesis the folic acid of about 17.15 mg kg^{-1}, other three micro-organisms folic acid content was not detected. In (*Mangifera indica* L) banganapalli the LAB strain was decreased the folic acid and able to synthesis in lower concentration. *S. cerevisiae* synthesis the folic acid of about 31.04 mg kg^{-1} in 10% of fruit pulp concentration whereas *S. thermophilus* synthesis the 0.05 mg kg^{-1} and in *L. mesenteroides* synthesis 0.03 mg kg^{-1}. The decrease in folic acid its chance of fructose which present in fruit further deplete the degradation of folates [25]. As compared to milk products like yoghurt and in banana juice [26–28] in fruits pulp LAB microbes with respect to different concentration of fruit pulp synthesis very less amount of folic acid.

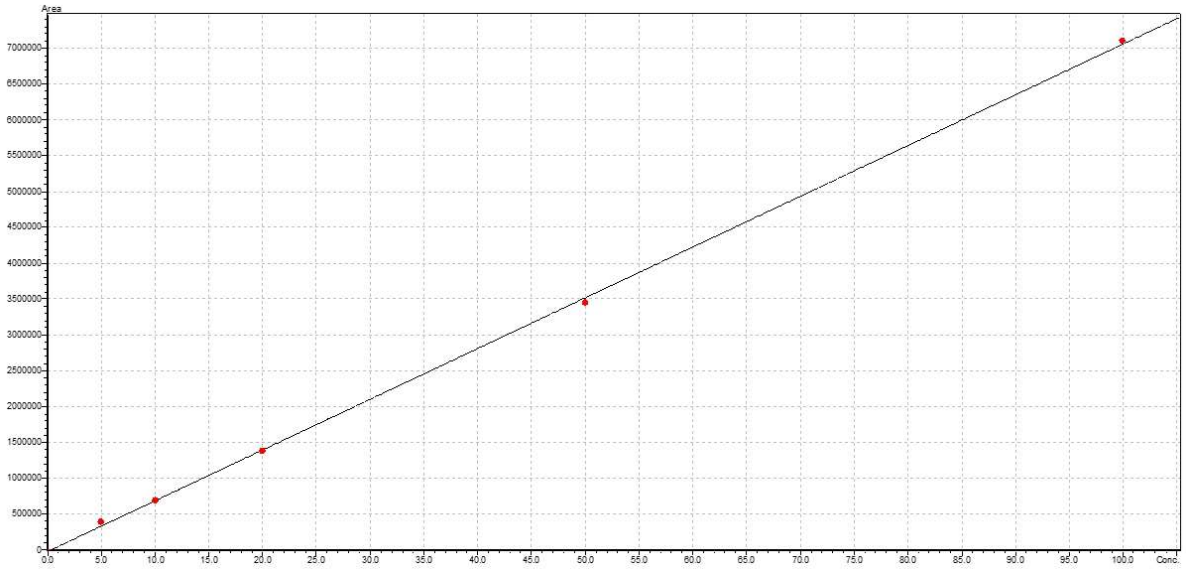

Figure 1. Calibration curve for HPLC result.

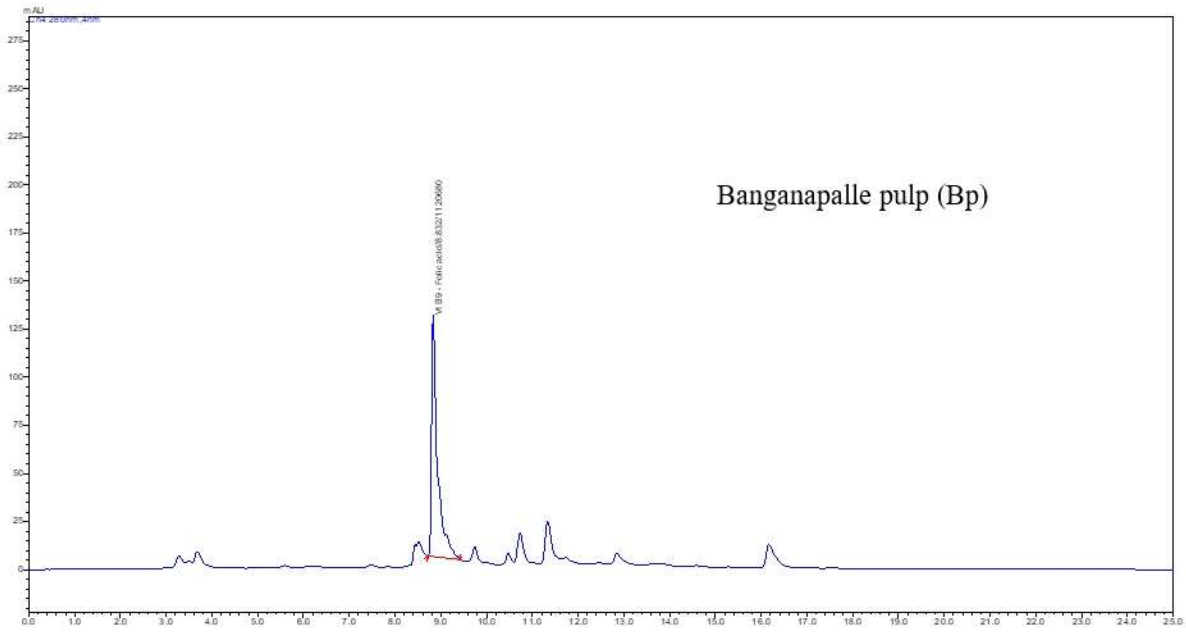

Figure 2. (*Continued*).

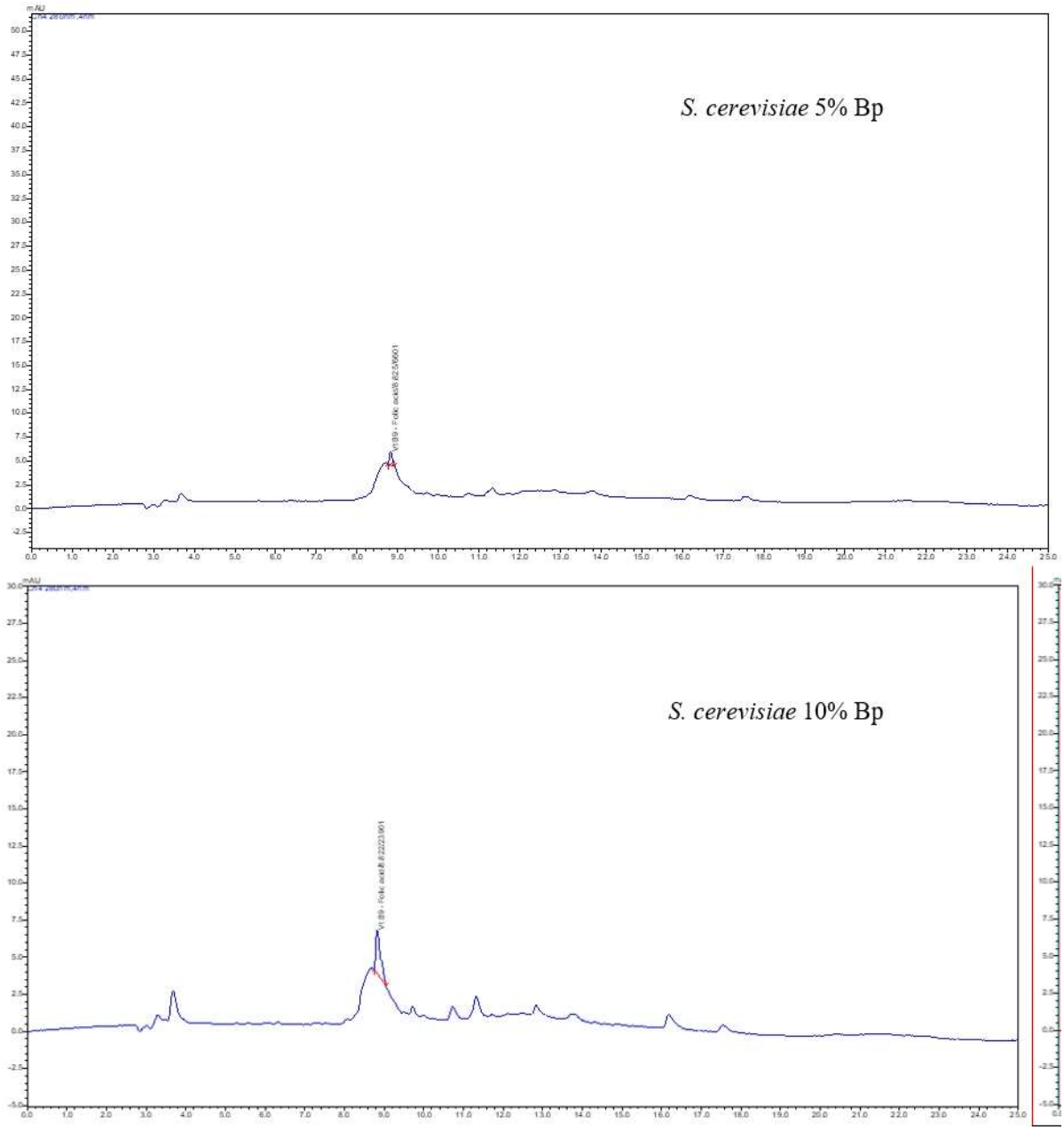

Figure 2. (*Continued*).

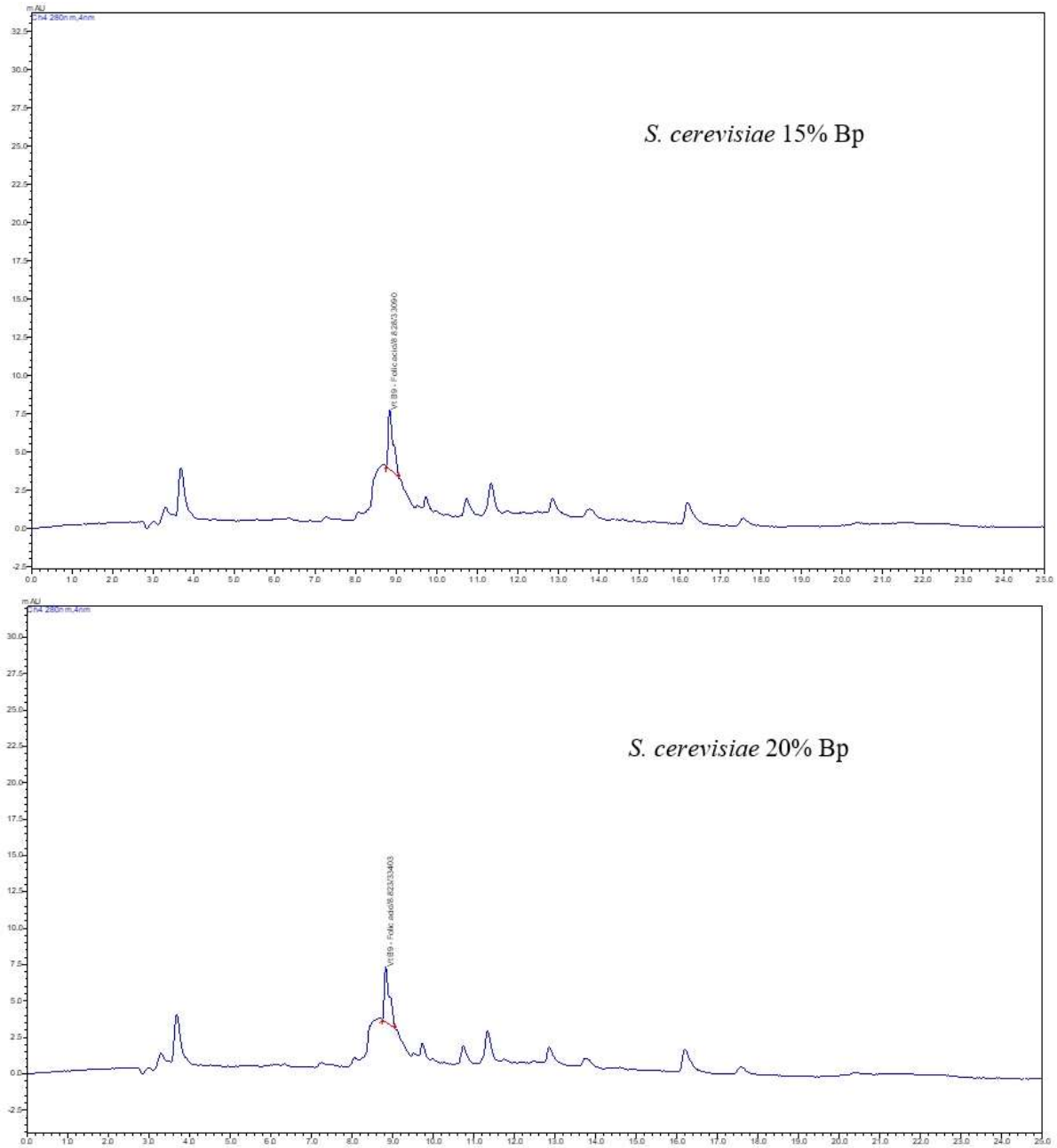

Figure 2. (*Continued*).

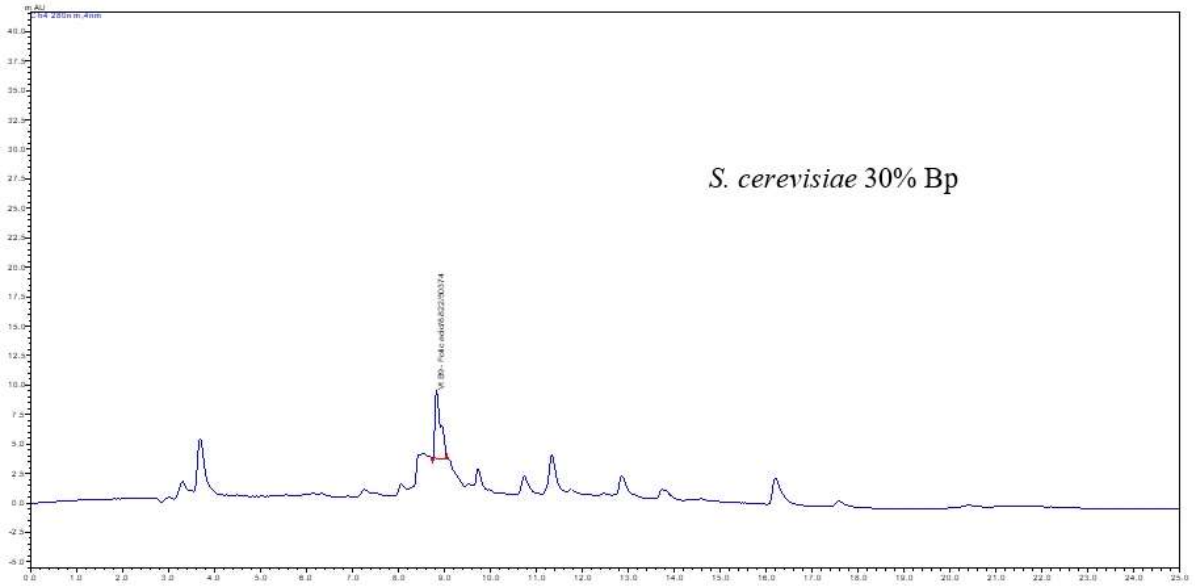

Figure 2. HPLC chromatogram for pulp and different pulp concentration.

Table 1. Folic acid concentration of banganapalli (*Mangifera indica* L) pulp after the fermentation.

Micro-organisms	Percentage of pulp and concentration of folic acid (mg kg^{-1})				
	5%	**10%**	**15%**	**20%**	**30%**
S. cerevisiae	17.15	31.04	28.62	21.70	21.78
S. thermophilus	BDL	0.05	0.05	0.05	0.03
L. acidophilus	BDL	0.02	0.02	0.03	0.02
L. mesenteroides	BDL	0.03	0.02	0.03	0.02

*BDL: below detectable limit.

3.2. Effects of in-situ fermentation on pH content of banganapalli (*Mangifera indica* L) pulp

It's one of the important parameters during 24 h fermentation of all four microbes. Every 6 h all the four microbes' pH were recorded and entitled in **Tables 2–5**. After 6h pH was gradually decrease in all four microbes, this same progressive pH decreases as observed in fruit juice which made from lactic acid starter culture [29,30]. The formation of acid during fermentation decreases the pH. In probiotic mango juice also showed decreasing the pH during 72 h of fermentation [31]. The least pH was observed in pulp 30% of *L. mesenteroides*. The pH range fall down in between 4.61 to 3.78, this pH value even supports to coco fermented milk after 24 h fermentations [32].

Table 2. Effects of in-situ fermentation on pH content of *L. acidophilus* in banganapalli (*Mangifera indica* L) pulp.

Pulp concentration	Initial	6 h	12 h	18 h	24 h
5%	4.61 ± 0.01^{a}	4.4 ± 0.01^{b}	4.3 ± 0.01^{c}	4.02 ± 0.00^{d}	3.78 ± 0.01^{e}
10%	4.56 ± 0.01^{a}	4.18 ± 0.01^{b}	4.18 ± 0.00^{b}	3.96 ± 0.05^{c}	3.94 ± 0.00^{c}
15%	4.52 ± 0.00^{a}	4.12 ± 0.01^{b}	4.12 ± 0.00^{b}	3.96 ± 0.01^{c}	3.9 ± 0.00^{d}
20%	4.51 ± 0.01^{a}	4.1 ± 0.01^{b}	4.09 ± 0.01^{b}	3.95 ± 0.00^{c}	3.85 ± 0.01^{d}
30%	4.52 ± 0.00^{a}	4.07 ± 0.00^{b}	4.03 ± 0.00^{c}	3.88 ± 0.00^{d}	3.99 ± 0.01^{e}

Letters in the same column represent statistically significant differences ($p < 0.05$).

Table 3. Effects of in-situ fermentation on pH content of *L. mesenteries* in banganapalli (*Mangifera indica L*) pulp.

Pulp concentration	Initial	6 h	12 h	18 h	24 h
5%	4.61 ± 0.01^{a}	4.4 ± 0.01^{b}	4.3 ± 0.01^{c}	4.02 ± 0.00^{d}	3.78 ± 0.01^{e}
10%	4.56 ± 0.01^{a}	4.18 ± 0.01^{b}	4.18 ± 0.00^{b}	3.96 ± 0.05^{c}	3.94 ± 0.00^{c}
15%	4.52 ± 0.00^{a}	4.12 ± 0.01^{b}	4.12 ± 0.00^{b}	3.96 ± 0.01^{c}	3.9 ± 0.00^{d}
20%	4.51 ± 0.01^{a}	4.1 ± 0.01^{b}	4.09 ± 0.01^{b}	3.95 ± 0.00^{c}	3.85 ± 0.01^{d}
30%	4.52 ± 0.00^{a}	4.07 ± 0.00^{b}	4.03 ± 0.00^{c}	3.88 ± 0.00^{d}	3.99 ± 0.01^{e}

Table 4. Effects of in-situ fermentation on pH content of *S. thermophilus* in banganapalli (*Mangifera indica L*) pulp.

Pulp concentration	Initial	6 h	12 h	18 h	24 h
5%	4.37 ± 0.01^{a}	4.36 ± 0.01^{a}	4.28 ± 0.01^{b}	4.1 ± 0.00^{c}	4.04 ± 0.01^{d}
10%	4.31 ± 0.01^{a}	4.22 ± 0.17^{ab}	4.27 ± 0.00^{b}	4.16 ± 0.01^{ab}	4.06 ± 0.00^{c}
15%	4.27 ± 0.01^{a}	4.27 ± 0.01^{a}	4.24 ± 0.00^{b}	4.15 ± 0.00^{c}	4.03 ± 0.00^{d}
20%	4.25 ± 0.00^{a}	4.26 ± 0.01^{a}	4.23 ± 0.01^{b}	4.22 ± 0.01^{c}	4.06 ± 0.00^{d}
30%	4.22 ± 0.00^{c}	4.24 ± 0.00^{b}	4.26 ± 0.00^{a}	4.21 ± 0.01^{d}	4.08 ± 0.00^{e}

Table 5. Effects of in-situ fermentation on pH content of *S. cerevisiae* in banganapalli *(Mangifera indica L)* pulp.

Pulp concentration	Initial	6 h	12 h	18 h	24 h
5%	4.25 ± 0.00^{a}	4.16 ± 0.00^{b}	4.06 ± 0.00^{d}	4.05 ± 0.00^{d}	4.10 ± 0.00^{c}
10%	4.22 ± 0.00^{a}	4.13 ± 0.00^{b}	4.04 ± 0.00^{c}	3.99 ± 0.00^{d}	3.95 ± 0.00^{e}
15%	4.19 ± 0.00^{a}	4.12 ± 0.00^{b}	4.02 ± 0.00^{c}	3.95 ± 0.00^{d}	3.87 ± 0.00^{e}
20%	4.18 ± 0.00^{a}	4.11 ± 0.00^{b}	4.01 ± 0.00^{c}	3.91 ± 0.00^{d}	3.89 ± 0.00^{e}
30%	4.16 ± 0.00^{a}	4.11 ± 0.00^{b}	4.02 ± 0.00^{c}	3.93 ± 0.00^{d}	3.90 ± 0.00^{e}

3.3. Effects of in-situ fermentation on titratable acidity content of banganapalli (*Mangifera indica L*) pulp

The titratable acidity one of the important parameters regarding taste of any RTS juice. There was increasing TA with increasing the pulp concentration was observed in all four microbes which is given in **Table 6**. The highest acidity was showed in *S. cerevisiae* of followed by *L. mesenteroides*, *L. acidophilus*. The least acidity value was recorded in *L. mesenteroides*, followed by, *L. acidophilus*. There was no significant different found among the different concentration of pulp. The acidity value gradually increased in probiotic mango juice that observed by Mwanzia et al. [31] after 72 h of fermentation studies.

Table 6. Effects of in-situ fermentation on titratable acidity (%) of banganapalli pulp.

Pulp concentration	*L. acidophilus*	*L. mesenteroides*	*S. thermophilus*	*S. cerevisiae*
5%	0.11 ± 0.02^{f}	0.10 ± 0.02^{f}	0.12 ± 0.02^{ef}	0.27 ± 0.02^{cd}
10%	0.26 ± 0.02^{cd}	0.24 ± 0.05^{d}	0.25 ± 0.01^{d}	0.35 ± 0.02^{c}
15%	0.30 ± 0.02^{cd}	0.21 ± 0.02^{de}	0.21 ± 0.02^{d}	0.55 ± 0.02^{b}
20%	0.35 ± 0.02^{c}	0.28 ± 0.04^{cd}	0.30 ± 0.02^{cd}	0.56 ± 0.02^{b}
30%	0.52 ± 0.04^{b}	0.55 ± 0.02^{b}	0.50 ± 0.02^{b}	1.19 ± 0.02^{a}

3.4. Effects of in-situ fermentation on vitamin C content of banganapalli (*Mangifera indica L*) pulp

The banganapalli pulp contain less vitamin C but after the fermentation it increased about 38.64 ± 1.24 mg/g in 30% of *L. acidophilus*. The least concentration of vitamin content observed in *L. mesenteroides*. The increasing the pulp concentration there was increasing in the vitamin content. The overall vitamin C content in different pulp concentration as given in **Table 7**. There was no significant difference between the microbes and different concentration of pulp.

Table 7. Effects of in-situ fermentation on vitamin C (mg/g) content of banganapalli *(Mangifera indica L)* pulp.

Pulp concentration	*L. acidophilus*	*L. mesenteroides*	*S. thermophilus*	*S. cerevisiae*
5%	9.20 ± 1.24^{f}	8.59 ± 2.12^{f}	12.27 ± 2.12^{ef}	9.81 ± 2.12^{f}
10%	12.88 ± 1.24^{def}	20.85 ± 2.12^{c}	18.40 ± 1.68^{cde}	20.24 ± 1.24^{cd}
15%	20.85 ± 2.12^{c}	23.92 ± 1.24^{bc}	23.92 ± 1.24^{bc}	20.85 ± 2.12^{c}
20%	31.28 ± 1.24^{ab}	23.31 ± 2.12^{c}	34.35 ± 2.12^{a}	31.84 ± 2.08^{a}
30%	38.64 ± 1.24^{a}	31.89 ± 2.12^{a}	34.96 ± 1.24^{a}	33.12 ± 1.37^{a}

3.5. Effects of in-situ fermentation on antioxidant activity and total phenol content of banganapalli (*Mangifera indica L*) pulp

The scavenging activity of free radical represent antioxidant present in pulp. Antioxidant activity was high in 10% of *S. cerevisiae*, followed by 5% of *L. acidophilus* and 30% of *S. cerevisiae*. The least antioxidant activity observed in 30% of *L. mesenteroides*. Several potential antioxidant forms which provide biological importance to body has mentioned by Rabie et al. [8]. The antioxidant activity of all different concentration of fruit pulp given in **Table 8**. The presence of antioxidants prevents oxidation of folic acid which formed during fermentation. Total phenols content of banganapalli pulp was contain 15.37 ± 0.20 mg GAE/g in *S. cerevisiae* of 30% of pulp. The least total phenol showed in *L. acidophilus* of about 13.15 ± 0.2 mg GAE/g. The total phenols from different concentration of pulp have similar result and no significant to each other. Total phenols of all four microbes with five different concentrations of pulp as given in **Table 9**.

Table 8. Effects of in-situ fermentation on antioxidant activity (%) of banganapalli *(Mangifera indica L)* pulp.

Pulp concentration	*L. acidophilus*	*L. mesenteroides*	*S. thermophilus*	*S. cerevisiae*
5%	38.28 ± 0.01^{a}	32.36 ± 0.00^{d}	30.95 ± 0.05^{c}	32.53 ± 0.01^{e}
10%	35.44 ± 0.01^{c}	30.55 ± 0.00^{e}	32.7 ± 0.00^{b}	43.94 ± 0.01^{a}
15%	34.24 ± 0.00^{d}	34.24 ± 0.00^{a}	34.76 ± 0.00^{a}	32.67 ± 0.06^{d}
20%	35.65 ± 0.05^{b}	33.32 ± 0.00^{c}	24.03 ± 0.00^{e}	34.26 ± 0.01^{c}
30%	32.61 ± 0.00^{e}	33.62 ± 0.03^{b}	24.63 ± 0.00^{d}	36.62 ± 0.01^{b}

Table 9. Effects of in-situ fermentation on total phenol (mg GAE/g) content of banganapalli *(Mangifera indica L)* pulp.

Pulp concentration	*L. acidophilus*	*L. mesenteroides*	*S. thermophilus*	*S. cerevisiae*
5%	13.15 ± 0.2^{g}	13.35 ± 0.21^{efg}	13.22 ± 0.30^{g}	13.37 ± 0.23^{efg}
10%	13.28 ± 0.30^{fg}	13.97 ± 0.20^{bcdef}	13.53 ± 0.24^{defg}	14.00 ± 0.20^{bcde}
15%	13.84 ± 0.23^{cdefg}	14.21 ± 0.14^{bcd}	13.79 ± 0.23^{cdfeg}	14.21 ± 0.14^{bcd}
20%	14.26 ± 0.18^{bc}	14.57 ± 0.10^{b}	14.10 ± 0.27^{bcd}	14.62 ± 0.30^{b}
30%	14.48 ± 0.23^{bc}	14.64 ± 0.20^{b}	13.99 ± 0.30^{bcdef}	15.37 ± 0.20^{a}

3.6. Effects of in-situ fermentation on color value of banganapalli (*Mangifera indica* L) pulp

In banganapalli mango pulp undergone colour changes during fermentation. The colour changes due to break down of pigments. Usually Mango contains carotene and chlorophyll (a and b). There were drastic changes in yellowness based on increasing the pulp concentration. The positive scale in b represent yellowness was observed in all concentration of fruit pulp except in 5%. The color value of pulp for yellowness and different concentration of pulp color slightly different. The lightness (L) all are above 50 it represents light color. The all-color value from four microbes tabulated from **Tables 10–13**.

Table 10. Effects of color value from *L. acidophilus* in banganapalli *(Mangifera indica L)* pulp.

Pulp concentration	L	a	B	Δ *E*
5%	34.45 ± 0.08^{p}	-2.08 ± 0.01^{h}	-2.13 ± 0.03^{t}	14.27 ± 0.20^{g}
10%	43.18 ± 0.02^{l}	-4.43 ± 0.04^{q}	20.44 ± 0.17^{m}	12.08 ± 0.08^{h}
15%	49.53 ± 0.01^{g}	-2.63 ± 0.01^{j}	32.96 ± 0.02^{g}	8.54 ± 0.04^{j}
20%	53.61 ± 0.00^{c}	-0.05 ± 0.01^{b}	41.53 ± 0.02^{b}	8.4 ± 0.02^{j}
30%	54.18 ± 0.01^{a}	-0.78 ± 0.00^{d}	36.93 ± 0.04^{d}	19.76 ± 0.45^{e}

Table 11. Effects of color value from *L. mesenteries* in banganapalli *(Mangifera indica L)* pulp.

Pulp concentration	L	a	b	Δ *E*
5%	35.73 ± 0.11^{o}	-2.17 ± 0.02^{i}	-1.09 ± 0.04^{s}	14.16 ± 0.23^{g}
10%	44.64 ± 0.01^{k}	-4.14 ± 0.03^{p}	23.41 ± 0.14^{k}	9.73 ± 0.08^{i}
15%	48.92 ± 0.00^{h}	-3.01 ± 0.00^{l}	32.66 ± 0.02^{h}	8.51 ± 0.06^{j}
20%	50.52 ± 0.00^{e}	-1.99 ± 0.01^{g}	35.37 ± 0.02^{e}	13.75 ± 0.03^{g}
30%	53.89 ± 0.00^{b}	0.12 ± 0.01^{a}	42.76 ± 0.01^{a}	13.88 ± 0.48^{g}

Table 12. Effects of color value from *S. thermophilus* in banganapalli *(Mangifera indica L)* pulp.

Pulp concentration	L	a	b	Δ *E*
5%	32.08 ± 0.16^{q}	-3.26 ± 0.03^{m}	4.69 ± 0.11^{q}	7.40 ± 0.27^{k}
10%	43.18 ± 0.06^{k}	-4.01 ± 0.02^{o}	24.14 ± 0.17^{j}	8.55 ± 0.12^{j}
15%	48.24 ± 0.00^{i}	-2.96 ± 0.01^{l}	32.16 ± 0.015^{i}	8.67 ± 0.04^{j}
20%	50.31 ± 0.01^{f}	-2.00 ± 0.01^{g}	34.71 ± 0.01^{f}	14.39 ± 0.00^{g}
30%	53.22 ± 0.00^{d}	-0.21 ± 0.00^{c}	40.44 ± 0.00^{c}	16.22 ± 0.48^{f}

Table 13. Effects of color value from *S. cerevisiae* in banganapalli *(Mangifera indica L)* pulp.

Pulp concentration	L	a	b	Δ *E*
5%	21.97 ± 0.02^{s}	-1.26 ± 0.01^{e}	-0.10 ± 0.00^{r}	11.27 ± 0.38^{h}
10%	30.68 ± 0.01^{r}	-2.82 ± 0.005^{k}	5.21 ± 0.00^{p}	28.65 ± 0.29^{d}
15%	36.85 ± 0.17^{n}	-3.50 ± 0.00^{n}	11.27 ± 0.00^{o}	30.01 ± 0.11^{c}
20%	40.87 ± 0.01^{m}	-3.45 ± 0.00^{n}	16.24 ± 0.01^{n}	33.97 ± 0.02^{b}
30%	45.40 ± 0.01^{j}	-1.79 ± 0.01^{f}	22.79 ± 0.00^{l}	34.96 ± 0.49^{a}

4. Conclusion

The folic acid synthesis by the beneficial micro-organisms in mango pulp had a good impact on the product development stream. This is the better option for those with lactose intolerance. The fermentation not only synthesizes folic acid but also increases vitamin C content of the pulp. All four micro-organisms have the ability to synthesize folic acid at different pulp concentrations. This variety is so popular due to its commercial importance. This study supports nutritional enrichment and also provides important cultivation.

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