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Development of fermented beverage from *Clitoria ternatea* and *Citrus lemon*

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Abstract
We have used the method of tepache preparation to develop innovative fermented drink infused with *Clitoria ternatea* and *Citrus lemon.* We developed three fermented beverages coded as TP1, TP2 and TP3; TP1 was the control or the basic tepache, TP2 was tepache infused with lemon and TP3 was the tepache infused with *Clitoria ternatea*. We analyzed the titratable acidity and the vitamin C content of the beverages. We applied two-way ANOVA to study the effect of treatment and period of storage on the titratable acidity and vitamin C content of the beverages. The titratable acidity of beverages ranged from 0.363% to 1.153%. Titratable acidity in the beverage increased on storage from day 1 to day 6 and there was a decrease in the titratable acidity on the 9th day except in the case of TP2 tepache infused with lemon where the titratable acidity kept on increasing. Lowest titratable acidity was reported in TP3. The vitamin C content ranged from 5.4 mg/100 g to 13.758 mg/100 g. Vitamin C increased from day1 to day 6 and then it reduced on day 9. Highest vitamin C content was reported in TP3 i.e tepache infused with *Clitoria ternatea* and vitamin C content increased on day 9 from 11.257 mg/100 g to 13.758 mg/100 g. The result of two-way Anova showed no effect of treatment and storage period on vitamin C but in case of titratable acideity storage showed some effect though it was not less than 0.05 but it was very close. Overall acceptability was highest for TP3.

Keywords: *Clitoria ternatea*, Citrus lemon, titratable acidity, vitamin C and organoleptic evaluation

Introduction
Tepache is a traditional Mexican fermented beverage made from pineapple peels, sweetened with unrefined cane sugar and flavoured with cinnamon or other spices. It's lightly fermented, so it has a mild alcohol content (usually under 2%), and is slightly fizzy, tangy, and refreshing. The foundational understanding of tepache's health benefits comes from detailed microbiological characterization studies. Comprehensive analysis revealed that tepache fermentation creates a complex ecosystem dominated by beneficial bacterial and fungal communities. After 72 hours of fermentation, the microbial community is primarily composed of *Lactobacillus* species, *Leuconostoc*, *Acetobacter*, and *Lactococcus* bacterial genera, alongside fungal species including *Saccharomyces*, *Gibberella*, *Zygosaccharomyces*, and *Candida* [1]. A particularly significant finding identified bacteriocin-producing lactic acid bacteria in tepache. Specifically, *Lactococcus lactis* and *Enterococcus faecium* were found to produce bacteriocins antimicrobial peptides encoding nisin and enterocin. These bacteriocins demonstrated inhibitory effects against pathogenic bacteria including *Micrococcus luteus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pneumoniae*. This antimicrobial capacity provides both natural preservation functions and potential protective effects for consumers [2]. We used the base tepache to develop innovative beverages using *Clitoria ternatea* and *Citrus lemon*. *Clitoria ternatea* L., commonly known as 'blue pea' [3], is a perennial twinning herbaceous plant which belongs to the family Fabaceae. The plant is mainly distributed in the tropical regions of India, Sri Lanka, Malaysia, Burma, and Philippine islands [4, 5]. It has two main varieties based on the colour of the petals, namely, white and blue flowered varieties. Different parts of this plant have been used in Indian traditional system of medicine and in folklore to treat variety of disorders such as anasarca, ascites, liver problems, hemicrania, irritation of urethra and bladder, and enlargement of abdominal viscera [6]. Further, the medicinal properties of this plant are scientifically validated particularly at international level and reported to have several biological activities such as antioxidant,

antidiabetic, and hepatoprotective properties [7, 8]. The main objective of the research was to develop probiotic rich innovative drinks suitable for Indian population.

Material and Methods

Preparation of fermented pineapple drink

Pineapple was purchased from the local market from Madhepura, Bihar. Pineapple was washed thoroughly using the tap water. Pineapple was cut and the rind was removed. The cut pineapple and the peel were transferred into a glass jar to which we added sugar, ginger, cinnamon stick and filtered water. The glass jar was covered using a muslin cloth and tied using an elastic. This was control and was coded as TP1.

To the same base ingredients, we infused lemon including its peel it was coded as TP2 and *Clitoria ternatea* flower commonly known as aprajita flower coded it as TP3. The glass jar was covered with muslin cloth and tied using an elastic and was kept for fermentation at 30 ºC for 2 days. It was filtered and then refrigerated.

- **Filtration process of tepache:** The Fermented beverage TP1, TP2 and TP3 was filtered using a muslin cloth and then it was filtered using a filter paper.
- **Estimation of titratable acidity:** The sample was extracted using distilled water, filtered and the supernatant was collected. The supernatant was titrated against 0.1N NaOH until a stable brownish pink colouration was obtained.
- **Estimation of vitamin C:** Ascorbic acid was analysed using the volumetric method. Ascorbic acid reduces the 2, 6 dichlorophenol indophenol dye to a colourless leuco-base. The ascorbic acid gets oxidised to dehydroascorbic acid. Though the dye is blue coloured compound, the end point is the appearance of pink colour, the dye is pink coloured in acidic medium. Oxalic acid was used as the titrating medium.

Organoleptic evaluation of control tepache and tepache infused with *Clitoria ternatea* and *Citrus lemon*

Semi-trained panel of 20 members evaluated the organoleptic feel of the drink. Appearance, colour, aroma, texture/ mouthfeel, flavour, aftertaste and the overall acceptability of the beverages were evaluated. Semi-trained panel evaluated the drink on 9-point scale. The panel members were unaware of the raw ingredients and the processing method.

Statistical analysis

Two-way ANOVA was performed to analyse the effect of treatment and storage on the titratable acidity and vitamin C. R studio was used for statistical analysis. We are presenting the initial result of the study undergoing in the University Department of Home Science, BNMU., Madhepura.

Results and Discussion

Method of Preparation

Table 1(a), (b) and (c) presents the amount of raw ingredients used in the preparation of beverage. Changes during fermentation is presented in plate 1(a), (b), (c), (d), (e) and (f) respectively. The filtration process is shown in plate 2(a) and (b) and the final product is shown in plate 3(a), (b) and (c).

Table 1(a): Raw ingredient used in the preparation of tepache (TP1)

S. No.	Ingredients	Quantity	Percentage
1	Pineapple	1kg	53.56%
2	Sugar	60 g	3.21%
3	Ginger	5 g	0.27%
4	Cinnamon Stick	2 g	0.11%
5	Water	800ml	42.85%

Table 1(b): Raw ingredient used in the preparation of tepache infused with lemon (TP2)

S. No.	Ingredients	Quantity	Percentage
1	Pineapple	1kg	52.44%
2	Sugar	60 g	3.15%
3	Ginger	5 g	0.26%
4	Cinnamon Stick	2 g	0.10%
5	Lemon	40 g	2.10%
6	Water	800ml	41.95%

Table 1(c): Raw ingredient used in the preparation of tepache infused with *Clitoria ternatea* flowers (TP3)

S. No.	Ingredients	Quantity	Percentage
1	Pineapple	1kg	52.99%
2	Sugar	60 g	3.18%
3	Ginger	5 g	0.26%
4	Cinnamon Stick	2 g	0.11%
5	Aprajita flowers	17g	1.06%
5	Water	800ml	42.40%

Titratable acidity: Titratable acidity of the tepache control TP1 was 0.662% which increased to 1.067% on day 6 and further decreased to 0.939% on 9th day. For the tepache infused with 2.1% *Citrus lemon* the titratable acidity was 0.576% on day 1 which increased to 1.067% on day 2 and increased further on 9th day to 1.153%. For the tepache infused with 1.06% *Clitoria ternatea* or aprajita flower coded as TP3 titratable acidity on day 1 was 0.363% which increased to 0.683% on 2nd day and decreased on 9th day to 0.597%. Increase in titratable acidity was observed only in the case of 2.1% lemon infused tepache. There was decrease in titratable acidity in case of TP1 and TP2 but the titratable acidity was lowest in TP3 i.e. tepache infused with 1.06% *Clitoria ternatea* flower (Table no. 2). Fig no. 1 presents the trendline in titratable acidity, only tepache infused with 2.1% citrus lemon showed an increase on storage.

Vitamin C: Vitamin C content of the sample was analysed using the titrimetric method using 2, 6 dichlorophenolindophenol dye. Vitamin C content of the beverage ranged from 5.4 mg/100 ml to 13.756 mg/100ml.Vitamin C content of tepache control was 10.8 mg/100ml on day 1 and 13.756 on 6th day and 11.257 mg/100ml on 9th day. Tepache infused with 2.1% Citrus lemon had the least vitamin C content of 5.4 mg/100ml on day 1 which increased to 8.755 mg/100ml on day 6 and then decreased to 7.505 mg/100 ml on 9th day. Tepache infused with 1.06% *Clitoria ternatea* flower or aprajita flower had vitamin C content of 10.2mg/100ml on day 1 which increased to 11.257 mg/dl on day 6 and increased further to 13.758 mg/dl on day 9 (Table no. 2). Fig no. 2 presents the trend in vitamin C content of the beverage on storage; only in TP3 i.e. tepache infused with 1.06% *Clitoria ternatea* flower extract showed increase in the vitamin C on storage whereas the TP1 and TP2 showed decrease in vitamin C on storage.

Effect of treatment and storage on the titratable acidity and vitamin C content of the beverage: There was no significant effect of treatment and storage on the titratable acidity and vitamin C content of the beverage. In case of titratable acidity storage did affect the titratable acidity as the p-value was very close to 0.05 (table no. 2).

Table 2: Effect of treatment and storage on the titratable acidity and vitamin C content of the beverage

Treatments	Temperature	Duration	Titratable acidity (%)	Vitamin C (mg/100g)
TP1	Refrigerated	1	0.661747	10.8
		6	1.06748	13.756
		9	0.939333	11.257
TP2	Refrigerated	1	0.57636	5.4
		6	1.067333	8.755
		9	1.153	7.505
TP3	Refrigerated	1	0.362893	*10.2*
		6	0.683093	11.257
		9	0.597333	13.758
		Treatments		
		F-value	0.1072	0.942
		Duration		
		F-value	0.0918	0.471

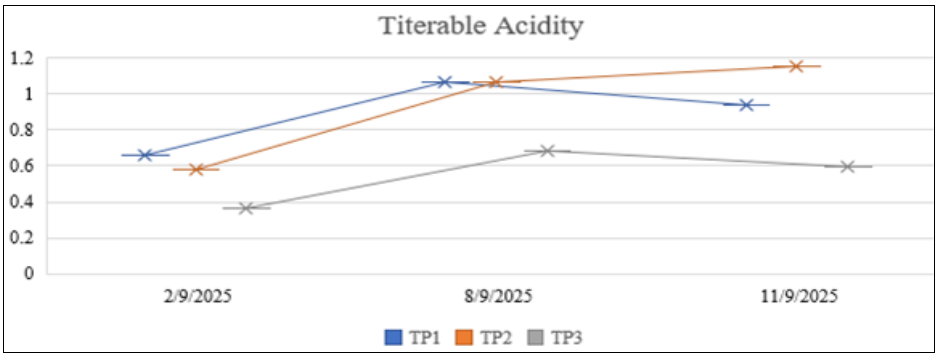

Fig 1: Trendline showing titratable acidity changes in beverages during storage period.

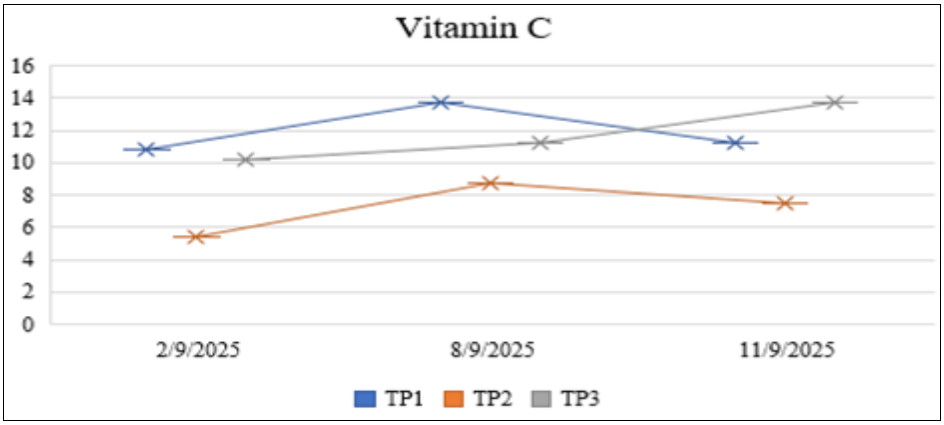

Fig 2: Vitamin C trends showing increase only in *Clitoria ternatea* infused tepache.

Organoleptic profile of the beverage: Tepache infused with Citoria terntea flowers (TP3) had a beautiful blue hue, the intensity of the colour increased on storage. All the panel member highly praised the blue colour. Table no 3 presents the scoring of all the beverage overall acceptability of TP3 was highest on day 1 followed by TP2; on 6th day the overall acceptability increased for TP1 and TP3 but decreased slightly for TP2, panel member said that it tasted bitter. On the 9th day overall acceptability decreased for all three beverages.

Table 3: Overall acceptability trends of beverages across storage days.

Duration	1			6			9		
Sample	**TP1**	**TP2**	**TP3**	**TP1**	**TP2**	**TP3**	**TP1**	**TP2**	**TP3**
Appearance	7.56	8.5	9	8	8	9	6	5	5.3
Colour	7.56	8.5	9	8	7	9	6	4	6
Aroma/Smell	7.22	8.5	8.5	8	8	9	7	5	4
Texture/ Mouthfeel	7.67	8.25	8.5	8	7	8	6	4	7
Flavour/ Taste	7.78	8.25	8.8	8.3	8	9	6	4	7
Aftertaste	7.33	8.25	8.8	8	8	9	5	4	6
Overall Acceptability	7.89	8.25	8.8	8	8	9	5	4	7

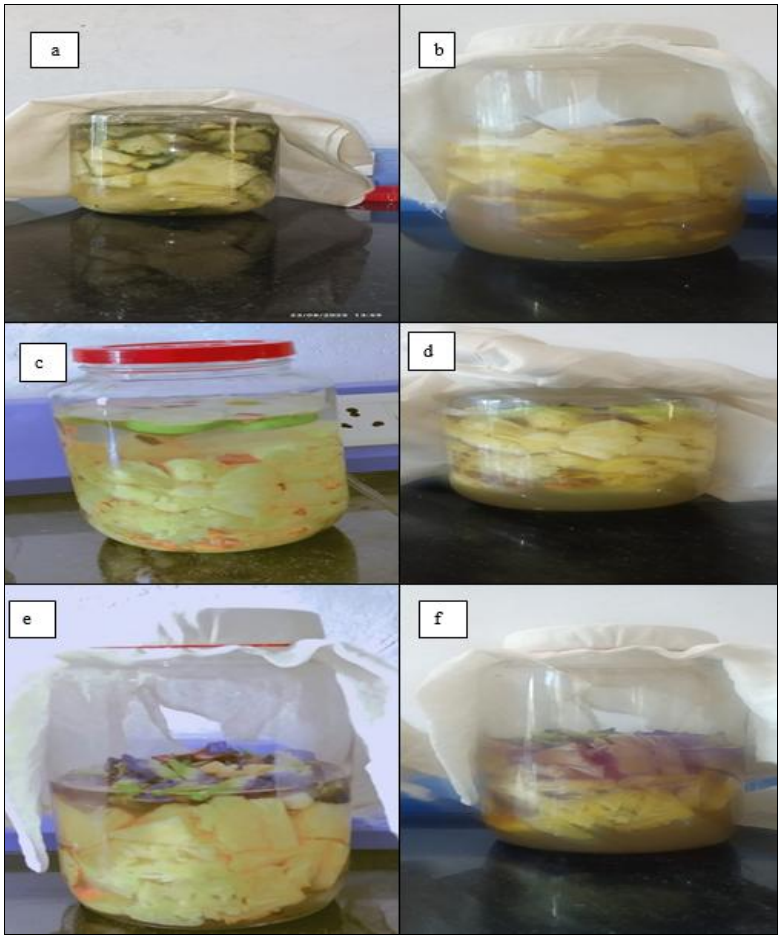

Plate 1: Changes in the appearance during the fermentation of TP1 a represent the freshly prepared tapache control (TP1) and b represents tapache control (TP 1) day-2; c represents the freshly prepared tapache infused with lemon (TP2) and d represents (TP 2) day 2; e represents the freshly prepared TP3 and f represents TP3 on day 2.

Plate 2: Filteration process of tapache
a: filteration using a muslin cloth, b: secondary filteration using filter paper

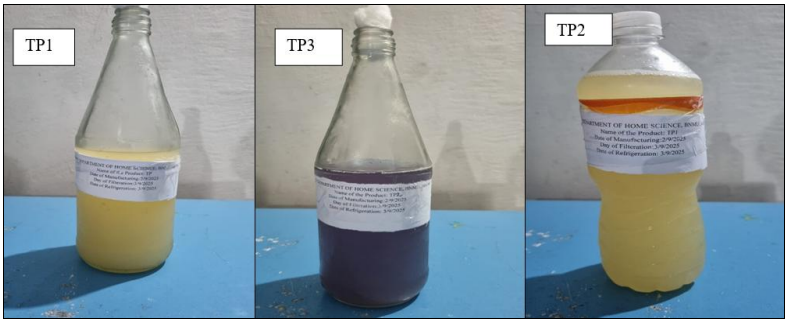

Plate 3: Final Product

Discussion

Plantain fermented wine exhibited the biphasic pattern matched TP1: starting at 0.4% titratable acidity on day 0, jumping significantly to 1.8% by day 4, then rising more slowly to 2.02% by day 8. This two-phase pattern reflected initial rapid acid production by lactic acid bacteria followed by secondary metabolic processes that stabilized or reduced acidity [9].

The exceptional performance of TP2 (lemon-infused tepache) reflected the direct contribution of citric acid from lemon juice to the titratable acidity pool. Research on fermented beverages demonstrated that citric acid, a triprotic acid with three ionizable protons, contributes substantially to total titratable acidity measurements. In addition to the exogenous citric acid provided by lemon juice, the added acidic environment (lower initial pH) created by citrus created optimal conditions for lactic acid bacteria (LAB) and acetic acid bacteria (AAB) proliferation. Studies shows that reduced initial pH (around pH 4-5) accelerates acid production in fermented beverages, as LAB are acid-tolerant organisms that thrive in acidic microenvironments [10] (Wilbert *et al*., 2022). Multiple studies confirm that fermented beverages with acidic substrates show sustained titratable acidity increase throughout fermentation. Blended cactus pear and Lantana camara wine achieved 0.8-0.83% titratable acidity, with titratable acidity increasing significantly when fermentation temperature was optimized at 24-25°C. Lemongrass kombucha demonstrated strong linear correlation (r = 0.914) between fermentation time and titratable acidity, showing increase from 0.6225% at 4 days to 2.1600% at 16 days, with fermentation time explaining 83.5% of acidity variation [11].

Clitoria ternatea (butterfly pea flower), also called aprajita in Ayurveda, contains high concentrations of anthocyanins (particularly ternatins and other polyacylated delphinidin derivatives), flavonoid glycosides, and phenolic compounds. These compounds exhibit documented antimicrobial properties against pathogenic and fermentative microorganisms. Research has shown that methanolic extracts of C. ternatea demonstrate inhibition zones ranging from 10.3 to 14.3 mm against various bacterial species through disruption of bacterial cell membranes and interference with metabolic pathways.

The lowest titratable acidity in TP3 suggests that polyphenolic compounds from the butterfly pea flowers suppressed or modulated the activity of acid-producing microorganisms, resulting in both lower initial acid production and potential acid consumption by secondary metabolic pathways. The colour-changing property of butterfly pea flowers (changing from blue to purple when exposed to acidic conditions) is driven by anthocyanin structural changes in response to pH shifts, indicating that the polyphenolic environment substantially alters the pH microenvironment within the fermenting system. Research on honeysuckle and pomegranate fermentation with aromatic plants showed that high-polyphenol substrates modify acid production patterns compared to simple sugar fermentation. This explains TP3's lowest titratable acidity values (0.363% → 0.683% → 0.597%) anthocyanins suppressed acid-producing microbial populations [12].

The pattern where only TP2 (lemon-infused) shows continued acidity increase during storage suggests that this formulation maintains highest probiotic viability and metabolic activity through day 9. The decline in TP1 and sustained low values in TP3 suggest that prolonged storage under these conditions may favor acid-consuming pathways or reduce fermentative capacity.

Vitamin C content of *Clitoria ternatea* extract and lemon infused herbal drink was 3.5% [13], Aeleti and Anil., 2023 [14] found vitamin C content of 14.64 mg/100g similar to the vitamin C content reported in our study. A study on fermented milk beverages demonstrated that vitamin C content varies with fermentation and storage: typically, vitamin C content either declines or fluctuates, influenced by microbial metabolism and substrate composition. Vitamin C in control milk started at 10.5 mg/100 g, rose after fermentation to 14 mg/100 g, but then decreased significantly during storage, with the largest losses in unfortified (control) samples. Notably, microbial synthesis or stabilizing effects were also observed depending on the starter culture and additives [15].

Similarly, research on fermented fruit beverages like blood fruit and aonla showed that titratable acidity and vitamin C content can increase or decrease with storage, and sometimes botanical or microbial interventions lead to increased vitamin C values during later storage periods, aligning with the increase seen for tepache infused with *Clitoria ternatea* in our data. Most studies emphasized that vitamin C losses during storage are common, but depending on the botanical infusion, synthesis or preservation may occur.

Conclusion: Further, work on the beverage is required to study its antioxidant effect and total microbial count.

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