



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 355-361
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www.thepharmajournal.com
Received: 26-04-2022
Accepted: 30-05-2022

Neekshitha Shetty
Ph.D. Scholar, Department of
Agricultural Microbiology,
Gandhi Krishi Vigyana Kendra
(GKVK), University of
Agricultural Sciences, Bangalore,
Karnataka, India

KB Munishamanna
Associate Professor (Retired),
AICRP on PHET Scheme,
Gandhi Krishi Vigyana Kendra
(GKVK), University of
Agricultural Sciences, Bangalore,
Karnataka, India

Lohith Kumar N
JRF, Department of
Agricultural Microbiology,
Gandhi Krishi Vigyana Kendra
(GKVK), University of
Agricultural Sciences, Bangalore,
Karnataka, India

Suvarna VC
Professor and Head, Department
of Agricultural Microbiology,
GKVK, University of
Agricultural Sciences, Bangalore,
Karnataka, India

Corresponding Author
Neekshitha Shetty
Ph.D. Scholar, Department of
Agricultural Microbiology,
Gandhi Krishi Vigyana Kendra
(GKVK), University of
Agricultural Sciences, Bangalore,
Karnataka, India

Development of fermented beverages from banana pseudo stem core juice enriched with honey and whey and their shelf-life assessment

Neekshitha Shetty, KB Munishamanna, Lohith Kumar N and Suvarna VC

Abstract

Banana pseudostem is an agriculture residue which is considered as absolute waste after harvest of the fruit. Edible pseudostem core is highly nutritious and known to have many health benefits, however, they are underutilized. By considering the importance of underutilized banana pseudostem core could be a good option for exploitation into fermented beverages. Hence this study aimed at developing yeast (*Saccharomyces ellipsoideus*, NCIM-3200) and lactic acid bacteria (*Lactobacillus plantarum*, MTCC 6161) fermented beverages from banana pseudostem core juice enriched with honey (5%) and liquid whey (10%). Results revealed that yeast fermented pseudo-stem core juice blended with 5% honey showed more reduction in terms of pH (4.12), TSS (5.33 °Brix) and total sugar (7.16%) with highest production of alcohol (7.05%) and highest sensory score value with respect to overall acceptability (16.00/ 20.00) compared to other yeast fermented treatments. LAB fermented pseudo-stem core juice blended with 10% whey showed moderate reduction in pH (3.61), TSS (12.98 °Brix) with optimum titrable acidity (0.76%) and highest lactic acid bacterial population (2.8×10^7 cfu/ml) and highest sensory score value with respect to overall acceptability (16.50/20.00) compared to other LAB fermented treatments. Shelf life and quality of selected two beverages were assessed at ambient and refrigerated temperature for 60 days. Results indicated that quality of yeast fermented beverage remained same upto 60 days of storage whereas LAB fermented beverage deteriorated after 7 days and 30 days under ambient and refrigerated temperature respectively.

Keywords: Banana pseudo-stem, whey, honey, *Saccharomyces ellipsoideus*, *Lactobacillus plantarum*, shelf life

Introduction

Banana crop produces large quantity of biomass (12 tones/ha) apart from fruit, e.g., pseudo-stem, leaves, suckers. These are considered to be absolute waste after the harvest of fruit. Farmers are spending about 8000 to 10000 Rupees/ hectare for the disposal of pseudo-stem (Anonymous, 2011) [2]. Pseudo-stem is the trunk of banana plant made of tightly packed overlapping leaf sheath. It consists of outer non-edible hard and soft fiber and inner edible fibrous stalk known as centre core. Banana pseudo-stem core is rich in fiber, potassium and vitamin B6 which help in treating ulcers. It is also used in development of a sport drink and provides many health benefits such as preventing kidney stones (Pillai, 1995) [14], treating diarrhea, dysentery, diabetes (Ghani, 2003) [7] and pain & snakebite (Coe and Anderson, 2005) [6].

Development of fermented beverages has gained more importance from the last two decades. Lactic acid fermentation is one of the oldest methods of preserving fruits and vegetables which contributes desirable physical and flavour characteristics. There are several reports on alcoholic and non-alcoholic beverages or wine preparation from different fruits like apple, plum, apricot, pineapple, strawberry, guava, jamun, sapota, litchi, amla, orange, carambola *etc* (Zeng *et al*, 2008; Saranraj *et al.*, 2017) [27, 20].

Quality of fermented beverages depends upon the nutrient composition of raw material used for the fermentation. Honey with fruits and vegetables gained a lot of importance with respect to nutrition and health point of view. Hence, 5 per cent of honey is explored by blending with center core juice in the present study. Similarly, whey is one of the important nutritious by-products obtained from the dairy industry milk processing waste composed of lactose (5%), water (93%), proteins (0.85%), minerals (0.53%) and a minimum amount of fat (0.36%) as reported by Pescuma *et al.* (2010) [13].

Shukla *et al.* (2013)^[23] reported on the possibility of utilizing the milk whey in the fruit beverage preparation. Hence, this can be explored by blending with pseudo-stem juice for the nutritional improvement of fermented banana center core juice beverage. With this background, an experiment was designed with the objective to develop yeast and lactic acid fermented beverages from banana pseudostem core juice enriched with honey and whey and assess the shelf life of developed beverages.

Material and Methods

The experiment was conducted in the Department of Agricultural microbiology, Gandhi Krishi Vigyana Kendra,



Plate 1: Banana pseudo-stem and its central core

Preparation of Yeast and LAB starter cultures:

The purified and authenticated yeast culture *Saccharomyces cerevisiae* var. *ellipsoideus* (NCIM-3200) and lactic acid bacteria (LAB) viz., *Lactobacillus plantarum* (MTCC- 6161) was maintained on yeast extract peptone dextrose agar media and MRS agar media respectively. A loopful of yeast and LAB culture were inoculated into sterilized pseudostem core juice and incubated at 28 °C and 37 °C. After incubation, when population reached upto 10⁶ cfu/ ml, it was used as a starter culture for the experiment.

Treatment details

The experiment set up consisted of 9 treatments with 3 replications under CRD design. Treatment details are as follows: T1: Pseudostem core juice (PSCJ) as control, T2: PSCJ + *Saccharomyces cerevisiae* var. *ellipsoideus*, T3: PSCJ + 5% honey + *Saccharomyces cerevisiae* var. *ellipsoideus*, T4: PSCJ+10% whey+ *Saccharomyces cerevisiae* var. *ellipsoideus*, T5: PSCJ+5% honey+10% whey+ *S. cerevisiae* var. *ellipsoideus*, T6: PSCJ+ *Lactobacillus plantarum*, T7: PSCJ+5% honey+ *Lactobacillus plantarum*, T8: PSCJ+10% whey+ *Lactobacillus plantarum*, T9: PSCJ +5% honey+10% whey+ *Lactobacillus plantarum*.

Assessment of Shelf-life and quality of the standardized fermented beverages

The experiment set up consisted of 2 treatments with 8 replications and two storage temperatures. Best adjudged two treatments were selected for the shelf-life assessment. The best developed two product samples (PY1- yeast fermented pseudo-stem core juice blended with honey and PL1- LAB fermented pseudo-stem core juice blended with whey) were prepared on a large scale. The prepared products were filled into the sterilized glass bottle of capacity 200 mL and yeast

UAS Bangalore and AICRP on the PHET Scheme, Gandhi Krishi Vigyana Kendra during the year 2017-18.

Collection of Banana Pseudo-stem and preparation of juice

Banana pseudo-stem required for the experiment were collected from the Department of Horticulture, University of Agricultural Sciences, GKVK, Bengaluru. The edible inner central core of the pseudo-stem was selected for study (Plate 1). The procedure for the preparation of banana pseudo-stem core juice was followed as per Bornare and Khan (2015)^[3] and Juice was pasteurized at 72 °C for 15 seconds.

fermented beverage bottles were pasteurized at 85-90 °C for 10 minutes and capped. The prepared product samples were stored for 60 days at ambient temperature (26-28°C) and refrigerated temperature (4 °C). Observations were recorded at a fixed interval of 15 days during storage period.

Biochemical analysis

The pH of the yeast and LAB fermented blended pseudo-stem core juice sample was analyzed using digital pH meter (Digital pH meter type MK-VI). Total Soluble Solids (TSS) of the fermented samples of different treatments were measured with the help of "ERMA " hand refractometer having a range of 0 to 35 °Brix at room temperature. Titrable acidity of the samples was determined as per the procedure followed by Srivastava and Kumar (1993)^[26]. Alcohol content was estimated colorimetrically as described by Caputi *et al.* (1968)^[4]. Total sugars were estimated by Fehling's method (Sadasivam and Manickam, 1996)^[19]. Vitamin C content of fermented pseudo-stem core juice samples were estimated by 2, 6 -dichlorophenolindophenol visual titration method (Ranganna, 1996)^[17].

Microbial analysis

The assessment of microbial population of the fermented samples was done by employing dilution plating method (Hoben and Somasegaran, 1982)^[8] and the results were expressed in terms of logarithms of colony forming units/ ml of the sample (log cfu/ ml).

Sensory analysis: The developed fermented beverages from core juice were evaluated by selected five panel members. Hedonic scale of 20 scores was considered to evaluate the product based on the appearance, color, aroma, taste and acceptability (Amerine *et al.*, 1972)^[1].

Statistical analysis

Analysis was carried out by completely randomized design using WASP -2 tool. Critical difference values were used to locate significant mean difference.

Results and Discussion

Biochemical quality

The results pertaining to changes in pH, TSS, titrable acidity and Vitamin C content of yeast and LAB fermented pseudo-stem core juice as influenced by honey and whey enrichment are presented in Table 1. After 6 days of fermentation, yeast and LAB fermented beverages showed reduction in pH and TSS but increase in titrable acidity. There was no significant difference between the treatment with respect to change in pH. The reduction in pH was found to be more in lactic acid bacteria fermented juices compared to yeast fermented juices. Increase in titrable acidity of fermented beverages and decreased pH might be due to the production of organic acid (lactic acid) during fermentation. These findings support the results of Priya *et al.* (2015) [15] in tomato fermented juice, Sabokbar and Khodayan (2015) [18] in LAB fermented beverage of pomegranate juice when blended with whey, Sasi Kumar (2015) in fermented whey blended aloe vera juice.

The initial vitamin C content of pseudo-stem core juice was 0.11 mg/ 100ml. The yeast fermented pseudo-stem core juice enriched with honey and whey (T5) showed highest vitamin C content (0.18mg/ 100ml) (Table 1). Similarly, LAB fermentation of pseudo-stem core juice enriched with honey and whey (T9) showed highest vitamin C content (0.38 mg/100ml). The results clearly indicated that addition of whey to pseudo-stem core juice and fermented by yeast and bacteria significantly enhanced the vitamin C content, whereas honey did not influence on vitamin C content.

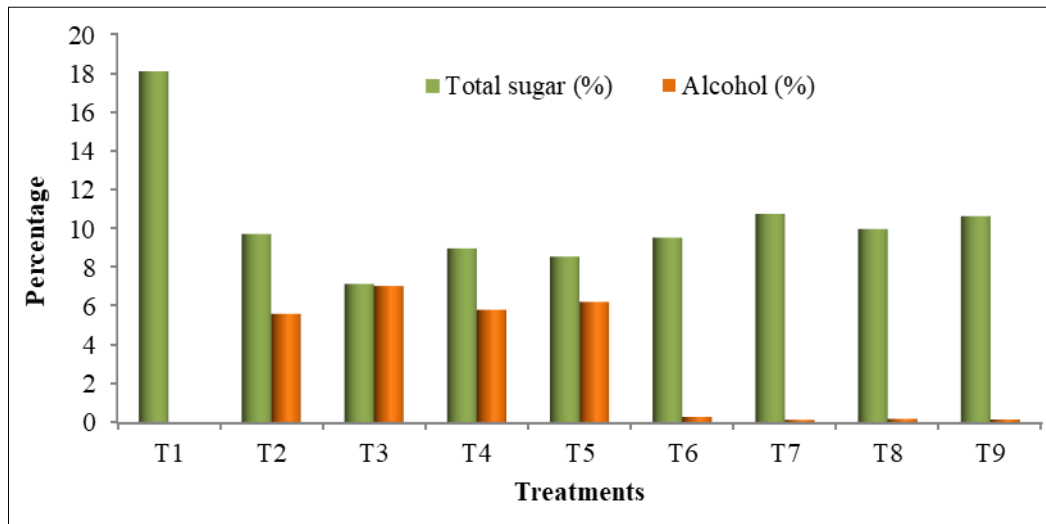
Fig1 shows the variation in total sugar and alcohol production in the yeast and LAB fermented of pseudo-stem core beverages as influenced by enriched with honey and whey. The initial total sugar content of enriched pseudo-stem core juice was 18.09%. Yeast fermentation of pseudo-stem core juice enriched with honey (T3) showed lowest total sugar content (7.16) (Fig. 1). This indicates that fermentation efficiency of yeast has been influenced by honey enrichment which supports the work of Joshi *et al.* (2014) [9] in plum wine. The decreasing trend of total sugar is also reported in papaya fermented beverages by Maragatham and Panneer Selvam (2011) [11]. Alcohol production ranged from 5.62 to 7.05% between treatments (Fig. 1). The yeast fermentation of pseudo-stem core juice enriched with honey (T3) showed highest alcohol content (7.05). The results indicated that addition of honey influence on alcohol content. Results supported the study conducted by Shraddha *et al.* (2021) [22]. Chaudhary *et al.* (2014) [5] reported similarly in fermented grape and jamun juice blend. LAB fermentation results in a negligible amount of alcohol production that ranged between 0.17% and 0.32%. The inoculum *Lactobacillus plantarum* is a hetero fermentative. Hence, alcohol has been produced by them as a primary metabolite other than lactic acid.

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Table 1: Changes in pH, TSS, Titrable Acidity and Vitamin C of Fermented Pseudo-Stem Core Juices enriched with Honey and Whey

Treatments		pH	TSS (°Brix)	Vitamin C (mg/ 100ml)	Titrable Acidity (%)
Yeast Fermentation	T1-PSCJ (control)	5.22 ^a	20.00 ^a	0.11 ^e	0.05 ^f
	T2- PSCJ + Yeast	4.12 ^b	6.97 ^{ef}	0.14 ^d	0.36 ^d
	T3- PSCJ+ Honey(5ml) + Yeast	4.12 ^b	5.33 ^f	0.15 ^d	0.31 ^e
	T4- PSCJ + Whey (10ml) + Yeast	4.22 ^b	7.10 ^e	0.17 ^c	0.34 ^{de}
	T5- PSCJ + Honey (5ml) + Whey (10ml) + Yeast	4.20 ^b	7.40 ^e	0.18 ^c	0.37 ^d
LAB Fermentation	T6- PSCJ + LAB	4.08 ^b	14.77 ^c	0.35 ^b	0.37 ^d
	T7-PSCJ+ Honey(5ml) + LAB	3.53 ^c	15.90 ^b	0.34 ^b	0.68 ^c
	T8- PSCJ+ Whey(10ml) + LAB	3.61 ^c	12.98 ^d	0.37 ^a	0.76 ^b
	T9- PSCJ + Honey (5ml) + Whey (10ml) + LAB	3.46 ^c	14.47 ^c	0.38 ^a	0.96 ^a

Note: Initial TSS maintained: Yeast Fermentation – 20 °Brix, LAB Fermentation – 16°Brix; Yeast - *Saccharomyces cerevisiae* var. *ellipsoideus* (NCIM-3200); LAB - *Lactobacillus plantarum* (MTCC- 6161); Values are presented as mean. The same lowercase letters within a column are not significantly different at $p < 0.05$



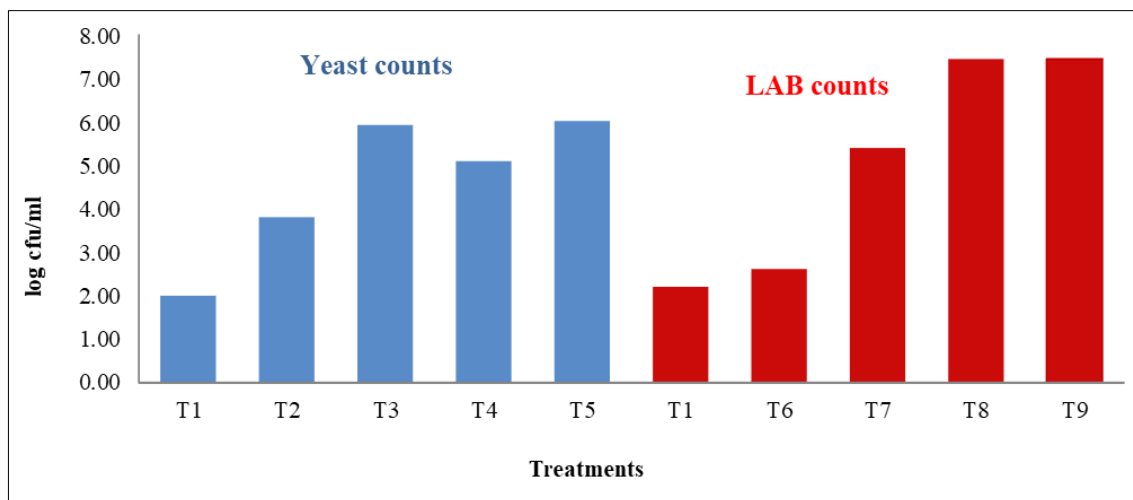
Note: T1- PSCJ (control), T2- PSCJ + Yeast, T3- PSCJ + Honey (5ml) + Yeast, T4- PSCJ + Whey (10ml) + Yeast, T5- PSCJ + Honey (5ml) + Whey (10ml) + Yeast, T6- PSCJ + LAB, T7- PSCJ + Honey (5ml) + LAB, T8- PSCJ + Whey (10ml) + LAB, T9- PSCJ + Honey (5ml) + Whey (10ml) + LAB

Fig 1: Variation in total sugar and alcohol production in the yeast and LAB fermented pseudo-stem core juice enriched with honey and whey;

Changes in Microbial Counts

Pseudo-stem core juice when enriched with honey (T3) as well as in combination with whey (T5) resulted in maximum yeast counts 5.93 log cfu/ml and 6.02 log cfu/ml respectively (Fig. 2). This indicated that honey and whey influenced the fermentative activity of yeast in pseudostem core juice. Similarly, Chaudhary *et al.* (2014) [5] reported on increased yeast in fermented grape and jamun blended beverage. LAB fermentation of pseudo-stem core juice enriched with 10 ml

whey (T8) recorded highest population of 7.45 log cfu/ml. Results clearly showed the influence of whey on LAB population which supports the work of Shukla *et al.* (2013) [23] pineapple enriched with whey fermented by LAB which recorded higher population (8.38×10^8 cfu/ml) after 24 hrs of fermentation. Nargis Fathima *et al.* (2021) [12] also reported the highest LAB population in foxtail millet based probiotic beverage when enriched with whey.



Note: T1- PSCJ (control), T2- PSCJ + Yeast, T3- PSCJ + Honey (5ml) + Yeast, T4- PSCJ + Whey (10ml) + Yeast, T5- PSCJ + Honey (5ml) + Whey (10ml) + Yeast, T6- PSCJ + LAB, T7- PSCJ + Honey (5ml) + LAB, T8- PSCJ + Whey (10ml) + LAB, T9- PSCJ + Honey (5ml) + Whey (10ml) + LAB

Fig 2: Yeast and bacterial population in the pseudo-stem core juice enriched with honey and whey fermented by yeast and lactic acid bacteria

Sensory Quality

Table 2 indicates the changes in sensory attributes of Yeast and LAB fermented pseudo-stem core juice enriched with honey and whey with respect to sensory quality. The highest score was obtained by LAB fermented beverage enriched with whey (T8) with the score 16.50/ 20.0 followed by yeast fermented beverage enriched with honey (16.0 /20.0 (T3). Srinivas *et al.* (2012) [25] reported 5 per cent level of incorporation of whey enriched probiotic Shrikand showed higher overall acceptability scores compared to control.

Shelf-life Assessment

Best adjudged two products *viz.*, Yeast fermented pseudo stem core juice enriched with honey (PY) and Lactic acid bacteria fermented pseudostem core juice enriched with whey (PL) were selected for the storage studies. The results pertaining to changes in pH and Titrable acidity during storage period is shown in Fig 3a and 3b. The initial pH of product PY and PL were 4.11 and 3.50 respectively. The pH gradually declined over the storage period in both the products at ambient and refrigerated

temperature (Fig 3a). The product PL showed significant decline in pH (2.50) after 15 days at ambient storage. These findings are similar to results of Profir *et al.* (2015) [16] in fermented vegetable juices. Sasikumar *et al.* (2015) [21] reported a decline in pH of whey-based pineapple juice RTS after 90 days at refrigerated storage.

The initial titratable acidity (TA) of PY and PL was 0.33% and 0.72% respectively. The acidity of PY increased during ambient storage from 0.33 to 0.46% and during refrigerated storage from 0.33 to 0.37% (Fig. 3b). Titratable acidity of PL increased at ambient and refrigerated temperature (Fig. 3b). Under ambient temperature, TA of PL was 1.31 per cent at 30 days of storage period. Increase in acidity during storage period may be due the production of acids as a metabolite by lactic acid bacteria. Similarly, Sasi Kumar (2015) reported the increase in acidity of LAB fermented whey blended with aloe vera at ambient temperature storage.

Fig 4a represents the changes in bacterial population of the product PL during storage period. The recorded lactic acid bacterial population was of 6.2×10^7 at initial 0 days storage. During storage period, there was reduction in LAB counts both at ambient temperature and refrigerated temperature (Fig. 4a). However, LAB fermented (PL) product showed more decline in bacterial counts (8.1×10^3 cfu/ml) under ambient storage at 15 days storage and sample got deteriorated within 7 days of storage under ambient temperature and there was significant decline in LAB counts

(10^4 cfu/ml) after 30 days of refrigerated storage.

Decline in LAB counts could be due to increase in acidity of products during storage period or depletion of nutrients. These results are similar to the results of Profir *et al.* (2015) [16] reported in fermented vegetable juices. Khatoon and Gupta (2015) [10] reported that *Lactobacillus acidophilus* could not survive in sweet lime after 1 week of cold storage due to the initial pH drop. Sasikumar (2015) [21] reported that fermented whey blended with aloe vera recorded with 1.3×10^7 cfu/ml at 4 °C after 30 days of storage.

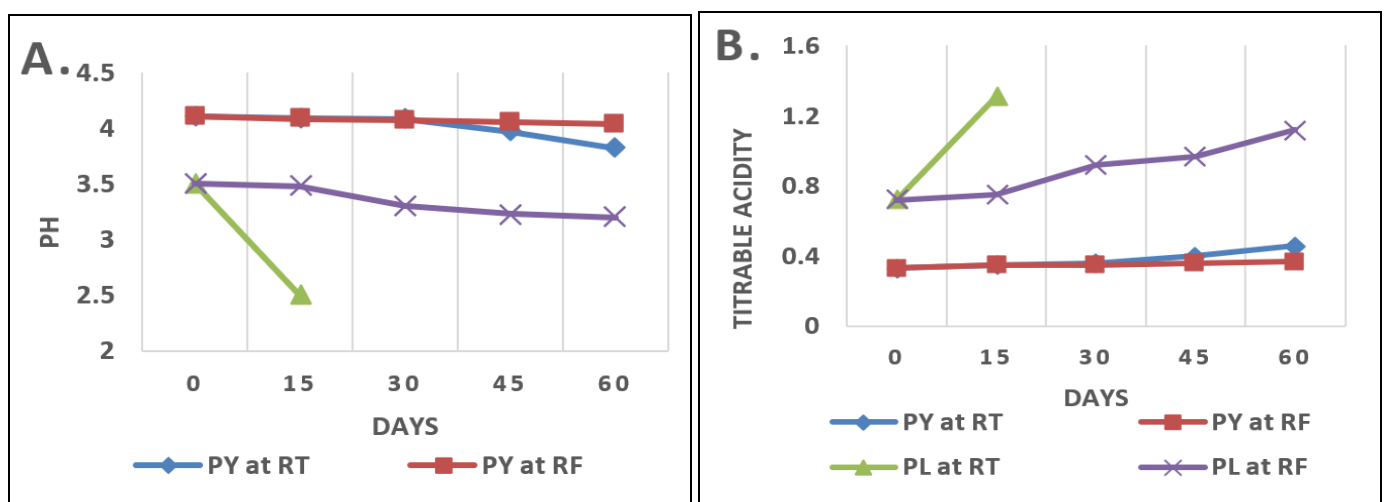
Yeast fermented beverage (PY) showed alcohol concentration of 7.02% at zero day's storage. The gradual increase in alcohol content was observed during ambient and refrigerated temperature storage (Fig. 4b). Soibam *et al.* (2016) [24] reported the gradual increase in alcohol content in watermelon and sugarcane blended fermented beverage stored for 6 months at ambient temperature.

The variation in sensory attributes on storage studies with respect to overall acceptability of yeast and LAB fermented (PY and PL) products stored at ambient and refrigerated temperature have been shown in Table 3. Sensory properties of PY did not show difference up to 30 days under ambient temperature and up to 45 days under refrigerated temperature and secured the highest score (16.0/ 20.0) with respect to over acceptability. Sensory property of PL reduced over the storage period.

Table 2: Sensory Attributes of yeast and LAB fermented Pseudo-Stem Core Juice enriched with Honey and Whey

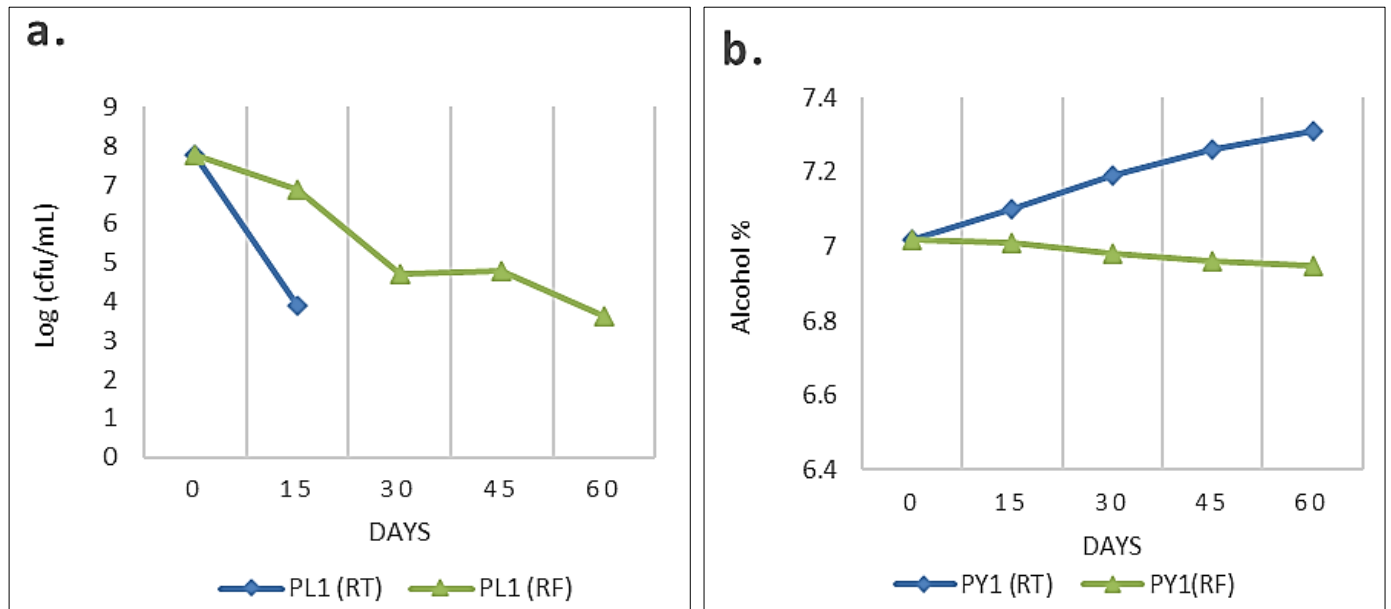
Treatments	Appearance (2)	Colour (2)	Aroma (2)	Bouquet (2)	Acidity (2)	Sweetness (2)	Body (2)	Astringency (2)	Flavour (2)	Quality (2)	Overall acceptability (20)	
	T1	1.00	1.00	1.00	1.00	1.00	1.25	1.00	1.00	1.00	10.25	
Yeast Fermentation	T2	1.50	1.50	1.50	1.25	1.00	1.25	1.25	1.25	1.25	13.00	
	T3	1.75	1.75	1.75	1.50	1.50	1.50	1.50	1.75	1.50	16.00	
	T4	1.25	1.25	1.00	1.00	1.00	1.00	1.50	1.00	1.00	11.75	
	T5	1.25	1.25	1.50	1.25	1.25	1.25	1.50	1.25	1.50	1.25	13.50
	T6	1.25	1.25	1.00	1.00	1.25	1.50	1.25	1.25	1.25	1.00	12.00
LAB Fermentation	T7	1.25	1.25	1.50	1.50	1.50	1.75	1.25	1.00	1.50	1.25	13.75
	T8	1.75	1.75	1.75	1.50	1.75	1.50	1.75	1.75	1.75	1.50	16.50
	T9	1.50	1.50	1.50	1.25	1.50	1.50	1.25	1.50	1.50	1.25	14.25

Note: T1- PSCJ (control), T2- PSCJ + Yeast, T3- PSCJ + Honey (5ml) + Yeast, T4- PSCJ + Whey (10ml) + Yeast, T5- PSCJ + Honey (5ml) + Whey (10ml) + Yeast, T6- PSCJ + LAB, T7- PSCJ + Honey (5ml) + LAB, T8- PSCJ + Whey (10ml) + LAB, T9- PSCJ + Honey (5ml) + Whey (10ml) + LAB; Initial TSS maintained: Yeast fermentation – 20 °Brix, LAB fermentation – 16 °Brix; Yeast - *Saccharomyces cerevisiae* var. *ellipsoideus* (NCIM-3200); LAB - *Lactobacillus plantarum* (MTCC- 6161)



Note: PY: Yeast fermented Pseudo-Stem Core Juice (PSCJ) blended with Honey (5ml), PL: LAB Fermented PSCJ blended with Whey (10ml), RT- Room Temperature; RF- Refrigerated Temperature

Fig 3: Effect of storage condition on a) pH and b) TA of fermented beverages



Note: PY1: Yeast fermented Pseudo-Stem Core Juice (PSCJ) blended with Honey (5ml), PL1: LAB Fermented PSCJ blended with Whey (10ml); RT- Room Temperature; RF- Refrigerated Temperature

Fig 4: a) Lactic acid bacterial population of PL1 and b) alcohol content in the PY 1 during storage under ambient and refrigerated temperature

Table 3: Sensory Attribute with respect to overall acceptability (20 point scale) Fermented Pseudo-Stem Core Juice during storage under Ambient and Refrigerated temperature

Best adjudged product/ Juice	Ambient Temperature (26 – 28 °C)					Refrigerated temperature (4 °C)				
	STORAGE DAYS									
	0	15	30	45	60	0	15	30	45	60
	Overall acceptability (20.0 point scale)									
PY	16.0	16.0	15.75	12.0	12.00	16.0	16.0	16.0	15.50	15.00
PL	16.0	ND*	ND*	ND*	ND*	16.0	15.5	13.75	11.00	11.0

Note: PY: Yeast Fermented Pseudo-Stem Core Juice blended with Honey (5ml); PL: LAB Fermented Pseudo-Stem Core Juice blended with Whey (10ml); ND*- Not Determined

Conclusion

The developed yeast fermented pseudo stem core juice enriched with honey (5%) and LAB fermented pseudo stem core juice enriched with whey (10%) were very much desirable and acceptable for consumption with respect to biochemical and sensory properties. Yeast fermented honey blended pseudo-stem core beverage product (PY) could be stored up to 30 and 60 days under ambient and refrigerated temperature respectively. LAB fermented whey blended pseudo-stem core juice product (PL) could be stored up to 7 days and 15 days under ambient and refrigerated temperature respectively.

Acknowledgement

I deeply acknowledge the Department of Agricultural Microbiology and AICRP on PHET Scheme, GKVK, University of Agricultural sciences, Bangalore for providing laboratory facilities and institutional support for carrying out the experiments.

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