DR-2283
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RESEARCH ARTICLE Asian Journal of Dairy and Food Research

Development of Barnyard Millet-Honey Blended Dietetic *Lassi*

Sahil M. Hake[1], Amit A. Kulthe[1], Fayaj L. Pathan[1], Prerana D. Shere[1]

10.18805/ajdfr.DR-2283

ABSTRACT

Background: *Lassi*, widely consumed as a fermented milk beverage, is characterized by a creamy consistency, smooth texture, glossy shine and a white colour with a yellowish tinge in good quality. It should also taste mild to highly acidic and have a rich, sweet aroma.

Methods: In present investigation, a probiotic dietetic *lassi* was formulated. The product was standardized for varying levels of Barnyard millet flour and honey added with 2% concentration of *Lactobacillus fermentum* (NCIM-2165) strain. The developed product was analysed for sensory, physico-chemical properties and microbial count in both fresh form as well as during the storage of 15 days at refrigeration temperature (5±2°C) in different packages *viz.* glass bottles, PET bottles and LDPE pouches.

Result: Chemical analysis of product showed antioxidant activity (4.65 to 9.36 mg/100 g), total phenol content (6.07 to 15.72 mg GAE/100 g), reducing sugar (4.12 to 8.19 mg/100 g), consistency (337.33 to 343.25 cP) and ascorbic acid (1.45 mg/100 g) respectively. Proximate analysis showed variations in fat, protein, fiber, ash, carbohydrate and total solid content among the variants. Storage studies revealed variable decrease in total viable count (TVC) over time, with the highest count (8.09 log cfu/ml) observed in the variant containing 7.5% Barnyard millet and 7.5% honey after 15 days storage at refrigeration temperature (5±2°C) in glass bottles.

Key words: Barnyard millet, Honey, *Lactobacillus, Lassi,* Probiotic.

INTRODUCTION

Lassi, widely consumed as a fermented milk beverage, is characterized by a creamy consistency, smooth texture, glossy shine and a white colour with a yellowish tinge in good quality. It should also taste mild to highly acidic and have a rich, sweet aroma. According to national tastes, the beverage is usually flavoured with either sugar or salt along with additional spices (Ben *et al.,* 2020). Being an ubiquitous lactic acid fermented milk beverage of the Indian sub-continent, *lassi* contains a significant amount of milk proteins and phospholipids, along with various metabolites produced by lactic acid bacteria (Sharma *et al.,* 2016). Though *lassi* is a favourite beverage for all age groups due to its refreshing, tasty and nutritious characteristics as well as its high therapeutic value along with ability to quench thirst, its short shelf life and whey syneresis make it challenging to sell on the market (Krishna *et al.,* 2019). Microorganism metabolism may occur while refrigerated storage, which leads to post-acidification and proteolysis which reduce product quality and reduce *lassi* shelf life. The quality and acceptability of *lassi* are determined by these processes based on the degree of acidity (Ashwani *et al.,* 2003).

Barnyard millet (*Echinochloa frumentacea*) belongs to the family *Poaceae* and is one of the oldest domesticated small millets. This millet is cultivated extensively in several countries, including India, China, Japan, Malaysia, the East Indies, Africa and the United States of America. Its nutritional richness and capacity to adapt to a variety of environmental situations make it a popular staple meal in many areas (Kaur and Sharma, 2020). Barnyard millet serves as a valuable source of protein, carbohydrates and fibre. Compared to other main grains, it notably has higher quantities of important micronutrients including zinc and iron. The nutritional composition of Barnyard millet includes protein (6-13 g), carbohydrates (55-65.5 g), fat (2-4 g), crude fiber (9.5-14 g), mineral matter (3.8-4.5 g), calcium (11-27.1 mg), phosphorus (280-340 mg) and iron (15-19.5 mg) (Ugare *et al.,* 2014). Furthermore, owing to low glycaemic index and gluten, barnyard millet provides extra health benefits including prevention and treatment against several kinds of illnesses, *viz.* blood pressure regulation, diabetes, obesity, celiac disorder and cardiovascular disease. Moreover, the major fatty acid profile of barnyard millet is linoleic acid, with palmitic and oleic acids (Singh *et al.,* 2022).

The Codex alimentarius commission (CAC) defines “honey” as a naturally sweet material produced by honeybees. The sweetness of honey is due to the presence of carbohydrates in the form of maltose, isomaltose, maltulose, sucrose, turanose, fructose, glucose and monosaccharides and disaccharides. It is like sucrose in terms of sweetness. Additionally, it has enzymes including

[1]MIT School of Food Technology, MIT Art, Design and Technology University, Pune-412 201, Maharashtra, India.

Corresponding Author: Amit A. Kulthe, MIT School of Food Technology, MIT Art, Design and Technology University, Pune-412 201, Maharashtra, India. Email: amitkulthe.ft@gmail.com

ORCIDs: 0009-0005-0022-6338; 0000-0002-9388-5244; 0000-0002-2467-3125; 0000-0002-0992-2947

How to cite this article: Hake, S.M., Kulthe, A.A., Pathan, F.L. and Shere, P.D. (2025). Development of Barnyard Millet-Honey Blended Dietetic *Lassi.* Asian Journal of Dairy and Food Research. 1-7. doi: 10.18805/ajdfr.DR-2283.

Submitted: 21-10-2024 **Accepted:** 12-02-2025 **Online:** 09-04-2025

amylase, oxidase peroxide, catalase and acid phosphorylase as well as oligosaccharides like anderose and panose. In addition to these nutrients, honey has antioxidants, minerals, iron, zinc, trace vitamin B, vitamin B_6, vitamin C, niacin and folic acid (Meo *et al.,* 2017). According to research by Kadam *et al.* (2018), honey may be useful in raising the populations of probiotic bacteria in the gut, which might help to improve digestion, lower cholesterol and prevent colon cancer in addition to boosting the immune system.

Hence, the present investigation was undertaken for the process standardization and quality assessment of Barnyard millet-honey blended dietetic lassi and effect of packaging materials on the storage stability of the prepared *lassi*.

MATERIALS AND METHODS

Raw materials and probiotic starter strains

Milk, Barnyard millet, sugar and honey were procured from local market of Pune. The *Lactobacillus fermentum* (NCIM-2165) strain was procured from National Chemical Laboratory (NCL), Pune. The culture was stored at 5±2°C. Prior to use, culture was activated in suitable medium by incubating at 37°C for 24 h.

Standardization of Barnyard millet-honey dietetic *lassi*

Preliminary trials were conducted to standardize the level of Barnyard millet(0, 5, 7.5, 10%) and honey (0, 5, 7.5, 10, 12.5%)in *lassi*. The standardization process was undertaken step wise first for Barnyard millet followed by honey in the lassi sample with acceptable level of Barnyard millet subjecting to sensory evaluation by 10 semi trained panel of judges.

Preparation of dietetic *Lassi*

The Barnyard millet-honey blended dietetic *lassi* was prepared as per method Pardhi *et al.* (2014) with some modification Fig 1.

Sensory evaluation

The prepared *lassi* samples were evaluated for sensory attributes by a panel of 10 semi-trained judges, as described by Amerine *et al*. (1965) using a 9-point Hedonic scale system for different parameters like colour and appearance, flavour, taste, texture, mouthfeel, consistency and overall acceptability. The mean values of 10 semi-trained judges were considered for evaluating the quality.

Physico-chemical parameters for raw material and *lassi*

Physico-chemical analysis of raw materials and dietetic *lassi* included several tests like total soluble solids (TSS), ash, fibre, total phenolic content (AOAC, 1990), pH (Jan *et al.,* 2016), titratable acidity, consistency (Ranganna, 1997), total solids, fat content, protein content (IS: SP-18 1981), ascorbic acid and antioxidant activity (Shimada *et al.,* 1992).

Energy value

The energy value of dietetic *lassi* was estimated using the sum of the product of respective physiological fuel values and contents of protein, carbohydrate and fat. It was expressed in Kcal (Kulthe *et al.*, 2018b).

Milk

↓

Heat treatment (85°C/10 min)

↓

Cooling (20°C)

↓

Inoculate (at 20°C) with *Lactobacillus fermentum* culture (2%)

↓

Incubation (43°C for 8hrs)

↓

Cooling (at 20°C) and breaking of Coagulum

↓

Addition of Barnyard millet flour and Honey

↓

Blending

↓

Packaging and Storage (5 ± 2°C)

Fig 1: Preparation of barnyard millet-honey blended dietetic *lassi.*

Microbial analysis of *lassi*

Microbial analysis of dietetic *lassi* included total viable count, yeast and mold counts and coliform count determined by standard method (Tharmaraj and Shah, 2003, Marshall, 1993 and Houghtby *et al.,* 1992).

Storage study

Dietetic *lassi* samples were packed in different packages *viz.* glass bottles, PET bottles and LDPE pouches and stored at refrigeration temperature (5±2°C) to assess the shelf life and to study the storage related changes in the products. The samples were analyzed periodically for sensory, chemical and microbiological parameters.

Statistical analysis

Each sample was analysed in triplicate. The figures then were averaged. The data obtained was analysed statistically using standard methods given by Snedecor and Cochran (1990) and by Duncan's multiple range test with the probability $p \leq 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

The research aimed to the process standardization and quality assessment of a dietetic *lassi* formulated with Barnyard millet and honey. The research progressed to standardizing the Barnyard Millet-honey blended dietetic *lassi* using the selected *LAB* strain, followed by rigorous sensory, physicochemical and microbial quality assessments. A techno-economic feasibility analysis, supported by product stability studies, demonstrated promising commercialization potential.

Physico-chemical analysis of raw materials

This study investigates the physico-chemical properties of milk, barnyard millet and honey, key ingredients in a nutritious *lassi* formulation Table 1. Milk, with its high moisture content (86.60%) and significant caloric value (68.82 kcal), provides essential hydration and energy, containing 3.49% fat, 2.42% protein, 0.65% ash and 6.93% carbohydrates, aligning with previous research by Ghule (2015) and Jadhav (2002). Barnyard millet, noted for its low moisture (12.21%) and high fiber content (8.25%), supports digestive health and offers a robust nutritional profile with 3.25% ash, 6.85% protein, 4.21% fat and 73.50% carbohydrates, contributing 359.29 kcal, consistent with Ugare *et al.* (2014).

Honey stands out with its low fat (0.26%) and protein (0.62%) levels but high carbohydrate content (82.83%) and substantial caloric value (336.17 kcal), enhanced by significant reducing sugars (54.65 mg/100 g) and ascorbic acid (19.65 mg/100 g), boosting its antioxidant properties. The total phenolic content and antioxidant activities of milk (4.97 and 3.45 mg/100 g, respectively), barnyard millet (22.63 and 48.31 mg/100 g) and honey (120.89 and 30.91 mg/100 g) underscore the combined nutritional and health benefits of these ingredients, particularly showing honey's role in enhancing the *lassi*'s nutritional value.

Standardization of Barnyard millet-honey blend of dietetic *lassi*

In the context of this study, sensory evaluation was conducted to evaluate the sensory characteristics of dietetic *lassi* formulations containing different concentrations of barnyard millet and honey Fig 2. The study standardized the curd-to-barnyard millet ratio in dietetic *lassi,* using samples with 0, 5, 7.5 and 10% barnyard millet, respectively. On the basis of sensory evaluation (Fig 2a), treatment B_2 (7.5% barnyard millet) secured the highest scores for colour (8.19), texture (8.19), flavour (7.97), mouthfeel (8.08) and overall acceptability (7.97). Thus, B_2 was selected for further research due to its superior sensory profile. These findings align with previous studies by Pardhi *et al*. (2014) and Das (2015).

The standardized barnyard millet *lassi* (B_2-7.5%) was added with different levels of honey (0, 5, 7.5, 10 and 12.5%) for further standardization using sensory evaluation (Fig 2b).

Table 1: Physico-chemical characteristic of raw materials.

Parameters	Milk	Barnyard millet	Honey
Moisture (%)	86.50±0.11	12.21±0.15	15.87±0.30
Fat (%)	3.49±0.12	4.21±0.09	0.26±0.06
Protein (%)	2.42±0.08	6.85±0.12	0.62±0.11
Fiber (%)	-	8.25±0.05	-
Ash (%)	0.65±0.07	3.23±0.06	0.42±0.06
Carbohydrate (%)	6.93±0.09	73.50±0.28	82.83±0.49
Energy (Kcal)	68.82±0.54	359.29±1.11	336.17±1.05
TSS (°Bx)	9.16±0.05	-	75.06±0.67
Acidity (%)	0.19±0.06	-	0.31±0.09
Reducing sugar (mg/100 g)	4.91±0.25	-	54.65±1.58
Ascorbic acid (mg/100 g)	-	-	19.65±1.08
Total phenolic content (mg GAE/100 g)	4.97±1.34	22.63±0.31	120.89±2.71
Antioxidant activity (mg/100 g)	3.45±2.96	30.91±1.85	48.31±2.39

Each value is average of three determinations ± standard deviation.

Sensory evaluation revealed that B_2H_2 (7.5% honey) had the highest scores for colour (8.33), texture (8.22) and overall acceptability (7.9), indicating optimal sensory performance. Comparatively, other samples showed good but slightly lower overall sensory performance compared to B_2H_2. The results were found consistent with literature (Riazi *et al.,* 2012 and Ghadge *et al.,* 2008).

The comparative sensory analysis of selected *lassi* formulations B_0, B_2 and B_2H_2 revealed distinct profiles (Fig 2 c). B_0 scored 8.2 for colour, 7.7 for flavour, 7.85 for texture, 7.65 for taste, 7.95 for mouthfeel and 8.03 for overall acceptability. B_2 had scores of 7.9 for colour, 7.9 for flavour, 7.85 for texture, 7.75 for taste, 7.95 for mouthfeel and 7.94 for overall acceptability. B_2H_2 achieved the highest scores with 8 for colour, 8.1 for flavour, 8.25 for texture, 8.15 for taste, 8.22 for mouthfeel and 8.01 for overall acceptability. These results indicate that B_2H_2 is superior in flavour, texture and overall acceptability.

Physico-chemical and microbial analysis of fresh *lassi*

The physicochemical and microbial analysis of selected *lassi* variants B_0, B_2 and B_2H_2 revealed significant compositional differences Table 2. It is clearly seen that addition of Barnyard millet and replacement of sugar with honey caused significant decrease in TSS (15.03 to 12.50°Bx), acidity (0.88 to 0.76%), carbohydrates (14.77 to 11.93%), calories (101.37 to 90.99Kcal), whereas significant increase in pH (3.81 to 4.93), proteins (2.52 to 2.92%), fibres (0.08 to 0.67%), ash (0.67 to 0.89%), total solids (21.47 to 22.26%), viscosity (337.33 to 343.25 cP),

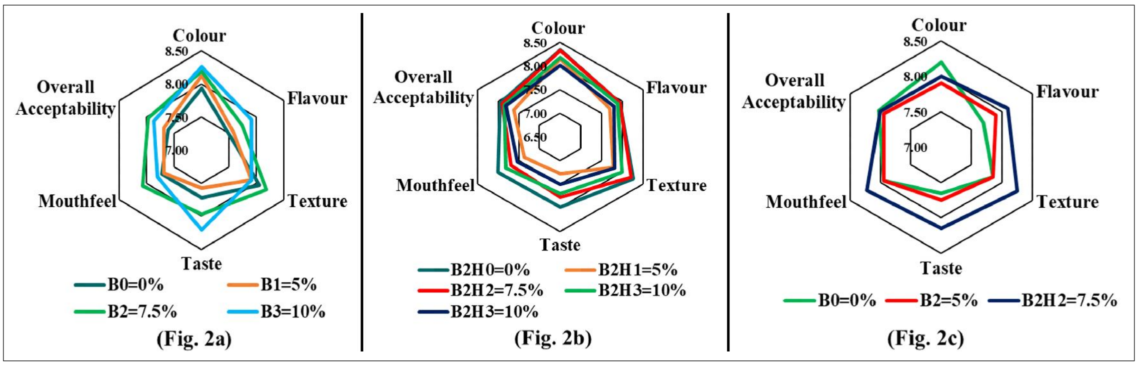

Fig 2: Standardization of formulation for dietetic *lassi*.

Table 2: Physico-chemical and microbial analysis of dietetic *lassi.*

Parameters	B_0	B_2	B_2H_2
TSS (°Bx)	15.03±0.21^a	15.07±0.15^a	12.50±0.12^b
Acidity (%)	0.88±0.04^a	0.83±0.02^b	0.76±0.02^c
pH	3.81±0.29^c	4.31±0.15^b	4.93±0.12^a
Fat (%)	3.57±0.15^a	3.45±0.10^a	3.51±.0.20^a
Protein (%)	2.54±0.08^c	2.75±0.04^b	2.92±0.07^a
Fiber (%)	0.08±0.01^b	0.64±0.04^a	0.67±0.03^a
Ash (%)	0.67±0.05^b	0.85±0.06^b	0.89±0.03^a
Carbohydrate (%)	14.77±0.40^a	15.74±0.34^a	11.93±0.30^b
Total solid (%)	21.47±0.16^c	22.79±0.20^a	22.26±0.25^b
Energy (Kcal)	101.37±0.62^b	105.01±0.52^a	90.99±0.74^c
Consistency (CP)	337.33±0.31^c	342.55±0.41^b	343.25±0.55^a
Reducing sugar (mg/100g)	4.12±0.23^b	4.36±0.25^b	8.19±0.26^a
Ascorbic acid (mg/100g)	-	-	1.45±0.03
Total phenolic content (mg GAE/100g)	6.07±0.02^c	7.69±0.03^b	15.72±0.05^a
Antioxidant activity (mg/100gm)	4.65±0.05^c	6.83±0.07^b	9.36±0.07^a
Total viable count (log cfu/ml)	8.25±0.02^c	8.36±0.01^b	8.45±0.03^a
Yeast and mold count (log cfu/ml)	Not detected	Not detected	Not detected
Coliform count (log cfu/ml)	Not detected	Not detected	Not detected

Each value are average of three determinations ± standard deviation.

Values not sharing a common superscript letter(s) in a row are significantly different at p<0.05 as assessed by Duncan's multiple range test.

reducing sugars (4.12 to 8.19 mg/100 g), total phenolics (6.07 to 15.72 mgGAE/100 g) and anti-oxidant activity (4.65 to 9.36 mg/100 g). There was non-significant effect on fat content. B_2H_2 uniquely contained detectable ascorbic acid (1.45 mg/100 g) due to presence of honey. Similar results were reported in the literature (Shuwu *et al.,* 2011; Pardhi *et al.,* 2014; Khupse *et al.,* 2017). The results also indicated significant increase in total viable count of *lassi* samples (8.25 to 8.45 log cfu/ml) suggesting that barnyard millet and honey influenced microbial growth. Yeast, mold and coliform counts were not detected in any samples, indicating no fungal or faecal bacteria contamination. These findings align with previous studies by Chick *et al.* (2001); Krishna *et al.* (2019); Patel *et al.* (2020).

Shelf-life study of dietetic *lassi*

The shelf-life study of dietetic *lassi* focused on monitoring its quality and stability over time, examining critical parameters during refrigerated storage (5±2°C) in conjugation with effectiveness of packaging materials *viz.* glass bottles, PET bottles and LDPE pouches.

Change in sensory analysis during storage

During the shelf-life study of dietetic *lassi*, sensory properties were analyzed and shown in Table 3. There was a significant decline in the scores of samples for all the sensory parameters under acceptable limits like colour (8.50 to 6.50), flavour (8.70 to 6.80), texture (8.00 to 6.00), taste (8.60 to 6.40) and overall acceptability (8.45 to 6.53).

Table 3: Effects of packaging materials on storage life of dietetic *lassi.*

Parameters	0 Day	Days	Glass bottle	PET bottle	LDPE pouch
Colour	8.50±0.11	5	8.20±0.08^{a}	7.90±0.11^{b}	6.50±0.11^{c}
		10	7.80±0.10^{a}	7.30±0.08^{b}	*
		15	7.10±0.11^{a}	6.70±0.10^{b}	*
Flavour	8.70±0.13	5	8.50±0.11^{a}	8.10±0.15^{b}	6.80±0.13^{c}
		10	8.00±0.13^{a}	7.60±0.14^{b}	*
		15	7.50±0.11^{a}	7.00±0.13^{b}	*
Texture	8.00±0.13	5	7.60±0.10^{a}	7.30±0.10^{b}	6.50±0.06^{c}
		10	7.20±0.08^{a}	6.80±0.16^{b}	*
		15	6.70±0.16^{a}	6.00±0.13^{b}	*
Taste	8.60±0.14	5	8.30±0.10^{a}	8.00±0.13^{b}	7.10±0.15^{c}
		10	7.80±0.08^{a}	7.10±0.20^{b}	*
		15	7.20±0.16^{a}	6.40±0.10^{b}	*
Overall acceptability	8.45±0.06	5	8.15±0.08^{a}	7.83±0.07^{b}	6.73±0.09^{c}
		10	7.70±0.07^{a}	7.20±0.07^{b}	*
		15	7.13±0.07^{a}	6.53±0.09^{b}	*
TSS (°Bx)	12.50±0.12	5	12.25±0.10^{a}	11.50±0.12^{b}	10.75±0.10^{c}
		10	11.75±0.10^{a}	11.25±0.10^{b}	*
		15	10.75±0.12^{a}	9.75±0.11^{b}	*
Acidity (%)	0.76±0.02	5	0.86±0.01^{c}	0.89±0.03^{b}	0.94±0.03^{a}
		10	0.92±0.01^{b}	1.04±0.17^{a}	*
		15	1.01±0.02^{b}	1.18±0.09^{a}	*
pH	4.93±0.02	5	4.63±0.03^{a}	4.28±0.03^{b}	3.95±0.03^{c}
		10	4.05±0.03^{a}	3.88±0.02^{b}	*
		15	3.85±0.02^{a}	3.53±0.02^{b}	*
Consistency (CP)	343.25±0.55	5	345.50±0.53^{c}	347.25±0.44^{b}	352.75±0.58^{a}
		10	349.50±0.26^{b}	352.00±0.31^{a}	*
		15	355.25±0.41^{b}	365.50±0.45^{a}	*
Total viable count (log CFU/ml)	8.40±0.05	5	8.22±0.03^{a}	8.17±0.03^{b}	7.98±0.04^{c}
		10	8.14±0.03^{a}	8.06±0.06^{b}	*
		15	8.09±0.04^{a}	7.73±0.03^{b}	*
Yeast and mold count (log CFU/ml)	Not detected	5	Not detected	Not detected	1.87±0.04^{a}
		10	1.18±0.06^{b}	1.32±0.08^{a}	*
		15	1.62±0.07^{b}	1.75±0.05^{a}	*

* Spoilage of product.

Each value is average of four determinations ± standard deviation.

Values not sharing a common superscript letter(s) in a row are significantly different at $p<0.05$ as assessed by Duncan's multiple range test.

Spoilage of *lassi* samples packed in LDPE pouches was observed indicating less efficiency of package. Among the other samples, *lassi* stored in glass bottles showed better retention of overall sensory scores (7.13) compared to that of PET bottles (6.53) indicating high efficiency of the package to store *lassi* under acceptable condition up to 15 days. These findings found to be consistent with previous studies by Patidar and Prajapati (1998); Satkar *et al*. (2013); Kulthe *et al*. (2018a) and Ahuja *et al.* (2017) who reported similar impact of both storage conditions and packaging materials on sensory quality of product during storage.

Physico-chemical analysis of dietetic *lassi* during storage

During the shelf-life study of dietetic *lassi*, TSS, acidity, pH and consistency were monitored shown in Table 3. Initially, the TSS of the *lassi* at day 0 was 12.50°Bx but decreased notably during storage in glass bottles (10.75°Bx), PET bottles (9.75°Bx) and LDPE pouches (10.75°Bx before spoilage occurred). This reduction in TSS reflects the consumption of sugars, likely due to microbial fermentation (Ding and Shah, 2008). There was significant increase in acidity of samples (0.76 to 1.18%) with decrease in pH (4.93 to 3.53) across all packaging types, indicative of ongoing fermentation and the production of lactic acid and other organic acids (Patidar and Prajapati, 1998 and Bagal *et al.,* 2007). Moreover, consistency increased during storage, indicating changes in the physical properties of the *lassi*, likely influenced by the conversion of lactose to lactic acid and subsequent protein denaturation, as noted by Amrinder (2018) in a similar context with aloe vera juice incorporated *lassi.*

Microbial analysis of dietetic *lassi* during storage

During the shelf-life study of dietetic *lassi*, microbial analysis revealed significant changes in the total viable count (TVC) and yeast and mold counts Table 3. Initially, the TVC was 8.40 log cfu/ml but over 15 days, decreased slightly in glass bottles (8.09 log cfu/ml), with more significant reduction in PET bottles (7.73 log cfu/ml) and LDPE pouches (7.98 log cfu/ml). Yeast and mold count initially was not detected but appeared in LDPE pouches (1.87 log cfu/ml) at 5 days, indicating higher susceptibility to contamination compared to that at 15 days in glass bottles (1.62 log cfu/ml) and PET bottles (1.75 log cfu/ml). Similar results were reported in the literature. Patel *et al.* (2020) reported a decrease in TVC in amaranthus flour incorporated *lassi*. Sharma *et al.* (2016) reported an increase in yeast and mold count during the storage period of synbiotic *lassi* containing honey, with a significant decrease in *LAB* during storage.

CONCLUSION

The study on process standardization of Barnyard millet honey blended dietetic *lassi* involved the physicochemical analysis of milk, Barnyard millet and honey, revealing distinct nutritional profiles. The antioxidant capacity of the Barnyard millet-honey blended *lassi* (B_2H_2) was measured, yielding an impressive value of 67.8%. Ascorbic acid content was determined to be 12.4 mg/100 g, reflecting the lassi's enhanced nutritional profile. The reducing sugar content was quantified at 8.2 g/100 g and the total phenolic content was 34.6 mg GAE/100 g, underscoring the *lassi* potential health benefits due to its high antioxidant and phenolic compounds.

Shelf-life studies assessed the stability and quality of dietetic *lassi* under refrigerated conditions using different packaging materials (glass bottles, PET bottles and LDPE pouches). Results indicated that glass bottles provided the best preservation of sensory attributes, total soluble solids, acidity, pH and consistency over 15 days, while LDPE pouches were the least effective. The microbial analysis showed significant differences in total viable counts, with the highest counts in the B_2H_2 variant, suggesting enhanced microbial growth with the addition of Barnyard millet and honey.

ACKNOWLEDGEMENT

The present study was supported by MIT Art, Design and Technology University, Pune.

Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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