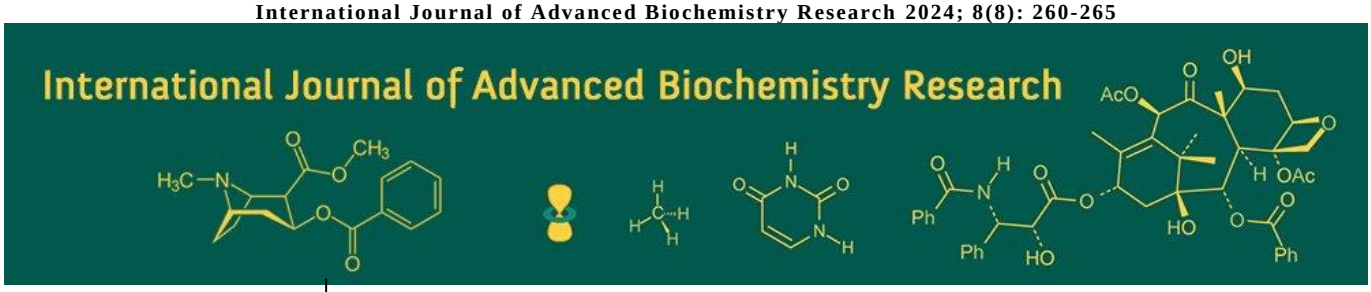

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Development of malted and fermented finger millet based beverage blended with beetroot juice

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Abstract
In a post-pandemic world, consumer profiles are continuing to change and new trends are forming, as more have begun working from home. These include direct-to- consumer (DTC), restaurant industry, fresh produce trends. A growing niche in the food and beverage industry is dairy-free and plant-based alternatives. With these considerations, the present study was focused to develop a new variety of blended beverage by utilizing finger millet (*ragi*) along with beetroot. Development of beverage was done in three steps *viz*., preparation of malted finger millet flour, preparation of fermented suspension as a base using malted finger millet flour, blending of vegetable juices with fermented finger millet suspension. The effect of different blending ratio and packaging materials for shelf life extension of developed beverage stored at 4±2 ℃, in terms of biochemical, microbial and sensory attributes were studied. From the analysis of observations taken, it was observed and concluded to blend malted and fermented finger millet suspension with beetroot juice (FB2P2) in the ratio of 1:1 and store at 4±20C temperature in glass bottle for 4 weeks to maintain freshness and quality of it with 0.56 of titratable acidity, 5 ºBrix of total soluble solids, 5.40 of pH, 6.09 g/100 ml of total sugar content, 75.30% of antioxidant activity, 9.58 g/100 ml of true protein, 48.46 mg/100 ml of calcium, 1.77 mg/100 ml of iron, 1.09 mg/100 ml of zinc, 935.63 mg/100 ml of total phenol content and 682 mg/l of betacyanin with minimum level of microbial infection and good score of acceptability. The cost required for developing this beverage stored at 4±2 ℃ was estimated to be 16 Rs per PET bottle and 21 Rs per glass bottle of 200 ml.

Keywords: Finger millet, Beetroot, malting, fermentation, beverages, blending ratio, pet and glass bottle

Introduction
Millets are small seeded grains with different varieties, taxonomically belonging to family *Poaceae*, considered as crop of food security because of their sustainability in adverse agro-climatic conditions. India is the largest producer of millets in the world. India is the global leader in production of millets with a share of around 15% of the world total production. In India, millets are cultivated majorly in 21 states in an area of 12.53 Mha, producing 15.53 MT with a yield of 1237 kg/ha. Finger millet or *ragi* (*Eleusine coracana*) having nutraceutical properties, is the oldest cereal grain in India which is fairly grown in extreme climatic conditions. *Ragi* is a rich source of calcium which is thirty times more than that of rice and wheat.

The fresh and edible portions of herbaceous plants are generally termed as vegetables, which are important component of a healthy diet as they are a good source of vitamins and minerals, dietary fibers and antioxidants. Beetroot (*Beta vulgaris* L.) belongs to the *Chenopodiaceae* family. Red beetroot is a rich source of minerals. It is also rich in valuable active compounds such as glycine, betaine, saponins, pigments and pool of phenolic compounds that includes phenolic acids, flavonoids and organic and inorganic acids. Beetroot juices have a high fiber content (0.7–1.1 g), folic acid, vitamins A, C, E, K and B, as well as mineral salts such as zinc, iron, sodium, potassium, magnesium, phosphorus and calcium.

Beverages can be classified as specialty foods that provide energy and some of the essential nutrients like proteins, minerals and vitamins. These foods offer energy and nutrition to the consumers and help them to overcome fatigue and convalescence in the form of liquid food

supplement. These foods are generally taken as refreshing beverages. Processing of millet beverages has largely involved two basic food processing methods that include fermentation and malting, both of which deliver a diversity of products. Fermentation is economically important because, through the microbial action, the process transforms substrates into new products. Biochemical changes during this process result in modification of substrate and production of volatiles (Kohajdova and Karovicova, 2007) [14]. Activating the enzymes with pH variations, the fermentation process enhances the enzyme performance of amylases, hemicellulases and proteases (Saleh *et al.*, 2013) [18].

Materials and methods

The experiment was carried out at Department of Processing and Food Engineering, College of Agricultural Engineering and Technology, Junagadh Agricultural University, Junagadh during the year of 2023 – 2024. Finger millet (*Eleusine coracana*) grains and Beetroot (*Beta vulgaris* L.) were procured from local market of Junagadh, Gujarat. Food grade *Saccharomyces Cerevisiae* (yeast) was purchased through online (amazon) from Bioven ingredients. Potassium metabisulphite, a food preservative was purchased through online from BakersVille India Pvt. Ltd.

The experiment was designed using Factorial Completely Randomized Design (FCRD) with two replications and had twelve treatments *viz*., first factor as different blending ratios and second factor as developed beverages stored in two different packaging materials.

Development of beverages

The process flow chart for the preparation of beverages and the procedural steps are explained in detail as below that takes place broadly under three steps.

Preparation of malted finger millet flour

Malting of finger millet was carried out according to the procedure standardized by Chilkawar *et al.*, (2010) [10] with necessary modifications. Finger millet grains were weighed, cleaned to remove dust, dirt etc., and washed 3 times with distilled water. The grains were kept for steeping in water for 16 h at room temperature (Grain:water - 1:3). During steeping, the water was changed after every 4 h. Excess water was removed. Steeped grains were put in jars lidded with muslin cloth and placed upside down and germinated at 25±2 ℃ for 48 h in an incubator. The seeds were then dried in a fluidized bed dryer maintained at 65 ℃ for 15 min. The malted, dried finger millet seeds were derooted with manual rubbing by hands and grinded into flour using commercial flour mill adjusted to a fine setting. Subsequently, the flour was sieved and stored in airtight containers at 4 ℃ until use.

Preparation of fermented suspension as a base using malted finger millet flour

Malted finger millet flour was mixed with distilled water in the ratio of 1:20 and centrifuged to collect the supernatant part. Submerged state fermentation (SmF) was carried out as the process mentioned in Khandelwal *et al.* (2012) [13] with little modifications as per requirements: 0.25% inoculum of actively growing *Saccharomyces cerevisiae*, followed by addition of sucrose @ 1% and to check any undesirable microbial contamination, potassium meta-bisulphite (KMS) at 180 ppm was added in the jars filled with supernatant collected from malted *ragi* flour and air tightened. Detailed steps are shown in plate 3.1. Fermentation was carried out at 30 ℃ in an incubator for 4 h. The cleared mash i.e., fermented suspension was further blended with filtered and pasteurized beetroot and carrot juices separately.

1.1.1

Blending of beetroot juice with fermented finger millet suspension

Juice was extracted from beetroot by centrifugal juice extractor and immediately pasteurized for 2-3 min at 90 ℃, consequently cooled in running tap water and filtered using a filter (100 µm) to separate clear juice from the pulp. Each filtered and pasteurized beetroot juice was blended to the fermented finger millet suspension at different ratios (v/v) with final volume being 150 ml and detailed steps are shown in plate 3.2. In order to set the control for finger millet based beverage, no beetroot juice was blended and all the samples were stored in refrigerator at 4±2 ℃ for 4 weeks.

Biochemical properties of developed beverages

Titratable acidity was calculated by titration against 0.1N sodium hydroxide (NaOH) to achieve pH 8.1 according to AOAC (1995) [5]. The pH was measured using pH meter by dipping the electrodes of pH meter into the sample. Total soluble solids (TSS) were determined by the procedure described by Ali *et al.* (2010) [1]. A digital refractometer was used to measure TSS. Total sugars were estimated by the modified method of Dubois *et al*. (1956) [11]. Calcium, iron and zinc content of finger millet fermented suspension, pasteurized beetroot juice and standardized beverages were determined by MP-AES diacid digestion method AOAC (2012b) [6]. Antioxidant activity was measured by DPPH free radical scavenging method as described by Chandra Shekhar and Anju (2014) [9]. True protein content was determined by Folin-Lowry method as described by Lowry's methods (Lowry *et al.*, 1951) [15]. Phenol content was estimated by the method suggested by Mahayothee *et al*. (2015) [16]. For betacyanin content, beetroot juice was diluted with McIlvaine buffer (pH 6.5) until a maximum absorption of 1.00±0.05 was reached (Herbach *et al.* 2007) [12] and for each sample, 0.1 ml sample was added with 3.9 ml McIlvaine buffer in a plastic cuvette prior spectrophotometric analysis and the readings were noted.

Microbial analysis

Total yeast and mould count (CFU/ml)

The total plate count in terms of log colony-forming unit per ml (CFU/ml) was calculated using the following formula (AOAC 2000b) [8]. The colonies of 10-3 and 10-7 were considered for calculations. The procedure was carried out in aseptic condition and with proper precaution.

$$\text{YMC}\ \frac{\text{CFU}}{(\text{ml})} = \frac{\log(\text{Mean Number of colony forming units})}{\text{Volume of samples} \times \text{Dilution factor}} \times 100$$

$$\text{TPC}\ \left(\frac{\text{CFU}}{\text{ml}}\right) = \log\left(\frac{\text{Number of colony forming units}}{\text{Volume of samples} \times \text{Dilution factor}}\right) \times 100$$

Sensory analysis

A panel of 15 semi trained panel lists of faculty members and post graduate students of College of Agricultural Engineering and Technology, Junagadh Agricultural University, Junagadh were asked to assess the samples and

mark them on a hedonic rating test (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely) in accordance with their opinion for colour, flavour, taste, and overall acceptability (Amerine *et al*. 1965) [3].

Storage analysis

In the last phase, storage stability was aimed to investigate the quality of developed beverages stored in glass and PET bottles. The samples were kept for 4 weeks storage at refrigerated condition at 4±2 ℃.

Cost analysis of developed beverages

For the economics of developed malted and fermented finger millet based beverage blended with beetroot juice, the cost analysis was carried out by calculating the cost of developing 1 bottle of 200 ml which included fixed and variable cost.

Statistical analysis

Statistical analysis was done to study the effect of two different factors like blending ratios (finger millet suspension: beetroot juice) and packaging materials (glass and PET) on dependent parameters, i.e., pH, TSS, titratable acidity, total sugar, total phenol content, true protein, calcium, iron, zinc, antioxidant activity, betacyanin, microbial and sensory analysis by Factorial Completely Randomized Design (FCRD by using Microsoft Office Excel 2013) (Pansee and Sukhatme, 1985) [17].

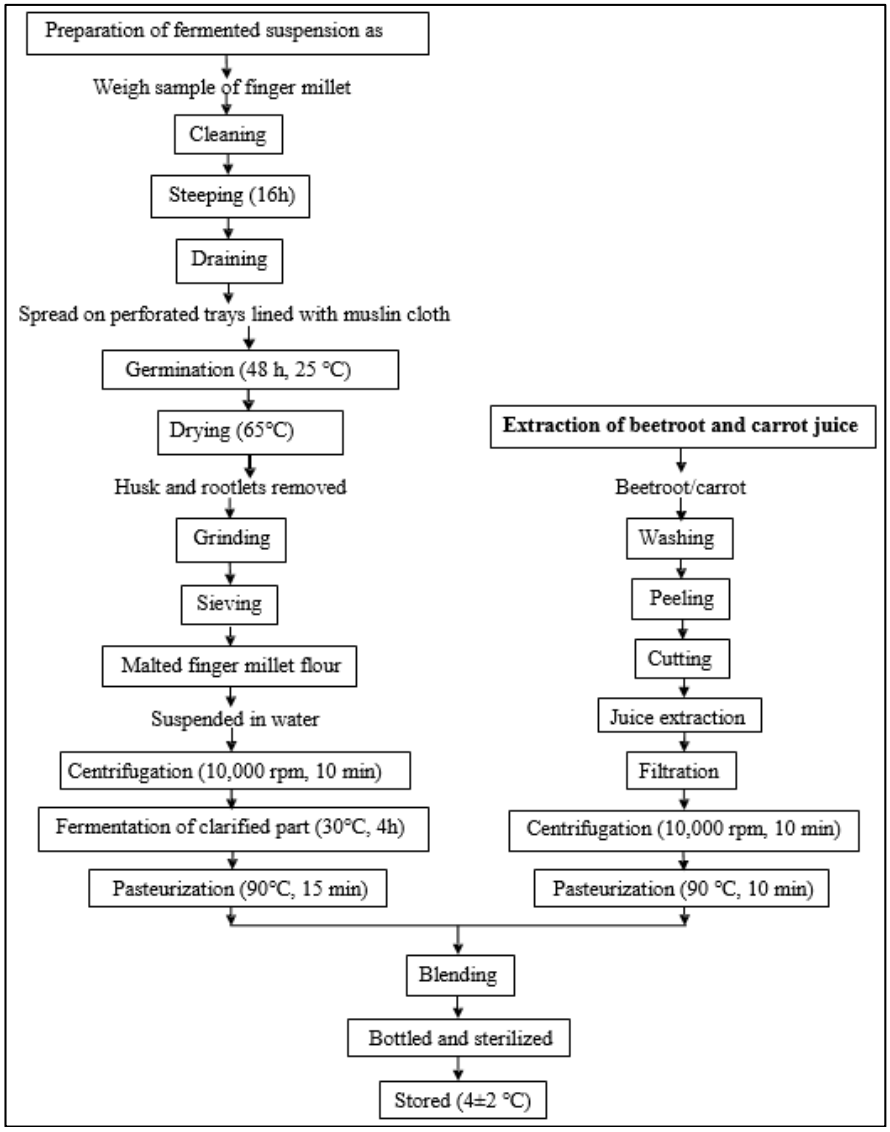

Fig 1: Process flow chart for the preparation of malted and fermented finger millet based beverage blended with beetroot juice

Results and discussion

Table 1: Average values of biochemical parameters of malted and fermented finger millet suspension and fresh and pasteurized beetroot juice

	pH	TSS, °Brix	Titratable acidity,% citric acid	Total sugar, g/100ml	Antioxi- dant activity,%	True protein, g/100ml	Total phenol, mg/100 ml	Betacya -nin, mg/l
Malted and fermented finger millet suspension	6.9	7.54	0.304	4.51	74.79	7.43	676.76	-
Fresh beetroot juice	7.8	8.85	0.059	6.34	64.93	0.947	949.56	774.13
Pasteurized beetroot juice	7.3	8.76	0.056	6.16	65.62	0.909	951.25	810.02

Effect of different blending ratio for beetroot juice and packaging materials on titratable acidity, total soluble solids (TSS), pH of developed beverages during storage period

Titratable acidity ranged from 0.41 to 0.89%. The maximum and minimum titratable acidity were observed for beverage having blending ratio 7:3 stored in PET bottle (FB3P1) and 3:7 stored in glass bottle (FB1P2) as 0.89 and 0.46% respectively by the end of 4th week of storage period. Results cleared that the individual and interaction effects were significant during entire storage period at 5% level of significance.

Total soluble solids (TSS) ranged from 2.93 to 8.13 ºBrix. The maximum and minimum TSS were observed for beverage having blending ratio 3:7 stored in glass bottle (FB1P2) and 7:3 stored in PET bottle (FB3P1) as 5.98 and 2.93 ºBrix respectively by the end of 4th week of storage period. Results cleared that the individual effect was significant except for 4th week. Also the interaction effect was found to be significant during entire storage period except for 1st and 4th week at 5% level of significance.

pH ranged from 5.06 to 7.13. The maximum and minimum pH were observed for beverage having blending ratio 7:3 stored in PET bottle (FB1P1) and 7:3 stored in PET bottle (FB3P1) as 5.55 and 5.06 respectively by the end of 4th week of storage period. Results cleared that the effect of different blending ratio (FB) was significant during entire storage period and the effect of different packaging material (P) was significant except for 3rd week. The interaction effect (FB×P) was found to be non significant during entire storage period except for 0th and 1st week at 5% level of significance.

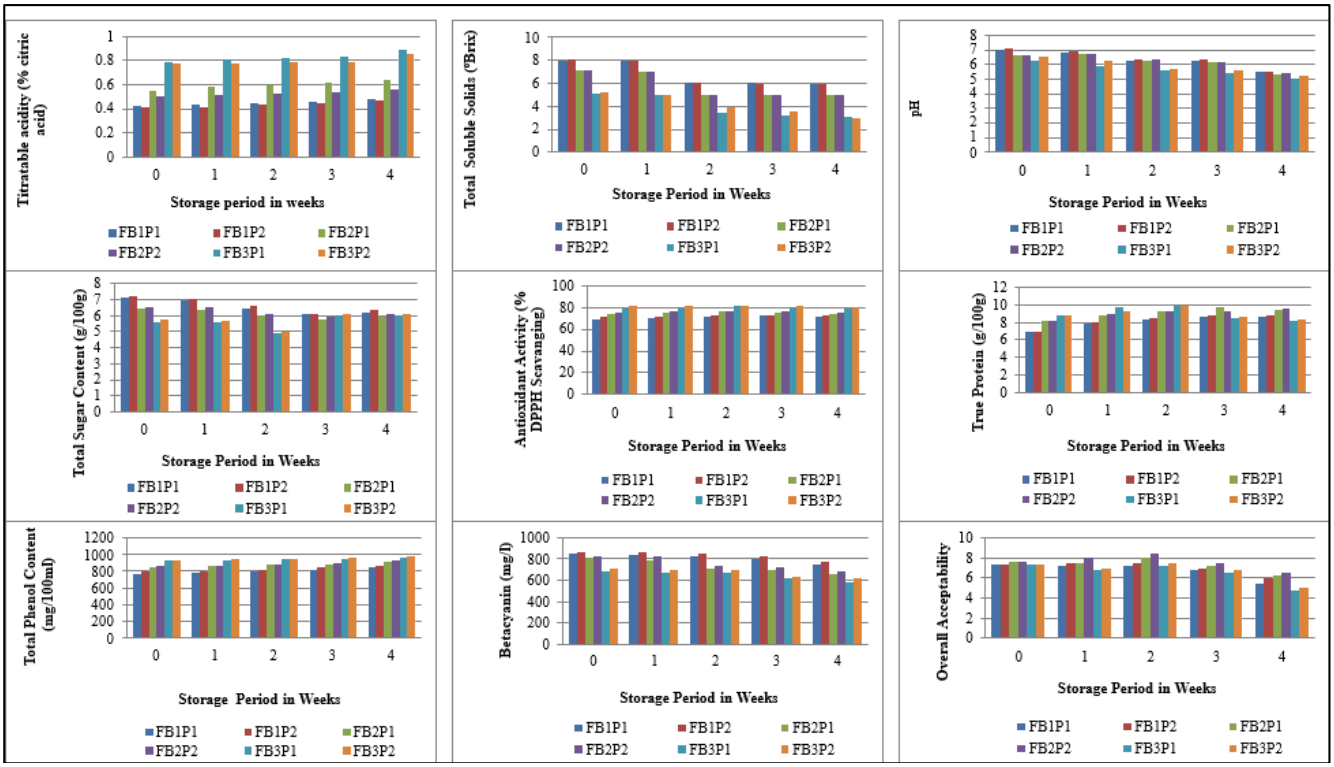

Fig 2: The figure presents the impact of different treatments on various quality parameters, such as titratable acidity, total soluble solids, pH, total sugar content, antioxidant activity, true protein content, total phenol content, betacyanin, and overall acceptability, during a 4-week storage period.

Effect of different blending ratio for beetroot juice and packaging materials on total sugar content, antioxidant activity (DPPH radical scavenging%), true protein of developed beverages during storage period

Total sugar content ranged from 4.93 to 7.23 g/100 ml during 4 weeks of storage period. The maximum and minimum total sugar content were observed for beverage having blending ratio 3:7 stored in glass bottle (FB1P2) and 7:3 stored in PET bottle (FB3P1) as 6.32 and 6.01 g/100 ml respectively by the end of 4th week of storage period. Results cleared that the individual effect was significant except for 3rd week. The interaction effect (FB×P) was found to be non significant during entire storage period at 5% level of significance.

Antioxidant activity ranged from 68.43 to 82.34% during 4 weeks of storage period. The maximum and minimum antioxidant activity were observed for beverage having blending ratio 7:3 stored in glass bottle (FB3P2) and 3:7 stored in PET bottle (FB1P1) as 80.42 and 71.78% respectively by the end of 4th week of storage period. Results cleared that the individual effect was significant whereas, the interaction effect was found to be non significant during entire storage period at 5% level of significance.

True protein ranged from 6.97 to 9.94 g/100 ml during 4 weeks of storage period. The maximum and minimum true protein were observed for beverage having blending ratio 1:1 stored in glass bottle (FB2P2) and 7:3 stored in PET bottle (FB3P1) as 9.58 and 8.16 g/100 ml respectively by the end of 4th week of storage period. Results cleared that the effect of different blending ratio (FB) was significant during entire storage period and the effect of different packaging material (P) was significant except for 0th and 1st week. Also the interaction effect (FB×P) was found to be non significant during entire storage period except for 2nd and 3rd week at 5% level of significance.

Effect of different blending ratio for beetroot juice and packaging materials on total phenol content, betacyanin of developed beverages during storage period

Total phenol content ranged from 773.12 to 975.10 mg/100 ml during 4 weeks of storage period. The maximum and minimum total phenol content were observed for beverage having blending ratio 7:3 stored in glass bottle (FB3P2) and 3:7 stored in PET bottle (FB1P1) as 975.10 and 845.08 mg/100 ml respectively by the end of 4th week of storage period. Results cleared that the individual effect was significant during entire storage period. The interaction effect was found to be non significant during entire storage period except for 0th and 3rd week at 5% level of significance.

Betacyanin ranged from 580.55 to 867.66 mg/l during 4 weeks of storage period. The maximum and minimum betacyanin were observed for beverage having blending ratio 3:7 stored in glass bottle (FB1P2) and 7:3 stored in PET bottle (FB3P1) as 772.44 and 580.55 mg/l respectively by the end of 4th week of storage period. Results cleared that the individual effect was significant during entire storage period. Also the interaction effect was found to be significant during entire storage period except for 0th and 2nd week at 5% level of significance.

Effect of microbiological parameters for stored beverages

The total plate count of developed beverages ranged from 0 CFU/ml to 737.9 CFU/ml, increasing with increase in storage period. The yeast and mould count of developed beverages ranged from 0 CFU/ml to 195.88 CFU/ml, increasing with increase in storage period. Alwis *et al.*, 2016 [2], concluded that the carrot-based synbiotic fermented beverage could be served as a ready to drink product for 6 weeks under refrigerated storage, meeting the standards (108 - 1010 CFU/ mL) of a functional drink.

Effect of different blending ratio for beetroot juice and packaging materials on overall acceptability of developed beverages during storage period

The overall acceptability score ranged from 5.00 to 8.50 during 4 weeks of storage period. The maximum and minimum overall acceptability were observed for beverage having blending ratio 1:1 stored in glass bottle (FB2P2) and 7:3 stored in PET bottle (FB3P1) as 6.5 and 4.75 respectively by the end of 4th week of storage period. Results cleared that the individual effect was significant during entire storage period. The interaction effect was found to be non significant during entire storage period at 5% level of significance.

Effect of blending ratio for beetroot juice and packaging materials on calcium, iron and zinc for 0th and 4th week of storage period

Based on sensory evaluation and microbial analysis of developed beverages, it was found that FB2P2 (1:1) gained higher scores in terms of overall acceptability and with lesser microbial infection during 4 weeks of storage period. Therefore, further analysis of this treatment for estimation of calcium, iron and zinc was carried out. It was found that at 0th week there were 37.07, 1.13 and 0.11 mg/100 ml of calcium, iron and zinc respectively and at the end of 4th week 48.46, 1.77 and 1.09 mg/100 ml of calcium, iron and zinc respectively. An increase was observed for calcium: 254 ppm in FPM1, 282 ppm in FPM2 and 156 ppm in the unfermented sample. Iron increased in FPM2 and FPM3 (approx. 100 ppm) with respect the unfermented sample (71 ppm) (Balli *et al.* 2023) [4].

Cost analysis for development and storage of beverages

Cost required for developing malted and fermented finger millet based beverage blended with beetroot juice includes fixed cost (depreciation cost of all the machines, interest on the machine cost, housing, insurance and other miscellaneous expenditure) and variable cost (repair and maintenance of machine, wages, rent of storage room, cost of raw materials, cost of energy consumed by machineries used). Therefore, cost required for developing malted and fermented finger millet based beverage blended with beetroot juice stored at 4±2°C was estimated to be 16 Rs per PET bottle and 21 Rs per glass bottle of 200 ml.

Conclusion

It is concluded to blend malted and fermented finger millet suspension with beetroot juice (FB2P2) in the ratio of 1:1 and store at 4±20C temperature in glass bottle for 4 weeks to maintain freshness and quality of it with 0.56% of titratable acidity, 5.00 ºBrix of TSS, 5.40, of pH, 6.09 g/100 ml of total sugar, 75.30% of antioxidant activity, 9.58 g/100 ml of true protein, 48.46 mg/100 ml of calcium, 1.77 mg/100 ml of iron, 1.09 mg/100 ml of zinc, 935.63 mg/100 ml of total phenol content and 682 mg/l of betacyanin with minimum level of microbial infection (satisfactory level) and good score of acceptability. The cost required for developing this beverage stored at 4±2°C was estimated to be 16 Rs per PET bottle and 21 Rs per glass bottle of 200 ml.

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