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Research Paper

Preparation of blended low alcoholic beverages from under-utilized millets with zero waste processing methods

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ABSTRACT

Low-alcoholic beverages, especially those produced from fruits, are increasing in demand and are being consumed in the form of table beverages. Present investigation features development of such new table beverages using the under-utilized regional millets- finger millet (ragi) and pearl millet (bajra). Alcoholic fruit beverages (fruits wines) are conventionally prepared from apples, peaches, oranges, blackberries, mangoes etc. In order to accommodate an ever-increasing demand, more wineries are starting to increase their versatility. Review of literature reveals that hardly any work that is documented on the ragi and millet based alcoholic beverages. Thus the present study was focused on utilization of underutilized millets and blending them with selected fruits to develop low alcoholic beverages with appreciable acceptability. Different blends of fruit juices (green grapes, black grapes and apple) with finger millet and pearl millet along with germinated wheat kernels as inducers were taken and fermentation was carried for a period of 8-10 days using brewer's yeast (*Saccharomyces cerevisiae*). Any undesirable microbial contamination was checked by adding potassium meta-bisulphite (KMS) @ 180 ppm. After completion, ethanol content in fermented product was analyzed and physico-chemical tests namely pH and % Titratable acidity (TA) were performed for all the samples. The microbial quality was also checked in all beverage samples. Such developed novel non alcoholic beverages were found to be acceptable and consumer friendly with appreciable shelf stability.

Keywords: Under-utilized millets, alcoholic beverages, SmF, *Saccharomyces cerevisiae*, Zero waste processing method

A large variety of fruits are grown in India, of which mango, banana, citrus, guava, grape, pineapple and apple are the major ones**.** In India, the major fruit growing states are Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, Uttar Pradesh and Gujarat **.** Grapes are non-climacteric fruit that grows on the perennial and deciduous woody vines of the genus *Vitis*, can be used for making jam, juice, jelly, vinegar ,wine and some kinds of confectionery**.** Ginger is the rhizome of the plant *Zingiber officinale*, consumed as a delicacy, medicine,

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or spice**.** India produces over 30% of the global share in the production of ginger, replacing China (~20.5%), followed by Indonesia (~12.7%), Nepal (~11.5%) and Nigeria (~10%). Apple is the pomaceous fruit of species *Malus domestica* in the rose family Rosaceae. Laboratory experiments say that apples possess phenolic compounds like quercetin, epicatechin, and procyanidin which are cancer-protective and posses antioxidant activity. Bajra (Pearl millet) (*Pennisetum glaucum*) is the most widely grown type of millet in India and is primarily consumed in the states of Karnataka, Haryana, Rajasthan, Gujarat and Madhya Pradesh. Pearl millet is good for bones and has higher energy content than other flours. It is rich in calcium and fats. In India, finger millet (*Eleusine coracana*) (locally called by various name including *ragi* and *nachani*) is mostly grown and consumed in Karnataka, Rajasthan, Andhra Pradesh, Tamil Nadu, Orissa, Maharashtra, Kumaon (Uttarakhand) and Goa. Finger millet is especially valuable as it contains the more content of methionine compared to common food grains such as cassava, plantain, polished rice, or maize meal. The grain is made into a fermented drink (or beer) in Nepal and in many parts of Africa. It is also used as a flavoured drink in festivals. It is rich in calcium and protein and also has good amount of iron and other minerals. It is low in fat content and most of which are unsaturated fats.

Alcoholic beverages (beers, wines, and spirits) are legally consumed in most countries, and over 100 countries have laws regulating their production, sale, and consumption**.** Beer is the world's most widely consumed and probably oldest of alcoholic beverages. Beer may be lager or ales, depending upon the type of fermentation; bottom fermentation leads to lager beers whereas top fermentation goes for ales. Wine is an alcoholic beverage, made of fermented grape juice. White wines are generally fermented at 10-18 ^{0}C for 7-14 days or more, Red wines are fermented for about 7days at 20-30 ^{0}C**.** Clarification and pretreatment of the juice, chemical composition of the fruits, temperature of the fermentation and influence of other microorganisms are the principle factors controls wine fermentation process. Killer strains of *Saccharomyces cerevisiae* and various species of *Candida*, *Pichia* and *Hansenula* have been isolated from the flora of wine fermentation. Fruits wines are prepared from fruits namely apples, peaches, oranges, bananas, blackberries, mangoes pumpkin etc. Use of under-utilized millets for making low (~ 4-9% ethanol, v/v) alcoholic beverages to increase versatility and add to novelty is not reported. The aspect of blending desired and nutritive fruit juices in such low alcoholic beverages could add more acceptability with nutrition. Market survey and published information reveal that the demand of such beverages is increasing significantly and there are green opportunities to develop such low alcoholic beverages, add to versatility and economy.

With these considerations, the present study was focused on utilization of underutilized millets namely finger millet (ragi) and pearl millet (bajra) along with selected and regionally available fruits to develop a new variety of blended low alcoholic beverages. The study suggests an alternative route of utilizing such under-utilized millets by blending the millet extracts with fruits for carrying out

fermentation.

Materials and methods

All the chemicals used in the study were of analytical grade.

Source of materials: Finger millet was obtained from fields in Tumkur district of Karnataka and pearl millet was procured from provisional stores in the months of February and March 2012. Fresh, fully ripened fruits (Green grapes, Black grapes and Apple) were procured from local market (K.R Market and Mysore Road) Bangalore during the same period. Lyophilized yeast *Saccharomyces cerevisiae* was also procured from the market of Bangalore.

Storage of fruits: Grapes (both green and black) were washed under running tap water and soaked overnight in salt water to remove pesticides residues. Apple was washed under running tap water. All the fruits were then refrigerated at temperature ~ 4 ^{o}C.

Optimization of finger millet and pearl millet: 50g each of finger millet and pearl millet were taken in different containers and soaked in water for 0, 24, 36, 48, 60, 72 and 84 hours. Soaked grains were then set for germination for 24 hours. Acceptability checks were performed qualitatively for odor and α-amylase enzyme concentration for each combination. Soaking for 24, 48, 72 hours along with germination for 24 hours was found to be favorable for fermentation. Then germinated finger millet and pearl millet were ground in fine powder with the help of mixer. Powder was sieved (100 µm) to separate out the fine powder with the bran (this bran that was used as natural fiber source after its size-reduction). The obtained finger millet and pearl millet fine powder was separately suspended with water and centrifuged at 10000 rpm for 10 mins. Supernatant was extracted as enzyme source and α-amylases enzyme activity was measured quantitatively using well established Lowry's method. Enzyme activity was calculated and tabulated.

Blending of germinated finger millet and pearl millet powders with different fruit juices: Juice was extracted from all the three fruits namely: green grapes, black grapes and apple by electrical juicer. All juices were immediately pasteurized (via in-bottle technique) for 2-3mins at 90^{0}C, consequently cooled in running tap water. The pasteurized juice was filtered using a filter (100 µm) to separate clear juice from the pulp. Each filtered and pasteurized fruit juices were blended to germinated finger millet and pearl millet suspension (each of 20% strength) at different ratios (v/v) with final volume being 100 ml.

In order to set the control for finger millet and pearl millet based alcoholic beverages, no fruit juice was blended. Thus, only respective millet suspension (20%) was maintained as control. Other combinations were worked out as follows:

- 70:30, 50:50 ratios for finger millet: green grape juice
- 70:30, 50:50 for finger millet: black grape juice.

- 70:30, 50:50 for finger millet: apple juice, and
- 0:100 as control, (with all three fruit juice in equal proportions)

On similar lines, combinations for pearl millet based beverages were worked out.

Submerged state fermentation (SmF): To each of sterilized flask with designated fruit juice and/or millet suspension combination, 5% inoculum of actively growing *Saccharomyces cerevisiae* was added. It was followed by addition of sucrose @ 11% and potassium meta-bisulphite (KMS) @ 350ppm. Few soaked wheat kernels were suspended as inducer and the flasks were made air tight. Fermentation was carried out at 30^0 C in an incubator. After the completion of fermentation, the fermented mash was stored at 4^0C in order to settle down the unfermented biomass, dead yeast cells and the suspended particles, and then beverage was decanted in sterile glass bottles. These cleared mash i.e. the beverage samples of the two millets were pasteurized in bottle and stored for product analysis and aging at low temperature (6-8^0 C).

Product analysis: The prepared green beverage samples were analyzed for pH and % of TA. Microbial analyses were performed for safety analysis and sensory attributes were calculated through un-trained and semi-trained panel members from the department.

pH of the beverage samples was measured directly by electronic/digital pH meter. Titratable acidity (% TA as tartaric acid) of beverage was estimated by Association of Official Analytical Chemists (AOAC) method. Ethanol content of wine was estimated using and specific gravity method. Sensory evaluation of the prepared wine samples was done on the 9 point Hedonic scale**.** The evaluation was done according to following characters i.e aroma, taste, appearance/color, overall acceptability and results were recorded in sensory sheets.

Microbial activity of beverage product: Microbial activity was done to enumerate the number of microorganism in the wine sample by using pour plate method.

Production of peptone and dietary fibers: In order to harness all valuable and discard no waste, the left over and separated biomass consisting of dead yeast and unfermented matter from clarified beverage were autolysed as per the standard procedures at temperatures 60-70^0C for 18 hours continuously until it dried. Extracted product (peptone) was thus collected and stored for its utilization in growth medium for microbial assay and allied work. Bran collected during millet processing were used as dietary fibers.

Results and discussion

Optimization of finger millet and pearl millet: Soaking for 24, 48, 72 hours and germination for 24 hours was found to be favorable for fermentation. The germinated finger millet and pearl millet were ground in fine powder and analyzed for enzyme (α-amylases) activity by using Lowry's method. Enzyme activity was calculated

and discussed in Table 1.

Table 1: α-Amylases enzyme activity of germinated finger millet and pearl millet powder

Sl. no	Test tubes	O.D. at 540 nm	α-Amylases Enzyme Activity (μM/min)	O.D. at 660 nm	Specific Activity (μM/mg min)
1.	Finger millet –0hrs	0.22	0.815	0.74	1.101
2.	Pearl millet -0hrs	0.19	0.703	0.35	2.008
3.	Finger millet-24hrs	0.16	0.593	1.78	0.333
4.	Pearl millet -24hrs	0.11	0.407	1.14	0.357
5.	Finger millet-48hrs	0.22	0.815	1.68	0.485
6.	Pearl millet -48hrs	0.20	0.740	1.35	0.548
7.	Finger millet 72hrs	0.68	2.518	1.56	1.614
8.	Pearl millet -72hrs	0.44	1.629	1.05	1.545

Product analysis: The Physico-chemical characteristics (such as pH and % of TA) of blended beverage produced from finger millet-grape juice (100:0, 70:30, 50:50); finger millet -apple (100:0, 70:30, 50:50); pearl millet-grapes (100:0, 70:30, 50:50); pearl millet -apple (100:0, 70:30, 50:50) were found to be different.

pH: Variations in pH of blended beverage samples were noted in time interval of 15 days. pH of the both blended beverage samples were found to be nearly stable throughout the period of investigation,stability was maintained in all the time intervals depicting the acidity of acids present in the sample (Figure 1).

%Titratable acidity (% TA): No regular trends were found in the variation of %TA during the fermentation periods of various blended beverage samples. It was observed that the %TA was slightly higher after few days of fermentation. The %TA of all sets of blended beverages was shown in (Figure 2).

The pH and % of TA of the finger millet and pearl millet based beverages depending on their soaking time was found to be as shown in the Table 2.

Ethanol content: The percent ethanol production in all the beverage samples was estimated by specific gravity method. The ethanol content production was more in black grapes juice blended product both finger millet and pearl millet induced beverages compared with that blended with green grape juice and apple juice; Finger millet control and pearl millet control % ethanol content in all samples were tabulated in Figure 3**.**

Time of fermentation: A time period of 15 days for fermentation of all the blended samples was found to be optimal for developing the desired attributes.

Sensory evaluation of wines: Keeping in view the quality of the beverage produced, Sensory test was conducted to rate the quality of product as per 9 point-Hedonic scale viz. taste, aroma, texture, colour, mouth feel etc. perspective. Analysis was carried out for all the samples to ensure the public acceptance of the product. The rating out of 9 for each blends of green grapes, black grapes and apple juices blended

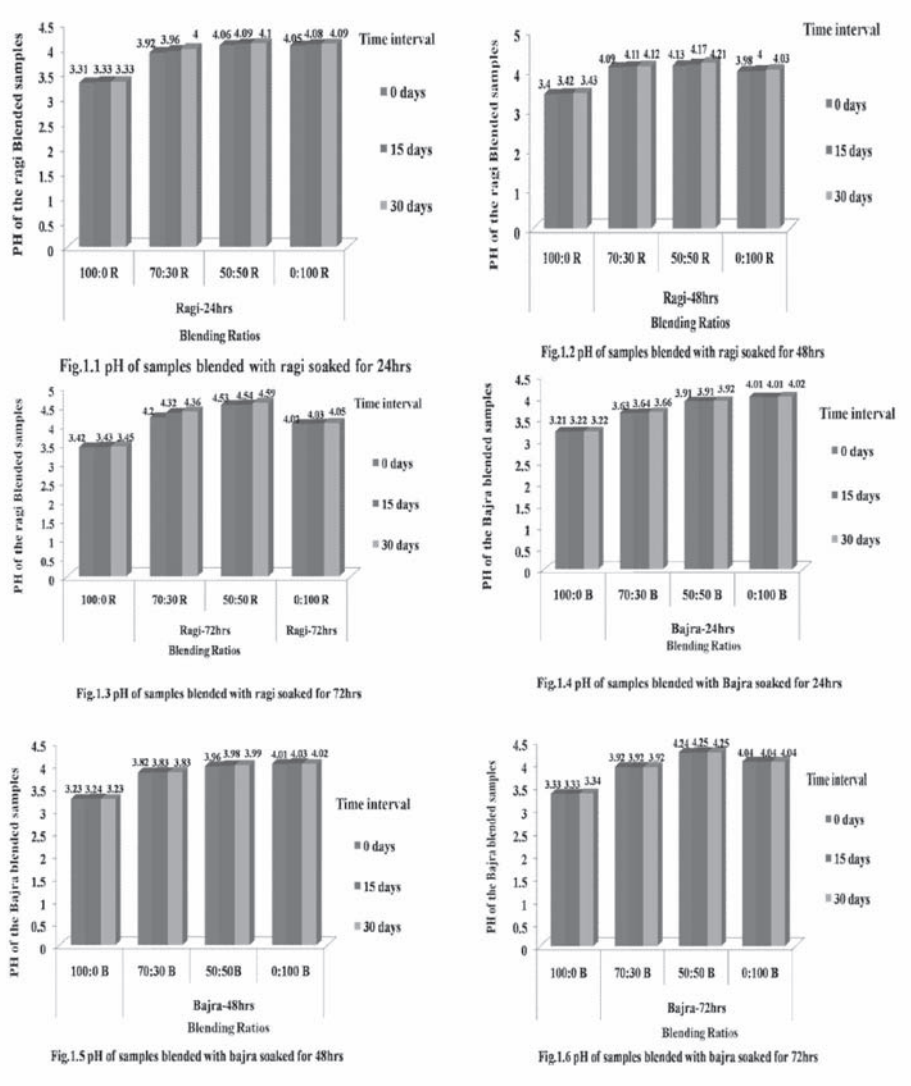

Figure 1: pH of finger millet (ragi) and pearl millet (bajra) based beverage samples

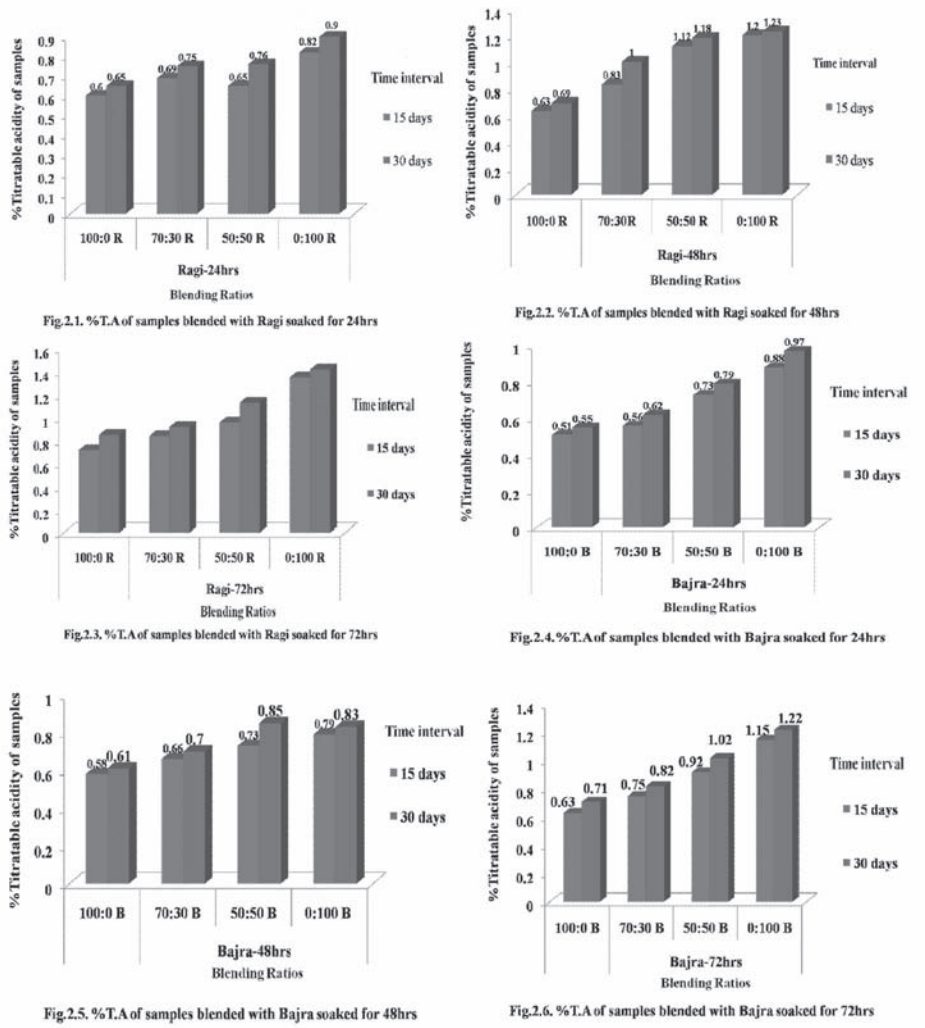

Fig.2.1. %T.A of samples blended with Ragi soaked for 24hrs

Fig.2.2. %T.A of samples blended with Ragi soaked for 48hrs

Fig.2.3. %T.A of samples blended with Ragi soaked for 72hrs

Fig.2.4. %T.A of samples blended with Bajra soaked for 24hrs

Fig.2.5. %T.A of samples blended with Bajra soaked for 48hrs

Fig.2.6. %T.A of samples blended with Bajra soaked for 72hrs

Figure 2: % TA of finger millet (ragi) and pearl millet (bajra) based beverage samples

Table 2: Physico-chemical parameters (pH and % of TA) of finger millet and pearl millet based blended beverage samples

Sl No	Type of beverage	pH	%TA
1.	Finger millet -24hrs	3.6-4.1	0.6-0.8
2.	Pearl millet -24hrs	3.4-4.2	0.5-1.0
3.	Finger millet -48hrs	3.8-4.4	0.6-1.2
4.	Pearl millet -48hrs	3.5-4.2	0.5-0.8
5.	Finger millet -72hrs	3.8-4.6	0.6-1.4
6.	Pearl millet -72hrs	3.6-4.5	0.6-1.2

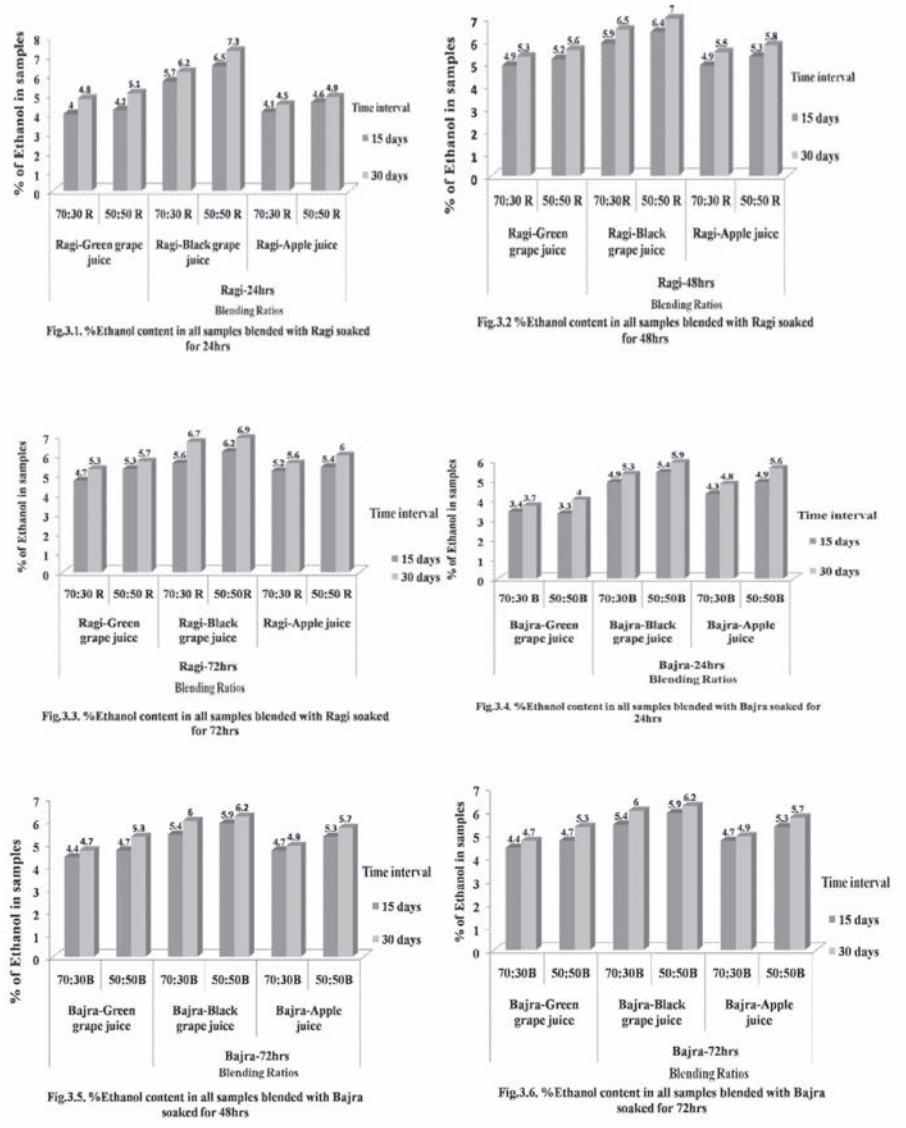

Fig.3.1. %Ethanol content in all samples blended with Ragi soaked for 24hrs

Fig.3.2 %Ethanol content in all samples blended with Ragi soaked for 48hrs

Fig.3.3. %Ethanol content in all samples blended with Ragi soaked for 72hrs

Fig.3.4. %Ethanol content in all samples blended with Bajra soaked for 24hrs

Fig.3.5. %Ethanol content in all samples blended with Bajra soaked for 48hrs

Fig.3.6. %Ethanol content in all samples blended with Bajra soaked for 72hrs

Figure 3: Ethanol content of finger millet (ragi) and pearl millet (bajra) based beverages

with finger millet and pearl millet were shown in the Table 3.

Table 3: Results pertained out of sensory analysis of all the beverage samples blended with finger millet and pearl millet

Sample code	Aroma	Colour	Taste	Mouth feel	Overall acceptability
Finger millet :G.G	8	6	8	7	8
Finger millet :B.G	8	7	8	8	8
Finger millet :Apple	8	7	7	7	7
Pearl millet :G.G	4	5	6	5	5
Pearl millet :B.G	5	6	6	6	6
Pearl millet :Apple	4	5	5	5	5

Microbial activity of Beverage: Microbial activity in all samples of both finger millet and pearl millet based blends before and after pasteurization were conducted. It was found that the microbial colonies seen on the Nutrient agar media, Potato dextrose agar media (PDA) and Eosin methylene blue (EMB) agar media before pasteurization at the end of 24-36 hours of incubation, were nearly zero when the beverage samples were analysed after the pasteurization.

Peptone and dietary fibers as by-products: Production of peptone using the biomass of fermented product was the novelty applied to the present research work. Solid biomass was the remains of fermentation which contained yeast live and dead cell, pulp of fruit juices was added. The resulted product after autolysis of the biomass for 18 hours continuously on application of temperatures of 60-70^{0}C was used in growth of microbes by adding small quantities of product in nutrient agar media. Excellent growth of colonies was reported when the dehydrated commercial peptone was substituted with the peptone so generated as by-product.

Conclusion

The present investigation deals with the preparation of blended millets based (finer and pearl) beverages as an alternative means of utilizing the high productivity of these millets especially in this part of the country. Nutritive value was increased after germination and blending the millet extract with fruits of choice like grapes and apple by application of fermentation technique. The entire process was developed in line of zero waste generation as dietary fibers and peptone were harnessed as useful by-products. It was concluded that an acceptable quality low alcoholic beverage could be prepared using under-utilized millets- finger millets (ragi) and pearl millets (bajra) with blending it with fruits of choice. The process is being scaled up to open up entrepreneurship opportunities through the Incubation Centre of the college (NHCE, Bangalore).

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