

Original Research Article

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Amla as a Potential Substrate for Production of Probiotic Drink

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ABSTRACT

This study intended to examine the growth and stability of *Saccharomyces boulardii* and *Lactobacillus acidophilus* in amla drink with the aim of formulation of probiotic drink and to analyze the nutritive value, sensory qualities and shelf life of the product. *S. boulardii* CNCM 1-745 and *L. acidophilus* MCC10307 were capable of growing in 15 per cent amla drink at natural pH, 30°C with 22°Brix and 5 per cent honey. Optimum fermentation time at which the highest population was observed (8-10 log CFU/ml), for *S. boulardii* CNCM 1-745 was 35 hr while *L. acidophilus* MCC10307, it was 64 hr. The sensory scores for fermented drinks were significantly ($P \leq 0.05$) lesser than the unfermented drink. In order to enhance the sensory qualities by reducing fermentation time while maintaining the highest population, initial pH and total soluble solids were adjusted and *L. acidophilus* (nu-trish LA-5) was used to replace *L. acidophilus* MCC 10307. With these modifications, amla drink with 3 hr of fermentation provided better sensory qualities with required cell population and 97-108 µg/ml of ascorbic acid and 937.5 µg/ml of antioxidant potential. With the consideration to both population and sensory qualities, amla drink with *S. boulardii* CNCM 1-745 could be stored at refrigerated conditions (4°C) for 30 days while *L. acidophilus* (nu-trish LA-5) could be stored for 12 days.

Keywords

Amla, *Saccharomyces boulardii*, *Lactobacillus acidophilus*, Probiotication

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Introduction

Food with required population of beneficial microorganisms in human health is known as probiotic food which is gaining a growing public awareness as a functional food recently. The term probiotic is defined as live microorganisms which can accord several health benefits beyond inherent general nutrition when access into the body in adequate amount (Anon, 2001). According to findings, to be a probiotic product, it should contain a minimum number of probiotic

population ranged from 10^5 to 10^6 CFU/ml or gram of the product (Vasudha and Mishra, 2013). Many authors suggested that ingestion of 10^7 - 10^{10} viable cells in a day could sufficiently promote the human health such as supplementation of food with probiotic is able to enhance several indices of acquired and natural immunity, probiotic treatment is an effective dietary means of averting or limiting diarrhoea and other stomach problems in human. Probiotics also can fight against pathogens like *Helicobacter pylori*, associated with the development of gastric cancer, peptic ulcers and chronic gastritis. Probiotics could

protect against allergies, it can increase natural killer cell activity and probiotic consumption may also have potential effects on delayed tumour development (Matsuzaki and Chin, 2000; Oxman *et al.*, 2001; Reid and Bruce, 2001).

Traditionally, probiotics have been added to yoghurt and other fermented dairy products since milk is the ideal source for probiotics growth. However, in recent years, a growing demand for non-dairy probiotic products comes due to several drawbacks related to probiotic foods based on dairy including lactose intolerance, high cholesterol content, vegetarianism, milk allergy and other factors (Priya and Khatkar, 2012). These facts have led to development of non-dairy probiotic products from various food matrices and also fruit based source would be a better option due to highly nutritive value of fruit itself.

Amla fruit is widely used as medicine as alone or in combination with other plants to treat common cold and fever, as a diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, anti-pyretic, hair tonic; to prevent ulcer and dyspepsia (Priya and Khatkar, 2012). Modern scientists have studied amla's unique nutrient blend proved to have positive influences on everything from metabolic syndrome and blood flow to anti-aging processes. Pharmacological research reports on amla reveal its analgesic, anti-tussive, anti-atherogenic, adaptogenic; cardio, gastro, nephron, neuroprotective and anticancer properties (Sharma *et al.*, 2004; Nosal *et al.*, 2003; Santoshkumar *et al.*, 2013). Amla is also reported to possess chemopreventive, radio, and immunomodulatory, free radical scavenging, antioxidant, anti-inflammatory, anti-mutagenic activities (Baliga *et al.*, 2013). These properties are efficacious in the prevention and treatment of various diseases like cancer, atherosclerosis, diabetes, peptic ulcer, anaemia, liver and heart diseases and

various other disorders (Muruganandam *et al.*, 2002).

Amla is highly nutritious and is one of the richest sources of vitamin-C (600 mg/100g) and minerals (50 mg/ 100 g). pH of fruit is normally 3.0 to 4.0 (Zhang *et al.*, 2003; Gopalan *et al.*, 2010). It contains several phytochemical constituents like tannins, alkaloids and phenols (Srivasuki, 2012). Amla fruit, owing to its high acidity and astringent taste, are not palatable and hence not suitable for direct consumption. It is consumed mainly in the processed form. Amla extracts are used in various food and beverage preparations such as nutritional bars, jams, yoghurts and dietary supplements.

The potential for amla extract as a food ingredient is increasing substantially, owing to the growing global nutraceuticals and functional food market. Increase in the demand of health drinks has given road for the development of various value-added beverages (Jain and Khurdiya, 2004). Hence, present study was carried to examine the growth of *Saccharomyces boulardii* and *Lactobacillus acidophilus* in amla drink with the aim of formulation of probiotic amla drink.

Materials and Methods

Procurement of samples

Commercially available fresh amla, sugar, honey and other ingredients for fruit drink were purchased from local market of Bangalore city, India. *Saccharomyces boulardii* CNCM 1-745 and *Lactobacillus acidophilus* MCC 10307 were purchased from local medical shop while *Lactobacillus acidophilus* (nu-trish LA-5) was from M/s CHR HANSEN Denmark, procured from CHR HANSEN India Pvt. Ltd. Mumbai were used for the fermentation.

Formulation of amla drink

Amla fruit was steam blanched and seeds were separated. It was blended and juice was obtained. Different concentrations (10%, 20%, 30% and 40%) of amla drink were prepared by mixing different proportions of the extracted juice and water. Honey (5% w/v) was added to each drink with a pinch of salt. Total soluble solids of each drink was adjusted to 22°Brix (Amaley *et al.*, 2016) by adding sugar and yield was about 611 ml.

Acceptability of amla drink

Acceptability of formulated amla drinks was studied by evaluating their sensory characteristics. Sensory qualities namely appearance, sweetness, sourness, consistency, colour, flavour, aroma and overall quality were tested using semi-trained panel (N=22) and the panelists were asked to record their observations based on a 9-point hedonic scale (Tuorila *et al.*, 2008). Out of four concentration variations, the most accepted variation was selected for carrying out the further fermentation process.

Preparation of substrate media

Formulated amla drink was used as a substrate media for fermentation drink at natural pH, 22°Brix with 5 per cent honey supplementation.

Preparation of inoculum

Lactobacillus acidophilus MCC 10307 was stored by sub-culturing every two weeks in de Man, Rogosa and Sharpe (MRS) agar. *Saccharomyces boulardii* CNCM 1-745 and *Lactobacillus acidophilus* (nu-trish LA-5) were stored in sachets and kept at 4°C in the refrigerator. About 25 ml of pure amla juice was measured and 75 ml of sterilized water was added into a sterilized conical flask and

mixed well. Five grams of D- glucose (5%) and honey (5%) were added to flask. It was pasteurized at 62°C for 30 min. Cultures were propagated in concentrated drink in aseptic condition. Prepared inoculums were kept at 30°C in the incubator for 24-36 hours.

Fermentation

Experiment was carried out as batch fermentation (in flasks). Pasteurized amla drink was inoculated with a 10% (v/v) of *Saccharomyces boulardii* CNCM 1-745 and *Lactobacillus acidophilus* MCC 10307 inoculums separately and incubated at 30°C for 72 hr for fermentation. Samples were collected at different time intervals for the analysis.

Effect of fermentation on microbial population

A change in population of organisms in amla drink during fermentation was measured through plate count technique (Dimitroski *et al.*, 2015) and microbial population was recorded in log CFU/ml. Fermentation time which showed maximum population was considered as the optimum fermentation time in further processes. Change in TSS and pH during fermentation was determined in order to study metabolic activity of organisms in the drink (Priyanka, 2014).

Effect of fermentation on sensory qualities

Effect of fermentation on sensory qualities of amla drink was studied by evaluating sensory characteristics of fermented drinks (Tuorila *et al.*, 2008).

Nutrition analysis of probiotic amla drink

Ascorbic acid content of unfermented and fermented amla drinks was analysed using spectrophotometer (Sadasivam and

Manickam, 1997). Ferric reducing antioxidant potential (FRAP) of unfermented and fermented amla drinks was measured using spectrophotometer according to the method proposed by Benzie and Strain (1999) and results were reported as μg of ascorbic acid equivalents (AAE) per ml.

Storage study of formulated probiotic fruit drinks

Shelf life of formulated probiotic amla drinks was studied by evaluating sensory characteristics and survivability of population in the drinks during 30 days of storage period.

Statistical analysis

Statistical tools such as mean, standard deviation, cubic curve fit and ANOVA were used in the analysis and interpretation of experimental data. The results of effect of fermentation on microbial population were fitted to cubic curve since it was better fit for experimental data (Pereira *et al.*, 2011). One way ANOVA was used to test the significance of experimental data from sensory evaluation (Dandin, 2010).

Results and Discussion

Acceptability studies of amla drink

The scores pertaining to sensory qualities of concentration variations of amla drink are presented in Table 1. Amla drink with 15 per cent concentration was recorded significant ($P \leq 0.05$) highest overall acceptability score of 8.0 compared to other three variations with highest sensory scores for all the parameters (ranged between 7.9-8.3 out of 9) except for colour. Therefore, among four variations, 15 per cent amla drink was selected for further fermentation process. A similar findings were reported by Priya and Khatkar (2012) who studied sensory acceptability of laboratory

prepared amla juice using 9-point hedonic scale and reported amla was awarded 6-7 score for all most all studied parameters except sourness and bitterness (sensory score 1-3) with overall acceptability of 6.8. Similarly, amla and kinnow mandarin juice was blended in proportions *viz.*, 0:100, 10:90, 20:80, 30:70, 40:60 and 50:50 to prepare ready-to-serve (RTS) beverages having 10 and 12 per cent TSS and 0.3 per cent acidity. The treatment involving blending of amla juice with kinnow mandarin juice in 40:60 proportion was found most suitable since it had maximum sensory qualities in terms of highest overall acceptance score (6.74/9.00) with bright yellow colour, pleasant aroma and taste (Jain and Meena, 2013).

Figure 1 and 2 depict the influence of fermentation time on population of *Saccharomyces boulardii* CNCM 1-745 and *Lactobacillus acidophilus* MCC10307 in amla drink respectively. Increment in viable cell of organisms during fermentation process indicates capability of growth and survival of organisms in the drink. Result shows that both the organisms were capable of growing in amla drink in natural pH level, at 30°C and 22°Brix conditions with 5 per cent honey supplementation. The experimental data was statistically analyzed by fitting to the cubic model since cubic model was better fit for the data. F-test was used as significance criteria for the fitted model.

Fitted cubic model for *Saccharomyces boulardii* CNCM 1-745 is presented in Figure 1 which was built using Equations 1.

The model was statistically significant at 5 per cent level since the calculated F value (97.45) was higher than the table F value. Good coefficients of determination was also obtained ($R^2 = 0.987$) which indicates the 98.7 per cent of the variation is explained by the model for time intervals.

$$\text{Population (log CFU/ml)} = 5.868 + 0.258 t - 0.005t^2 + 2.693E-5t^3 \quad (1)$$

Where t is fermentation time (h)

Cubic curve showed (Figure 1) a fast growth of *Saccharomyces boulardii* CNCM 1-745 up to 35 hr of fermentation and the population increased from 5.85 to 10.03 log CFU/ml. At 35 hr, it showed the peak level of population and after that gradual decreased was observed. It may be due to either depletion of one or more essential nutrients or the accumulation of toxic by-products of growth (Duygu, 2004).

Even though *Saccharomyces boulardii* CNCM 1-745 could be able to maintain more than 9.0 log CFU/ml population upto 64 hr of fermentation time, 35 hr was the ideal fermentation time for amla drink with highest computed population 10.03 log CFU/ml. Because extended fermentation time could affect to the sensory qualities and commercial value. Chiang *et al.*, (2006) and Ganzle *et al.*, (2007) also observed similar behaviour of different microorganisms in different fermentation media.

Cubic fit for *Lactobacillus acidophilus* MCC10307 was built using Equations 2 (Figure 2). The model was statistically significant at 5 per cent level since the calculated F value (74.5) was higher than the table F value. Good coefficients of determination was also obtained ($R^2=0.982$) which indicates the 98.2 per cent of the variation is explained by the model for time intervals.

$$\text{Population (log CFU/ml)} = 6.340 + 0.048t + 4.072 E-4t^2 - 8.582E-6t^3 \quad (2)$$

Where t is fermentation time (h)

Cubic curve showed a fast growth of *Lactobacillus acidophilus* MCC10307 upto 64

hr of fermentation and the population increased upto 8.83 log CFU/ml. At 64 hr of fermentation, it showed the peak growth and after that population showed a decreasing trend. Therefore, 64 hr was the ideal fermentation time for amla drink with highest computed population.

Effect of fermentation time on total soluble solids (TSS)

Change in TSS of amla drink due to fermentation is depicted in Figure 3. The initial TSS of amla drink at 22°Brix was decreased upto 19.4 and 19.1°Brix by *Saccharomyces boulardii* CNCM 1-745 and *Lactobacillus acidophilus* MCC10307 respectively within 72 hr fermentation. This may be due to utilization of sugar by the microorganisms.

The changes in TSS in the developed fruit drinks proved the metabolic activity of organisms during fermentation. Initially TSS in the fruit drinks was adjusted to 22°Brix in order to supply the optimum growth condition for microorganisms. These results are supported by the findings of Amaley *et al.*, (2016) who reported the optimum TSS level for microorganism's is 20-22°Brix. Total soluble solids of a solution indicate sugar, mostly sucrose content in the solution. Decreased sugar content may be a reason for reduction in cell growth after a certain time of fermentation. According to Charalampopoulos *et al.*, (2002), all microbes attained maximum cell populations when there is sufficient availability of total fermentable sugars in the fermentation media. Substrate deficiency in sugars contributed to growth limitation.

Effect of fermentation time on pH

pH plays a very important role in enhanced growth of the cells. Decrease in pH will affect the cell contents adversely.

Table.1 Sensory scores for concentration variations of amla drink

Treatments	Appearance	Sweetness	Sourness	Consistency	Colour	Flavour	Aroma	Overall quality
10%	7.2 ^a	7.0 ^a	6.6 ^a	7.3	7.5	6.5	7.8	6.9 ^a
12%	8.0 ^b	7.3 ^a	7.0 ^a	7.9	7.8	7.5	8.0	7.3 ^b
15%	8.3 ^c	8.1 ^b	7.9 ^b	8.1	8.2	8.2	8.0	8.0 ^c
20%	7.7 ^a	6.8 ^a	6.5 ^a	7.4	8.4	6.5	7.7	6.8 ^a
Mean	7.8	7.3	7.0	7.7	8.0	7.1	7.9	7.3
F value	*	*	*	NS	NS	NS	NS	*
SEm±	0.083	0.079	0.08	0.08	0.06	0.75	0.06	0.07
CD	0.081	0.077	0.078	-	-	-	-	0.068

Figures with the same superscript in a column indicates no significant difference at $P \leq 0.05$

*Significant at 5% level

NS-Non-significant

Table.2 Sensory scores for fermented amla drink

Treatments	Appearance	Sweetness	Sourness	Consistency	Colour	Flavour	Aroma	Overall quality
Before fermentation	8.3	8.1 ^a	7.9 ^a	8.1	8.2	8.2 ^a	8.0 ^a	8.1 ^a
<i>L. acidophilus</i>	8.2	4.7 ^b	5.5 ^b	7.8	7.8	5.2 ^b	3.8 ^b	5.7 ^b
<i>S. boulardii</i>	7.8	5.0 ^b	4.7 ^b	8.2	8.0	5.0 ^b	4.5 ^b	4.8 ^b
Mean	8.1	5.9	6.0	8.2	8.0	6.0	5.4	6.2
F value	NS	*	*	NS	NS	NS	*	*
SEm±	0.41	1.09	0.96	0.16	0.11	1.03	1.29	0.98
CD	-	0.71	0.63	-	-	0.67	0.84	0.64

Figures with the same superscript in a column indicates no significant difference at $P \leq 0.05$

*Significant at 5% level

NS-Non-significant

Table.3 Ascorbic acid and antioxidant potential of probiotic amla drink

Treatments	Ascorbic acid (µg/ml)	Antioxidant potential (µg/ml)
Before fermentation	96	938
<i>L. acidophilus</i>	109	938
<i>S. boulardii</i>	98	938
Mean	101	938
F value	NS	NS
SEm±	0.09	0.11

NS-Non-significant 5% level

Table.4 Effect of storage time on population in probiotic amla drink

Storage period (days) at 5 ± 1°C	<i>S. boulardii</i> Population (CFU/ml)	<i>L. acidophilus</i> Population (CFU/ml)
Initial	1.4×10 ⁹	2.6×10 ¹⁰
6	2.9×10 ⁹	6.9×10 ¹⁰
12	6.7×10 ⁹	8.2×10 ⁹
18	3.3×10 ⁹	5.5×10 ⁵
24	5.4×10 ⁹	**
30	2.3×10 ⁹	**
Mean	3.7×10 ⁹	5.6×10 ¹⁰
F value	NS	NS
SEm±	8.7×10 ⁹	1.2×10 ¹⁰

**Due to loss of cell viability, discontinued the experiment
NS-Non significant at 5% level

Fig.1 Effect of fermentation time on population of *Saccharomyces boulardii* CNCM 1-745 in amla drink

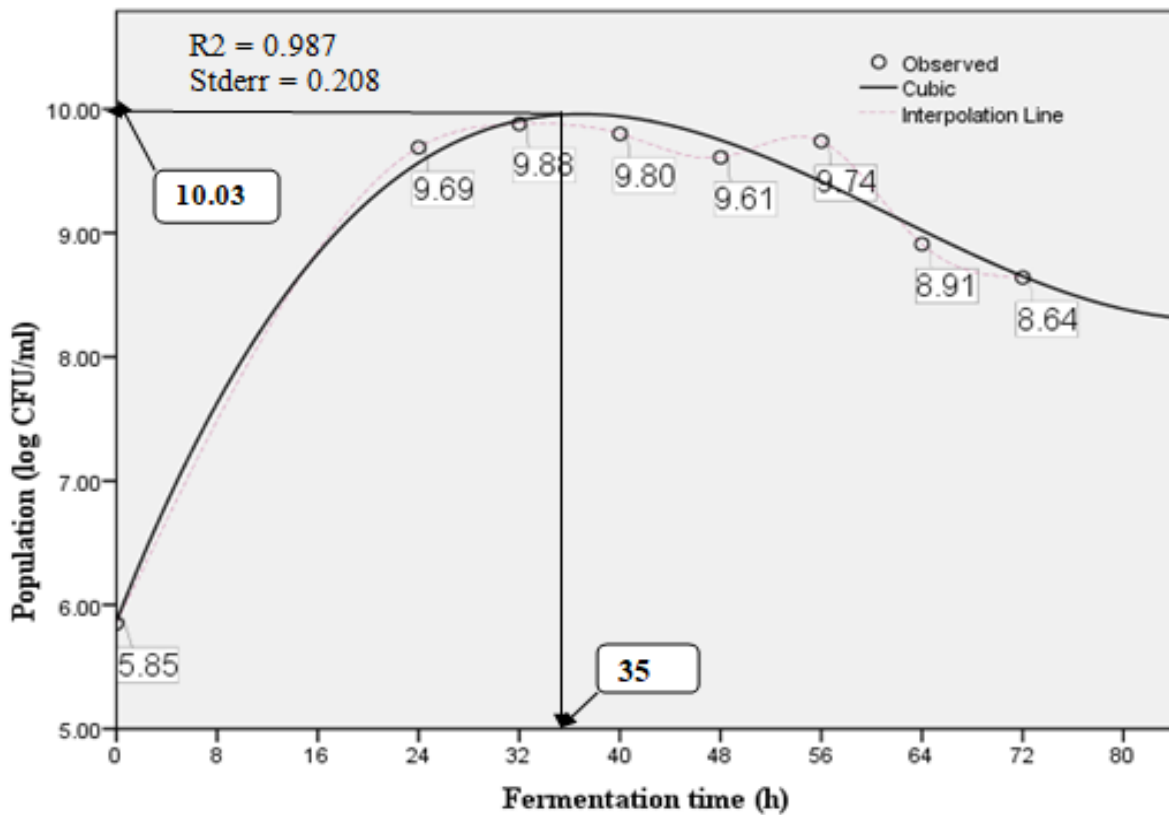


Fig.2 Effect of fermentation time on population of *L. acidophilus* MCC10307 in amla drink

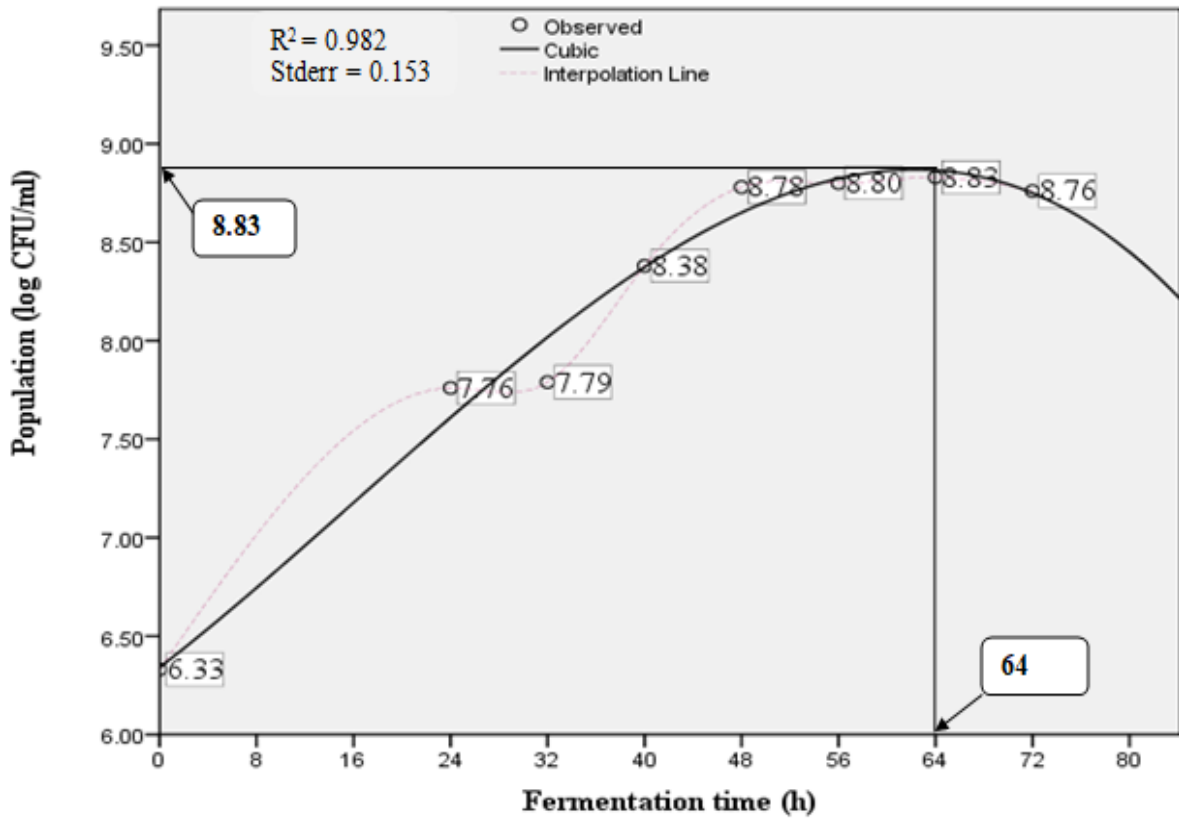


Fig.3 Total soluble solids of amla drink during fermentation

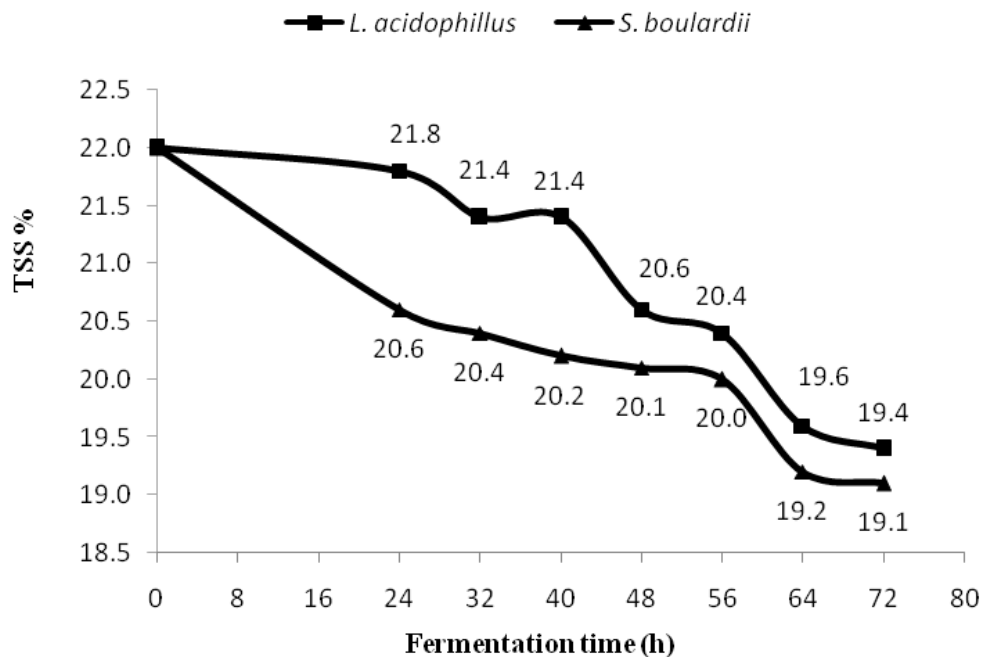
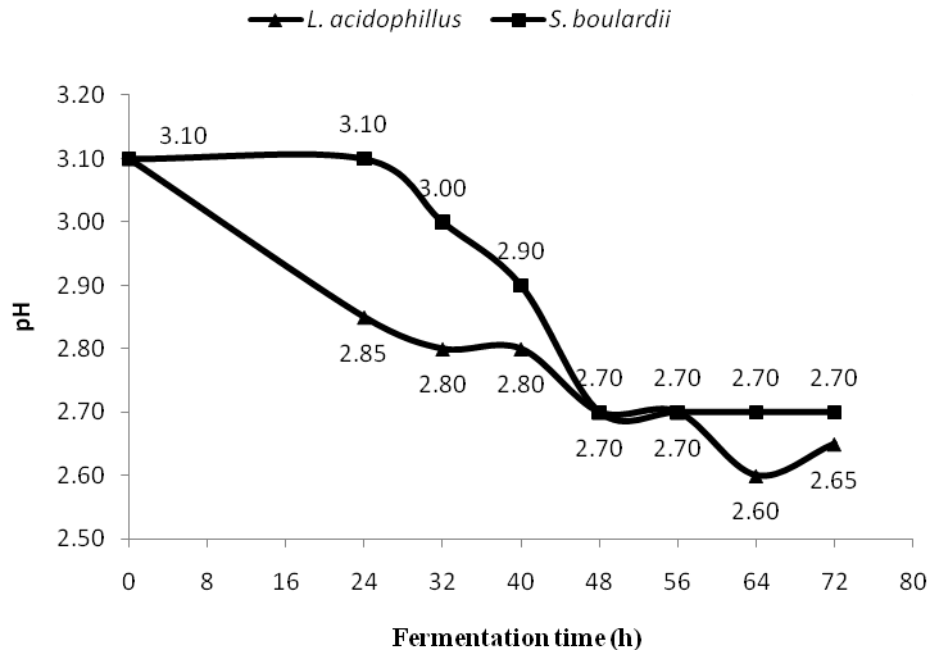


Fig.4 pH of amla drink during fermentation



Change in pH with fermentation time in amla drink is shown and the data is presented in Figure 4. With the production of acid during fermentation by both the cultures, as expected initial pH of 3.1 of amla drink was gradually decreased. According to the literature studies, pH value of the fermented media significantly changed with fermentation time, substrate, and strain. pH value significantly affected further microorganism activity of the media (Steinkraus, 1992).

These organisms are grown in fruit drinks and as a result, acid and alcohol production occur and lower the pH rapidly to a point where competing organisms are no longer able to grow. Therefore, the most significant factor in growth limitation is probably the pH value after the certain time of fermentation. Prado *et al.*, (2008) reported that *Lactobacillus casei* DN-114 001, *Lactobacillus rhamnosus* GG and *Lactobacillus paracasei* remained viable in orange (pH 3.65) and pineapple (pH 3.40) juices at the levels greater than 10^7 CFU/ml and 10^6 CFU/ml.

Acceptability of fermented amla drink

Sensory scores for fermented amla drink by *Saccharomyces boulardii* CNCM 1-745 and *Lactobacillus acidophilus* MCC10307 upto their optimum fermentation time with unfermented fruit drink is presented in Table 2. Fifteen per cent unfermented amla drink was used as the control. The scores pertaining to sensory qualities of fermented drinks are significantly ($P \leq 0.05$) lower than the unfermented drinks and there were no significant ($P \leq 0.05$) differences between *S. boulardii* CNCM 1-745 fermentation and *L. acidophilus* MCC10307 fermentation on sensory properties. Hence the experiment drinks needed modification in sensory parameters. This may be due to the development of undesirable flavour and aroma during fermentation due to production of undesirable by-products such as alcohol. Unpleasant perfumery aromas, as well as sour and savoury flavours have been observed by Luckow *et al.*, (2006) in orange drinks inoculated with *Lactobacillus Plantarum* and

has been reported that the perceptible off-flavors of probiotic orange drink, that often contribute to consumer dissatisfaction. These findings support the present study.

Improvement action in order to enhance sensory quality of probiotic amla drinks

In order to enhance the organoleptic properties while maintaining highest cell growth, some improvement action was carried out as follows,

Adjustment of initial pH of the drink to organism's optimum pH level.

Adjustment of total soluble solids of the fruit drink at 25° Brix.

Lactobacillus acidophilus (nu-trish LA-5) organism was used to replace *Lactobacillus acidophilus* MCC10307 for fermentation.

Average pH of amla drink was 3.1 and at these pH level growth rates of organisms was less. Generally, optimum pH level for both organisms' is ranged from 3.5 to 5. In order to increase the pH of amla drink to optimum level for organisms, calcium propionate, a buffer was used. Minimum optimum buffer concentration for maintaining pH of amla drink at 4.4 was 0.5 per cent and it was suitable to achieve desired pH range for optimum cell growth with better sensory parameters. This level was acceptable by the panel members and was within safe limits also. Total soluble solids were adjusted at 25° Brix to increase pH and nutritional source for the growth of organisms while maintaining sweetness of drinks at acceptable levels. *Lactobacillus acidophilus* (nu-trish LA-5), a freeze dried ready-made culture with higher viability was needed. Amla drink adjusted for pH and TSS were inoculated with a 10% (v/v) of *S. boulardii* CNCM 1-745 and *L. acidophilus* (nu-trish LA-5) inoculums

separately and incubated at 30°C for 3 hr for fermentation. Since cultures are having high growth rate in this condition, the population was analyzed in order to confirm the significant growth of them in amla drink. All these improvement actions were used to bring down the fermentation time while maintaining highest cell count since probiotic fruit drinks with less fermentation period provide better organoleptic properties.

Nutritive value of probiotic amla drink

Ascorbic acid content and antioxidant potential of unfermented and fermented amla drinks is tabulated in Table 3. Ascorbic acid content of both fermented and unfermented amla drink ranged from 96-109 µg/ml and there was no significant difference between fermented and unfermented amla drink. Similarly, antioxidant potential of both fermented and unfermented amla drink is 938 µg/ml and there was no significant difference of antioxidant potential of fermented and unfermented amla drink proving that probiotication has maintained antioxidant potential of the developed drink.

Mishra *et al.*, (2011) reported that the ascorbic acid content of the fresh amla fruit was 463 mg/100 g. When the fruit was made into powder there was an increase in the vitamin C content. Freeze dried powder had the maximum vitamin C content a 5432.75 mg/100 g whereas the sun dried amla powder contained the vitamin C as 3006.00 mg/100 g. An investigation involving blends of anola and kinnow mandarin juice for their value addition to prepare a RTS beverage was carried out by Jain and Meena (2013). It had 27.92 mg/ 100 ml of ascorbic acid content. The minimum vitamin C content of 85.47 mg/100g was reported in amla *murabbas*, whereas the maximum of 166.46 mg/100g in the product was reported by Patel *et al.*, (2013). Puranik *et al.*, (2012) reported the

DPPH per cent scavenging activity in the amla candy, bar and toffee. Fresh amla showed DPPH scavenging activity of 83.24 whereas candy (42.77%) showed highest DPPH scavenging activity among the processed products and the toffee (18%) showed the lowest value among all. The reason for low bioactive component retention in the toffee when compared to candy and bar was attributed to the heat processing. The free radical scavenging activity of the fresh amla fruit ranges from 80.3 per cent to 86.8 per cent (Prakash *et al.*, 2012).

Storage study of probiotic amla drink

The data in Table 4 illustrates the effect of cold storage on viability of *Saccharomyces boulardii* CNCM 1-745 and *Lactobacillus acidophilus* (nu-trish LA-5) in fermented amla drink. Initial pH was adjusted to 4.4 by using a buffer. Total soluble solids were adjusted at 25° Brix as well. After 3 hr of fermentation, a superior population of both the organisms was observed. The initial total viable count of the drink was 10^9 and 10^{10} CFU/ml, in case of *Saccharomyces boulardii* CNCM 1-745 and *Lactobacillus acidophilus* (nu-trish LA-5) respectively

Saccharomyces boulardii CNCM 1-745 grown at 30°C could survive for several weeks in the fermented amla at 4°C storage, while *Lactobacillus acidophilus* (nu-trish LA-5) could survive for 12 days. During 30 days storage period, there was no significant change in population of *Saccharomyces boulardii* CNCM 1-745 upto 30 days and there was no significant change in population of *Lactobacillus acidophilus* (nu-trish LA-5) upto 12 days. However, after 12 days, *Lactobacillus acidophilus* (nu-trish LA-5) has lost its viability and there was no convenient cell growth after that. The viability of probiotic organisms is dependent on many factors, such as level of oxygen in products,

oxygen permeation of the package, fermentation time and storage temperature (Soccol *et al.*, 2007) and also it is affected by lactic acid produced during production and cold storage. Contrary to the study results, some researchers (Wang *et al.*, 2002) have reported a decline in total viable count of *Lactobacillus reuteri* and *Bifidobacterium bifidum* of whey based probiotic beverage stored at $4 \pm 1^\circ\text{C}$ and *Lactobacillus acidophilus* in commercial yoghurt during refrigerated storage.

Fermented (for 3 hours) amla drink by *Saccharomyces boulardii* CNCM 1-745 and *Lactobacillus acidophilus* (nu-trish LA-5) did not show sensory differences throughout the studied storage period for all sensory attribute with overall acceptability scores of 7.3 and 8.0 respectively. Storage studies of amla drink with *Saccharomyces boulardii* CNCM 1-745 was continued for 30 days while with *Lactobacillus acidophilus* (nu-trish LA-5) was discontinued after 12 days since loss of cell viability. With the consideration, both population and sensory quality, amla drink with *Saccharomyces boulardii* CNCM 1-745 can be stored at refrigerated conditions for 4 weeks while *Lactobacillus acidophilus* (nu-trish LA-5) can be stored for 12 days.

These findings are in line with the findings of Kumar *et al.*, (20013) who reported the changes in sensory attributes of whey based probiotic beverage. According to the researchers it did not show sensory differences for the first two weeks at refrigerated storage. But after the second week, difference was perceived in colour and flavour.

However, amla drink suffers from severe browning during storage, particularly at a higher temperature, which adversely affects the appearance and overall acceptability of juice (Bhattacharjee *et al.*, 2011).

Formulation of probiotic fruit drink using amla fruit was possible with *Saccharomyces boulardii* and required cell count, good sensory qualities having 30 days storage period was achieved in the present study. Formulation of probiotic fruit drinks using amla fruit was possible with *Lactobacillus acidophilus* with required cell count, good sensory qualities with 12 days storage period. These probiotic drinks as the best alternatives to dairy based probiotic products. Exploitation of antioxidant rich foods as a substrate for beneficial micro flora is achieved.

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