

## Evaluation of Different Strains of Yeast and Lactic Acid Bacteria for Fermentation of Kokum Juice

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Kokum (*Garinia indica* cv.Choicy) is an important spice fruit, which is used in preparation of many value added products. Four different yeast strains viz. *Saccharomyces cereviceae* (MTCC 6008, MTCC 4780, UCD 522 and isolate K.Sc) and lactic acid bacteria viz. *Streptococcus thermophilus* (MTCC1928), *Lactobacillus brevis* (MTCC1750), *Lactobacillus plantarum* (MTCC6161) and isolate K.LAB were screened for the efficiency of fermentation of kokum juice. The results revealed that the kokum juice fermented by yeast strain *Saccharomyces cereviceae* (UCD 522) recorded as lowest total soluble solids (7.0° brix), highest titable acidity (1.46 %), vitamin C (3.50 mg/100ml), lowest total sugars (6.55 %), highest alcohol production (6.65 %) and highest fermentation efficiency (93.39%) and highest organoleptic score (13.75 / 20.0 ) compared to other yeast strains. Similarly, among LAB strains, the strain *Lactobacillus plantarum* MTCC 6161 showed higher values with respect to titrable acidity (1.60 %), vitamin C (0.42 mg), least total sugar (10.17 %) and highest organoleptic score (13.00/20.0). The results indicated that the yeast strain UCD 522 and LAB strain MTCC 6161 found to be efficient in fermentation of kokum juice.

**Key words:** Kokum, Yeast, Bacteria, Fermentation.

Kokum is a seasonal fruit which ripens during April – May; fruit yields are very high during summer in the Western Ghats. Traditionally the fruit is dried to preserve rinds and can be used for processing to develop many value added products. Kokum is unique in that both the rind and the pulp of the fruit can be used in several ways. Kokum fruit rind is rich source of hydroxyl citric acid (-HCA) which is unique, potent, metabolic regulator of obesity and helpful in treating many cardiovascular risk factors associated with abdominal obesity (Verghese J, 2000). Kokum juice is widely used to prepare kokum syrup or sherbet

or squash known as amrut kokum which is extensively used in summer months for body cooling effects.

Traditionally, the fruit juices are used to ferment by modified strains of *Saccharomyces cereviceae* are currently used for the production of wine or fermented beverages. The quality of wine or fermented beverage is depends upon a number of factors like cultivars, adequate sugar level, acid content, color, aroma and strains used Ethiraj *et al.* (19930), Wahab O. oknowo *et al.*, (2005) evaluated four different yeast strains from different sources and examined for fermentation of orange juice. Singh *et al.* (2009) screened four yeast strains for alcoholic fermentation of litchi juice and reported that *Saccharomyces cerevisiae* (MTCC 178) was found to be the most potent strain. Yoon *et al.* (2005) screened four lactic acid bacterial species (*Lactobacillus acidophilus*, *L. caesi*, *L.*

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*delbrueckii* and *L. plantarum*) for fermentation of red beets as a potential substrate for the production of probiotic beet juice. Lactic acid fermented beverage from kokum juice using reference strain of lactic acid bacteria *Lactobacillus acidophilus* has been developed (Dushyantha, *et al.*, 2008). However, there is paucity of information on yeast and lactic acid bacterial strains for the fermentation of kokum juice. Hence the present investigation was carried out.

## MATERIAL AND METHODS

Kokum dried rind samples were collected from Madhu multiples, Puttur taluk, Uttara kannada district for the experimentation. The kokum rind was processed into kokum juice, the preparation of kokum juice done as per wasker, 2002 and the same was used for the evaluation of yeast and lactic acid bacterial strains for the fermentation of kokum juice. The proven and authenticated yeast and lactic acid bacterial cultures were procured from Microbial Type Culture Collection Center (MTCC), Chandigarh, India in the form of lyophilized cultures. These proven strains along with the isolates obtained from kokum rind were used in the fermentation studies.

### Preparation of yeast starter culture

Purified and authenticated loop full of inoculums of different strains of yeast culture were transferred to conical flasks containing 100 ml of YEPDA broth. The inoculated flasks were kept for 2-3 days incubation at 28°C. These broth cultures of yeast were inoculated with 10<sup>7</sup> cfu/ml at 5 per cent to 300 ml kokum juice in a 500ml conical flask for fermentation.

### Preparation of LAB starter culture

Loop full inoculums of purified and authenticated different lactic acid bacteria were transferred to conical flasks containing 100 ml of MRS broth. The inoculated flasks were incubated for 2 to 3 days at 37<sup>0</sup> C. These broth cultures of LAB were inoculated with 10<sup>8</sup> cfu/ml at 5 per cent to 300ml kokum juice in a 500ml conical flask for fermentation.

The experiment was conducted to evaluate the efficiency and potentiality of different strains of yeast and lactic acid bacteria for the

fermentation of kokum juice.

The treatments are kokum juice without inoculant as control, Y1: Kokum juice + *Saccharomyces cerevisiae* (MTCC 6008), Y2: Kokum juice + *Saccharomyces cerevisiae* (MTCC 4780), Y3: Kokum juice + *Saccharomyces cerevisiae* (USD 522), Y<sub>4</sub>: Kokum juice + K Sc (Isolate yeast), L<sub>1</sub>: Kokum juice + *Streptococcus thermophilus* (MTCC 1938), L<sub>2</sub>: kokum juice + *Lactobacillus brevis* (MTCC 175), L<sub>3</sub>: Kokum juice + *Lactobacillus plantarum* (MTCC 6161) and L<sub>4</sub>: Kokum juice + KLAB (isolate LAB).

The inoculated flasks and control flasks were plugged with rubber cork with bent glass tube as air trap and kept for fermentation for 7 days under room temperature (27 to 30°C). After 7 days of fermentation the fermented juice was filtered through muslin cloth and the filtrate was kept in sterilized glass bottles. The fermented filtrate juice was subjected for biochemical and microbiological analysis by standard procedures. PH of the kokum juice was measured using digital pH meter of analog model Pocket Refractometer (Sadasivam and Manickam, 1996). Total soluble solids of juice were determined with the help of "ERMA" Hand Refractor meter having a range of 0 to 32°Brix. Titrable acidity (%) was carried out as per Srivastava and Kumar, 1993, Ascorbic acid produced from fermented kokum juice was determined by 2, 4 dinitrophenyl hydrazine dye solution by spectrophotometer method and Estimation of reducing sugars by the standard method of Fehling's method (Sadasivam and Manickam, 1996), Ethanol was estimated calorimetrically as described by Caputi *et al.* (1968).

The percentage fermentation efficiency of different yeast and LAB strains were calculated on the basis of the relationship between the alcohol content in wine and alcohol obtainable from total sugar following the fermentation stichiometry. The developed kokum fermented beverages by the influence of different yeast and LAB strains were evaluated for organolyptic characters by selected 5 panel members with twenty point hedonic scales (Amerine *et al.*, 1972) was taken into consideration, which was based mainly on the appearance, color, aroma, taste and acceptability.

## RESULTS AND DISCUSSION

The influence of different yeast and lactic acid bacterial strains on changes in PH, TSS and titrable acidity is presented in the Table 1. The initial pH was reduced from 2.59 to 2.15 between yeast and LAB strains. The range of PH by yeast and bacterial strain varied from 2.15 to 2.39 and 2.42 to 2.55 respectively. The yeast strain UCD 522 (Y1) recorded lowest pH (2.42) compare to other strains. Among LAB strains, highest pH (2.55) was recorded in isolate K LAB (L4). The pH of the fermented beverage depends upon the acids and sugar contents of juice. Similar results were reported by Dushyanth et al. (2010) in kokum juice fermented by yeast and lactic acid bacteria.

Sugars are the main source to ferment into alcohol. The amount of sugar present is measured in terms of total soluble solids. Yeast strains used more of sugars during fermentation, hence reduction in TSS after 7 days (Table 1). LAB strains showed less fermentative activity with low fermentation of both the kokum isolates of K Sc and K LAB. These results were supported by studies of Ayoga (1999) in pineapple fruits and similar results obtained by Girish (2006) in fermented kokum juice.

Upon completion of fermentation, production of titrable acidity is more important in fermented products. The highest titrable acidity (1.46 %) is produced in the juice fermented by yeast strain (Y3) UCD 522 followed by MTCC 6008 (1.26 %). The lowest titrable acidity was recorded in other processed juices fermented by yeast isolate K Sc. Among LAB strains, higher acidity (1.6 %) was noticed in juice fermented by *Lactobacillus plantarum* (MTCC 6161) (L3) followed by MTCC 1938 (1.47 %) The results indicating that the juice inoculated with yeast UCD 522 and LAB (L3) are able to produce more titrable acidity. Similar results were reported in jamun fermented juice by Chowdhury and Ray (2007) by yeast fermentation in kokum juice. The increase in titrable acidity in fermentation may be due to the production of certain organic acids mainly lactic acid. This trend was similar to the study conducted by Sapna et al., (2002). The influence of different yeast and lactic acid bacterial strains on changes in total sugars, alcohol and fermentation efficiency is presented in the Table 1 and Fig 1.

The results indicated that yeast strains used more of sugars during fermentation, hence reduction in total sugars after 7 days of fermentation. LAB strains showed less fermentative activity with low fermentation of both the kokum isolates K Sc and K LAB Utilization of sugars during fermentation was varied among microbial strains. The highest utilization was by yeast strains MTCC 4780 followed by UCD 522 compared to other strains. The lower levels of utilization by bacterial strains indicative of the reduced efficiency of fermentation. This may be due to presence of fructose in fruit, initial sugar level of the fruit and sugar utilization capacity of the strains. Similar results reported by Dushyanth *et al.* (2010) in fermented kokum juice.

Alcohol production is one of the parameters to test the efficiency of strains, since alcohol is a major solvent in wine. Alcohol production varies between efficiency of yeast strains. Among the yeast strains, the highest alcohol (6.65 %) was observed in kokum juice fermented by *Saccharomyces cerevisiae* (UCD 522) followed by MTCC 4780 (5.65 %) (Table1). Alcohol production by LAB strains was in the range of 0.75 to 2.8 per cent. The variation in alcohol production by different yeast strains may be due to the variation in their rate of sugar utilization from the fermentation medium and alcohol tolerance limits Table 1 and Fig 2.

Similar report has been published by Wahab O kunowo *et al.* (2005) in the study of alcoholic fermentative efficiency of indigenous yeast strains on orange juice and reported that the highest alcohol produced by *Saccharomyces carlsbergensis* (6.80%) and least was produced by *Saccharomyces cereviceae* (3.19 %) also confirmed with the results of Choudhari and Chincolkar (1996) who reported that among 30 yeast strains.

Efficiency of fermentation with reference to the alcohol concentration was in the range of 76.54 to 93.39 for yeast strains while it was very less for isolate K Sc (58.98 %). The highest (93.39%) fermentation efficiency was with yeast strain UCD 522 than other strains. The bacterial fermentation efficiency was very low in the range of 10.53 to 39.39 per cent least with isolate K LAB (10.53%). This could be due to the utilization of sugar for the formation of other products apart from the alcohol.

**Table 1.** Influence of yeast and LAB on pH, TSS (°Brix) and Titrable acidity, Total Sugar, Alcohol, Fermentation efficiency, Reducing sugar, Non reducing sugar and Vit-C content of Kokum juice

Yeast and LAB Strains	pH (°Brix) (%)	TSS acidity (%)	Titrable sugar (%)	Total (%)	Alcohol efficiency (%)	Fermentation	Reducing sugar	Non reducing Sugar (%)	Vit-C (mg/100ml)
Control (Initial value)									
Yeast strains									
Y <sub>1</sub>	2.59 <sup>a</sup>	19.0	0.90 <sup>g</sup>	15.45 <sup>ab</sup>	0.0 <sup>d</sup>	-	14.00 <sup>a</sup>	6.20 <sup>bc</sup>	1.40
Y <sub>2</sub>	2.15 <sup>f</sup>	9.0	1.26 <sup>de</sup>	10.25 <sup>c</sup>	5.45 <sup>ab</sup>	76.54	6.50 <sup>ab</sup>	6.55 <sup>cd</sup>	3.70
Y <sub>3</sub>	2.25 <sup>e</sup>	9.5	1.24 <sup>e</sup>	6.40 <sup>d</sup>	5.65 <sup>a</sup>	79.35	7.40 <sup>ab</sup>	5.20 <sup>de</sup>	1.20
Y <sub>4</sub>	2.39 <sup>d</sup>	7.0	1.46 <sup>b</sup>	6.55 <sup>d</sup>	6.65 <sup>a</sup>	93.39	7.70 <sup>a</sup>	3.05 <sup>e</sup>	3.50
LAB strains									
L <sub>1</sub>	2.16 <sup>f</sup>	13.0	1.13 <sup>f</sup>	11.25 <sup>e</sup>	4.20 <sup>b</sup>	58.98	6.30 <sup>b</sup>	8.70 <sup>bc</sup>	2.55
L <sub>2</sub>	2.49 <sup>bc</sup>	13.5	1.47 <sup>b</sup>	11.65 <sup>c</sup>	2.80 <sup>c</sup>	39.32	3.30 <sup>d</sup>	11.05 <sup>ab</sup>	0.60
L <sub>3</sub>	2.41 <sup>d</sup>	12.5	1.41 <sup>bc</sup>	12.42 <sup>bc</sup>	2.20 <sup>c</sup>	30.89	6.50 <sup>ab</sup>	11.0 <sup>ab</sup>	1.42
L <sub>4</sub>	2.45 <sup>cd</sup>	10.5	1.60 <sup>a</sup>	10.17 <sup>c</sup>	2.50 <sup>c</sup>	35.10	6.50 <sup>ab</sup>	9.75 <sup>b</sup>	0.42
SEm±	2.55 <sup>ab</sup>	16.5	1.35 <sup>cd</sup>	16.00 <sup>a</sup>	0.75 <sup>d</sup>	10.53	4.90 <sup>d</sup>	11.00 <sup>ab</sup>	5.00
CD (at 5 %)	0.02	0.48	0.03	1.10	0.43	-	1.08	1.06	4.67
	0.06	1.43	0.10	3.27	1.27	-	3.20	3.16	13.88

**Table 2.** Organoleptic evaluation score of the fermented Kokum beverages.

Treatments	Appearance (2)	Color (2)	Aroma (2)	Bouquet (1)	Vinegar (2)	Total acidity (2)	Sweetness (2)	Body (1)	Flavour (2)	Astringency (2)	General quality (2)	Overall Acceptability (20)
Y <sub>1</sub>	1.50	1.00	1.00	0.50	0.5	1.0	0.50	1.50	1.50	0.75	0.75	10.50
Y <sub>2</sub>	1.50	1.25	1.25	0.50	0.5	1.0	1.00	0.50	1.00	1.25	1.25	11.00
Y <sub>3</sub>	1.50	1.50	1.50	0.75	1.0	1.0	1.25	0.75	1.50	1.50	1.50	13.75
Y <sub>4</sub>	1.00	1.00	0.50	0.50	1.0	1.0	1.00	0.50	1.00	1.00	1.00	9.50
L <sub>1</sub>	1.25	1.00	0.50	0.50	1.0	1.0	0.75	0.50	1.00	1.00	1.00	9.50
L <sub>2</sub>	1.25	1.25	1.50	0.50	1.0	1.0	1.25	0.50	1.25	1.00	1.00	11.50
L <sub>3</sub>	1.50	1.50	1.50	0.50	1.0	1.0	1.50	0.50	1.50	1.25	1.25	13.00
L <sub>4</sub>	1.00	1.00	1.00	0.50	1.0	1.0	0.75	0.50	1.00	1.00	0.75	9.50

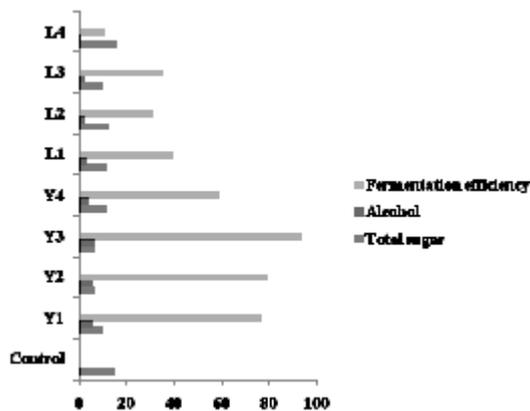
Note: 1=Not acceptable, 2=Poor, 3=Good, 4=Very good, 5=Excellent

However the efficiency of individual strain in the fermentation is carried out at their respective pH. These results are in conformity with data reported by Wahab O Okunowo *et al* (2005) in orange juice fermentation by different strains of yeast. The highest fermentation efficiency was 99.46 per cent with *S. carlsbergensis* and least (48.05 %) was *S. cereviceae var ellipoids*.

In the present study, residual sugar concentration in the form of reducing sugars was very low in the range of 3.05 to 8.7 per cent among yeast strains. Whereas the reducing sugar in bacterial fermented kokum juice was high in the range of 9.75 to 11.05 per cent indicative of the fermentative efficiency of both yeast and bacterial

strains. Whereas, non reducing sugar levels are comparatively low in bacterial fermentation than yeast strains. As a result, the bacterial fermented kokum product will have more sweetness than yeast fermentation. The yeast and LAB cannot utilize 100 per cent sugar some amount of sugar will be left in the wine after 7 days of fermentation. In the present study also highly significant difference between the microbial strains. These results support the work of Kulkarni *et al.* (1980).

Vitamin C content of the yeast fermented kokum juice was significantly higher than the bacterial fermentation. The highest vitamin C content was recorded in products fermented by yeast strains UCD 522 and MTCC 4780. Whereas,

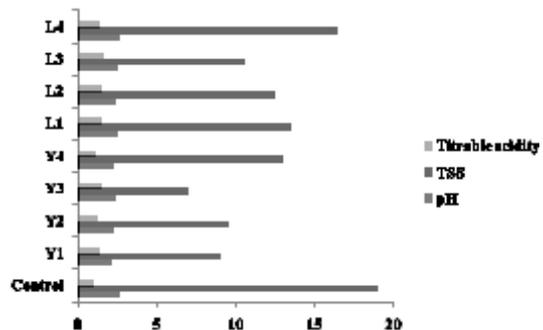


Y<sub>1</sub> = *Saccharomyces cerevisiae* (MTCC 6008)  
 Y<sub>2</sub> = *Saccharomyces cerevisiae* (MTCC 4780)  
 Y<sub>3</sub> = *Saccharomyces cerevisiae* (UCD 522)  
 Y<sub>4</sub> = Isolated yeast from kokum (K.Sc)  
 L<sub>1</sub> = *Streptococcus thermophilus* (MTCC 1938)  
 L<sub>2</sub> = *Lactobacillus brevis* (MTCC 1750)  
 L<sub>3</sub> = *Lactobacillus plantarum* (MTCC 6161)  
 L<sub>4</sub> = Isolated LAB from kokum (K.LAB)

**Fig. 1.** Influence of yeast and LAB fermentation for pH, TSS (°Brix) and Titrable acidity (%) of kokum juice

bacterial strain MTCC 6161 had highest of 120.0 mg/100ml. These results are conformity with data reported by Sapna *et al.* (2002) in spice fermented beverage and Wahab O. Okunowo *et al.* (2005) in fermented orange juice.

Sensory evaluation was done by selected panel of member through organoleptic procedures. Results of organoleptic evaluation (Table 2) showed that juice inoculated with yeast strain UCD 522 recorded highest score (13.75 out of 20.0) followed



Y<sub>1</sub> = *Saccharomyces cerevisiae* (MTCC 6008)  
 Y<sub>2</sub> = *Saccharomyces cerevisiae* (MTCC 4780)  
 Y<sub>3</sub> = *Saccharomyces cerevisiae* (UCD 522)  
 Y<sub>4</sub> = Isolated yeast from kokum (K.Sc)  
 L<sub>1</sub> = *Streptococcus thermophilus* (MTCC 1938)  
 L<sub>2</sub> = *Lactobacillus brevis* (MTCC 1750)  
 L<sub>3</sub> = *Lactobacillus plantarum* (MTCC 6161)  
 L<sub>4</sub> = Isolated LAB from kokum (K.LAB)

**Fig. 2.** Influence of yeast and LAB fermentation for total sugar (%), alcohol (%) and fermentation efficiency (%) of kokum juice

by juice inoculated *Lactobacillus plantarum* MTCC-6161 (13.0 out of 20.0) with respect overall acceptability. These findings are similar to the results reported by Sapna *et al.* (2002) in fermented spice beverage and Girish (2006) in LAB fermented kokum juice and Priya (2010) in yeast and LAB fermented tomato juice beverage. Hence, the results of this experiment conclude that the yeast strain UCD 522 and lab strain MTCC 6161 found to be efficient in fermentation of kokum juice.

## REFERENCES

1. Amerine, M. A., Berg, H. W. and Cruess, W. V., The technology of wine making **3rd Ed.** Publ: AVI Co. West Port, Connecticut, 1972.
2. Ayoga, T. E., Evaluation and performance of yeast isolated from Nigerian palm wine production from pineapple fruits. *Biores. Technol.*, 1999; **69**: 189-190.
3. Caputi, A., Ueda, J. M. and Brown, T., Spectrophotometric determination of chromic complex formed during oxidation of alcohol. *Amer. J. Enol. Vitic.*, 1968; **19**: 160-165.
4. Chaudary, A.B., and Chincholkar, S.B., New osmotolerant *Schizosaccharomyces* for ethanol production. *J. Food. Sci. Technol.*, 1996; **36**: 166-169.
5. Chowdhury, P. and Ray, R. C., Fermentation of janum (*Syzygium cumini L.*) fruits to form red wine. *ASEAN Food Journal.*, 2007; **14**(1): 15-23.
6. Dushyantha, D. K., Girish, D. N. and Suvarna, V. C., Native lactic acid bacterial isolates of kokum for preparation of fermented beverage. *EJBS*, 2010; **2**(1): 21-24.
7. Ethiraj, S. and Suresh, E. R., Fruits Wines. *Advances in Horticulture*, 1993; **4**: 1957-1966.
8. Girish, Native lactic acid bacterial isolates of kokum for preparation of fermented beverage. Thesis submitted to University of Agricultural Sciences, Bangalore, 2010.
9. Kulkarni, J. H., Singh, H. and Chadha, K. L., Preliminary screening of mango variety for wine making. *J. Food. Sci. Technol.*, 1980; **17**: 218-220.
10. Priya, R. Mathapati., Nitin V. Ghasghase, Mayuri K. Kulkarni, Study of *Saccharomyces cerevisiae* 3283 for the production of tomato wine. *Int. J. Chem. Sci.*, 2010; 5-15.
11. Sadasivam, S and A. Manickam., Biochemical methods. Second Edition, *New Age International (P) Limited, Publishers*, 1996.
12. Sapna, V., Vasundhara, M. and Annapurna, M. L., Fermented beverages from spices- a new nutraceutical drink. *J. Spices Aromatic Crops.*, 2002; **11**: 106-111.
13. Singh and Preetinder Kaur, Evaluation of litchi juice concentrates for the production of wine. *Natural product Radiance*. 2009; **8**(4): 386-391.
14. Srivastava, R. P., and Kumar, S., Important methods for analysis of fruits/vegetable and their products. *Fruit and Vegetable preservation principles and practices.*, 2<sup>nd</sup> ed. 1993; 321-229.
15. Verghese, J., Hydroxy citrates a unique potent metabolic regulators of obesity, *Indian spices*, 2000; **33**(2): 10-11.
16. Wahab O. Okunowo, Rufus O. Okotore and Akinniyi. A Osuntoki, 2005. The alcoholic fermentative efficiency of indigenous yeast strain of different origin on orange juice. *African J. of biotechnology.*, **4**(11): 1290-1296.
17. Wasker, D, P., 2000. Standardization of method of juice extraction from Kokum fruit. *Indian Food Packer.*, **45**(1):122-124.
18. Yoon, K.Y., Edward E. Woodams, and Yong Hang, 2006. Production of Probiotic cabbage juice by lactic acid bacteria. *Bioresources Technology*, **97**: 1427-1430
19. Zeng xa, Chen xd, Qin fgf and ZHANG L, 2008. Composition analysis of litchi juice and litchi wine, *Intet J. Food Engg.*, **4**(4): 1-16.