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Microbial Growth on Fermented Kokum Juice by Different Yeast and Lactic Acid Bacterial Strains

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Kokum (*Garcinia indica* Choisy) is underutilized fruits are grow which are known for their therapeutic and enhanced nutritive value and ferment the kokum juice by different yeast and lactic acid bacterial strains. Most of the fruit juices have wholesome therapeutic effect and are consumed as refreshing drinks. Because of their acid flavour, attractive and appealing color, they are welcome addition to the table. Commonly used fruits for making juice are orange, grape, pomegranate, melon, mango *etc.* and vegetables such as carrot, beetroot and cucumber *etc.* The highest yeast population was recorded in kokum juice fermented by yeast strain UCD 522 (1.80 x 10^3) and LAB strain MTCC 1750 (1.8 x 10^3) followed by MTCC 6161(1.5 x 10^3). UCD 522 and LAB strain MTCC 6161 performed better for fermentation of kokum juice. Kokum fruits / kokum rind can be used as raw material for production of fermented kokum beverage through yeast and lactic acid fermentation.

Key words: Kokum, YEPDA, MRS, yeast, lactic acid Bacteria, Fermentation.

Kokum (*Garcinia indica* Choisy) is an important minor fruit spice crop which finds an application in many culinary preparation of syrups used for healthy soft drinks and in ayurveda as therapeutic agent and its effect includes curing of burns, piles, allergies, sunstroke, diarrhea, dysentery, tumor and cardiac diseases, *etc*. Kokum is a rich source of beneficial compounds like HCA (hydroxyl citric acid), garcinol, citric acid, malic acid, anthocyanin pigments and ascorbic acid (Mishra *et al.*, 2006).

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Fermentation is a biological activity, where sugar is converted to carbon dioxide and alcohol, catalysed by microorganism or enzyme. Glucose is considered as a typical substrate for fermentation, as it is the most widely distributed sugar and the most bacteria and yeast can utilize it. The initial steps in the metabolism of most other substrate converts them to some intermediate product of glycolytic pathway. Pyruvic acid, during fermentation, instead of getting converted into acetyl CoA entering into the end product of fermentation thus, play a key role in principal fermentation.

Probiotics are live microorganisms available to consumers mainly in the form of dietary supplements, foods and used as complementary and alternative medicine (CAM) (Prado *et al.*, 2008).

Fermented and unfermented milk, miso, tempeh, and some juices and soy beverages.

Streptococcus thermophilus was known to promote gastrointestinal health. *Streptococcus thermophilus* is used as a starter culture for dairy foods besides yogurt, including Mozzarella cheese and also considered one of the most commercially important of the lactic acid bacteria. (Netiyuliana *et al.*, 2010). *Saccharomyces cervisiae* are currently used for production of wine/fermented beverages. The quality of wine/fermented beverage depends upon number of factors like cultivars, adequate sugar level, acid content, color, aroma and strains used (Ethiraj *and* Suresh.,1993)

Lactobacillus brevis was recognized as crucial member of the probiotic microorganisms that actively promote the health of the human gut and subsequent introduction into the human digestive system (Jungh and Wadston 2009). *Lactobacillus plantarum* is a beneficial bacterium that can be used for improved health and most versatile probiotics found in plant material and the gastrointestinal tract of animals, including humans, fermentation of foods like sauerkraut, kimchi, pickles, and sourdough bread and has ability to destroy pathogens and to preserve critical nutrients, vitamin and antioxidants. (Tayler *et al.*, 2007)

MATERIALS AND METHODS

The Experiment was conducted at AICRP on Post Harvest Technology Scheme, UAS Bangalore during the year 2012 to study the microbial analysis of fermented kokum juice by different yeast and lactic acid bacterial strains. Kokum dried rind samples were collected from Puttur taluk, Uttara kannada district for the experimentation.

The treatments are Y_1= Kokum juice + Saccharomyces *cerevisiae* (MTCC 6008), Y_2 Kokum juice + =*Saccharomyces cerevisiae* (MTCC 4780) Y_3= Kokum juice + *Saccharomyces cerevisiae* (UCD 522), Y_4= Kokum juice + Isolated yeast from kokum (K.Sc) L_1= Kokum juice + Streptococcus *thermophillus* (MTCC 1938), L_2= Kokum juice + Lactobacillus *brevis* (MTCC 1750), L_3= Kokum juice + *Lactobacillus plantarum* (MTCC 6161) , L_4= Kokum juice + Isolated LAB from kokum (K.LAB) Inoculated flasks and control flasks were plugged with rubber cork with bent tube and kept for fermentation for 7 days under room temperature (27– 30^0 C). After 7 days of fermentation the fermented juice was filtered through muslin cloth and the filtrate was kept in sterilized glass bottles. The filtrate fermented juice was microbiological analysis of yeast and LAB population was analyzed as per standard procedures.

Isolation of yeast and lactic acid bacteria

The lactic acid bacteria (K LAB) and yeast (K Sc) were isolated from dried kokum rind by following standard procedure. The isolate yeast and LAB labeled as K Sc and K LAB respectively.The pure cultures of yeast and lactic acid bacteria were obtained in the form of freeze dried ampoules and these cultures were revived using YEPDA and MRS media for yeast and lactic acid bacteria respectively and maintained in the form of agar slant cultures for further use.

Growth of different yeast and LAB strains on YEPD and MRS broth

The experiment was conducted to measure the growth rate of different yeast strains. The growth rate of the yeast and LAB strains was measured by inoculating a loop full of yeast culture from the slant to YEPD and MRS broth contained in 250 ml conical flask and incubated at 28^0 C and 30^0 C for 72 hours. The growth was determined by OD values taken at 24 hrs intervals 48 hrs, 72 hrs, at 600 nm.

Ethanol tolerance of different yeast strains

The experiment was conducted to evaluate the ethanol tolerance by different yeast strain at different ethanol concentration at 2.5, 5.0 and 7.5 per cent (v/v).

Microbiological analysis of yeast and Lactic acid bacteria

7 days of fermentation, the samples were subjected for microbiological analysis of yeast and lactic acid bacterial counts by employing standard dilution plate count method (Hoben and Somasegaran, 1982).10 ml of sample was diluted in 90 ml water blank (10^{-1}) then subsequent dilutions were made up to 10^{-4} dilution. 1 ml 10^3 and 10^4 dilutions of were used for plating. YEPDA and MRS media were poured into the Petri dishes containing respective dilutions of the different samples and rotate the media poured plates both clock and anti clock wise direction for uniform mixing of the sample

allowed to solidify. Later kept for incubation at 28-30^{0}C for yeast and LAB at 32-37^{0}C.

RESULTS AND DISCUSSION

Growth of different Yeast and LAB strains on YEPD and MRS broth

The results on the growth of different yeast strains on YEPD broth at 24 hrs intervals are presented in (Table 1, Fig 1&2). Highest OD value was recorded by the yeast strain (Y3) *Saccharomyces cerevisiae* UCD 522 (0.621, 0.850, 1.560 at 24, 48, and 72 hrs respectively) followed by yeast Y1 (MTCC 6008) (0.481, 0.510, 0.810 at 24, 48, and 72 hrs respectively), yeast strain MTCC 4780 (0.420, 0.480, 0.720 at 24, 48, and 72 hrs respectively) and least by kokum yeast isolates (0.400, 0.490, 0.690 at 24, 48, and 72 hrs respectively). The highest OD value was recorded *Lactobacillus plantarum* MTCC 6161 (0.90, 1.63, and 1.78 at 24, 48, and 72 hrs respectively) followed by *Lactobacillus brevis* MTCC 1750 (0.85, 1.25, 1.52 at 24, 48, and 72 hrs respectively) , kokum LAB isolate (0.72, 0.94, 1.75 at 24, 48, and 72 hrs respectively) and least OD value observed in case of *Streptococcus thermophillus* MTCC 1938 (0.54, 1.12, and 1.60 at 24, 48, and 72 hrs respectively).

The growth activity of different strains of yeast and lactic acid bacteria differs with genera and species of yeast and lactic acid bacterial strains. The maximum growth (1.56) was noticed with *Saccharomyces cerevisiae* UCD 522 (Y3). Whereas, Y_1 (MTCC 6008) showed minimum growth (0.81) at 72 hrs concurrence with the findings of Saigal (1994) who reported that growth and activity varies with isolates.

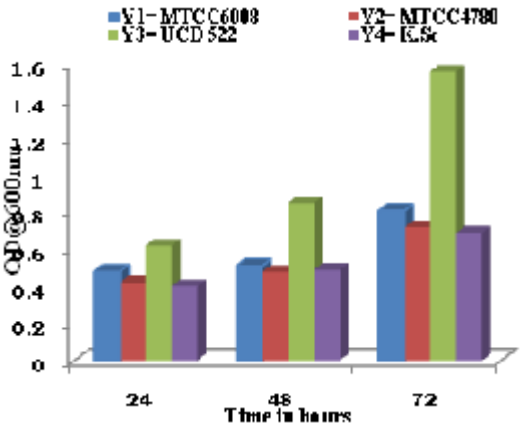

Fig. 1. Growth of yeast strains in YEPD broth

Table 1. Growth of different strains of yeast and lactic acid bacteria on YEPDA and MRS broth

Yeast and LAB Strains			Growth of yeast and LAB (OD at 600 nm)		
Yeast strains			At 24hrs	At 48 hrs	At 72hrs
	Y_1	MTCC 6008	0.481	0.510	0.810
	Y_2	MTCC 4780	0.420	0.480	0.720
	Y_3	UCD 522	0.621	0.850	1.560
	Y_4	K.Sc	0.400	0.490	0.690
LAB strains	L_1	MTCC 1938	0.54	1.12	1.60
	L_2	MTCC 1750	0.85	1.25	1.52
	L_3	MTCC 6161	0.90	1.63	1.78
	L_4	K.LAB	0.72	0.94	1.75

Table 2. Ethanol tolerance of different yeast strains

Yeast Strains		Ethanol tolerance at 600 nm					
		2.5%		5%		7.5%	
		24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Y_1	MTCC6008	0.78	0.81	0.54	0.77	0.26	0.32
Y_2	MTCC4780	0.72	0.80	0.62	0.79	0.23	0.38
Y_3	UCD 522	0.80	0.87	0.51	0.86	0.40	0.54
Y_4	K.Sc	0.55	0.6	0.61	0.67	0.10	0.11

The highest growth (1.78) in MRS broth was noticed with *Lactobacillus plantarum* MTCC-6161 (L3). Whereas, L_2 (MTCC 1750) showed least OD growth (1.52) at 72 hrs. These results in concurrence with the findings of Deepak (1994) who reported that growth and activity lactic acid bacteria varies with isolates and strains.

Ethanol tolerance of different yeast strains

The ethanol tolerance by different strains of yeast was studied the effect of initial added ethanol concentration at 2.5, 5.0 and 7.5 per cent (v/v) (Table 2 & Fig 3). The results showed that all the strains of yeast grown better at 2.5 per cent ethanol concentration, highest OD (600 nm) was recorded by yeast strain Y3 (UCD 522) (0.80, and 0.87 at 24 and 48 hrs respectively) followed by *Saccharomyces cerevisiae* MTCC 6008 (0.78 and 0.81 at 24 and 48 hrs respectively) by *Saccharomyces cerevisiae* MTCC 4780 (0.72 and 0.80 at 24 and 48 hrs respectively) least by kokum yeast isolates (0.55 and 0.60 at 24 and 48 hrs respectively).Ethanol is the end product of fermentation by yeast. Ethanol in higher concentration inhibited the growth of yeasts. Some strains tolerate the higher concentration of ethanol and showed their activity. In the present study, ethanol tolerance varies between the yeast strains. Yeast strain UCD 522 (Y3) showed maximum tolerance OD (0.87) at 48 hrs in 2.5 per cent alcohol concentration and less tolerance in presence of 5 per cent and 7.5 per cent ethanol. The least tolerance was recorded in isolate from kokum K Sc compare to other strains.

The ethanol tolerance capacity of yeast is mainly due to its unsaturated fatty acid content and also heat shock proteins produced in yeast cells. Ethanol inhibition is also linked to denaturation and inhibition of glycolytic and fermentative enzymes and modification of cell membranes (Bajaj *et al.,* 2001)

Biochemical analysis of kokum juice

The results of bio-chemical analysis juice of kokum juice are presented in the Table 3.

The pH of the kokum juice was 2.59 with a TSS content of (19^0 brix), titrable acidity of 0.90 per cent, reducing sugar of 14.05 per cent, total sugar of 15.45 per cent, non-reducing sugar of 1.40 per cent, and Vitamin-C of 73.0 mg/100m . 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. Kokum RTS beverage has been

Table 3. Biochemical properties of kokum

Particulars	Values
Ph	2.59
TSS(ÚBrix)	19.00
Titrable acidity (%)	0.90
Reducing sugar (%)	14.05
Total sugar (%)	15.45
Nonreducing sugar(%)	1.40
Vit-c(mg/100ml)	73.00
Pigment(mg/100ml)	6.20

Table 4. Yeast and Bacterial population in the fermented kokum juice

Yeast and LAB Strains			Population of Yeast Cfu.×10^3/ ml.	Population of LAB Cfu.×10^3/ ml.
Yeast strains	Y_1	MTCC 6008	0.22	0.00
	Y_2	MTCC 4780	1.20	0.00
	Y_3	UCD 522	1.80	0.16
	Y_4	K.Sc	1.50	0.00
LAB strains	L_1	MTCC 1938	0.50	2.10
	L_2	MTCC 1750	1.00	1.80
	L_3	MTCC 6161	0.00	1.50
	L_4	K.LAB	0.00	2.10

Note:
Y_1= Saccharomyces *cerevisiae* (MTCC 6008); L_1= Streptococcus *thermophillus* (MTCC 1938)
Y_2=*Saccharomyces cerevisiae* (MTCC 4780); L_2= Lactobacillus *brevis* (MTCC 1750)
Y_3= *Saccharomyces cerevisiae* (UCD 522); L_3= *Lactobacillus plantarum* (MTCC 6161)
Y_4= Isolated yeast from kokum (K.Sc); L_4= Isolated LAB from kokum (K.LAB)

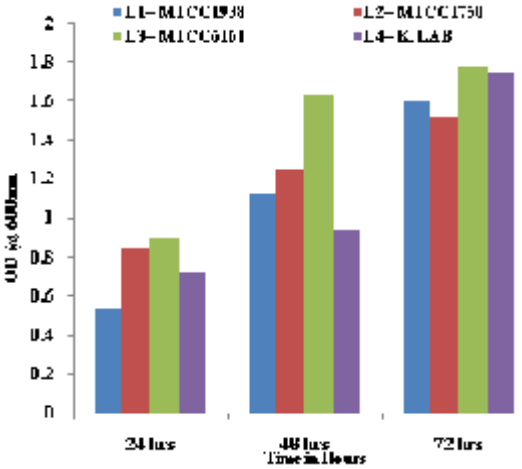

Fig. 2. Growth of Lactic acid bacteria MRS broth

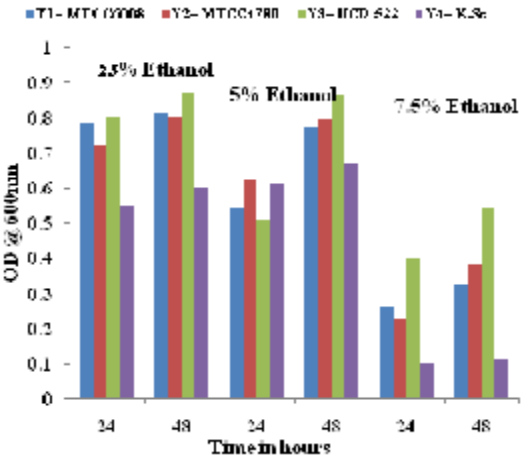

Fig. 3. Growth of yeast strains at different Ethanol levels

developed and very well accepted for consumption (Wasker 2000, Jagadeesh, 2006). In the present study, the kokum juice has been prepared by processing of dried kokum rind as per the standard procedure and biochemical characteristic of the kokum juice were studied and the same juice was used for the fermentation studies.

Population of yeast and LAB counts in the fermented kokum beverages

The yeast and lactic acid bacterial population was analyzed in the kokum juice fermented by different strains of yeast and bacteria after 7 days of fermentation and results are presented in Table 4. The initial inoculum of different yeast strains were added to the kokum juice at 10^7 cfu / ml and bacterial strains at 10^8 cfu/ ml to the kokum juice. After 7 days of fermentation, the population of yeast strains decreased from 10^7 to 10^3 cfu/ml, No. LAB population was detected in yeast inoculated treatments. Yeast population was greatly reduced in the kokum juice inoculated with LAB strains. The highest yeast population was recorded in the kokum juice fermented by yeast strain UCD 522 (1.80×10^3) and LAB strain MTCC 1750 (1.8×10^3) followed by MTCC 6161(1.5×10^3). The alcohol content in the fermented product along with low pH. No LAB population was detected in yeast inoculated treatments. Similarly, there was reduction of LAB strains in the fermented product which is also due to the low pH of the product. Yeast population was greatly reduced in the kokum juice inoculated with LAB strains. The highest yeast population was recorded in the kokum juice fermented by yeast strain UCD 522 (1.80×10^3) and LAB strain MTCC 1750 (1.8×10^3) followed by MTCC 6161(1.5×10^3) respectively. The significant reduction in population was mainly due to the production of higher acidity and pH, alcohol and carbon dioxide on completion of the fermentation by yeast isolates. These results are supported by the work of Pushpa Priya (2011) who reported that viable cell counts were reduced from $5x10^7$ to $3x10^2$ CFU/ml after fermentation in tomato fermented beverage juice.

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