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Fermentation of Blended Kokum Juice by Yeast and Lactic Acid Bacteria for Nutritional Improvement

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Kokum (*Garcinia indica* cv. *choicy*) 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. Fermentation of blended kokum juice by yeast *Saccharomyces cerevisiae* (UCD 522) and lactic acid bacteria *Lactobacillus planatarum* (MTCC 6161) was studied for nutritional improvement. The results revealed that the kokum juice blended with 5 per cent honey and 20 per cent sweet potato fermented by yeast and lactic acid bacteria recorded lowest total sugar (4.05%) and TSS (7.0° brix), titrable acidity (1.22%), vitamin C (290.0mg/100ml), alcohol (7.75%) and organoleptic score (13.50/20.0) when compared to other treatments. The results concluded that the microbial processing of kokum fruits in to alcoholic/non alcoholic fermented beverages can minimize the extent of post harvest losses in kokum fruits during glut season.

Key words: Kokum, Yeast, Lactic acid Bacteria, Fermentation, Honey, Sweet potato.

Fermentation is one of the oldest forms of food preservation technology in the world. Lactic acid fermentation is one of the oldest methods of preserving fruits and vegetables which contributes desirable physical and flavor characteristics. Fermentation is a potent tool in the development of new products with modified physical and chemical properties, improved sensory quality of flavor, nutrition besides therapeutic value. There are several reports on alcoholic beverages or wine preparation from different fruits like apple, plum, apricot, pomegranate, strawberry, guava, jamun, sapota, litchi, amla, orange, carambola etc (Sandhu *et al*., 1995; Joshi and Sharma, 1994; Adsule and Kadam, 1992; Joshi *et al*., 2005; Zeng *et al*., 2008). Lactic acid fermented beverage from kokum juice using reference strain of lactic acid bacteria *Lactobacillus acidophilus* has been developed (Dushyantha, *et al.*, 2010). Kokum juice fermented by yeast (*Saccharomyces ellipsoideus* No 101) is very good therapeutic agent and good antioxidant activity with 60.32 per cent (Dandin, 2010).

Kokum (*Garcinia indica*) is an important spice fruit which finds application in many culinary preparations. There is high potential for the development of fermented product which contains symbiotic effects from kokum fruits. Sugar content

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in kokum is low, and required to add an external source of sugar to facilitate the fermentative activity of both bacteria and yeasts. Therefore, raw materials rich in sugars and carbohydrates are blended to improve the quality parameters flavor, chemical characters and nutrient content through fermentation. Hence honey and sweet potato powders were used as source of sugar and carbohydrates to improve the quality of kokum juice.

Honey with fruits and vegetables gained lot of importance in improving nutrition and health. It is very rich in sugars and many medicinal properties, 5 per cent of honey is blending to kokum juice in the present study. Similarly, sweet potato is one of the important starchy food vegetable rich in carbohydrates (27 %), protein (1.5–2.0%), sugar (3–6%) and minerals. Hence, it was blended (20 %) with kokum juice to improve nutritional quantity of fermented kokum beverage.

MATERIALS AND METHODS

The Experiment was conducted at AICRP on Post Harvest Technology Scheme, UAS Bangalore during the year 2012 to evaluate the nutritional improvement upon fermentation of kokum juice. Kokum dried rind samples were collected from Madhu multiples, Puttur taluk, Uttara kannada district for the experimentation. It was processed into kokum juice the preparation of kokum juice done as per Wasker, 2002 and the same was used for evaluation of yeast and lactic acid bacterial strains for fermentation of kokum juice.

The treatments are T• = Kokum juice only; T , = Kokum juice + yeast; T*f* = Kokum juice + 5 % honey + yeast; T„ = Kokum juice + 20 % sweet potato + Yeast; T… = Kokum juice + 5 % honey +20 % sweet potato + Yeast; T_6 = Kokum juice + LAB; T_7= kokum juice +5 % honey + LAB; T_8 = Kokum juice + 20 % sweet potato + LAB; $T_{9=}$ Kokum juice +5 % honey + 20 % sweet potato + LAB; T_{10}= Kokum juice + yeast + LAB; T_{11}= Kokum juice + 5 % honey + yeast + LAB; T_{12}= Kokum juice + 20 % sweet potato + Yeast + LAB and T_{13}= Kokum juice + 5 % honey + 20 % sweet potato + Yeast + 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 subjected for biochemical pH TSS, titrable acidity, vitamin C, total sugars, reducing sugars, non reducing sugars, alcohol, and microbiological analysis of yeast and LAB population and organoleptic evaluation was analyzed as per standard procedures.

RESULTS AND DISCUSSION

Before blending of kokum juice, initial pH and TSS was 2.51 and 18^0 Brix respectively. Kokum juice blended with 20 per cent sweet potato increased the pH from 2.51 to 2.87; blending with only 5 per cent honey recorded 2.6 pH. Similarly the TSS showed variation due to blending, addition of sweet potato reduced the TSS value while addition of only honey increased TSS to 22^0B. Addition of honey and sweet potato increased the pH of kokum juice because of the neutralizer factor of sweet potato and honey which are slightly higher than kokum juice. The decrease in TSS by blending with sweet potato may be due to adsorption of solutes by potato slurry, but honey increased TSS by 4^0 Brix.

Fermentation of kokum juice by yeast for 7 days resulted in slightly decreases in pH with blending of honey (2.51) and sweet potato (2.68) compared to the initial pH level (Table 1). Similar effect was observed with mixed blending (2.64). Yeast fermentation of kokum juice with and without blending showed more decrease in p^H compared to LAB fermentation and combined inoculation. The kokum juice blending with 20 per cent sweet potato fermented by yeast recorded 2.68 from 2.87, similarly LAB fermentation of kokum juice blended with 20 per cent sweet potato recorded 2.73 from 2.87. LAB inoculation or combined inoculation did not much influence on decrease in pH of blended kokum juice. Bacterial fermentation also reduced pH slightly margin in kokum fermentation either alone or with the blending of honey and sweet potato. The slightly variation in pH among different treatments both by yeast and bacteria was due to the fermentation potential of the microorganisms fermentative activity of yeast only reduced the pH level compared to un inoculation, mainly due to conversion of sugar to alcohol and acids. The p^H level depends the acids and sugar content of the

Table 1. Combined effect of yeast and lactic acid bacterial strains on pH, TSS, titrable acidity (%), reducing sugar, total sugar, alcohol (%),Vitamin-C (mg/100ml) and Non reducing sugar (%) of fermented kokum juice blended with 5% honey and 20% sweet potato

Inoculants	Treatments	pH	TSS (°Brix)	Titrable acidity (%)	Reducing Sugar (%)	Total sugar (%)	Alcohol (%)	Vit-C (mg/ 100ml)	Non Reducing sugar (%)
Control	T_1	2.51^{ef}	18.00^{b}	0.90^{l}	11.45^{a}	16.25^{a}	0.00^{f}	80.4^{k}	4.80^{bc}
Yeast inoculation	T_2	2.47^{fg}	9.00^{f}	1.27^{j}	1.90^{fghi}	6.45^{d}	6.15^{ab}	290.5^{c}	4.55^{bc}
	T_3	2.51^{e}	10.50^{de}	1.28^{i}	1.85^{ghi}	5.90^{d}	5.80^{ab}	292.5^{b}	5.95^{a}
	T_4	2.68^{b}	7.00^{g}	1.22^{k}	1.35^{i}	5.90^{d}	7.75^{a}	290.0^{c}	$4.60b^{c}$
	T_5	2.64^{c}	9.50^{ef}	1.28^{i}	1.45^{hi}	4.05^{e}	7.25^{bc}	297.5^{a}	2.60^{d}
LAB inoculation	T_6	2.51^{e}	17.00^{b}	1.37^{h}	7.70^{b}	8.15^{c}	0.40^{ef}	176.5^{i}	0.45^{e}
	T_7	2.59^{d}	19.50^{a}	1.51^{g}	11.50^{a}	16.00^{a}	0.40^{ef}	182.7^{h}	4.50^{c}
	T_8	2.73^{a}	14.00^{c}	1.61^{f}	2.45^{efg}	7.95^{c}	0.80^{def}	172.5^{j}	5.50^{abc}
	T_9	2.63^{c}	11.50^{d}	1.64^{e}	6.60^{c}	7.95^{c}	3.40^{cde}	196.6^{e}	1.35^{e}
Co-inoculation	T_{10}	2.45^{g}	7.50^{g}	1.68^{d}	2.05^{fgh}	8.10^{c}	1.35^{def}	230.5^{d}	6.05^{a}
	T_{11}	2.51^{e}	9.50^{ef}	1.70^{c}	2.85^{e}	8.25^{c}	3.00^{bc}	192.5^{f}	5.40^{abc}
	T_{12}	2.70^{ab}	7.00^{g}	1.71^{b}	2.55^{ef}	8.15^{c}	6.50^{ab}	187.0^{g}	5.60^{ab}
	T_{13}	2.63^{c}	13.00^{c}	1.75^{a}	4.15^{d}	9.00^{b}	4.00^{bcd}	231.5^{d}	4.85^{bc}
	SEm±	0.00013	0.49	0.001	0.24	0.22	0.36	0.23	0.36
	CD (at 5 %)	0.03	1.44	0.01	0.69	0.64	1.05	1.41	1.05

Note: Sc = *Saccharomyces cerevisiae*, LAB = *Lactobacillus plantarum* , 5%H =Honey, 20% = Sweet potato

juice. These results supports the work of Yoon *et* al., (2004) in tomato juice fermentation by lactic acid bacteria results in reduction of pH from 6.5 to 4.1. Similarly, Priya *et al.* 2010 reported that tomato juice fermentation by yeast *Saccharomyces cerevisiae* 3283 strain resulted in reduction of pH from 4.4 to 3.1.

The yeast fermentation of blended kokum juice showed more reduction in TSS content compared to lactic acid fermentation. The kokum juice blended with 20 per cent sweet potato fermented by yeast (T4) showed maximum reduction in TSS (7.0^0 Brix) followed by yeast fermented kokum juice without blending (T2) recorded 9.0^0 Brix. Addition of both honey and sweet potato (T5) did not much influence much on reduction of TSS (9.56^0 Brix) (Table 1and Fig 1). The reduction in TSS content varies from 3.36^0 to 6.56^0 brix. The TSS content was slightly reduced in the kokum juice fermented by yeast, addition of honey and sweet potato slightly enhanced the TSS content in both yeast and lactic acid bacterial fermentation. This could be due to the fact that bacterial and yeast strains vary in their fermentation efficiency and sugar conversion capacity.

The combined fermentation of kokum juice with and without blending showed higher titrable acidity compared to single inoculation of yeast or LAB. Yeast fermentation increased the acidity of kokum juice from 0.90 to 1.28 per cent with a highest by blending honey alone or alone with sweet potato. LAB fermentation of blended kokum juice blending with honey and sweet potato (T9) showed maximum titrable acidity (1.64 %) compared to other treatments. Combined

Table 2. Population of yeast and lactic acid bacteria in fermented blended kokum juice at 7 and 30 Days of storage

Inoculant	Treatments	Yeast population (10^4cfu/ml)		LAB population (10^5cfu/ml)	
		7 days	30 days	7 days	30 days
	T_1	-	-	-	-
	T_2	0.9	0.4	ND	ND
Yeast inoculation	T_3	1.9	0.2	ND	ND
	T_4	4.2	2.5	ND	ND
	T_5	5.5	3.4	ND	ND
LAB inoculation	T_6	ND	ND	0.5	0.1
	T_7	ND	ND	4.0	1.4
	T_8	ND	ND	7.6	1.6
	T_9	ND	ND	8.0	1.6
Co-inoculation	T_{10}	2.2	0.1	1.2	0.1
	T_{11}	3.9	2.5	3.2	0.3
	T_{12}	7.6	0.2	1.2	0.1
	T_{13}	6.0	1.5	2.1	2.1

ND= Not determined

T1= Kokum juice only; T2 = Kokum juice + yeast; T3 = Kokum juice + 5 % honey + yeast; T4 = Kokum juice + 20 % sweet potato + Yeast; T5 = Kokum juice + 5 % honey +20 % sweet potato + Yeast; T6 = Kokum juice + LAB; T 7= kokum juice +5 % honey + LAB; T8 = Kokum juice + 20 % sweet potato + LAB; T9 = Kokum juice +5 % honey + 20 % sweet potato + LAB; T10= Kokum juice + yeast + LAB; T11= Kokum juice + 5 % honey + yeast + LAB; T12= Kokum juice + 20 % sweet potato + Yeast + LAB and T13= Kokum juice + 5 % honey + 20 % sweet potato + Yeast + LAB.

fermentation of blended kokum juice showed slight enhancement of titrable acidity. The highest titrable acidity (1.75 %) was recorded in the kokum juice blended with honey and sweet potato (T13) by combined inoculation of yeast and LAB The highest titrable acidity (1.28 %) was produced in the kokum juice blended with honey and fermented by yeast (T3) (Table 1and Fig 1). Yoon *et al.* (2006) developed a probiotic cabbage juice using lactic acid bacteria resulted in decreased amount of titrable acidity which was expressed as lactic acid.

Lacto-juice processed by lactic acid

Table 3. Organolyptic evaluation score of the blended fermented kokum beverage by yeast and lactic acid bacteria

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)
T_1	-	-	-	-	-	-	-					
T_2	1.00	1.50	1.00	0.50	0.25	0.75	1.50	1.00	1.00	1.00	1.0	10.50
T_3	1.25	1.25	1.25	0.75	1.00	1.00	1.00	0.50	1.00	1.00	1.0	11.00
T_4	1.00	1.50	1.00	1.00	0.50	0.25	0.25	0.50	0.50	0.50	0.5	7.50
T_5	1.50	1.50	1.25	1.00	0.50	1.00	1.50	0.75	1.50	1.50	1.5	13.50
T_6	1.25	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.0	8.25
T_7	1.25	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.0	8.25
T_8	1.25	1.00	1.00	1.00	1.00	1.00	0.50	0.50	0.50	1.50	1.0	10.25
T_9	1.50	1.50	1.50	1.00	0.75	1.00	1.50	1.00	1.50	1.25	1.5	14.00
T_{10}	1.50	1.50	1.50	0.50	0.50	1.00	1.50	1.00	1.25	1.00	1.0	12.25
T_{11}	1.50	1.50	1.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.5	13.00
T_{12}	1.00	1.00	1.00	1.00	1.00	1.00	1.25	1.00	1.00	1.00	1.5	11.75
T_{13}	1.50	1.75	1.50	1.00	1.00	1.00	1.50	1.00	1.75	1.25	1.5	14.75

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

fermentation brings changes in the beverage assortment for their high nutrient value, vitamins and minerals which are beneficial to human health. Blending with sweet potato at 20% and 5% honey with kokum juice produced higher acidity (1.64 %) by bacterial fermentation and was further enhanced when ferment with yeast. This indicated the availability of fermentable sugar favoring lactic acid bacteria. In sweet potato there is no significant variation in biochemical constituents. This was also in confirmity for *L. plantarum* fermentation with (Panda and Roy 2007)

The Kokum juice blended with honey and sweet potato (T5) showed highest vitamin C content (297.5 mg/100ml) followed by treatment T3 (292.5 mg/100ml) and others are not significantly differed indicating addition of honey or sweet potato did not influence much on vitamin C content (Table 1 and Fig 2). The vitamin-C concentration was higher in yeast fermentation than bacterial fermentation. Blending of honey or sweet potato did not enhance much the production of vitamin C in all the treatments studied. The combined inoculation yeast and bacteria or yeast alone increased vitamin C content of kokum juice 230.5 and 297.5 mg/100ml respectively.

The reducing sugar content varied from 1.35 to 11.5 per cent between the treatments and found to be significant each other (Table 1 Fig 2). The kokum juice blended with 20 per cent sweet potato fermented by yeast (T4) recorded lowest total sugar (1.35 %) indicating that addition of sweet potato helps in utilization of more sugar by yeast during fermentation. Kokum juice blended with 5 per cent honey fermented by LAB (T7) showed highest total sugar (11.50 %) indicating that not utilized more sugar by lactic acid bacteria during fermentation. Blending of both honey and sweet potato did not much influence on utilization of sugar by yeast or lactic acid bacteria during fermentation. The yeast cannot utilize 100 per cent sugar; some amount of sugar was left in the fermentation juice after fermentation process. The highest reducing sugar was observed in the blended juice fermented by lactic acid bacteria indicating sugar utilization efficiency is low when compared to yeast fermentation. The kokum juice blending with 5 % honey by yeast fermentation showed highest reducing sugar (11.5 %) followed by without blending (T6). Combined inoculation as well as blending material did not influenced much on utilization of sugar for alcohol conversion. This might be due to the presence of fructose in fruit and sugar utilization capacity of the strain and also increase in alcohol content of beverage inhibits activity of strains. Similar results were reported by in fermented kokum juice by Girish (2008).

Total sugar per cent varied from 4.05 to 16.0 per cent among the treatments and found to be significant each other. Kokum juice blended with

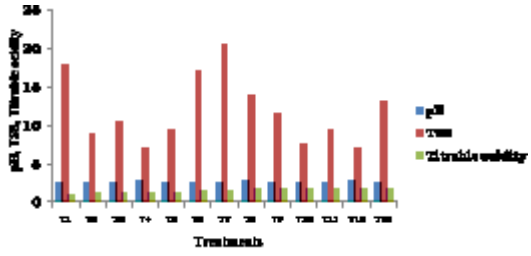

Fig 1. Combined effect of yeast and lactic acid bacterial strains for pH, TSS and Titrable acidityof kokum juice blended with 5% honey 20% sweet potato

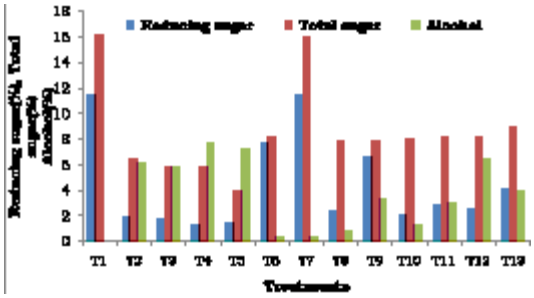

Fig. 2. Combined effect of yeast and lactic acid bacterial strain for reducing sugar, total sugar (%), alcohol (%), of kokum juice blended with5% honey and 20% sweet potato

Note:

T_1 = kj
T_2 = kj + Sc
T_3 = kj + 5% H+ Sc
T_4 = kj + 20 % SP + Sc
T_5 = kj + 5% H +20 % SP + Sc
T_6= kj + LAB
T_7= kj + 5% H + LAB
T_8= kj + 20% SP + LAB
T_9= kj + 5% H + 20 % SP + LAB
T_{10} = kj+ Sc + LAB
T_{11}= kj + 5 % H + Sc + LAB
T_{12}= kj + 20 % SP + Sc + LAB
T_{13}= kj + 5 % H + 20 % SP + Sc + LAB

Note: Sc = *Saccharomyces cervisiae*, LAB = *Lactobacillus plantarum* 5% H = Honey, 20% = Sweet potato

5 per cent honey and 20 per cent sweet potato fermented by yeast (T5) recorded lowest total sugar (4.05%) indicating that highest sugar was utilized by the yeast during fermentation (Table 1 and Fig 2). Kokum juice blended with 5 per cent honey fermented by LAB (T7) showed highest total sugar (16.0%) indicating utilization of small amount of sugar by lactic acid bacteria during fermentation. Blending of both honey and sweet potato did not much influence on utilization of sugar by yeast or lactic acid bacteria during fermentation. In bacteria fermentation treatment, there was reduction in total sugar from 16.25 per cent to a lowest of 7.95 per cent. The sugar utilization was significant in all the treatments with bacteria except with 5% honey.

Utilization of sugar is indicating of growth and fermentative efficiency of yeast and lactic acid bacteria. Low total sugar was due to high fermentative character of the yeast than lactic acid bacteria. Blending with sweet potato (20%) has further enhanced the yeast activity during fermentation. Lactic acid bacteria in kokum fermentation has reduced sugar utilization rate. However co-inoculation also recorded higher total sugar in the fermented product. Bacterial fermentation will have sweetness comparatively more than yeast fermentation which will be having a bearing on over all acceptability.

Yeast fermentation with increased fermentative activity and higher levels of sugar utilization produced maximum alcohol. Added advantage was with addition of 20 per cent sweet potato which produced highest alcohol (7.75 %). This may be due to higher release of sugar from carbohydrates of sweet potato by enzymatic activities of yeast (Table1 and Fig 2).

Bacterial production of alcohol was greatly reduced as there was limited fermentative activity as indicated by the higher residual sugar in final kokum fermented product. However, combined blending under co-inoculation increased alcoholic content to 6.5 per cent compared to other treatments. This result was in accordance with Ayoga (1999).

Population of yeast and lactic acid bacteria in blended kokum juice at 7^{th} day of fermentation and 30^{th} day storage is presented in (Table 2). Population of yeast on 7^{th} days was high in the range of 10^5 cfu/ml of the product in all treatments. Blending of kokum juice with sweet potato (T5) favored the multiplication of yeasts and was highest in combined blending (5.5×10^4 cfu/ml), similarly, the population density in combined-inoculated (T12) fermentation with a maximum population of 7.6×10^4 cfu/ml with sweet potato blending but, combined blending (T13) reduced the yeast population.

Increase in the yeast and bacterial population was mainly due to the blending materials. Sweet potato at 20 per cent has provided very good nutrients for the multiplication at 7^{th} day (Table 2). But further storage has reduced the population level mainly due to stabilization of the nutrient along with developed alcohol and acidity. Population of yeast on 7^{th} day was high in the range of 10^5 cfu/ml of the product in all treatments. Blending of kokum with sweet potato favoured the multiplication of yeasts and was highest in combined blending (5.5×10^4cfu/ml) similarly, the population density in co-inoculated fermentation with a highest population of 7.6×10^5 cfu/ml) with sweet potato blending but, combined blending reduced the yeast population. At 30^{th} day, that is about 23 days storage the population of yeast and lactic acid bacteria was very significantly reduce in all the treatments. These results supports the work of Pushpa Priya *et al.*, (2011) reported that viable cell counts were reduced from 5×10^7 to 3×10^2 cfu after fermentation in fermented tomato beverage.

Results of organoleptic evaluation (Table 3) showed that kokum juice blending with 20 per cent sweet potato and 5 per cent honey fermented by combined inoculation (T13) showed highest score (14.75 out of 20.0) followed by kokum juice blending with 20 per cent sweet potato and honey fermented by LAB (T9) with 14.0 out of 20 and followed by treatment T5 with (13.5 out of 20). The least score was recorded with juice without blending and single inoculation.

Organolyptic evalution of fermented kokum product indicated that in general, the product highly acceptance is good for combined inoculation, with blending of sweet potato at 20 per cent and honey at 5 per cent. Over all acceptability was also due to yeast ferment and LAB fermentation particularly with sweet potato blending. Sweet potato may be helping in modulating acidity as well as alcohol production

and creating sweetness for better acceptability (Table 3). Fermented beverage produced from kokum juice blending with sweet potato and honey fermented by yeast was found to be superior in good quality parameters like appearance, aroma, colour, flavor and general quality. These findings are similar to the results reported by Sapna *et al.*, (2002) in fermented alcoholic spice beverage and Chowdhury and Ray (2007) in jamun wine, Girish 2008 in kokum juice fermented by lactic acid bacteria.

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