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RESEARCH PAPER

Effect of T.S.S. and pH levels on chemical composition of kokum (*Garciniaindica*) must and it's fermentation

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SUMMARY : The present study focused on effect of T.S.S. and pH levels on chemical composition and fermentation of kokum must. In case of kokum must, the reducing sugars (6.12 to 8.56 %) showed increasing trend with increase in T.S.S. levels, while the anthocyanin (3655 to 2911.33 mg/100g) and tannin content (0.205 to 0.172 %) showed a decreasing trend. However, the acidity do not showed any increasing or decreasing trend with increase in T.S.S. levels of the must. Considering the effect of pH levels on must,the acidity (2.34 to 0.99%) showed a decreasing trend with increase in pH levels. However, reducing sugars, anthocyanin and tannin content did not show any increasing or decreasing trend with increase in pH levels of must. The interaction T_2P_3 showed better fermentation ability and hence this interaction can be used for preparing must for preparation of kokum wine.

KEY **W**ORDS : Kokum must, Fermentation, T.S.S., pH

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In India, kokum is grown widely in tropical rain forest of western ghat mainly Konkan region of Maharashtra, Goa, South Karnataka, Coorg, Wyanand, the lower slopes of Nilgiri hills and also in Andaman. In these areas, kokum is planted in the backyard or in orchards. In Konkan region of Maharashtra, it is planted in almost every household though the exact area is not known, it is estimated to be on the area of 1000 ha which produces 4,500 tonnes of kokum fruits (Anonymous, 2008).

The fresh kokum rind contains moisture 80 per cent, protein 1.92 per cent, crude fibre 14.28 per cent, total ash 2.57 per cent, tannin 2.85 per cent, pectin 5.71 per cent, starch 1 per cent, crude fat 10 per cent, acid (as hydroxy citric acid) 22.80 per cent, pigment 2.40 per cent, ascorbic acid 0.06 per cent, carbohydrades by difference 35 per cent (Anonymous, 2005).

Being fruit based fermented and undistilled product, wine contains most of the nutrients present in the original fruit juice. The nutritive value of wine is increased due to release of amino acids and other nutrients from yeast during fermentation. Fruit wines contain 8 to 11 per cent alcohol and 2 to 3 per cent sugar with energy value ranging between 70 and 90 Kcal per 100 ml.

The therapeutic properties of the kokum fruit have been described in traditional medicine Ayurveda. These include its usefulness to relieve sunstroke, very good appetizer, as a cardiotonic, for tumours and heart diseases. It is also known to contain hydroxy citric acid (HCA), a potential anti-obesity agent and fights cholesterol. The juice of ripe fruit have appealing red colour. It was therefore, thought to utilize ripe kokum fruits for wine preparation. By developing such technology the post harvest losses in kokum fruit can be reduced. This will also help to generate rural employment in addition to higher returns to the farmers. Hence, in the present investigation efforts were made to study the effect of T.S.S. and pH levels on chemical composition and fermentation of kokum must.

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EXPERIMENTAL METHODS

Well ripe, sound, healthy and disease free kokum fruits were collected from kokum trees present on the educational farm of Dr. BalasahebSawant Konkan KrishiVidyapeeth, Dapoli,Dist. Ratnagiri (M.S.). After washing fruits were cutted with kokum cutter machine, rind and seeds are separated. The juice was extracted from kokum rind using basket press. The juice was kept overnight under cold storage (12 ^{0}C) after treating with 0.1 per cent pectinase enzyme. Next dayclear juice was obtained by decanting and used for preparation of must.

For preparation of must a known volume of juice was taken and its T.S.S. was adjusted (*i.e.* 20, 25, 30, 35 and 40 ^{0}Brix) by addition of sugar while the pH was adjusted (*i.e.* 3.0, 3.5 and 4.0) by addition of calcium carbonate as per requirement. The juice was supplemented with 0.1 per cent diammonium hydrogen phosphate (DAHP) and potassium metabisulphite (KMS) equivalent to 30 ppm SO_2.

The must after pasteurization at 82 ^{0}C for 20 minutes was inoculated with yeast powder(*Saccharomyces cerevisiae*var. bayanus) at the rate of 0.3g/kg of must,after activating it in 10 times quantity of luke warm water and then must was kept for fermentation at room temperature. As the fermentation was not started in the P_1 and P_2 levels of pH with all T.S.S. levels 2nd inoculation was done in these interactions on 5th day of fermentation. Then on 11th day 3rd inoculation was done in P_1 level of pH with all T.S.S. levels as fermentation was not started in these interactions. The end of fermentation was indicated by the cessation of foaming and bubblingand even from constant T.S.S. recorded by the must during fermentation.The fermentation flasks were heated at 78^0 to 79 ^{0}C for 20 minutes to inactivate yeast to stop further fermentation.During this study the chemical composition of prepared must and changes in T.S.S. and pH during fermentation were studied.

The T.S.S. content was determined using Erma hand refractometer (A.O.A.C., 1975). The reducing sugar, titratable acidity, anthocyanin and tannin content were determined as per the procedures described by Ranganna (1977). The pH was measured by Systronics μpH system 361.

EXPERIMENTAL FINDINGS AND ANALYSIS

The results of the chemical composition of kokum must are presented in Table 1 to 2.The reducing sugars (6.12 to 8.56 %) showed increasing trend with increase in T.S.S. levels.It may be the impact of T.S.S. levels adjusted in must by addition of sugar. Results of this investigation are in agreement with the results obtained by Sapkal (2011) in ripeAlphonso mango must.While the anthocyanin (3655 to 2911.33 mg/100g) and tannin content (0.205 to 0.172 %) showed decreasing trends. Decrease in anthocyanin and tannin with increase in T.S.S.

Table 1 : Effect of T.S.S. and pH levels on reducing sugarand titratable acidity of kokum must

pH levels	Reducing sugar (%)						Titratable acidity (%)					
	T.S.S. levels											
	T_1 (20^{0}B)	T_2 (25^{0}B)	T_3 (30^{0}B)	T_4 (35^{0}B)	T_5 (40^{0}B)	Mean	T_1 (20^{0}B)	T_2 (25 ^{0}B)	T_3 (30^{0}B)	T_4 (35^{0}B)	T_5 (40 ^{0}B)	Mean
P_1(3.0)	5.78	7.53	8.45	8.56	8.96	7.86	2.56	2.24	2.88	1.92	2.08	2.34
P_2(3.5)	7.62	7.35	8.68	8.82	8.90	8.27	1.60	1.28	1.28	1.44	1.28	1.38
P_3(4.0)	4.96	6.72	7.37	7.65	7.81	6.90	0.78	1.12	0.96	1.28	0.80	0.99
Mean	6.12	7.20	8.17	8.34	8.56	7.68	1.65	1.55	1.71	1.55	1.39	1.57
			S.E. ±		C.D. at 1%			S.E. ±			C.D. at 1%	
T.S.S. levels (T)			0.020		0.083			0.020			0.083	
pH levels (P)			0.016		0.067			0.015			0.063	
Interactions (T×P)			0.035		0.146			0.034			0.142	

Table 2: Effect of T.S.S. and pH levels on anthocyanin and tannin of kokum must

pH levels	Anthocyanin (mg 100 g^{-1})						Tannin (%)					
	T.S.S. levels											
	T_1 (20 ^{0}B)	T_2 (25 ^{0}B)	T_3 (30 ^{0}B)	T_4 (35 ^{0}B)	T_5 (40 ^{0}B)	Mean	T_1 (20 ^{0}B)	T_2 (25 ^{0}B)	T_3 (30 ^{0}B)	T_4 (35 ^{0}B)	T_5 (40 ^{0}B)	Mean
P_1(3.0)	3825	3346	3281	3074	3013	3307.8	0.199	0.162	0.180	0.174	0.164	0.176
P_2(3.5)	3709	3218	3139	3093	2853	3202.4	0.211	0.195	0.176	0.180	0.176	0.188
P_3(4.0)	3431	3343	3335	3065	2868	3208.4	0.206	0.190	0.186	0.184	0.175	0.188
Mean	3655	3302.33	3251.67	3077.33	2911.33	3239.53	0.205	0.182	0.181	0.179	0.172	0.184
			S.E. ±		C.D. at 1%			S.E. ±			C.D. at 1%	
T.S.S. levels (T)			10.450		43.548			0.0015			0.0063	
pH levels (P)			8.094		33.730			0.0012			0.0050	
Interactions (T×P)			18.100		75.427			0.0026			0.0108	

level may be due to, dilution of native anthocyanin and tannin content of juice by addition of sugar in increasing order to maintain T.S.S. levels. However, the acidity did not show any increasing or decreasing trend with increase in T.S.S. levels of the must, similar results for acidity were reported by Jagtap (2010) in jamun must and More (2010) in karonda must.

Table 3: Changes in T.S.S. during fermentation (*Brix)

Treatments	T.S.S.(^{0}Brix)								
	Initial*	1st day	3rd day	5th day**	7th day	9th day	11th day***	13th day	15th day
T_1P_1	20.0	20.0	20.0	20.0**	20.0	20.0	20.0***	20.0	20.0
T_1P_2	20.0	20.0	20.0	19.0**	16.2	15.8	15.4	14.6	14.2
T_1P_3	20.0	20.0	10.0	9.8	9.6	9.4	9.0	9.0	-
T_2P_1	25.2	25.2	25.2	25.2**	25.2	25.2	25.2***	25.2	25.2
T_2P_2	25.0	25.0	25.0	25.0**	23.0	15.4	15.0	14.8	14.4
T_2P_3	25.0	25.0	11.4	10.8	10.6	10.4	10.2	10.2	-
T_3P_1	30.0	30.0	30.0	30.0**	30.0	30.0	30.0***	30.0	30.0
T_3P_2	30.2	30.2	30.2	23.4**	19.4	19.4	-	-	-
T_3P_3	30.0	30.0	16.6	16.0	15.8	15.6	15.4	15.4	-
T_4P_1	35.0	35.0	35.0	35.0**	35.0	35.0	35.0***	35.0	35.0
T_4P_2	35.0	35.0	35.0	29.0**	27.2	25.8	25.4	25.4	-
T_4P_3	35.2	35.2	23.6	23.0	22.6	22.4	22.2	22.2	-
T_5P_1	40.0	40.0	40.0	40.0**	40.0	40.0	40.0***	40.0	40.0
T_5P_2	40.0	40.0	40.0	40.0**	40.0	40.0	37.0	35.0	34.0
T_5P_3	40.0	40.0	30.4	28.8	28.4	28.2	28.2	-	-

*1st Inoculation **2nd Inoculation ***3rd Inoculation

Table 3 : Contd……..

Treatments	T.S.S. (^{0}Brix)										
	17th day	19th day	21st day	23rd day	25th day	27th day	29th day	31st day	33rd day	T.S.S. at the end of fermentation	Decrease in T.S.S. during fermentation
T_1P_1	20.0	20.0	20.0	19.8	19.6	19.6	-	-	-	19.6	0.4
T_1P_2	13.8	12.8	12.0	11.0	11.0	-	-	-	-	11.0	9.0
T_1P_3	-	-	-	-	-	-	-	-	-	9.0	11.0
Average										13.20	6.80
T_2P_1	25.2	25.2	25.0	25.0	21.0	19.0	18.8	18.4	18.4	18.4	6.6
T_2P_2	14.2	13.4	13.0	12.6	12.4	12.2	12.0	11.8	11.8	11.8	13.2
T_2P_3	-	-	-	-	-	-	-	-	-	10.2	14.8
Average										13.47	11.53
T_3P_1	30.0	30.0	30.0	28.8	23.6	23.2	23.0	22.8	22.8	22.8	7.2
T_3P_2	-	-	-	-	-	-	-	-	-	19.4	10.6
T_3P_3	-	-	-	-	-	-	-	-	-	15.4	14.6
Average										19.20	10.80
T_4P_1	35.0	35.0	35.0	35.0	35.0	-	-	-	-	35.0	0.0
T_4P_2	-	-	-	-	-	-	-	-	-	25.4	9.6
T_4P_3	-	-	-	-	-	-	-	-	-	22.2	12.8
Average										27.53	7.47
T_5P_1	40.0	40.0	40.0	40.0	40.0	-	-	-	-	40.0	0.0
T_5P_2	33.8	33.6	33.4	33.2	33.2	-	-	-	-	33.2	6.8
T_5P_3	-	-	-	-	-	-	-	-	-	28.2	11.8
Average										33.80	6.20

In case of pH levels, acidity (2.34 to 0.99%) showed a decreasing trend with increase in pH levels, it may be the impact of pH levels which were adjusted while preparing must. Similar results were reported by Roodagi (2010) in pineapple must. However, reducing sugar, anthocyanin and tannin content did not show any increasing or decreasing trend with increase in pH levels of must.No trend in reducing sugarmay be due to conversion of added sucrose into reducing sugars in varying

Table 4: Changes in pH during fermentation

Treatments	pH								
	Initial*	1^{st} day	3^{rd} day	5^{th} day**	7^{th} day	9^{th} day	11^{th} day***	13^{th} day	15^{th} day
T_1P_1	3.01	2.98	2.95	3.00**	2.98	2.96	2.94***	2.91	2.92
T_1P_2	3.54	3.70	3.69	3.66**	3.63	3.65	3.65	3.65	3.65
T_1P_3	4.00	4.10	3.85	3.90	3.98	3.89	3.86	3.88	-
T_2P_1	3.0	2.78	2.76	2.78**	2.77	2.74	2.73***	2.71	2.72
T_2P_2	3.50	3.52	3.53	3.51**	3.53	3.47	3.48	3.48	3.49
T_2P_3	4.00	4.21	3.89	4.00	4.01	3.94	3.91	3.93	-
T_3P_1	3.01	3.00	3.00	2.98**	2.99	2.99	2.95***	2.94	2.94
T_3P_2	3.50	3.64	3.63	3.56**	3.56	3.61	-	-	-
T_3P_3	4.00	4.30	4.01	4.03	4.01	4.05	4.01	4.03	-
T_4P_1	3.04	3.15	3.12	3.09**	3.06	3.12	3.11***	3.11	3.12
T_4P_2	3.54	3.78	3.76	3.70**	3.68	3.74	3.74	3.73	-
T_4P_3	4.00	4.30	4.04	4.07	4.07	4.09	4.06	4.08	-
T_5P_1	3.04	3.00	2.98	2.97**	2.96	2.96	2.98***	2.95	2.95
T_5P_2	3.53	3.77	3.73	3.76**	3.75	3.75	3.75	3.75	3.77
T_5P_3	4.00	4.50	4.13	4.15	4.09	4.18	4.14	-	-

Table 4 : Contd……..

Treatments	pH									
	17^{th} day	19^{th} day	21^{st} day	23^{rd} day	25^{th} day	27^{th} day	29^{th}day	31^{st} day	33^{rd} day	pH at the end of fermentation
T_1P_1	2.91	2.92	2.88	2.88	2.86	2.86	-	-	-	2.86
T_1P_2	3.65	3.64	3.58	3.58	3.59	-	-	-	-	3.59
T_1P_3	-	-	-	-	-	-	-	-	-	3.88
Average										3.44
T_2P_1	2.73	2.74	2.69	2.69	2.68	2.72	2.67	2.68	2.71	2.71
T_2P_2	3.50	3.49	3.42	3.40	3.39	3.37	3.34	3.35	3.37	3.37
T_2P_3	-	-	-	-	-	-	-	-	-	3.93
Average										3.34
T_3P_1	2.93	2.92	2.89	2.86	2.91	2.93	2.91	2.93	2.95	2.95
T_3P_2	-	-	-	-	-	-	-	-	-	3.61
T_3P_3	-	-	-	-	-	-	-	-	-	4.03
Average										3.53
T_4P_1	3.10	3.10	3.00	3.02	3.02	-	-	-	-	3.02
T_4P_2	-	-	-	-	-	-	-	-	-	3.73
T_4P_3	-	-	-	-	-	-	-	-	-	4.08
Average										3.61
T_5P_1	2.96	2.97	2.90	2.89	2.90	-	-	-	-	2.90
T_5P_2	3.77	3.78	3.70	3.69	3.71	-	-	-	-	3.71
T_5P_3	-	-	-	-	-	-	-	-	-	4.14
Average										3.58

degree at different pH levels.Results analogous to present findings are reported byMore (2010) in karonda must with different pH levels.

The highest reducing sugar (8.96%) was recorded in the interaction T_5P_1 and it was at par with T_4P_2 (8.82%) and T_5P_2 (8.90%). Considering the titratable acidity, the interaction T_3P_1 recorded significantly highest (2.88%) titratable acidity. The highest anthocyanin content was observed in the interaction T_1P_1 (3825 mg/100gm) and the lowest tannin content was recorded in the interaction T_2P_1 (0.162%) which was at par with T_5P_1 (0.164%).

The T.S.S. of the must was found to be decreased till the end of fermentation, except T_1P_1, T_4P_1 and T_5P_1. The decrease in T.S.S. during fermentation may be due to conversion of sugars into alcohol by yeast. The yeast convert the sugars into alcohol by forming enzymes,pyruvic decarboxylase andalcohol dehydrogenase. These results are in agreement with the results obtained by Patil (1994) in grape andPawar (2009) in sapota must during fermentation.From the average figures of T.S.S. it was observed that the T.S.S. content of fermented must increased with increase in T.S.S. levels, irrespective of pH levels. This increase in T.S.S. with increase in T.S.S. level may be the impact of increase in osmatic pressure due to more addition of sugar to maintain T.S.S. levels, which adversely affected the fermentation process by hindering the growth and activity of yeast. Present findings are in conformity with the findings of More (2010) in karonda must. Among different T.S.S. levels, treatment T_2 recorded more reduction in T.S.S. (11.53 ^{0}B), followed by T_3(10.80 ^{0}B), irrespective of pH levels. In case of interactions of T.S.S. and pH levels, the interaction T_2P_3 recorded more reduction in T.S.S. (14.8 ^{0}B), followed by T_3P_3 and T_4P_3 (Table 3).

The pH of must at the end of fermentation showed increasing trend with increase in pH levels in all the T.S.S. levels. This may be the impact of original adjustment of pH levels. Similar findings have been reported by Roodagi (2010) in pineapple must.From the average values of pH at the end of fermentation, no specific trend of pH with respect to T.S.S. levels was observed. Similar findings have been reported by More (2010) in karonda must. In case of all the interactions studied, interaction T_2P_1(2.71) recorded minimum pH followed by T_1P_1, T_5P_1, T_3P_1, and T_4P_1 (Table 1).

Conclusion :

From the present study it was observed that interaction T_2P_3(25 ^{0}B T.S.S. and 4.0 pH) recorded maximum reduction in T.S.S. (14.8 ^{0}B) during fermentation with 3.93 pH of fermented must. Even the must prepared with this interaction showed satisfactory chemical composition. Hence, this interaction can be used to prepare must for quality wine production from kokum fruits.

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