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Original Research Article

Effect of Different Dilution and pH Levels on Chemical Composition and Fermentation of Kokum Must

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Keywords

Kokum must, Dilution levels, pH levels, Fermentation

A B S T R A C T

An investigation was carried out to study the effect of dilution of kokum juice (1:0, 1:0.5. 1:1.0, 1:1.5 and 1:2) and pH levels (3.5 and 4.0) on chemical composition and fermentation of kokum must. From the study it was observed that reducing sugar, titratable acidity, anthocyanin and tannin content of kokum must decreased, while total sugars increased with increase in dilution level. Total sugars and tannins showed increasing trend with increase in pH levels while the titratable acidity, reducing sugars and anthocyanin showed a decreasing trend in must. During fermentation T.S.S. and pH found to be decreased throughout the fermentation period and titratable acidity was increased. The yeast count increased rapidly upto the 3rd day and later on it showed decreasing trend. Among the ten interactions of dilution and pH levels, interaction D_4P_2 recorded better reduction in T.S.S. i.e. better fermentation of must to prepare wine.

Introduction

Kokum *(Garcinia indica* Choisy) a tropical fruit, is a native of India can be viewed as a wonder berry that has a pleasant, tangy-sweet taste and a myriad of health benefits. It is mostly found in Konkan region of Maharashtra, Goa, Karnataka, Kerala and Surat district of Gujarat on the West Coast of India and to some extent in the forests of Assam, Meghalaya, and West Bengal. As per a base line survey in 2010, kokum is grown on about 1000 ha area in the Konkan region with production of about 4500 MT fruits (Annon., 2012). It is seen from the base line survey 2014, harvesting of kokum fruit coincides in Western Ghat with the monsoon and more than 70 per cent of harvesting trapped in heavy rains and hence lost (Annon., 2014). Majority of yield in kokum used only for syrup and juice preparation during summer months, some parts are dried and stored. Remaining part is not harvested and goes as waste. So it is great economic loss. It can be used to produce fermented beverages like wine. Fruit wines are undistilled alcoholic beverages which are nutritive, more tasty and mild stimulants (Darby, 1979). Being fruit based fermented and undistilled product, wine contains most of the nutrients present in the original fruit juice. The kokum fruits can be used for the manufacture of wine and liquor and could be a good substitute of grapes in the wine industry. The juice of ripe fruit is appearing red colour. It was therefore, thought to utilize kokum fruits for wine preparation. The kokum juice is having dark colour and more acidity, hence in order to reduce colour and acidity of wine and to get good amount of quality wine with light alcohol, the wines were prepared from kokum juice by diluting the juice and adjusting the pH levels of must. The efforts were made to study the effect of dilution and pH levels on chemical composition and fermentation of kokum must.

Materials and Methods

The present investigation was conducted during the period April 2015 to July 2015 at Pomology laboratory and Fruit processing unit of Department of Horticulture, College of Agriculture, Dapoli and Fruit Beverage Research Centre of Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri. The experiment was conducted with five dilution levels D_1 to D_5 (1:0, 1:0.5. 1:1.0, 1:1.5 and 1:2) and two pH levels P_1 and P_2 (3.5 and 4.0) in factorial completely randomized design. For this study, well ripe, sound, healthy and disease free kokum fruits were collected from kokum tree present on educational farm of the university. Juice was extracted from fruits and then kept overnight under cold storage (12 ± 1^0C). Next day clear juice was obtained by decanting and used for preparation of must by diluting the juice as per the treatments with addition of distilled water and T.S.S. of each treatment was adjusted to 25^0Brix by addition of powdered sugar. Then the pH of must was adjusted to two different levels i.e. 3.5 and 4.0 as per the treatments by addition of calcium carbonate. After adjustment of T.S.S. and pH, must was transferred to conical flasks (fermentation assembly) separately. The must was supplemented with 0.1 per cent diammonium hydrogen phosphate (DAHP) and 52 mg/kg potassium metabisulphide (KMS). Two hours after addition of KMS the must was inoculated with yeast culture (*Saccharomyces cerevisiae* var. bayanus- red wine yeast) @ 0.30 g/kg and kept for fermentation at room temperature. The end of fermentation was indicated by the cessation of foaming and bubbling and even constant T.S.S. recorded by must during fermentation. During this study the chemical composition of must and changes in total soluble solids, pH, titratable acidity and viable yeast count during fermentation were studied. The TSS content of kokum must was determined with the help of Hand refractometer (Erma Japan, 0 to 32^oBrix) and value was corrected at 20°C with the help of temperature correction chart (A.O.A.C., 1975). The pH of the must was determined with the help of pH meter (Model Systronics µ pH system 361). The titratable acidity, reducing sugars, total sugars, anthocyanin and tannins were estimated as per the methods suggested by Ranganna (1977). The viable yeast count in the fermenting must was taken at alternate day till the end of fermentation by aseptic serial dilution and plate technique on MGYP media as per the method described by Ranganna (1977).

Results and Discussions

The fresh kokum juice used for preparation of must contained T.S.S., reducing sugars, total sugars, titratable acidity, pH, anthocyanin and tannins, 13.45^oB, 5.23 per cent, 7.56 per cent, 4.8 per cent, 2.42, 3380 mg/100g, 1.73 per cent, respectively.

Chemical composition of kokum must

Effect of dilution and pH levels on chemical composition of must is presented in Table 1 and 2. With increase in dilution level, reducing sugars (9.47 to 6.55 %), titratable acidity (0.87 to 0.35 %), anthocyanin (3290 to 1405 mg/100g) and tannin content (1.34 to 0.58 %) of kokum must decreased, while total sugars increased with increase in dilution level (16.04 to 20.25%). Decrease in reducing sugars, titratable acidity, anthocyanin and tannins with increase in dilution level may be the impact of dilution of juice before fermentation. The increase in total sugars with increase in dilution may be due to addition of sugars in increasing amount with increase in dilution level to maintain the T.S.S. level (25^0B) in the must. Results of this investigation are in agreement with the results obtained by Sapkal (2011) in ripe mango must. With respect to pH levels, total sugars (18.08 to 18.88 %) and tannins (0.94 to 1.06 %) showed increasing trend with increase in pH levels while the titratable acidity (1.0 to 0.32 %), reducing sugars (8.18 to 6.90) and anthocyanin (2348 to 2050 mg/100g) showed a decreasing trend in must. The decrease in acidity with increase in pH may be the impact of pH levels which are adjusted while preparing must. Similar results were found by Fernandes (2010) in cashew apple must and Sarkale (2012) in kokum must. While considering interactions, maximum reducing sugars were recorded by D_1P_1 (10 %) while, D_5P_1 recorded maximum total sugars (20.33 %) and D_5P_2 (20.16 %) was at par with it. Considering the titratable acidity the interaction D_1P_1 recorded significantly higher (1.28 %) titratable acidity. The highest anthocyanin content was observed in the treatment D_1P_1 (3350 mg / 100 g) which was at par with D_1P_2 (3230 mg / 100 g) and the lowest tannin content was recorded in the treatment D_5P_1 (0.53 %) which was at par with D_4P_1 and D_5P_2.

Changes in chemical composition and yeast count during fermentation of kokum must

The data on changes in TSS during fermentation is presented in Table 3 (a) and (b). During fermentation of kokum must, T.S.S. was found to decrease throughout the fermentation period. A rapid decrease in T.S.S. was observed during initial three days of fermentation and later on it decreased at slower rate till the end of fermentation. The T.S.S. of must was found to be decreased with increase in dilution levels from dilution level D_1 to D_4 and thereafter increased at D_5 irrespective of pH levels at the end of fermentation. Among the pH levels the T.S.S. of the fermented must was found to be decreased with increase in pH levels from P_1 to P_2. In case of interactions, D_4P_2 recorded more reduction in T.S.S. The sharp decreased in T.S.S. in the initial period of fermentation may be due to faster conversion of sugars into alcohol which is available in maximum amount in the beginning of fermentation. The yeast converts the sugars into alcohol by forming enzymes, pyruvic decarboxylase and alcohol dehydrogenase. Faster rate of fermentation at initial period also may be due to low alcohol levels in the beginning of fermentation. The fermentation rate declines later due to increased quantity of alcohol exerting effect on fermentation process by hindering the activity of yeast. These results are in agreement with the results obtained by Patil (1994) in grape must during fermentation. The data regarding T.S.S. content at the end of fermentation of must is given in Table 3 (b). The T.S.S. of must was found to be decreased with increase in dilution levels from D_1 (9.7^0B) to D_4 (8.4^0B) and thereafter increased at D_5 (8.95^0B), irrespective of pH levels. Maximum decrease in T.S.S. during fermentation was 16.60^0B at D_4 dilution level, irrespective of pH levels. Among the pH levels the T.S.S. of the fermented must was found to be decreased with increase in pH levels from P_1 (9.62^0B) to P_2 (8.36^0B). This indicates that there was better fermentation by yeast at pH 4.0 and reduction in T.S.S. was 16.62^0B. In case of interactions of dilution and pH levels the interaction D_4P_2 recorded more reduction in T.S.S. (17.74^0B) closely followed by D_3P_2 (16.80^0B), D_5P_2 (16.60^0B) and D_2P_2 (16.40^0B).

Table.1 Effect of dilution and pH levels on reducing sugars, total sugars and titratable acidity of kokum must

Reducing sugars (%)			
Dilution levels	pH levels		
	P_1	P_2	Mean
D_1	10.00	8.93	**9.47**
D_2	8.10	7.80	**7.95**
D_3	6.94	6.41	**6.68**
D_4	8.93	5.20	**7.07**
D_5	6.94	6.15	**6.55**
Mean	**8.18**	**6.90**	**7.54**
	S.Em ±	C.D. at 1%	
Dilution levels (D)	0.045	0.182	
pH levels (P)	0.029	0.115	
Interaction (D x P)	0.064	0.257	

Total sugars (%)			
Dilution levels	pH levels		
	P_1	P_2	Mean
D_1	15.63	16.45	**16.04**
D_2	16.13	18.30	**17.22**
D_3	18.66	19.69	**19.18**
D_4	19.66	19.80	**19.73**
D_5	20.33	20.16	**20.25**
Mean	**18.08**	**18.88**	**18.48**
	S.Em ±	C.D. at 1%	
Dilution levels (D)	0.077	0.308	
pH levels (P)	0.048	0.195	
Interaction (D x P)	0.108	0.436	

Titratable acidity (%)			
Dilution levels	pH levels		
	P_1	P_2	Mean
D_1	1.28	0.45	**0.87**
D_2	1.22	0.38	**0.80**
D_3	1.15	0.32	**0.74**
D_4	0.83	0.25	**0.54**
D_5	0.51	0.19	**0.35**
Mean	**1.00**	**0.32**	**0.66**
	S.Em ±	C.D. at 1%	
Dilution levels (D)	0.010	0.041	
pH levels (P)	0.006	0.026	
Interaction (D x P)	0.014	0.057	

D : Dilution levels (Juice : Water) D_1 - 1.0 : 0.0 D_2 - 1.0 : 0.5 D_3 - 1.0 : 1.0 D_4 - 1.0 : 1.5 D_5 - 1.0 : 2.0

P : pH levels P_1 - 3.5 P_2 - 4.0 **NS :** Non-significant

Table.2 Effect of dilution and pH levels on anthocyanin and tannins of kokum must

Anthocyanin (mg / 100 g)				Tannins (%)			
Dilution levels	**pH levels**			**Dilution levels**	**pH levels**		
	P_1	**P_2**	**Mean**		**P_1**	**P_2**	**Mean**
D_1	3350.00	3230.00	3290.00	**D_1**	1.37	1.31	**1.34**
D_2	2810.00	2110.00	2460.00	**D_2**	1.18	1.22	**1.20**
D_3	2530.00	2020.00	2275.00	**D_3**	1.02	1.16	**1.09**
D_4	1610.00	1520.00	1565.00	**D_4**	0.61	0.97	**0.79**
D_5	1440.00	1370.00	1405.00	**D_5**	0.53	0.62	**0.58**
Mean	2348.00	2050.00	2199.00	**Mean**	**0.94**	**1.06**	**1.00**
	S.Em ±	**C.D. at 1%**			**S.Em ±**	**C.D. at 1%**	
Dilution levels (D)	42.485	170.957		**Dilution levels (D)**	0.018	0.072	
pH levels (P)	26.870	108.123		**pH levels (P)**	0.011	0.046	
Interaction (D x P)	60.083	241.770		**Interaction (D x P)**	0.025	0.102	

D : Dilution levels (Juice : Water) D_1 - 1.0 : 0.0 D_2 - 1.0 : 0.5 D_3 - 1.0 : 1.0 D_4 - 1.0 : 1.5 D_5 - 1.0 : 2.0

P : pH levels P_1 - 3.5 P_2 - 4.0 **NS :** Non-significant

Table.3 (a) Changes in T.S.S. (^{0}Brix) during fermentation of the must

Interactions	0 Day	1st Day	3rd Day	5th Day	7th Day	9th Day	11th Day	13th Day	15th Day
D_1P_1	25.20	23.4	15.0	12.0	11.4	11.2	10.6	10.6	10.4
D_1P_2	24.90	21.0	12.8	10.3	9.8	9.6	9.4	9.0	9.0
D_2P_1	24.80	23.0	14.8	10.6	11.0	10.4	10.0	9.6	9.6
D_2P_2	25.00	21.8	12.0	10.1	9.2	8.8	8.7	8.6	8.6
D_3P_1	24.90	22.4	14.8	11.6	11.0	10.0	9.8	9.6	9.4
D_3P_2	25.00	20.2	11.8	9.7	8.9	8.5	8.4	8.2	8.2
D_4P_1	25.00	21.8	15.6	11.4	11.2	10.4	9.8	9.4	9.2
D_4P_2	25.00	21.0	12.0	9.4	8.2	8.0	7.7	7.6	7.6
D_5P_1	24.90	22.0	15.0	11.2	10.6	9.9	9.5	9.4	9.5
D_5P_2	25.00	22.0	14.2	10.0	9.8	9.0	8.6	8.4	8.4

Table.3(b) Effect of dilution and pH levels on T.S.S. of fermented must (at the end of fermentation)

T.S.S.(^{0}Brix)						
pH Levels	Dilution Levels					
	D_1	D_2	D_3	D_4	D_5	Mean
P_1	10.4 (14.80)	9.6 (15.20)	9.4 (15.50)	9.2 (15.80)	9.5 (15.40)	**9.62 (15.34)**
P_2	9.0 (15.20)	8.6 (16.40)	8.2 (16.80)	7.6 (17.40)	8.4 (16.60)	**8.36 (16.62)**
Mean	**9.7 (15.35)**	**9.1 (15.80)**	**8.8 (16.15)**	**8.4 (16.60)**	**8.95 (16.00)**	**8.99 (15.98)**

Table.4 Changes in titratable acidity (%) during fermentation of the must

Interactions	0 Day	1st Day	3rd Day	5th Day	7th Day	9th Day	11th Day	13th Day	15th Day
D_1P_1	1.28	2.02	1.89	1.92	2.24	1.92	1.76	1.53	1.41
D_1P_2	0.45	0.60	0.57	0.65	0.78	0.77	0.75	0.72	0.71
								Average	**1.06**
D_2P_1	1.22	1.50	1.31	1.47	1.57	1.54	1.44	1.44	1.32
D2P2	0.38	0.58	0.54	0.64	0.70	0.68	0.67	0.70	0.69
								Average	**1.01**
D_3P_1	1.15	1.18	1.15	1.28	1.31	1.34	1.31	1.31	1.22
D_3P_2	0.32	0.54	0.48	0.48	0.58	0.61	0.67	0.62	0.60
								Average	**0.91**
D_4P_1	0.83	0.93	0.83	0.90	1.06	1.12	1.06	0.99	0.93
D_4P_2	0.25	0.42	0.40	0.48	0.55	0.54	0.58	0.55	0.53
								Average	**0.73**
D_5P_1	0.51	0.93	0.80	0.80	0.96	1.02	0.96	0.96	0.91
D_5P_2	0.19	0.40	0.38	0.48	0.51	0.45	0.51	0.50	0.46
								Average	**0.69**

Table.5 Changes in PH during fermentation of the must

Interactions	0 Day	1st Day	3rd Day	5th Day	7th Day	9th Day	11th Day	13th Day	15th Day
D_1P_1	3.48	3.32	3.35	3.30	3.29	3.31	3.34	3.42	3.42
D_1P_2	4.00	3.90	3.93	3.90	3.87	3.91	3.93	3.95	3.95
								Average	**3.69**
D_2P_1	3.50	3.35	3.41	3.36	3.33	3.36	3.40	3.42	3.43
D_2P_2	3.98	3.91	3.96	3.92	3.90	3.93	3.97	3.96	3.96
								Average	**3.70**
D_3P_1	3.50	3.37	3.47	3.40	3.35	3.33	3.42	3.49	3.49
D_3P_2	4.00	3.95	3.96	3.95	3.92	3.91	3.91	3.98	3.98
								Average	**3.74**
D_4P_1	3.55	3.38	3.55	3.44	3.41	3.39	3.46	3.54	3.53
D_4P_2	4.06	3.97	3.98	3.95	3.92	3.94	3.93	4.00	4.00
								Average	**3.77**
D_5P_1	3.56	3.41	3.57	3.54	3.48	3.45	3.57	3.55	3.55
D_5P_2	4.16	4.04	4.06	4.01	3.98	4.10	4.08	4.09	4.09
								Average	**3.82**

Table.6 Changes in yeast count (colonies × 10^4/ml) during fermentation of the must

Treatment	0 Day	1st Day	3rd Day	5th Day	7th Day	9th Day	11^{th} Day	13^{th} Day	15^{th} Day
D_1P_1	0.00	505	638	690	723	347	304	28	16
D_1P_2	0.00	219	705	632	541	237	200	41	15
$D2P_1$	0.00	328	1684	1085	991	668	632	29	23
D_2P_2	0.00	347	778	649	480	292	214	16	13
D_3P_1	0.00	717	1082	890	729	318	298	26	3
D_3P_2	0.00	851	1763	1040	985	371	292	15	3
D_4P_1	0.00	967	1010	600	219	188	134	63	5
D_4P_2	0.00	784	1295	1188	1163	289	261	26	5
D_5P_1	0.00	517	571	481	413	359	274	26	6
D_5P_2	0.00	626	997	688	595	501	426	46	20

Except few fluctuations, titratable acidity of must was found to be increased during fermentation (Table 4). The increased in titratable acidity during fermentation may be due to formation of lactic, acetic and succinic acid due to alcoholic fermentation. Also bacterial involvement might have produced lactic or acetic acid. Increase in acidity during fermentation was also reported by Roodagi (2010) in pineapple wine. Titratable acidity was decreased with increase in dilution level, irrespective of pH levels. While considering pH levels also the titratable acidity was found to be decreased with increase in pH levels at the end of fermentation. In case of interactions highest acidity was recorded by interaction D_1P_1 (1.41%) and lowest by D_5P_2 (0.46%).

It is observed from Table 5 that, at the end of fermentation, pH of the must was decreased during fermentation. This decrease in pH may be due to formation of different acids during fermentation. Observations analogous to these findings were reported by Sarkale (2012) in kokum must during fermentation. The pH of the different dilution levels recorded increasing trend with increase in dilution level, irrespective of pH levels. This increase in pH with increase in dilution may be due to decrease in acidity due to dilution of juice. Similar results were also reported by Pawar (2009) in sapota and Sapkal (2011) in mango. Among the interactions the lowest pH at the end of fermentation was recorded by interaction D_1P_1 (3.42) and highest was recorded by D_5P_2 (4.09).

The yeast count increases rapidly on the very first day of fermentation while it tends to increase at an alarming rate up to the third day (Table 6). Later on the number of colonies decreased till the end of fermentation. The maximum yeast count was observed in D_3P_2 (1763 × 10^4 colonies / ml) at the third day of fermentation. These results are in agreement with the results obtained by Patil (1994) in grape and Pawar (2009) in sapota must during fermentation.

In conclusion, looking to the chemical composition of must and reduction in T.S.S. during fermentation interaction D_4P_2 found to be good followed by D_3P_2, D_5P_2 and D_2P_2.

References

Anonymous (2012). Resource Book on Kokum (*Garcinia indica* Choisy) Western Ghats Kokum Foundation, Panaji – Goa.

Anonymous (2014). NAIP proposal for project “A value chain for kokum, karonda, jamun and jackfruit” Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. pp: 20.

Darby, W. J. (1979). The nutrient contribution of fermented beverages. In, fermented food beverages in nutrition (Eds. Gastineau, C. F., Darby, W. J. and Turner, T. B.) Academic Press Pub., New York. pp. 179.

Fernandes, B. L. (2010). Effect of variety and pH on quality of cashew apple wine. A M.Sc. (Agri.) thesis (Unpublished) submitted to Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.).

O. A. C., (1975). Official Methods of Analysis. Association of official analytical chemistry, Washington, D. C. 12lh Edn. pp. 15-18.

Patil, D. S. (1994). Studies on preparation of wine from commercially grown varieties of grape (*Vitis vinifera* L.). A M.Sc. (Agri.) thesis (Unpublished) submitted to Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahemednagar, Maharashtra.

Pawar, C. D. (2009). *Standardization* of wine making technology *in sapota (Manilkara achras.)* A thesis (Unpublished) submitted to University of Agricultural Science, Dharwad.

Prajkta A. Sapkal, (2011). Effect of T.S.S. and dilution levels of juice on quality of ripe mango (CV. *Alphonso.)* wine A M.Sc. (Agri.) thesis (Unpublished) submitted to the Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.).

Ranganna. S. (1977). Manual of analysis of fruit and vegetable products. Tata Me. Craw Hill Publishing Company Ltd., New Delhi, p: 9-82.

Roodagi. M.B. (2010). Effect of different levels of T.S.S. and pH on quality of pineapple (Ananas comosus Linn.) wine. A M.Sc. (Agri.) thesis (Unpublished) submitted to the Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.).

Sarkale A. N. (2012). Effect of different levels of T.S.S. and pH on quality of kokum *(Garcinia india)* wine. A M.Sc. (PHM.) thesis (Unpublished) submitted to the Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.).