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Research Article

PERIODICAL CHANGES IN TSS, PH, TITRATABLE ACIDITY AND YEAST COUNT DURING FERMENTATION OF SAPOTA WINE DUE TO DIFFERENT LEVELS OF TSS ADJUSTED BY DIFFERENT SUGAR SOURCES

KASTURE M.C.*[1] AND KADAM J.J.[2]
[1]Wine Chemist, Fruit Beverages Research Centre, Dr Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, 415712, India
[2]Wine Microbiologist, Fruit Beverages Research Centre, Dr Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, 415712, India
*Corresponding Author: Email - kasturemc@gmail.com

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Abstract- The powdered yeast (*Saccharomyces cerevisiae*) is one of the important yeast found to be superior in the fermentation technology. The present study was undertaken to study the periodical changes in TSS, pH, acidity and yeast count during the fermentation process from wine preparation from Sapota fruits with different TSS levels (25, 30, 35 and 40°Brix) adjusted with different sugar sources (Sucrose, Fructose, Dextrose and Jaggery). The TSS content of must decreased continuously till the end of fermentation. However, the TSS decreased sharply in the initial period of fermentation and later on, the rate of fermentation was found declined but continued at a much slower rate till the end of fermentation, in all the interactions of TSS levels adjusted by different sources of sugars. It was observed that the TSS level does not affect the pH of must. It was also noticed that the titratable acidity was found maximum in the treatment in which Jaggery was used for adjusting the TSS levels. The higher the TSS levels yields high titratable acidity during fermentation. After inoculation, 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 later on the yeast count found decreased up to the end of fermentation. The quality sapota wine was prepared from the juice of fully ripped fruits of sapota added with sucrose as source of sugar and pH adjusted to 3.5 using powdered yeast (*Saccharomyces cerevisiae* var. bayanus (No. 8906).

Keywords- *Sapota wine, TSS, pH, acidity, yeast count*

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Introduction

Fermentation is the unique process used in wine technology to sustain the nutritional parameters present in the fruits. Alcohol, acetic and lactic acids fermentation are very important from the point of view of quality in food production. Out of these, alcoholic fermentation is widely adopted for the preparation of wine in which alcohol is major constituent. Fermented beverages have been known to mankind from ancient period. Sapota is one of the important sub-tropical fruits and belongs to family *Sapotaceae*. In India, Maharashtra and Gujarat together account for the largest area under this crop out of total area of 69.1 thousand ha and production 298.0 MT under sapota in Maharashtra with productivity of 4.3 MT/ ha. Sapota fruits are good source of sugar which ranges between 12 to 14 percent. A 100 g of edible portion of fruit contains 73.7 g - moisture, 21.49 g - carbohydrates, 0.7 g - protein, 1.1 g - fat, 28 mg - calcium, 27 mg - phosphorus, 2 mg - Iron and 6 mg - ascorbic acid [5]. The plantations of sapota in some parts of Gujarat and Thane district of Maharashtra are facing the problem of seed borer attack. Even though the borer attacks only the seed, the hole made in the fruit affects the appearance of the fruit and such fruits (15 to 20%) are rejected in the markets. Such fruits can be used for quality wine preparation. Even glut in the market affects the rate considerably. Extensive research has been conducted on changes in pH, TSS, acidity and yeast count during production of wine from various fruits such as grapes [18 & 23]), guava [3], mango [16], banana [12], apples [17], plum [24], cashew apple [10], ber [1], pomegranate [2], mulberry [13], jamun [22], sapota [7], apricot [6] and karonda [4]. However, sapota under which area is increasing rapidly very little work has been done in this regard. Considering the huge wastage of sapota fruits and making value addition to get maximum returns from the crop by the farming community, the fermentation technology should be studied in systematic manner so, the present study was undertaken.

Materials and Methods

The present investigation on "Effect of different sources of sugars and TSS levels on quality of sapota (Cv. Kalipatti) wine was conducted at Fruit Beverages Research Centre, Department of Soil Science and Agricultural Chemistry, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli (M.S). Changes in TSS, pH, titratable acidity and yeast count during fermentation were discussed in this article. Sapota fruit juice was prepared from fully ripped fruits which was further diluted with distilled water in 1:1(w/w) proportion. The fruit juice so obtained was used for preparation of must and wine. For preparation of yeast inoculum, initially total must was dispensed in fermentation flask as per the treatments & then quantity of yeast powder required for inoculation of each treatment flask was calculated @ 0.3g/kg of must. Powdered yeast (*Saccharomyces cerevisiae* var. bayanus No. 8906) was then added to sterilized test tube containing 10 times luke warm water under aseptic conditions in laminar air flow bench. Separate test tubes were maintained for each treatment. These test tubes were incubated at room temperature for 1 hour for activation of yeast cells. After 1 hour, prepared inoculums was added to must in fermentation flask separately and allowed to ferment. The 1 kg clear diluted sapota fruit juice was taken in different vessels and the TSS content of juice was adjusted to 25° (T_1), 30°(T_2), 35°(T_3) and 40°(T_4) Brix by addition of different sources of sugars *viz*., sucrose (S_1), fructose (S_2), dextrose(S_3) and Jaggery (S_4), separately. The pH juice was adjusted to 3.5 by addition of citric acid and calcium carbonate. Further, the juice was supplemented with 0.1% diammonium hydrogen phosphate (DAHP) and potassium metabisulphate (KMS) equivalent to 30 ppm SO_2.

Table-1 Changes in TSS during fermentation of must

Tr.No	Treatment	Duration of fermentation								
		Day 0	Day 1	Days 3	Days 5	Days 7	Days 9	Days 11	Days 13	Days 15
1	T_1S_1	25	22.4	16.0	12.8	10.4	9.20	8.80	8.00	8.0
2	T_1S_2	25	22.8	16.2	13.0	10.8	9.80	8.80	8.20	8.0
3	T_1S_3	25	23.0	16.0	12.4	10.4	8.80	8.40	8.00	8.0
4	T_1S_4	25	22.0	12.8	10.0	9.20	8.20	8.00	8.60	8.0
5	T_2S_1	30	27.8	22.4	19.0	18.0	16.4	15.0	14.6	14.0
6	T_2S_2	30	28.0	23.0	20.0	18.2	16.8	15.6	15.0	14.2
7	T_2S_3	30	28.2	22.4	19.4	17.8	16.2	15.6	15.0	14.2
8	T_2S_4	30	27.4	19.0	15.6	14.0	13.0	12.0	13.0	14.0
9	T_3S_1	35	33.0	28.6	26.6	24.8	23.0	22.4	22.0	22.0
10	T_3S_2	35	31. 0	26.4	24.0	22.2	21.0	20.0	19.6	19.0
11	T_3S_3	35	29.8	25.0	22.8	20.2	19.2	17.8	17.2	17.0
12	T_3S_4	35	29.6	21.0	18.0	16.2	15.0	14.0	16.0	15.0
13	T_4S_1	40	35.5	31.2	29.8	28.0	27.0	24.6	25.2	25.0
14	T_4S_2	40	35.0	33.0	31.0	29.0	28.0	27.2	27.0	27.0
15	T_4S_3	40	35.2	33.0	31.0	29.2	28.2	28.2	27.2	27.0
16	T_4S_4	40	25.2	28.6	26.2	23.3	22.0	22.0	22.2	23.0

Table-2 Changes in pH during fermentation of must

Tr.No	Treatment	Duration of fermentation								
		Day 0	Day 1	Days 3	Days 5	Days 7	Days 9	Days 11	Days 13	Days 15
1	T_1S_1	3.50	3.36	3.11	3.30	3.45	3.31	3.40	3.82	3.61
2	T_1S_2	3.50	3.29	3.04	3.27	3.49	3.26	3.29	3.66	3.49
3	T_1S_3	3.50	3.58	3.15	3.42	3.51	3.37	3.25	3.81	3.52
4	T_1S_4	3.50	3.52	3.48	3.67	3.79	3.67	3.68	3.99	3.67
5	T_2S_1	3.50	3.78	3.20	3.52	3.60	3.51	3.43	3.85	3.67
6	T_2S_2	3.50	3.50	3.12	3.36	3.42	3.33	3.37	3.65	3.49
7	T_2S_3	3.50	3.64	3.16	3.36	3.49	3.40	3.44	3.75	3.56
8	T_2S_4	3.50	3.60	3.50	3.73	3.75	3.71	3.70	3.98	3.79
9	T_3S_1	3.50	3.70	3.25	3.43	3.44	3.38	3.50	3.80	3.61
10	T_3S_2	3.50	3.53	3.16	3.24	3.39	3.34	3.38	3.97	3.45
11	T_3S_3	3.50	3.78	3.27	3.50	3.45	3.47	3.48	3.79	3.60
12	T_3S_4	3.50	3.51	3.40	3.62	3.63	3.62	3.59	3.86	3.66
13	T_4S_1	3.50	3.66	3.24	3.48	3.46	2.42	3.42	3.73	3.54
14	T_4S_2	3.50	3.75	3.40	3.51	3.49	3.46	3.45	3.70	3.53
15	T_4S_3	3.50	3.78	3.38	3.51	3.47	3.45	3.47	3.72	3.49
16	T_4S_4	3.50	3.63	3.49	3.52	3.69	3.63	3.66	3.90	3.69

Table-3 Changes in Acidity during fermentation of must

Tr.No	Treatment	Duration of fermentation								
		Day 0	Day1	Day 3	Days 5	Days 7	Days 9	Days 11	Days 13	Days 15
1	T_1S_1	0.53	0.64	0.61	0.62	0.47	0.65	0.60	0.67	0.64
2	T_1S_2	0.51	0.69	0.71	0.67	0.47	0.67	0.67	0.66	0.69
3	T_1S_3	0.43	0.55	0.78	0.53	0.37	0.56	0.56	0.57	0.58
4	T_1S_4	0.69	0.80	0.64	0.84	0.51	0.75	0.76	0.72	0.80
5	T_2S_1	0.38	0.51	0.44	0.64	0.37	0.53	0.53	0.53	0.56
6	T_2S_2	0.46	0.62	0.65	0.69	0.46	0.64	0.75	0.64	0.67
7	T_2S_3	0.83	0.65	0.58	0.61	0.39	0.56	0.62	0.72	0.56
8	T_2S_4	0.87	0.78	0.83	0.81	0.55	0.79	0.83	0.70	0.83
9	T_3S_1	0.40	0.62	0.56	0.58	0.39	0.56	0.57	0.56	0.56
10	T_3S_2	0.47	0.51	0.69	0.57	0.46	0.70	0.67	0.69	0.69
11	T_3S_3	0.34	0.56	0.56	0.57	0.40	0.56	0.55	0.57	0.57
12	T_3S_4	0.81	1.01	0.97	0.97	0.64	0.93	0.94	1.06	0.96
13	T_4S_1	0.39	0.51	0.57	0.62	0.40	0.56	0.61	0.47	0.64
14	T_4S_2	0.38	0.60	0.61	0.60	0.42	0.61	0.62	0.65	0.64
15	T_4S_3	0.37	0.49	0.47	0.58	0.39	0.58	0.58	0.56	0.58
16	T_4S_4	0.85	0.98	0.98	1.01	0.71	0.99	1.02	1.02	1.10

The pH of the juice was adjusted to 3.5 and TSS was adjusted as per the treatment with different sugar sources. The calculated quantity of the DAHP and KMS was added to prepare must for fermentation. The prepared must was transferred into fermentation flasks and after pasteurization the powered yeast (*Saccharomyces cerevisiae var*. bayanus No. 8906) was inoculated. During fermentation process, changes in the TSS, pH and titratable acidity were recorded at an interval of 2 days. On completion of fermentation of fruit juice, the TSS of the raw wine showed constant readings. The viable yeast count in the fermenting must was taken by following standard serial dilution and plate technique in sterile 0.1 percent peptone [20]. After arriving at 10^{-4} dilution, 1 ml of aliquot from 10^{-4} dilution was placed in center of sterile Petri plate and 10-15 ml of molten (40-42ºC) malt glucose yeast peptone agar medium was added and mixed thoroughly by rotating plates clockwise and anticlockwise. The medium was allowed to solidify and then such plates were incubated at room temperature in inverted position for 48-72 hrs. The colonies from 1 cm^2 area were counted using a colony counter. Five random observations per plate were recorded and averaged to obtain number of colonies per cm^2. The results have been reported as number of colonies per ml of must.

Colony count/ml = No. of colonies per plate × Reciprocal of dilution

Result and Discussion

Changes in TSS during fermentation (ºBrix)

Table-4 Changes in yeast count at 10-4 during fermentation of must

Tr.No.	Treatment	Duration of fermentation							
		Day 1	Day 3	Days5	Days 7	Days 9	Days 11	Days 13	Days 15
1	T_1S_1	221	201	188	112	34	22	12	-
2	T_1S_2	266	212	174	90	58	34	10	5
3	T_1S_3	190	145	132	74	60	41	7	-
4	T_1S_4	141	166	127	45	22	18	5	-
5	T_2S_1	187	173	155	98	34	26	13	4
6	T_2S_2	200	189	176	103	23	16	9	7
7	T_2S_3	211	197	134	87	40	34	17	-
8	T_2S_4	170	190	180	56	34	29	6	-
9	T_3S_1	154	180	167	39	25	21	11	6
10	T_3S_2	190	198	170	43	33	22	15	9
11	T_3S_3	157	205	210	37	27	20	10	-
12	T_3S_4	102	185	189	76	56	43	19	10
13	T_4S_1	122	200	207	88	60	40	16	12
14	T_4S_2	130	177	183	59	45	30	22	17
15	T_4S_3	150	200	209	66	62	38	31	22
16	T_4S_4	202	245	256	76	59	42	28	20

The data regarding the changes in TSS during fermentation of the must are presented in [Table-1]. The initial TSS of the must was adjusted to 25, 30, 35 and 40 0Brix as per the treatments by addition of different sources of sugars and pH was adjusted to 3.5, to study the effect of different sources of sugars and TSS levels on quality of sapota wine. After inoculation the fermentation was allowed to continue till must showed constant TSS *i.e.* ^{0}Brix readings. Data from [Table-1] revealed that TSS content of must decreased continuously till the end of fermentation. However, it decreased sharply in the initial period of fermentation and later on, the rate of fermentation was found declined but continued at a much slower rate till the end of fermentation, in all the interactions of TSS levels adjusted by different sources of sugars. The sharp decrease in TSS in the initial period of fermentation might be due to faster conversion of sugars into alcohol which was 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 may also 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 in grape [18], in peach [8] and in sapota [11 & 19] must during fermentation. It was evident from the average figures of TSS levels adjusted with different sources of sugars at the end of fermentation that, the TSS of must was found to be increase with increase in TSS levels. This may be due to addition of sugars in increasing amount with increase in TSS levels. However, sugars levels did not show much difference within specific sources of sugars and even within the average of TSS levels, considering the different TSS levels. The lowest TSS (8 ^{0}Brix) at the end of fermentation was recorded by interaction T_1S_1 *i.e.*, 25^{0}B TSS adjusted by sucrose sugar and highest by (27^{0}Brix) in T_4S_2 and T_4S_3 treatment combination. The lowest TSS (8 ^{0}Brix) recorded by using sucrose sugar for adjustment of TSS at 25^{0}B. Similar findings were confirmed in sapota, pineapple and jamun must [9, 11 and 22] respectively during fermentation.

Changes in pH during fermentation of must

The pH of must [Table-2] showed slight increase in pH at the end of fermentation in case of must with initial adjustment of pH to 3.5. Recall the findings reported with changes in titratable acidity during fermentation of must. Therefore, it was mentioned that titratable acidity of must increase during fermentation. Generally when acidity is increased pH shows decreasing trend. However, this trend was not observed in must adjusted with 3.5 pH considering the pH values at the end of fermentation. Increase in pH with increase in acidity was also reported in cashew apple must [15]. However, it was observed that the TSS levels adjusted with Jaggery (S_4) recorded higher pH values as compared to other sources of sugars. It might be due to the chemical composition of Jaggery. It was also observed that pH of the must decreased slightly in the initial period of fermentation and later on it showed increased till the end of fermentation. With respect to different TSS levels, the pH content of must at the end of fermentation showed increasing trend with increase in pH levels. This may be the impact of original adjustment of pH levels. However, with respect pH levels at the end of fermentation, the must showed increase in pH of all the TSS levels tried at the end of fermentation. Lowest pH (3.45) was recorded by treatment combination T_3S_2 and highest (3.79) was recorded by T_2S_4 i.e. 30ºB TSS adjusted by Jaggery. From the average values of pH at the end of fermentation, irrespective of TSS levels, it was noticed that the TSS levels does not affect the pH of must. Similar findings were reported in carambola wine [14].

Changes in Titratable acidity during fermentation

The titratable acidity of the must was found to increase during fermentation which is presented in [Table-3]. The increase in titratable acidity during fermentation of must might be due to conversion of sugar into organic acids such as lactic acid, acetic acid and succinic acid. There was no definite pattern observed in change in titratable acidity during process of fermentation. However, at the end of fermentation the titratable acidity of must was found to be decreased with increase in pH. However, on an average, it was observed that the titratable acidity was found maximum in the treatment in which Jaggery was used for adjusting the TSS levels. It was also observed that the higher the TSS levels yields high titratable acidity. Similar results regarding TSS levels and acidity were recorded in different studies on wine [9, 11, 15 & 18].

Changes in yeast count during fermentation

The data on changes in yeast count during fermentation was presented in [Table-4]. It was observed that after inoculation, 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. This rapid increase in yeast count was due to availability ample nutrition at early stage of fermentation which helped the yeast to multiply at faster rate. But later on the yeast count was found decreased till the end of fermentation and this might be due to exhaustion of nutrients from must and accumulation of alcohol which cause the death of cells. The yeast count was taken up to the 15th day of fermentation *i.e.*, till the TSS of fermenting must became constant and did not show any further decrease/increase. Though, the yeast cells were observed up to the 13th and 15th day of fermentation their activity was found to be almost ceased. Similar findings were observed in wine prepared from karonda [4], jamun [9] and pineapple [21] fruits.

Conclusion

From the results obtained in the present study it is concluded that quality sapota wine can be prepared from the juice of fully ripped fruits of sapota added with sucrose as source of sugar and pH adjusted to 3.5 using powdered yeast (*Saccharomyces cerevisiae* var. bayanus (No. 8906).

Application of research: The quality sapota wine was prepared from the juice of fully ripped fruits of sapota

Research Category: Sapota wine

Abbreviations:
TSS: total soluble sugar

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***Principle Investigator or Research Guide: Dr M C Kasture**
University: Dr Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, 415712, India
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