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Development of a Process for Preparation of Pure & Blended Kinnow Wine without Debittering Kinnow Mandarin Juice

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The single most hindrance in the popularity and processing of kinnow mandarin juice is the development of bitterness due to enzymatic conversion of a non-bitter precursor LARL in to intensely bitter limonin. Although quality research work has been undertaken for debittering kinnow juice by physico-chemical/chemical/biotechnological means in order to utilize its immense potentiality in processed juice industry, these methods have met with variable and incomplete success. In transforming kinnow mandarin juice to wine, till date, only some work has been reported for the preparing kinnow wine and has been indicated that direct fermentation of kinnow mandarin juice yields bitterness in the wine. Hence, attempts were directed towards extracting bitterless kinnow mandarin juice via a 'modified method' of juice extraction. It comprised of enzyme inactivation of juice to control limonin development and its further processing to blended wine. The bitterless juice so obtained was centrifuged to get sera and pulp. After analyzing for any perceivable bitterness (≥ 5-6 ppm), sera was fermented with cane juice in various proportions (Control-100:0, A-90:10, B-80:20, C-70:30, D-60:40 and E-50:50) to obtain kinnow wine and kinnow-cane wines. The fermentation was carried out by *Saccharomyces cerevisiae* MTCC 178 & MTCC 180. Optimization of the inoculum size showed that 5% inoculum contributed to the highest fermentation rate and corresponding highest % ethanol production. The fermentation was completed in 10 days with kinnow mandarin juice as substrate and 8 days when kinnow-cane juice blend was the substrate, indicating positive effect of addition of cane juice. Of the two yeasts, strain 180, a secondary fermentation strain, generated higher % ethanol and effervescence with corresponding lower final TSS. The ethanol content (v/v) in wines was determined by gas chromatography method. Highest % ethanol (12.2%) was produced in the control wine. It is noteworthy that the limonin content in all the wines was found to be range between 4-6 ppm. Thus, acceptable pure and cane juice blended kinnow wine could be prepared without debittering the kinnow juice. Kinnow pulp, the generated byproduct in the study, was further processed to squash like product.

Key words: Kinnow mandarin juice, debittering, bitterless juice, fermentation, pure and blended kinnow wine

Kinnow mandarin is one of the major citrus fruit crops of India and the national production is in tune of over 0.4 Million Metric Tonnes[12]. Owing to the poor post-harvest infrastructure facilities and its availability in large quantities over a short period, its efficient marketing and utilization get affected. The single most hindrance in the popularity and processing of kinnow mandarin juice is the development of (delayed) bitterness due to enzymatic conversion of a non-bitter precursor Limonoate A ring Lactone (LARL) in to intensely bitter limonoid-limonin via limonoate-D-ring lactone hydrolase. It is worthwhile to note that quality research work has been undertaken for debittering (physico-chemical/chemical/biotechnological) the kinnow mandarin juice in order to utilize its immense potentiality in processed kinnow juice industry[2, 5, 6, 7,-9].

The goal has been to debitter the juice and thus make it usable for processing, but efforts have met with incomplete and variable success. Apart from utilizing the juice in processed non-alcoholic forms, another worthy alternative is its biotransformation to wine. This can also solve the problems of over production and related spoilage, apart from development of a new variety of wine. Till date, only some work has been reported for the development of method to prepare kinnow wine. But it has been indicated that direct fermentation of kinnow mandarin juice yields bitterness in the wine[3, 10]. The researchers have also investigated wine prepared from kinnow serum, either debittered prior to fermentation or during fermentation and subsequently reported wine of acceptable physico-chemical

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and sensory quality in comparison to that prepared from bitter kinnow serum. A survey of literature reveals that no successful attempt has been made to produce acceptable kinnow wine without applying the principles of debittering. Hence, attempts were directed towards extracting bitterless kinnow mandarin juice so as to overcome debittering process completely. This could substantially circumvent the debittering step before or during wine making. Reports of blending kinnow juice with other fruit juices, particularly cane juice, prior to fermentation for improving the quality and economics of product in hand are lacking. The cane juice is slightly acidic, sweet and a cheap source of fermentation, which can be also used to mask the bitterness in blended wine, if any. The value addition of the by-product in the process-kinnow pulp could easily be transformed in to a non-alcoholic squash like beverage. Thus keeping all these facts in view, the current investigation was carried out with application of novel technique for development of acceptable pure and blended kinnow-cane wine without attempting debittering of juice.

MATERIALS AND METHODS

Fresh, fully ripened kinnow mandarin fruits were procured from the local market Patiala (India) and Bangalore (India) in the months from January to April. Fresh cane juice was also obtained from the local market. The freeze-dried cultures of *Sacchromyces cerevisiae* MTCC 178 and *S. cerevisiae* MTCC 180 were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. All the chemicals used in the study were of analytical grade, except sucrose, which was procured fro the local market. Autoclavable and polypropylene screw capped glass bottles were used during the whole investigation of wine making. The lyophilized cultures were activated according to the procedure recommended by the suppliers and were grown in sterile Yeast Extract Peptone Dextrose broth. The cultures were maintained by fortnightly transfers on glucose yeast extract agar plates and stored at 4 ± 1ºC. These were sub cultured 2-3 times prior to use. Kinnow mandarins were cleaned thoroughly with running water so as to remove the adhered dirt; washed; dried in air and then packed in perforated polyethylene bags. Kinnow mandarin juice was extracted through FMC citrus juicer (FMC Corporation-Citrus Machinery and Services Division, Lakeland, Florida, USA) via a 'modified method' of juice extraction (Patent[4] filed). The bitterless juice so obtained was centrifuged to get sera and pulp. Healthy and sound sugar canes were selected, and juice was extracted through neat and clean cane juicer. The juice so obtained was filtered, pasteurized at 95ºC for 5 minutes at 1 atm then immediately cooled, and stored at 3-5ºC till further use. The kinnow juice sera and cane juice were analyzed for the physico-chemical attributes namely pH (Cyber-Scan 510, EUTECH Instruments, Singapore), Total soluble solids (by Hand refractometer, ERMA, Japan), titratable acidity[8], ascorbic acid[8] and limonin content[11]**.** The kinnow-cane wine was prepared according to the method shown in Figure 1. During fermentation, observations were made for noting the fall in degree brix after every 48 h till a constant brix was obtained. The ethanol content in wine was measured by gas chromatograph (5765 Nucon Gas Chromatograph, Nucon Engineers, New Delhi, India) according to the method described by D'Arcy[1]. The prepared wine samples were also analyzed for various physico-chemical and sensory attributes. Sensory evaluation of the prepared wine samples was done on the 9 point Hedonic scale[8] by a group of semi trained panel. Alcohol tolerance study was also undertaken for the inocula used in investigation. Pulp, the by-product generated was later processed into squash like beverage.

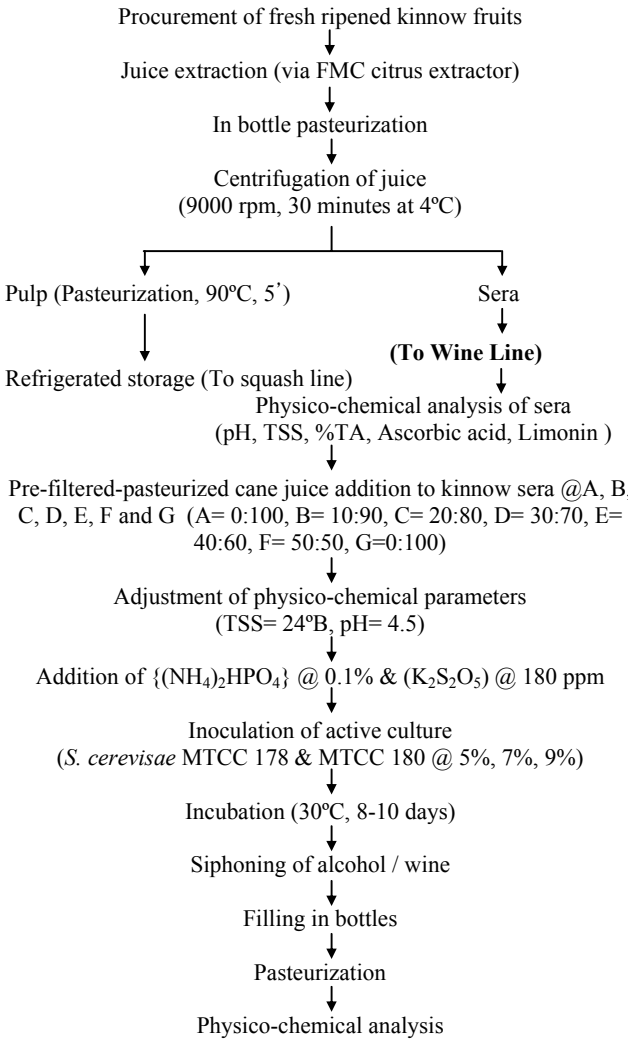

FIGURE 1. Flow diagram for the preparation of pure and blended kinnow-cane wine.

RESULTS AND DISCUSSION

Throughout the investigation, kinnow juice was extracted via FMC citrus extractor. The extracted juice did not turn bitter under low temperature storage for a period of about 4 hours and limonin content in such juice was found to be around 4.6-6.2 ppm. The juice extracted via conventional method had developed substantial bitterness (limonin, 60 ppm) under similar refrigerated conditions.

Effect of type of yeast strain and level of inoculum on various physico-chemical characteristics of kinnow wine, cane wine, and kinnow-cane wine (50:50). The pH, Total soluble solids (ºBrix), titratable acidity (% citric acid), and ascorbic acid (mg/100ml) in the kinnow mandarin juice was found to be respectively 3.95-4.1, 10.0-11.8, 0.64 and 22.8 respectively. The corresponding values in cane juice were respectively 5.43-5.78, 1 6.8-18.0, 0.1. The limonin content in kinnow mandarin juice was found to be 4.6 ppm. The various physico-chemical characteristics of wines produced from kinnow, cane and different kinnow-cane blends were found to be different with respect to type of yeast strain and level of inoculum. The results obtained are presented in Table 1.

pH. It can be inferred that during fermentation of kinnow sera, cane and kinnow-cane juices, strain *S. cerevisiae* MTCC 180 showed practically no variation in pH in comparison to *S. cerevisiae* MTCC 178. When only cane juice was being fermented using MTCC 178, a fall in pH from 4.8 to 4.5 was required. The level of inoculum had no effect on the pH of fermenting juice.

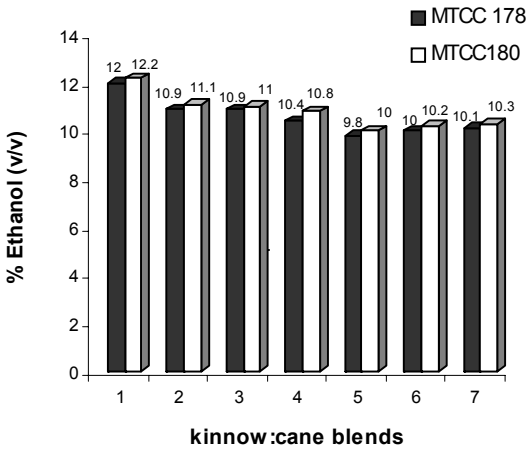

FIGURE 2. Percent ethanol (v/v) produced by two different strains of *Saccharomyces cerevisiae* (MTCC 178 and MTCC 180) in different kinnow: cane blends. Scales on X-axis, 1=100:0; 2=90:10; 3=80:20; 4=70:30; 5=60:40; 6=50:50; 7=0:100.

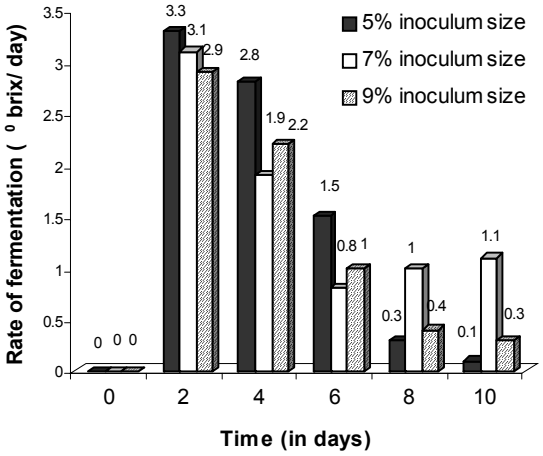

FIGURE 3. Rate of fermentation of kinnow juice at different inocula of *Saccharomyces cerevisiae* MTCC 180.

TSS (Total Soluble Solids). The maximum TSS reduction during the fermentation of kinnow sera, cane and kinnow-cane juice was found with 5% level of inoculum and strain MTCC 180 reduced more TSS in comparison to MTCC 178. The least final TSS was found to be 8ºB in fermentation of kinnow sera.

%TA. No regular trend was observed in the %TA during fermentation of all combinations kinnow, cane and kinnow-cane (50:50) juices. The final TA was found to increase in some cases and decrease in other cases and no correlation could be drawn. Also, the inoculum level had no effect on the titratable acidity of the fermenting juice

AA content. With respect to strain type and level of inoculum, there was no significant change in ascorbic acid (mg/100ml). Some loss of ascorbic acid was observed that could be attributed to fermenting temperature.

Limonin content. It was worth noticing that no change in limonin content (ppm) of various sera/juice was observed during and at end of fermentation.

% Ethanol. The percent ethanol production in all the wines was found to be maximum at 5% (v/v) level of inoculum and strain MTCC 180 produced more ethanol in comparison to *S. cerevisiae* MTCC 178. Maximum ethanol i.e. 12.2% (v/v) was produced when only a kinnow juice serum was used for fermentation by strain MTCC 180 as shown in Figure 2 and 3. This is in contrast to observations were made by Singh *et al.*[10], who reported a maximum ethanol of 11.3% (v/v) in kinnow juice fermentation at 14% inoculum level. The increasing order of ethanol content in various blends was observed in the given fashion of 60:40, 70:30, 80:20, 90:10 respectively.

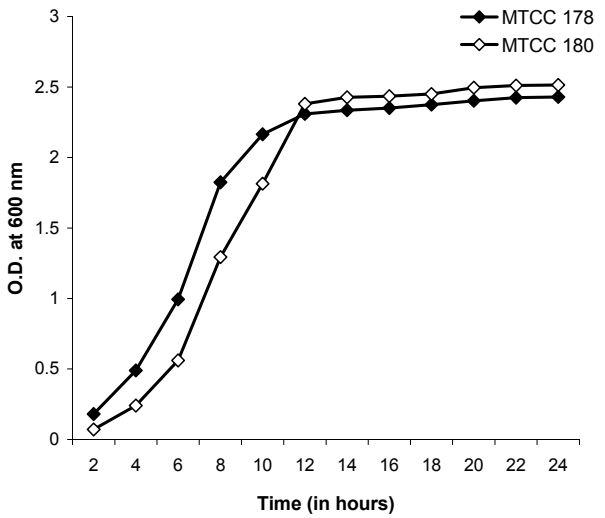

FIGURE 4. Growth curve of two different strains of *Saccharomyces cerevisiae* MTCC 178 and 180 in YEPD medium.

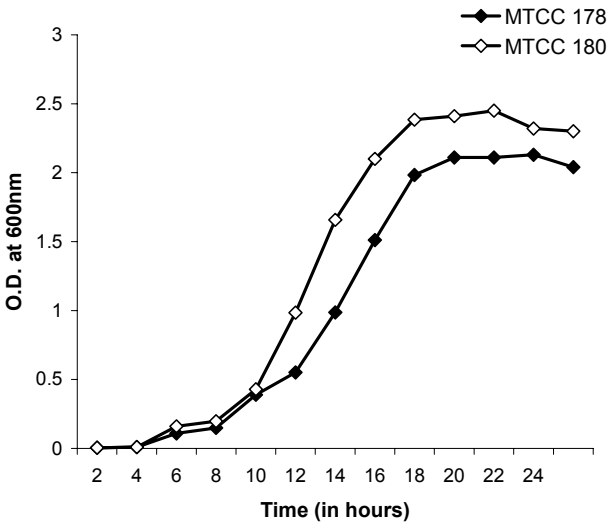

FIGURE 5. Growth curve of two different strains of *Saccharomyces cerevisiae* MTCC 178 and 180 in YEPD medium containing 12% ethnol (v/v).

Fermentation period. There was no effect of level of inoculum and type of strain used on the time period of fermentation. Moreover, it was found that fermentation in kinnow cane (50:50) blend was found to be complete in 8 days in comparison to 10 days, when the two juices were separately fermented. Thereafter, 8-day period of fermentation was taken optimal for different blends of the two juices. Thus, it was observed that blending resulted in decreasing the time period of fermentation from 10 to 8 days.

Sensory Analysis. The sensory analysis of various quality attributes of wine samples (kinnow/kinnow-cane/cane wines) were carried out as per the 9-point Hedonic scale viz. aroma, taste, appearance and over-all acceptability. The scores were used to evaluate the overall quality of wine. The wine produced from the blend of kinnow and cane juice in the ratio of 80:20 was found to score highest. This was followed by 100:0, 50:50, 60:40 and 70:30 and 90:10, respectively, in the decreasing order of overall acceptability. Thus, no regular trend was observed in relation to the amount of kinnow and cane juices used for blending for preparation of wine

Alcohol tolerance study. Firstly, growth pattern of both the yeast strains was observed and it was found that that both are more or less same in terms of their growth (Fig. 4). Then, alcohol tolerance study was carried out to observe the behavior of both the strains of *S. cerevisiae* MTCC 178 and MTCC 180 in the growth medium (Yeast Extract Peptone Dextrose) containing 12% absolute ethanol (v/v).

S. cerevisiae MTCC 180 was found to be more tolerant to ethanol in comparison to that of strain MTCC 178, and had lesser lag time (Fig. 5). It can be concluded that kinnow juice could be easily blended with sugar cane juice in different levels (up to 50:50) for the preparation of acceptable kinnow-cane wine. The effect of temperature treatment on juice/sera has a significant effect on the control of development of bitterness in wine due to limonin. Thus, a satisfactory wine could be prepared without undertaking debittering step

By-product utilization. The by-product (pulp) obtained in the present investigation was processed into a squash like product. The product so prepared was found to be acceptable and shelf stable for over a period of 2 months under refrigerated conditions. No bitterness could be detected in the product through out the storage period. It can be concluded that kinnow juice could be easily blended with sugar cane juice in different levels (up to 50:50) for the preparation of acceptable kinnow-cane wine. The effect of temperature treatment on juice/sera has a significant effect on the control of development of bitterness in wine due to limonin. Thus, a satisfactory wine could be prepared without undertaking debittering step

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TABLE 1. Physico-chemical parameters of pure and blended kinnow cane wine

Wine Sample (K:C)[1]	Inoculum used (Strain)	Day	Parameters of Pure and Blended Kinnow Wine					
			pH	TSS[2] (ºB)	%TA[3]	AA[4] (mg/100ml)	Limonin (ppm)	Ethanol content (%, v/v)
100:0 (control)	MTCC 178	0	4.50	24.0	0.64	22.8	4.6	---
		10	4.32	8.2	0.63	21.4	5.1	12.0
	MTCC 180	0	4.50	24.0	0.64	22.8	4.6	---
		10	4.41	8.0	0.61	22.7	4.8	12.2
0:100	MTCC 178	0	4.80	24.0	0.10	NA[5]	NA	---
		10	4.58	9.0	0.45	NA	NA	10.1
	MTCC 180	0	4.50	24.0	0.10	NA	NA	---
		10	4.42	9.2	0.42	NA	NA	10.3
90:10	MTCC 178	0	4.50	24.0	0.51	22.3	4.8	---
		10	4.42	9.8	0.57	22.1	4.3	10.9
	MTCC 180	0	4.50	24.0	0.51	22.3	4.8	---
		10	4.45	9.4	0.54	21.8	4.6	11.1
80:20	MTCC 178	0	4.50	24.0	0.45	20.6	4.2	---
		8	4.39	9.0	0.38	20.3	4.8	10.9
	MTCC 180	0	4.50	24.0	0.45	20.6	4.2	---
		8	4.44	8.8	0.49	20.5	5.6	11.0
70:30	MTCC 178	0	4.50	24.0	0.40	20.4	4.6	---
		8	4.46	9.4	0.45	20.1	5.8	10.4
	MTCC 180	0	4.50	24.0	0.40	20.4	4.6	---
		8	4.42	8.8	0.42	20.1	3.9	10.8
60:40	MTCC 178	0	4.50	24.0	0.38	18.6	5.2	---
		8	4.45	10.1	0.41	17.7	4.5	9.8
	MTCC 180	0	4.50	24.0	0.38	18.6	5.2	---
		8	4.44	9.2	0.39	18.2	4.9	10.0
50:50	MTCC 178	0	4.50	24.0	0.34	17.6	4.8	---
		8	4.59	9.0	0.42	16.8	4.3	10.0
	MTCC 180	0	4.50	24.0	0.34	17.6	4.8	---
		8	4.54	9.2	0.40	17.2	4.9	10.2

[1] K:C= Kinnow: Cane juice ratio.
[2] TSS: Total soluble Solids.
[3] %TA: Titratable acidity (as citric acid).
[4] AA: Ascorbic Acid.
[5] NA: Not Applicable.

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