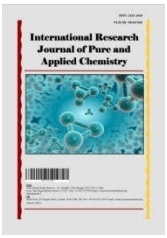

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Study on Optimization of Yeast Level and Duration for Aerobic and Anaerobic Fermentation for Production of Jack Fruit (*Artocarpus heterophyllus* L.) Wine

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Authors' contributions

This work was carried out in collaboration among all authors. Author SKA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PG and RH managed the analyses of the study. Author RH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation on Optimization of yeast level and duration for aerobic and anaerobic fermentationfor production of jack fruit (*Artocarpus heterophllyus* L.) wine was carried out in the department of Post Harvest Technology, Kittur Rani Channamma College of Horticulture (University of Horticultural Sciences, Bagalkot), Arabhavi, during the year 2010-2011. It consisting of different treatments *viz* T_1 –20 g of yeast for 100 Kg of *must*+24 hrs aerobic and 7 days anaerobic fermentation, T_2-30 g of yeast for 100 Kg of *must*+24 hrs aerobic and 7 days anaerobic fermentation, T_3–20 g of yeast for 100 Kg of *must*+24 hrs aerobic and 14 days anaerobic fermentation, T_4 –30 g of yeast for 100 Kg of *must*+24 hrs aerobic and 14 days anaerobic fermentation, T_5 –20 g of yeast for 100 Kg of *must*+48 hrs aerobic and 7 days anaerobic fermentation, T_6 –30 g of yeast for 100 Kg of *must*+48 hrs aerobic and 7 days anaerobic

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fermentation, T_7 –20 g of yeast for 100 Kg of *must*+48 hrs aerobic and 14 days anaerobic fermentation and T_8 –30 g of yeast for 100 Kg of *must*+48 hrs aerobic and 14 days anaerobic fermentation. The experiment was laid out in a completely randomized design with three replications. The main objective was to standardize the optimum yeast level and duration required for aerobic and anaerobic fermentation and also to study various biochemical and organoleptic quality of wine recorded at regular interval during the storage of wine. The highest TSS was maintained in treatment T_1 (10.47) and T_4 (10.47) in cold condition and in ambient condition T_1 (10.43) followed by T_2 (10.35) shows highest TSS. The pH value increases from 3.36 (fresh wine) to 3.89 (6 MAS in cold) and 3.84 (6 MAS in ambient) and acidity will decrease from 0.59 to 0.49 (Cold) and 0.52 (ambient). Alcohol content increase from 7.46 to 8.12 percent (Cold) and 8.04 (Ambient). Tannin per cent were showed non significant difference and decreasing trend can be seemed over period of aging, T_8 (30 g of yeast + 48 hrs aerobic and 14 days of anaerobic fermentation) observe the highest per cent of tannins throughout the investigation.

Keywords: Pomegranate; fermentation; must; TSS; acidity.

1. INTRODUCTION

Jackfruit is quite popular in Eastern and Southern India and is cultivated widely in many states. The total area under jackfruit in India is approximately 1.02 lakh hectares. It is cultivated in an area of about 0.11 lakh hectares mostly in Southern Plains and Western Ghats of Karnataka producing about 2.6 lakh tonnes of fruits per annum Anon. [1]. This difficulty has its origin in morphological and biochemical hindrances associated with the fruit. Therefore, it is urged that indigenous or underutilized fruits which are not easily marketed in the fresh form should be processed into acceptable products Jagadeesh et al. [2]. The jackfruit is classified into two groups, one with firm to crisp flesh; generally highly flavoured, while other is generally mushy or soft fleshed (Singh, 1995). Jackfruit is nutritious, rich in vitamins (A and B), minerals (Ca, K and Fe) and contains considerable amounts of carotene and vitamin C. It is a rich source of pectin and protein Nandini et al. [3].

Jackfruit is a poor man's fruit which is available in plenty during the season (April-August) and firm flesh bulbs are marketed and relished as dessert fruit. However, the soft flesh type does not find utility in the ripe form as it is not liked due to mashy texture. More than 60-70 per cent of the soft flesh form is wasted without utilization in the Western Ghats area where jackfruit is grown in plenty. This form being rich in sugars can be suitably used for the production of fermented beverage like wine. This endeavour helps not only to utilize the underutilized soft flesh jackfruit type but also to exploit excess produce of the jackfruit during the season.

2. MATERIALS AND METHODS

2.1 Total Soluble Solids (Brix)

The total soluble solids of the wine were determined with the help of "ERMA" hand refractometer having a range of 0 to 32 ^{o}Brix at room temperature.

2.2 pH

The pH of the wine was measured using digital pH meter (Model: Analog, research, USA). Standard buffer solution of pH 4.01 and 9.08 were used as reference to calibrate the instrument.

2.3 Titratable Acidity (%)

Ten ml of sample was taken in a100 ml volumetric flask and the volume was made up. From this 10 ml of aliquot was taken in a 100 ml conical flask and titrated against 0.1 N NaOH using one or two drops of Phenolphthalein indicator. Appearance of light pink color denoted the end point. Total titratable activity was expressed as per cent citric acid Srivastava and Kumar, [4].

$$\% \text{ of acidity} = \frac{\text{Titrable value x Normality of NaOH x Vol. made up x Equivalent wt. of acid}}{\text{Vol. of sample for estimation x Weight or volume of sampel taken x 1000}}$$

2.4 Estimation of Ethanol

Determination of ethanol concentration in aqueous solutions by using alcohol meter. Alcohol meter was directly dipped in the sample taken in a 500 ml measuring cylinder and the scale in the meter where it coincides with the meniscus of the sample indicates per cent v/v alcohol present in the sample.

2.5 Estimation of Tannins

Tannins were estimated by determining their oxidisability of potassium permanganate solution. A known volume of aliquot was taken in a porcelain dish. Indigo carmine solution (20 ml) and water (about 500-750 ml) were added and titrated against potassium permanganate solution until the colour turns to bright yellow or to a faint pink at the rim. Milliliters of potassium permanganate used (A) was noted.

A known volume of sample was taken and added with 25 ml of gelatin solution and the volume was made up to 500 ml by adding acid sodium chloride solution. From that, a 50 ml sample was taken and added with 20 ml of indigo carmine solution. Again the volume was made up to 500 ml by adding water and titrated against potassium permanganate solution as before (B).

The amount of milliliters of $KMnO_4$ used in this case (B) subtracted from (A) gave the quantity of $KMnO_4$ solution required to oxdise the tannins. The percentage of tannins in the sample was calculated by the formula given below.

A: Total tannin like materials
B: Non- tannin materials
A– B: True tannins

$$\% \text{ of tannin} = \frac{\text{Titrable value x g of tannins/ml of KMnO4}}{\text{Vol. of sample for estimation}} \text{x} 100$$

ml of 0.1 N $KMnO_4$ = 0.0042 g of tannin

3. RESULTS AND DISCUSSION

3.1 TSS (^{o}Brix)

In fresh wine the TSS had a range from 9.63 to 11.87 ºBrix. A comparatively lower TSS was found in the treatments (T_5, T_6, T_7 and T_8) associated with 48 hours of aerobic fermentation (Table 1). The treatment T_8 (30 gof yeast + 48 hrs aerobic and 14 days of anaerobic fermentation) recorded significantly lower TSS (9.63 ºB) and the treatment T_1 exhibiting significantly higher level of TSS (11.87 ºB).

Juice oxidation during stemming and crushing is usually sufficient to promote adequate yeast growth during vinification. The amount oxygen absorbed also depends on the duration of skin contact during maceration Jackson, [5]. During fermentation of red wine, oxygen may be absorbed during pumping over. The resulting incorporation of 10 g of oxygen per litre often speeds up the process of fermentation. This is more marked when aeration occurs at the end of exponential phase of yeast growth (Sablayrolles and Barre, 1986). In this experiment, aerobic fermentation for 48 hours might have resulted in increase in the number of yeast cells thus encouraging the utilization of sugars. Srivastava and SanjeevKumar [4] also recommend 48 hours of *must* aeration.

The jack fruit wine in the present study witnessed further, but slight decrease in the TSS during ageing with the same trend in all the treatments indicating slow activity of wine yeasts. At 3 and 6 months of ageing under cold condition, the treatment T_8 (30 g yeast 48 hrs and 14 days of aerobic and anaerobic fermentation) documented the lowest TSS of 9.20 and 9.03 ^{o}B. Under ambient condition (27±1ºC), the treatment T_5 (30 gyeast 48 hrs and 14 days of aerobic and anaerobic fermentation) recorded the lowest TSS of 9.17 and 9.07 ^{o}B at 3 and 6 months after aging respectively.

3.2 pH

The mean pH of fresh wine and aged wines indicate an increase in the pH from the original *must* pH (3.2) level. Similar observations of increase in pH after fermentation and during ageing have been recorded by Shankar et al. [6] in guava wine. In the fresh wine, the highest pH of 3.93 was noticed in T_8 (20 g yeast 48 hrs and 14 days of aerobic and anaerobic fermentation) (Table 2).

Increase in pH was due to reduction in acidity through precipitation of potassium tartarate salts from wine or due to enhanced synthesis of esters from ethyl alcohol and volatile acids. The changes in the pH were not correlated with the changes in total acidity because of the buffering capacity of the wines and the relative amount of various acids influencing the acidity Shankar et

al. [4]. After 3 and 6 months of ageing in both cold and ambient condition the same treatment T_8 was recorded the highestpH. However, a pH range between 3.1 and 3.4 is favoured for white wine, and between 3.3 and 3.6 for most of red wines Jackson, [5]. Shukla et al. [7] analysed the pH of the wine samples prepared from different varieties of jambal fruit and the pH of these wines ranged from 3.50 to 3.40. Attri et al. [8] opined that sand pear based wine had a pH of 3.99 whereas in vermouth pH decreased to 3.95.

3.3 Titrable Acidity

The titrable acid in all the samples decreased after fermentation to ageing (Table 3). However, the changes in pH were not correlated with the changes in titratable acidity because of the buffering capacity of the wines and the relative amounts of various acids influencing the acidity Amerine et al. [9]. The decrease in the acidity during ageing might be due to combination of acids with alcohol to form esters which adds aroma to the wine during ageing Shankar et al. [6]. The titrable acidity is an important parameter used to measure the quality of wine Olasupo and Obayori, [10]. The ideal acidity in the wine is dependent on the style and preferences of the consumer. Nevertheless, the acceptable range for total acidity in most wines is between 5.5 and 8.5 mg/l (0.55 to 0.85%). From fresh wine to ageing, the titrable acidity level in T_8 decreased and was found to be the lowest acidity under both the conditions at 6 months of ageing.

Table 1. Effect of yeast level, duration required for aerobic and anaerobic fermentation on TSS of fresh jackfruit wine and after ageing

Tr. no	TSS (°Brix)					
	Must	Ageing in cold condition			Ageing in ambient condition	
		Fresh wine	3MA	6 MA	3MA	6 MA
T_1	24	11.87	11.20	10.47	10.83	10.43
T_2		11.03	10.67	10.06	10.53	10.35
T_3		10.97	10.23	9.62	10.50	10.10
T_4		10.70	10.50	10.47	10.50	10.17
T_5		10.37	10.27	9.49	9.17	9.07
T_6		10.53	9.80	9.72	10.03	9.97
T_7		10.33	10.12	9.27	9.87	9.05
T_8		9.63	9.20	9.03	9.40	9.26
Mean		**10.67**	**10.82**	**10.25**	**10.14**	**9.77**
SEm ±		**0.39**	**0.24**	**0.18**	**0.19**	**0.15**
CD 1%		**1.15**	**0.84**	**0.58**	**0.61**	**0.45**

3MA = 3 months of ageing; 6MA = 6 months of ageing
Ageing in cold at 14±1ºC; Ageing in ambient condition 24±1ºC

Table 2. Effect of yeast level, duration required for aerobic and anaerobic fermentation on pH of fresh jackfruit wine and after ageing

Tr. no	pH					
	Must	Fresh wine	Ageing in cold condition		Ageing in ambient condition	
			3MA	6 MA	3MA	6 MA
T_1	3.2	3.09	3.64	3.73	3.80	3.83
T_2		3.13	3.83	3.83	3.77	3.83
T_3		2.86	3.79	3.81	3.51	3.55
T_4		3.15	3.74	3.93	3.81	3.87
T_5		3.59	4.01	4.19	3.74	3.81
T_6		3.26	3.72	3.75	3.83	3.90
T_7		3.85	3.87	3.93	3.95	4.01
T_8		3.93	4.02	4.09	4.01	4.11
Mean		**3.36**	**3.83**	**3.89**	**3.78**	**3.84**
SEm ±		**0.16**	**0.07**	**0.05**	**0.06**	**0.07**
CD 1%		**0.65**	**0.29**	**0.20**	**0.25**	**0.28**

3MA = 3 months of ageing; 6MA = 6 months of ageing
Ageing in cold at 14±1ºC; Ageing in ambient condition 24±1ºC

Table 3. Effect of yeast level, duration required for aerobic and anaerobic fermentation on acidity of fresh jackfruit wine and after ageing

Tr. No	Acidity (%)					
	Must	Fresh wine	Ageing in cold condition		Ageing in ambient condition	
			3MA	6 MA	3MA	6 MA
T_1	0.64	0.69	0.67	0.64	0.68	0.63
T_2		0.71	0.69	0.63	0.64	0.59
T_3		0.48	0.44	0.41	0.42	0.38
T_4		0.53	0.49	0.36	0.49	0.41
T_5		0.56	0.52	0.49	0.76	0.71
T_6		0.83	0.76	0.71	0.74	0.70
T_7		0.46	0.41	0.34	0.41	0.36
T_8		0.49	0.43	0.35	0.45	0.39
Mean		**0.59**	**0.55**	**0.49**	**0.57**	**0.52**
SEm ±		**0.03**	**0.04**	**0.02**	**0.03**	**0.02**
CD 1%		**0.12**	**0.14**	**0.09**	**0.10**	**0.08**

3MA = 3 months of ageing; 6MA = 6 months of ageing
Ageing in cold at 14±1ºC; Ageing in ambient condition 24±1ºC

3.4 Ethanol (% v/v)

In this experiment, significantly higher ethanol content in the fresh wine was recorded in the T_7 (20 g of yeast + 48 hrs aerobic and 14 days of anaerobic fermentation). A comparatively higher ethanol levels were noted in T_7 and T_8 indicating positive effect of increase in the duration of aerobic and anaerobic fermentation on conversion of sugars in to alcohol (Table 4). The variation in alcohol production depends on several factors such as, initial sugar content, initial pH, amount of by product formed, temperature maintained during fermentation, amount of quality sugar, pH maintained during fermentation and alcohol tolerance limits of the yeasts could cause variation in the alcohol production Thippesha et al. [11]. The TSS (adjusted to 24ºB) was constant thus making the same amount of sugar available in all the treatments. Though the sugar content, dilution and pH were same, the duration of fermentations and yeast level were different. This dissimilarity in the *must* in the factors during fermentation might have affected the rate of fermentation, the rate of yeast growth and efficacy during fermentation resulting in variation in the ethanol produced in different treatments.

During ageing, the alcohol level increased slightly in most of the treatments. This increase might have been due to very slow fermentation that might have occurred during ageing. The treatment T_8 (30 gof yeast + 48 hrs aerobic and 14 days of anaerobic fermentation) evidenced a significantly higher ethanol content after 3 months (8.44%) and 6 months (8.53%) of ageing in cold condition whereas in ambient condition of aging T_5 obtained higher per cent of alcohol after 3 months and T_7 obtained higher after 6 months. As evident from the mean, a slightly higher alcohol levels were observed under ambient aging than by aging under cold. This variation might be due to the influence of temperature on aging. Aging process is primarily physico-chemical, higher temperature speeds and activates most the reactions involved Jackson, [5].

3.5 Tannins (%)

Tannins were found to be non significant over the treatments and also showed the decreasing trend over the period of ageing in both ambient and cold condition. Treatment T_8 (30 g yeast + 48 hrs aerobic and 14 days of anaerobic fermentation) recorded the highest per cent of tannins in fresh (0.21) and at 3 months (0.91) and at 6 months (0.16) of ageing under cold condition (Table 5). However, the same treatment continued to record highest per cent of tannins (0.21 ad 0.19) under ambient condition at 3 and 6 months of ageing. There was a decrease in the per cent of tannins has seen in all the treatments, this may be due to complexing of tannins with protein and polymerization takes place (Sharma et al. 2009). The per cent of tannins may vary in the treatments depending upon the type of wine, yeast, fermentation conditions, containers and the maturation period was observed in litchi wine (Singh and Kaur, 2009).

Table 4. Effect of yeast level, duration required for aerobic and anaerobic fermentation on ethanol of fresh jackfruit wine and after ageing

Tr. no	Alcohol (%V/V)				
	Fresh wine	Ageing in cold condition		Ageing in ambient condition	
		3MA	6 MA	3MA	6 MA
T_1	6.91	7.30	7.71	7.51	7.73
T_2	7.40	7.60	7.95	7.68	7.78
T_3	7.42	7.85	8.28	7.70	7.92
T_4	7.58	7.70	7.71	7.66	7.88
T_5	7.77	7.83	8.27	8.45	8.51
T_6	7.67	8.09	8.14	7.96	7.55
T_7	7.79	7.91	8.41	8.05	8.58
T_8	7.18	8.44	8.53	8.32	8.40
Mean	**7.46**	**7.84**	**8.12**	**7.91**	**8.04**
SEm ±	**0.08**	**0.10**	**0.06**	**0.05**	**0.07**
CD 1%	**0.26**	**0.29**	**0.19**	**0.17**	**0.24**

3MA = 3 months of ageing; 6MA = 6 months of ageing
Ageing in cold at 14±1ºC; Ageing in ambient condition 24±1ºC

Table 5. Effect of yeast level, duration required for aerobic and anaerobic fermentation on tannins of fresh jackfruit wine and after ageing

Tr. no	Tannins (%)				
	Fresh wine	Ageing in cold condition		Ageing in ambient condition	
		3MA	6 MA	3MA	6 MA
T_1	0.11	0.09	0.07	0.09	0.08
T_2	0.14	0.12	0.10	0.10	0.09
T_3	0.09	0.08	0.07	0.09	0.07
T_4	0.13	0.12	0.11	0.12	0.10
T_5	0.14	0.14	0.10	0.14	0.13
T_6	0.12	0.11	0.10	0.12	0.10
T_7	0.16	0.14	0.13	0.16	0.14
T_8	0.21	0.19	0.16	0.21	0.19
Mean	**0.14**	**0.12**	**0.11**	**0.13**	**0.11**
SEm ±	**0.06**	**0.04**	**0.08**	**0.07**	**0.05**
CD 1%	**NS**	**NS**	**NS**	**NS**	**NS**

3MA = 3 months of ageing; 6MA = 6 months of ageing
Ageing in cold at 14±1ºC; Ageing in ambient condition 24±1ºC

3.6 Colour (OD value)

Treatment T_7 (20 g of yeast + 48 hrs aerobic and 14 days of anaerobic fermentation) was found to be with significantly higher OD value in fresh wine but after ageing in cold condition treatment T_6 (30 g of yeast + 48 hrs aerobic and 7 days of anaerobic fermentation) recorded highest OD value whereas in ambient aged wines the treatment T_8 (30 g of yeast + 48 hrs aerobic and 14 days of anaerobic fermentation) obtained higher OD values. The wines that were aged under ambient condition obtained less OD value when compared to those aged under cold condition. The greater wine clarity allows for more transmission of light resulting in smaller OD value whereas deeper colour of wine allows lesser transmission of light and increased OD value. A slight but insignificant variation in wine colour observed in this study may have its roots in the biochemical interaction between the sugars, pH and other constituents of pulp, in addition to the effect of conditions on fermentation and ageing (Table 6).

The change in colour of wine made from guavas at different stages of maturity was attributed to variation in the pigentation of fruits Anderson and Badrie, [12]. Joshi et al. [13] observed a variation in the colour intensity of wine made from peach cultivars where in the higher colour intensity was recorded in cv. Redhaven (OD of 0.19) and lower in cv. Stark Early Gaint (OD of 0.86) which is attributed to the variation in pulp colour [14].

Table 6. Effect of yeast level, duration required for aerobic and anaerobic fermentation on wine recovery and colour of fresh jackfruit wine and after ageing

Tr. No	Wine recovery (%)	Colour OD value 540 nm				
		Fresh wine	Ageing in cold condition		Ageing in ambient condition	
			3MA	6MA	3MA	6MA
T_1	55.77	1.36	1.45	1.46	0.33	0.45
T_2	54.63	1.32	1.02	1.12	1.05	1.12
T_3	55.27	1.26	1.25	1.31	0.52	0.57
T_4	51.13	1.28	2.11	2.14	1.19	1.23
T_5	39.90	1.25	2.68	2.71	0.93	1.02
T_6	54.13	1.45	3.44	3.45	0.27	0.29
T_7	40.20	2.37	3.31	3.32	0.82	0.92
T_8	53.23	2.14	3.16	3.21	1.53	1.56
Mean	**50.53**	**1.55**	**2.30**	**2.34**	**0.83**	**0.89**
SEm±	**3.01**	**0.24**	**0.46**	**0.42**	**0.25**	**0.31**
CD 1%	**9.94**	**0.98**	**1.84**	**1.26**	**1.03**	**1.10**

3MA = 3 months of ageing; 6MA = 6 months of ageing
Ageing in cold at 14±1ºC; Ageing in ambient condition 24±1ºC

4. CONCLUSION

The results revealed that addition of 30 g yeast and allowing 48 hours of aerobic and 14 days anaerobic fermentation (T_8) was found significantly better when compared to all the treatments for the parameters *viz*., TSS, pH, acidity, sugars, tannins and organoleptic scores.In the present study, *must* with 1:2.5 dilution and 3.5 pH (T_8) in the first experiment and the *must* added with 30 g yeast to and allowed for 48 hours of aerobic and 14 days anaerobic fermentation (T_8) in the second experiment was found statistically superior with respect to biochemical parameters. However, *must* prepared with 1:2.5 dilution and 3.3 pH (T_6) as well as *must* prepared with 1:2.5 dilution and 3.4 pH (T_7) in fist experiment were found organoleptically better. In the case of second experiment, *must* with 30 g of yeast and allowed for 24 hours of aerobic in and 14 days anaerobic fermentation (T_3) and that with 30 g of yeast and allowed for 48 hours of aerobic and 14 days anaerobic fermentation (T_8) were found score better for sensory quality.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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