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Standardization technology of papaya wine making and quality changes in papaya wine as influenced by different sources of inoculums and pectolytic enzyme

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ABSTRACT

Investigations on the preparation of wine from papaya are reported. All the inoculum was given good result for papaya wine making using clarified juice, non clarified juice and pulp. Among this the wine prepared from either the clarified or non clarified papaya juice is highly acceptable using the inoculum pure culture and sediment of secondary fermentation. It is quite possible to utilize papaya fruits successfully to make an acceptable quality of wine as per the procedure developed.

Key words: papaya fruits, *Saccharomyces cerevisiae*, wine, microbial and physico-chemical analysis.

INTRODUCTION

Papaya is a sugar crop with soluble saccharides in the form of glucose, fructose, sucrose and it's widely cultivated in several countries. In tropical climates such as Nigeria, the Papaya trees continue bearing fruits throughout the year, and the fruit turn follow the same pattern of maturity. Its display rapid growth and high yield of 100kg plant per year or 154,000kg per hectare per year, even during from fourth year of growth. The average yield per hectare is about 22000 fruits weighing 34tons. Sugars represent that part of the fruits which is used by Microorganisms for wine production. Ayanaru *et al.*, who showed that it has a capacity of generation of ethanol by microbial conversion of sugar in the papaya fruit [1]. Fermentation is a relatively low energy preservation process which increases the self life and decreases the need for refrigeration or other forms of food preservation technology. Wine is considered to be the oldest fermented alcoholic beverage. The term wine is applied to the product made by alcoholic fermentation by yeast of fruits or fruit juice, with an aging process. The present investigation was undertaken to develop a suitable methodology for making papaya wine of an acceptable quality using different sources of inoculum (*Saccharomyces cerevisiae*) using clarified and non clarified papaya juice.

MATERIALS AND METHODS

Sources of inoculums:

Pure culture

In this experiment the pure culture of *Saccharomyces cerevisiae* used were isolated from rotten papaya fruits and it was stored at 4°C were used for the preparation of inoculums. Two slant of pure culture was inoculated into 1 litre of papaya juice which was extracted enzymatically and pasteurized at 90°C for 15minutes. Two days old actively growing yeasts were used as inoculums at 0.5% level to the papaya pulp and juice.

Primary must dry

Dry primary must was obtained by using filtering previously fermented pulp through muslin cloth and drying the pomace under shade.

Primary must fresh

The fresh primary must was obtained by filtering the fermented pomace through muslin cloth and was used as fresh without drying.

Sediment of secondary fermentation

The sediment of secondary fermentation was the yeast sediment obtained from the wine after secondary fermentation by decanting the wine.

Fermentation process

17 kg of variety Co II papaya fruits was taken and it was completely peeled off. This yielded 15.5 kg of papaya pulp. The pulp was macerated in mixie/blender and pasteurized at 85-90°C for 5 minutes. After cooling the pulp required amount of cane sugar was added to adjust the final TSS to 24°Brix. Using this pulp, three types of treatment are done, using various processes.

There are as follows:

- I with bio pectinase CCM plus enzyme + Pulp(non clarified) +
 - 1. Pure culture
 - 2. Primary must (fresh and dry)
 - 3. Sediment of secondary fermentation

- II with out enzyme + Pulp (non clarified) +
 - 1. Pure culture
 - 2. Primary must (fresh and dry)
 - 3. Sediment of secondary fermentation

- III Juice (clarified) +
 - 1. Pure culture
 - 2. Primary must (fresh and dry)
 - 3. Sediment of secondary fermentation

In treatment number I the enzyme was added at a rate of 5 ml/kg pulp and the pure culture of the wine yeast *Saccharomyces cerevisiae* was added and mixed thoroughly and was allowed to ferment at a controlled temperature of 24 to 26°C. Potassium metabisulphite (KMS) at a rate of 200 ppm added to avoid growth of wild yeast and Diammonium orthophosphate at a source of nitrogen (N₂) and phosphorus to yeast. During the primary fermentation the must was aerated daily up to 9 days. Similarly in place of pure culture fresh primary must obtained from earlier

fermentation was added at a rate of 100g/1kg. The dry primary must was obtained by drying the fresh primary must under shade. This dry must was added to the pulp at a rate of 100g/1kg. Thirdly the sediment of secondary fermentation was added to the pulp at a rate of 100ml/1kg pulp.

Similarly in treatment number II the pure culture, primary must and sediment of secondary fermentation were added to the pulp with out enzyme.

In treatment III the pulp was treated with biopectinase CCM plus enzyme at a rate of 5ml/kg. And the pulp was incubated at 50°C for 2 hours. After incubation the juice from the pulp was separated by filtration through muslin cloth. This clarified juice was inoculated with pure culture at a rate of 2 slants/litre, the primary must and sediment of secondary must were added as explained earlier. All the treatment was kept for primary fermentation at 24 to 26° C for 9 days with periodic aeration. After 9 days all the treatments were filtered through muslin cloth and filtrate was kept secondary fermentation in plastic carboys with air lock/water seal the carbon dioxide developed during fermentation. The secondary fermentation was carried out for a period of 2 weeks at same temperature. After two weeks the evolution of CO₂ ceased and the wine was clarified by centrifugation at 5000 rpm. The sediment was discarded and the clear wine was filled into sterile bottles of 200ml capacity and crown corked. The same bottles were pasteurized at 50°C for 15 minutes. The pasteurized bottles of wine were kept for aging at ambient temperature.

Physico – chemical analysis

Physico – chemical analysis was carried out once in five days during primary fermentation and once after secondary fermentation. The observations were also recorded once after aging for one month. The parameters of observation recorded were total soluble solids, acidity, pH, alcohol, microbial count, clarity, sensory evaluation [2].

RESULTS AND DISCUSSION

Total soluble solids:

The TSS of must on the initial day of fermentation was 24° Brix. It kept on decreasing in all the treatments during fermentation and aging. The fall or decline in TSS was rapid up to 7 days fermentation in most of the treatments. In treatment without enzyme the decline in TSS was slow relatively. After secondary fermentation the least TSS was recorded in treatment using pure juice. Subsequently during aging there was further decrease in the TSS content in all the treatments. The final TSS after one month of aging varied between 8.00°Brix to 13.20°Brix (Table 1). Similar results was observed by various authors [3]-[6]. Maximum levels of these sugars were found in the wines from non clarified juice and pulp due to slow rate of fermentation. The TSS of the must on the initial day of fermentation was 24°Brix. It kept on decreasing during fermentation and aging observed in all banana varieties [7]. As the alcohol content increase, the content of TSS decreases [8].

Acidity (Total and Volatile):

In treatment E₁ (with pectinase enzyme + inoculums) the acidity was 0.68% initially which rose to a range of 1.060% to 1.120% on 5th day subsequently it decreased slightly towards aging. In E₀ (without enzyme + inoculums) the initial acidity ranged between 0.540 – 0.530% which showed a gradual decrease in the acidity during fermentation storage. The decrease in the acidity during fermentation in the juice could be due to its utilization by the yeast for production of carbon dioxide and water. While in E₁ & E₀ treatments in the increase in acidity with progress in

the duration of fermentation could be due to release of intracellular electrolytes into the medium (Table 2). The volatile acidity constituted majority of the total acidity (Table 3). In all the treatment the trend of volatile acidity was similar to that of total acidity. The non volatile acidity showed an initial increase followed by decrease in all the treatments (Table 4). The acidity of wine was observed coincides with other reports [7]-[12].

pH:

The pH of the must varied 4.14 to 4.80 initially. Subsequently this value is decreased in all the treatments indicating an increase in acidity (Table 5). Similar results was observed by various authors [8], [11]-[13].

Alcohol content:

The alcohol content in papaya wine showed an increasing trend during fermentation in all the treatments. However some treatment showed rapid alcohol conversion in comparison to other treatments. Maximum development of alcohol was found with in the period of primary fermentation subsequently during secondary fermentation, the alcohol development was sluggish. With regard to different treatments the E₁ (Enzyme + inoculum) showed rapid development during first 9 days as compared to E₀ treatments & clarified juice. During aging there was no considerable variation in alcohol content except in a few treatments (Table 6). Similar was observed by various authors [7], [13]-[18].

Total sugars:

The total sugars of papaya wine showed a decreasing trend during fermentation in all the treatments. This could be due to utilization of sugars in production of alcohol. The base of declined of total sugars was faster in treatment E₁ (Enzyme + inoculum) followed by juice and E₀ (without enzyme + inoculum) (Table 7). Similar results was observed by various authors [8], [19]-[21].

Microbial count (pour plate method)

The microbial population showed logerthemic increase during the primary fermentation subsequently there was decrease in its populations. This could be due to the fact that higher concentration of sugar substrates inhibited the growth and multiplication of yeast during secondary fermentation. With recorded to source of inoculums the pure culture had the least number of CFU/ml (184×10^3) while the maximum was found in dry pomace (320×10^3). In comparison to E₁ treatment, E₀ treatment and juice had relatively highest CFU units n 7 day. While on 9th day E₁PC was found to have the highest number of cfu/ml. (Table 8). Similar result was observed by various authors [22]-[26].

Microbial count:**(Yeast cell count by heamocytometer)**

Similar to pour plate method E₁PMD was found to have highest number of microbial cell on 5th day. Subsequently it decreases till 30th day. The highest number of cell count was observed in E₀SSF (560×10^3 /ml). After secondary fermentation the yeast cell count decreased significantly due to inhibition by low pH and high alcohol (Table 9).

Clarity

The clarity of the wine with E₀ treatment showed an increase as reflected by higher transmittance and lower optical density. However in other treated (E₁ & Juice) the clarity decreased with increase in the duration of fermentation (Table: 10) Koffi *et al.*, showed that pectic enzyme can

reduce viscosity and increase filterability of banana puree [27]. Clarification of must prior to the onset of alcoholic fermentation improves sensory characteristics of white wine [28].

Table: 1 Effect of different sources of yeast inoculum on changes in TSS of papaya wine during fermentation. (Means \pm SD (Standard deviation) triplicate results).

| TREATMENT | DURATION OF FERMENTATION | | | | |
|--------------------|--------------------------|---------------------|---------------------|----------------------|----------------------|
| | 5 th day | 7 th day | 9 th day | 30 th day | 60 th day |
| E ₁ PC | 12.14 \pm 0.01 | 9.86 \pm 0.02 | 9.44 \pm 0.01 | 9.53 \pm 0.03 | 9.36 \pm 0.03 |
| E ₁ PMD | 16.53 \pm 0.26 | 11.48 \pm 0.09 | 10.18 \pm 0.01 | 10.60 \pm 0.05 | 9.47 \pm 0.03 |
| E ₁ PMF | 13.61 \pm 0.21 | 12.62 \pm 0.02 | 10.86 \pm 0.04 | 9.84 \pm 0.02 | 9.52 \pm 0.12 |
| E ₁ SSF | 16.44 \pm 0.02 | 10.35 \pm 0.01 | 9.91 \pm 0.02 | 9.62 \pm 0.03 | 9.32 \pm 0.17 |
| E ₀ PC | 17.23 \pm 0.04 | 11.76 \pm 0.03 | 9.45 \pm 0.02 | 8.98 \pm 0.03 | 8.1 \pm 0.12 |
| E ₀ PMD | 17.36 \pm 0.01 | 17.09 \pm 0.01 | 14.55 \pm 0.04 | 13.53 \pm 0.03 | 08.65 \pm 0.02 |
| E ₀ PMF | 20.5 \pm 0.05 | 16.44 \pm 0.01 | 13.25 \pm 0.03 | 9.41 \pm 0.04 | 8.18 \pm 0.30 |
| E ₀ SSF | 22.33 \pm 0.02 | 10.16 \pm 0.03 | 12.16 \pm 0.01 | 9.56 \pm 0.03 | 8.05 \pm 0.40 |
| JPC | 11.25 \pm 0.02 | 9.45 \pm 0.02 | 08.65 \pm 0.02 | 8.51 \pm 0.02 | 8.11 \pm 0.29 |
| JPMD | 10.44 \pm 0.02 | 10.32 \pm 0.01 | 08.65 \pm 0.02 | 8.44 \pm 0.03 | 8.49 \pm 0.03 |
| JPMF | 10.46 \pm 0.01 | 9.75 \pm 0.01 | 9.46 \pm 0.02 | 8.62 \pm 0.02 | 8.51 \pm 0.29 |
| JSSF | 13.46 \pm 0.02 | 9.43 \pm 0.01 | 9.30 \pm 0.04 | 8.59 \pm 0.02 | 8.05 \pm 0.01 |

Table: 2 Effect of different sources of yeast inoculum on changes in Acidity (%) of papaya wine during fermentation. (Means \pm SD (Standard deviation) triplicate results).

| TREATMENT | DURATION OF FERMENTATION | | | | |
|--------------------|--------------------------|---------------------|---------------------|----------------------|----------------------|
| | 5 th day | 7 th day | 9 th day | 30 th day | 60 th day |
| E ₁ PC | 1.07 \pm 0.03 | 0.96 \pm 0.028 | 0.8 \pm 0.082 | 0.90 \pm 0.025 | 0.59 \pm 0.007 |
| E ₁ PMD | 1.11 \pm 0.02 | 0.81 \pm 0.013 | 0.93 \pm 0.082 | 0.84 \pm 0.025 | 0.53 \pm 0.009 |
| E ₁ PMF | 0.11 \pm 0.01 | 1.09 \pm 0.083 | 0.76 \pm 0.075 | 0.63 \pm 0.025 | 0.78 \pm 0.011 |
| E ₁ SSF | 1.23 \pm 0.021 | 1.09 \pm 0.05 | 0.94 \pm 0.083 | 0.96 \pm 0.012 | 0.57 \pm 0.018 |
| E ₀ PC | 0.85 \pm 0.02 | 0.984 \pm 0.001 | 1.13 \pm 0.090 | 0.97 \pm 0.016 | 0.54 \pm 0.015 |
| E ₀ PMD | 0.86 \pm 0.04 | 0.996 \pm 0.002 | 1.09 \pm 0.082 | 0.98 \pm 0.010 | 0.55 \pm 0.019 |
| E ₀ PMF | 0.94 \pm 0.04 | 1.22 \pm 0.008 | 1.25 \pm 0.167 | 0.83 \pm 0.016 | 0.56 \pm 0.022 |
| E ₀ SSF | 1.07 \pm 0.05 | 1.13 \pm 0.006 | 0.91 \pm 0.087 | 0.86 \pm 0.013 | 0.55 \pm 0.019 |
| JPC | 1.90 \pm 0.09 | 0.99 \pm 0.006 | 1.04 \pm 0.042 | 0.85 \pm 0.017 | 0.58 \pm 0.012 |
| JPMD | 0.94 \pm 0.04 | 1.94 \pm 0.059 | 0.82 \pm 0.058 | 0.68 \pm 0.067 | 0.57 \pm 0.016 |
| JPMF | 1.06 \pm 0.06 | 0.976 \pm 0.008 | 0.93 \pm 0.033 | 0.84 \pm 0.008 | 0.56 \pm 0.012 |
| JSSF | 0.94 \pm 0.020 | 1.01 \pm 0.011 | 0.82 \pm 0.051 | 0.55 \pm 0.033 | 0.57 \pm 0.017 |

Table: 3 Effect of different sources of yeast inoculum on changes in Volatile acidity (%) of papaya wine during fermentation. (Means \pm SD (Standard deviation) triplicate results).

| TREATMENT | DURATION OF FERMENTATION | | | | |
|--------------------|--------------------------|---------------------|---------------------|----------------------|----------------------|
| | 5 th day | 7 th day | 9 th day | 30 th day | 60 th day |
| E ₁ PC | 1.07 \pm 0.057 | 0.96 \pm 0.028 | 0.8 \pm 0.082 | 0.90 \pm 0.081 | 0.56 \pm 0.081 |
| E ₁ PMD | 1.11 \pm 0.02 | 0.81 \pm 0.013 | 0.92 \pm 0.040 | 0.64 \pm 0.115 | 0.066 \pm 0.008 |
| E ₁ PMF | 0.11 \pm 0.01 | 1.02 \pm 0.055 | 0.76 \pm 0.075 | 0.63 \pm 0.025 | 0.14 \pm 0.030 |
| E ₁ SSF | 0.96 \pm 0.008 | 0.68 \pm 0.057 | 0.92 \pm 0.033 | 0.63 \pm 0.105 | 0.12 \pm 0.010 |
| E ₀ PC | 0.85 \pm 0.02 | 0.97 \pm 0.093 | 0.79 \pm 0.088 | 0.59 \pm 0.086 | 0.09 \pm 0.013 |
| E ₀ PMD | 0.64 \pm 0.017 | 0.996 \pm 0.002 | 1.09 \pm 0.082 | 0.57 \pm 0.100 | 0.05 \pm 0.009 |
| E ₀ PMF | 0.94 \pm 0.04 | 0.98 \pm 0.017 | 1.08 \pm 0.080 | 0.68 \pm 0.068 | 0.05 \pm 0.008 |
| E ₀ SSF | 0.67 \pm .092 | 1.04 \pm 0.045 | 0.91 \pm 0.087 | 0.69 \pm 0.083 | 0.06 \pm 0.010 |
| JPC | 1.08 \pm 0.016 | 0.99 \pm 0.006 | 1.04 \pm 0.042 | 0.57 \pm 0.090 | 0.08 \pm 0.008 |
| JPMD | 0.90 \pm 0.06 | 0.88 \pm 0.078 | 0.82 \pm 0.058 | 0.54 \pm 0.098 | 0.09 \pm 0.009 |
| JPMF | 0.93 \pm 0.007 | 0.77 \pm 0.081 | 0.66 \pm 0.118 | 0.66 \pm 0.090 | 0.09 \pm 0.007 |
| JSSF | 0.94 \pm 0.020 | 0.94 \pm 0.046 | 0.79 \pm 0.090 | 0.53 \pm 0.075 | 0.08 \pm 0.007 |

Table: 4 Effect of different sources of yeast inoculum on changes in Non Volatile Acidity (%) of papaya wine during fermentation. (Means \pm SD (Standard deviation) triplicate results).

| TREATMENT | DURATION OF FERMENTATION | | | | |
|--------------------|--------------------------|---------------------|---------------------|----------------------|----------------------|
| | 5 th day | 7 th day | 9 th day | 30 th day | 60 th day |
| E ₁ PC | 0.02 \pm 0.008 | 0.120 \pm 0.008 | 0.133 \pm 0.005 | 0.023 \pm 0.012 | 0.024 \pm 0.004 |
| E ₁ PMD | 0.776 \pm 0.009 | 0.137 \pm 0.012 | 0.017 \pm 0.009 | 0.023 \pm 0.047 | 0.447 \pm 0.009 |
| E ₁ PMF | 0.64 \pm 0.008 | 0.076 \pm 0.005 | 0.167 \pm 0.047 | 0.057 \pm 0.005 | 0.643 \pm 0.005 |
| E ₁ SSF | 0.13 \pm 0.008 | 0.320 \pm 0.016 | 0.023 \pm 0.005 | 0.34 \pm 0.008 | 0.453 \pm 0.005 |
| E ₀ PC | 0.266 \pm 0.009 | 0.247 \pm 0.012 | 0.347 \pm 0.009 | 0.38 \pm 0.005 | 0.467 \pm 0.012 |
| E ₀ PMD | 0.24 \pm 0.022 | 0.150 \pm 0.008 | 0.060 \pm 0.014 | 0.42 \pm 0.008 | 0.513 \pm 0.012 |
| E ₀ PMF | 0.173 \pm 0.017 | 0.220 \pm 0.014 | 0.160 \pm 0.014 | 0.16 \pm 0.014 | 0.510 \pm 0.008 |
| E ₀ SSF | 0.423 \pm 0.026 | 0.080 \pm 0.008 | 0.213 \pm 0.005 | 0.163 \pm 0.009 | 0.480 \pm 0.008 |
| JPC | 0.83 \pm 0.012 | 0.077 \pm 0.005 | 0.150 \pm 0.008 | 0.276 \pm 0.004 | 0.510 \pm 0.008 |
| JPMD | 0.053 \pm 0.009 | 1.060 \pm 0.012 | 0.313 \pm 0.005 | 0.14 \pm 0.008 | 0.477 \pm 0.005 |
| JPMF | 0.14 \pm 0.014 | 0.223 \pm 0.012 | 0.280 \pm 0.008 | 0.18 \pm 0.008 | 0.450 \pm 0.035 |
| JSSF | 0.15 \pm 0.016 | 0.510 \pm 0.031 | 0.037 \pm 0.009 | 0.03 \pm 0.008 | 0.480 \pm 0.008 |

Table: 5 Effect of different sources of yeast inoculum on changes in pH of papaya wine during fermentation. (Means \pm SD (Standard deviation) triplicate results).

| TREATMENT | DURATION OF FERMENTATION | | | | |
|--------------------|--------------------------|---------------------|---------------------|----------------------|----------------------|
| | 5 th day | 7 th day | 9 th day | 30 th day | 60 th day |
| E ₁ PC | 4.06 \pm 0.72 | 4.15 \pm 0.21 | 4.17 \pm 0.24 | 3.85 \pm 0.22 | 3.80 \pm 0.30 |
| E ₁ PMD | 4.17 \pm 0.76 | 4.18 \pm 0.17 | 4.14 \pm 0.29 | 3.98 \pm 0.21 | 3.75 \pm 0.44 |
| E ₁ PMF | 4.06 \pm 0.49 | 4.29 \pm 0.24 | 4.26 \pm 0.18 | 3.92 \pm 0.23 | 3.78 \pm 0.16 |
| E ₁ SSF | 3.17 \pm 0.25 | 4.12 \pm 0.20 | 4.18 \pm 0.22 | 3.66 \pm 0.25 | 3.68 \pm 0.25 |
| E ₀ PC | 3.17 \pm 0.27 | 3.82 \pm 0.32 | 3.80 \pm 0.47 | 3.73 \pm 0.19 | 3.34 \pm 0.20 |
| E ₀ PMD | 4.20 \pm 0.36 | 4.29 \pm 0.31 | 4.27 \pm 0.27 | 4.18 \pm 0.20 | 3.38 \pm 0.16 |
| E ₀ PMF | 4.06 \pm 0.29 | 3.83 \pm 0.22 | 3.75 \pm 0.20 | 3.47 \pm 0.22 | 3.65 \pm 0.40 |
| E ₀ SSF | 3.89 \pm 0.27 | 3.87 \pm 0.20 | 3.82 \pm 0.20 | 3.36 \pm 0.22 | 3.37 \pm 0.25 |
| JPC | 3.98 \pm 0.28 | 4.10 \pm 0.27 | 4.12 \pm 0.27 | 3.36 \pm 0.22 | 3.74 \pm 0.28 |
| JPMD | 3.93 \pm 0.22 | 4.02 \pm 0.30 | 4.04 \pm 0.19 | 3.53 \pm 0.20 | 3.64 \pm 0.17 |
| JPMF | 4.05 \pm 0.16 | 4.14 \pm 0.19 | 4.15 \pm 0.13 | 3.69 \pm 0.23 | 3.64 \pm 0.11 |
| JSSF | 4.07 \pm 0.123 | 3.10 \pm 0.23 | 4.13 \pm 0.23 | 3.62 \pm 0.31 | 3.54 \pm 0.27 |

Table: 6 Effect of different sources of yeast inoculums on changes in Alcohol content (%) of papaya wine during fermentation. (Means \pm SD (Standard deviation) triplicate results).

| TREATMENT | DURATION OF FERMENTATION | | | | |
|--------------------|--------------------------|---------------------|---------------------|----------------------|----------------------|
| | 5 th day | 7 th day | 9 th day | 30 th day | 60 th day |
| E ₁ PC | 10.92 \pm 0.64 | 12.43 \pm 0.31 | 12.59 \pm 0.18 | 12.14 \pm 0.22 | 12.36 \pm 0.30 |
| E ₁ PMD | 9.08 \pm 0.21 | 10.30 \pm 0.30 | 11.18 \pm 0.20 | 11.24 \pm 0.28 | 12.24 \pm 0.20 |
| E ₁ PMF | 8.16 \pm 0.34 | 9.08 \pm 0.21 | 11.17 \pm 0.19 | 12.05 \pm 0.37 | 12.87 \pm 0.38 |
| E ₁ SSF | 7.36 \pm 0.025 | 8.74 \pm 0.20 | 10.62 \pm 0.30 | 12.08 \pm 0.19 | 12.41 \pm 0.30 |
| E ₀ PC | 5.11 \pm 0.19 | 10.37 \pm 0.30 | 10.64 \pm 0.29 | 11.18 \pm 0.19 | 11.40 \pm 0.29 |
| E ₀ PMD | 8.74 \pm 0.20 | 12.46 \pm 0.25 | 12.52 \pm 0.24 | 12.90 \pm 0.41 | 12.75 \pm 0.27 |
| E ₀ PMF | 3.24 \pm 0.20 | 4.80 \pm 0.15 | 8.63 \pm 0.29 | 11.24 \pm 0.19 | 11.35 \pm 0.20 |
| E ₀ SSF | 4.10 \pm 0.16 | 6.82 \pm 0.42 | 9.67 \pm 0.19 | 11.76 \pm 0.32 | 11.65 \pm 0.24 |
| JPC | 10.47 \pm 0.30 | 10.74 \pm 0.17 | 10.95 \pm 0.47 | 11.40 \pm 0.28 | 12.09 \pm 0.41 |
| JPMD | 11.37 \pm 0.25 | 11.49 \pm 0.26 | 11.64 \pm 0.27 | 11.67 \pm 0.16 | 11.96 \pm 0.48 |
| JPMF | 10.64 \pm 0.15 | 10.90 \pm 0.31 | 11.13 \pm 0.16 | 11.61 \pm 0.32 | 11.93 \pm 0.38 |
| JSSF | 8.65 \pm 0.25 | 10.44 \pm 0.34 | 10.82 \pm 0.32 | 11.26 \pm 0.25 | 11.67 \pm 0.16 |

Table: 7 Effect of different sources of yeast inoculums on changes in total sugar (%) of papaya wine during fermentation. (Means \pm SD (Standard deviation) triplicate results).

| TREATMENTS | DURATION OF FERMENTATION | | | | |
|--------------------|--------------------------|---------------------|---------------------|----------------------|----------------------|
| | 5 th day | 7 th day | 9 th day | 30 th day | 60 th day |
| E ₁ PC | 1.05 \pm 0.09 | 1.35 \pm 0.22 | 1.53 \pm 0.23 | 0.82 \pm 0.13 | 0.75 \pm 0.10 |
| E ₁ PMD | 1.84 \pm 0.34 | 1.13 \pm 0.16 | 0.80 \pm 0.12 | 0.66 \pm 0.10 | 0.64 \pm 0.03 |
| E ₁ PMF | 2.20 \pm 0.29 | 1.33 \pm 0.27 | 0.79 \pm 0.12 | 0.52 \pm 0.01 | 0.30 \pm 0.06 |
| E ₁ SSF | 1.45 \pm 0.34 | 1.15 \pm 0.11 | 0.37 \pm 0.08 | 0.35 \pm 0.08 | 0.35 \pm 0.01 |
| E ₀ PC | 1.38 \pm 0.22 | 1.28 \pm 0.21 | 0.93 \pm 0.14 | 0.55 \pm 0.01 | 0.49 \pm 0.14 |
| E ₀ PMD | 1.37 \pm 0.27 | 1.36 \pm 0.26 | 1.33 \pm 0.25 | 1.07 \pm 0.09 | 0.32 \pm 0.01 |
| E ₀ PMF | 1.47 \pm 0.30 | 1.43 \pm 0.29 | 1.39 \pm 0.23 | 0.97 \pm 0.16 | 0.43 \pm 0.02 |
| E ₀ SSF | 1.44 \pm 0.28 | 1.33 \pm 0.11 | 1.30 \pm 0.24 | 0.63 \pm 0.10 | 0.55 \pm 0.03 |
| JPC | 2.77 \pm 0.29 | 1.46 \pm 0.26 | 1.21 \pm 0.16 | 0.54 \pm 0.02 | 0.44 \pm 0.11 |
| JPMD | 2.14 \pm 0.25 | 1.31 \pm 0.14 | 0.78 \pm 0.10 | 0.66 \pm 0.15 | 0.26 \pm 0.04 |
| JPMF | 1.09 \pm 0.09 | 1.32 \pm 0.14 | 0.78 \pm 0.17 | 0.67 \pm 0.002 | 0.54 \pm 0.16 |
| JSSF | 1.53 \pm 0.23 | 1.33 \pm 0.13 | 0.65 \pm 0.02 | 0.53 \pm 0.09 | 0.35 \pm 0.07 |

Table: 8 Effect of different sources of yeast inoculum on changes in microbial count (Pour plate method) of papaya wine during fermentation.

| TREATMENT | DURATION OF FERMENTATION | | | |
|--------------------|--------------------------|-----------------------|-----------------------|-----------------------|
| | 5 th day | 7 th day | 9 th day | 30 th day |
| E ₁ PC | 184 x 10 ³ | 384 x 10 ³ | 800 x 10 ³ | 1.4 x 10 ³ |
| E ₁ PMD | 320 x 10 ³ | 480 x 10 ³ | 432 x 10 ³ | 4.2 x 10 ³ |
| E ₁ PMF | 204 x 10 ³ | 392 x 10 ³ | 576 x 10 ³ | 1.9 x 10 ³ |
| E ₁ SSF | 196 x 10 ³ | 256 x 10 ³ | 352 x 10 ³ | 1.2 x 10 ³ |
| E ₀ PC | 276 x 10 ³ | 288 x 10 ³ | 312 x 10 ³ | 0.9 x 10 ³ |
| E ₀ PMD | 312 x 10 ³ | 712 x 10 ³ | 672 x 10 ³ | 0.8 x 10 ³ |
| E ₀ PMF | 104 x 10 ³ | 416 x 10 ³ | 392 x 10 ³ | 1.1 x 10 ³ |
| E ₀ SSF | 188 x 10 ³ | 584 x 10 ³ | 544 x 10 ³ | 0.8 x 10 ³ |
| JPC | 304 x 10 ³ | 648 x 10 ³ | 216 x 10 ³ | 1.1 x 10 ³ |
| JPMD | 200 x 10 ³ | 616 x 10 ³ | 480 x 10 ³ | 3.5 x 10 ³ |
| JPMF | 244 x 10 ³ | 456 x 10 ³ | 248 x 10 ³ | 0.4 x 10 ³ |
| JSSF | 152 x 10 ³ | 376 x 10 ³ | 504 x 10 ³ | 0.3 x 10 ³ |

Table: 9 Effect of different sources of yeast inoculums on changes in microbial count (Heamocytometer method) of papaya wine during fermentation.

| TREATMENT | DURATION OF FERMENTATION | | | |
|--------------------|--------------------------|-----------------------|-----------------------|-----------------------|
| | 5 th day | 7 th day | 9 th day | 30 th day |
| E ₁ PC | 360 x 10 ³ | 520 x 10 ³ | 480 x 10 ³ | 160 x 10 ³ |
| E ₁ PMD | 480 x 10 ³ | 440 x 10 ³ | 400 x 10 ³ | 120 x 10 ³ |
| E ₁ PMF | 320 x 10 ³ | 400 x 10 ³ | 320 x 10 ³ | 120 x 10 ³ |
| E ₁ SSF | 400 x 10 ³ | 480 x 10 ³ | 400 x 10 ³ | 160 x 10 ³ |
| E ₀ PC | 320 x 10 ³ | 440 x 10 ³ | 440 x 10 ³ | 200 x 10 ³ |
| E ₀ PMD | 360 x 10 ³ | 400 x 10 ³ | 320 x 10 ³ | 080 x 10 ³ |
| E ₀ PMF | 280 x 10 ³ | 400 x 10 ³ | 440 x 10 ³ | 240 x 10 ³ |
| E ₀ SSF | 400 x 10 ³ | 560 x 10 ³ | 520 x 10 ³ | 40 x 10 ³ |
| JPC | 360 x 10 ³ | 520 x 10 ³ | 480 x 10 ³ | 160 x 10 ³ |
| JPMD | 240 x 10 ³ | 400 x 10 ³ | 400 x 10 ³ | 120 x 10 ³ |
| JPMF | 440 x 10 ³ | 480 x 10 ³ | 440 x 10 ³ | 40 x 10 ³ |
| JSSF | 320 x 10 ³ | 400 x 10 ³ | 520 x 10 ³ | 80 x 10 ³ |

Table: 10 Effect of different sources of yeast inoculums on changes in clarity of papaya wine during fermentation. (Means \pm SD (Standard deviation) triplicate results).

| TREATMENT | DURATION OF FERMENTATION | | | | |
|--------------------|--------------------------|---------------------|---------------------|----------------------|----------------------|
| | 5 th day | 7 th day | 9 th day | 30 th day | 60 th day |
| E ₁ PC | 0.674 \pm 0.001 | 0.854 \pm 0.002 | 0.785 \pm 0.026 | 0.755 \pm 0.008 | 0.864 \pm 0.002 |
| E ₁ PMD | 0.966 \pm 0.004 | 0.985 \pm 0.002 | 0.864 \pm 0.002 | 0.650 \pm 0.024 | 0.354 \pm 0.002 |
| E ₁ PMF | 1.092 \pm 0.004 | 1.044 \pm 0.004 | 0.712 \pm 0.003 | 0.504 \pm 0.003 | 0.435 \pm 0.016 |
| E ₁ SSF | 1.781 \pm 0.008 | 1.634 \pm 0.002 | 0.857 \pm 0.002 | 0.504 \pm 0.003 | 0.458 \pm 0.010 |
| E ₀ PC | 1.536 \pm 0.012 | 1.734 \pm 0.004 | 1.917 \pm 0.005 | 0.247 \pm 0.018 | 0.243 \pm 0.002 |
| E ₀ PMD | 1.438 \pm 0.016 | 1.094 \pm 0.002 | 0.449 \pm 0.007 | 0.323 \pm 0.002 | 0.216 \pm 0.002 |
| E ₀ PMF | 2.924 \pm 0.010 | 1.543 \pm 0.021 | 0.507 \pm 0.004 | 0.126 \pm 0.002 | 0.236 \pm 0.001 |
| E ₀ SSF | 1.652 \pm 0.007 | 0.418 \pm 0.004 | 0.273 \pm 0.004 | 0.164 \pm 0.002 | 0.176 \pm 0.002 |
| JPC | 0.895 \pm 0.002 | 0.366 \pm 0.008 | 0.362 \pm 0.007 | 0.346 \pm 0.013 | 0.234 \pm 0.001 |
| JPMD | 0.562 \pm 0.001 | 0.279 \pm 0.015 | 0.330 \pm 0.004 | 0.371 \pm 0.003 | 0.165 \pm 0.003 |
| JPMF | 0.914 \pm 0.010 | 0.257 \pm 0.001 | 0.254 \pm 0.001 | 0.239 \pm 0.006 | 0.215 \pm 0.004 |
| JSSF | 0.945 \pm 0.005 | 0.725 \pm 0.003 | 0.673 \pm 0.008 | 0.387 \pm 0.001 | 0.214 \pm 0.002 |

Organoleptic evaluation:

The sensory evaluation was done using 8 judge panels after aging for 1 month. Observations were recorded for color, clarity, body & taste on a 5 point scale with 5 points for excellent quality & 1 point for bad quality. The data recorded showed that the color was best in all the juice followed by E₀ treatments and was least liked in E₁ treatment. The scores for clarity, body & taste were also higher for juice treatment. The overall acceptability was found to be very good for juice treatments good for E₀ treatment and average for E₁ treatment [29] (Table: 11).

Wine yield & Economics

Among the different treatment JPC (With Enzyme + Juice + Pure culture) & E₁SSF (With Enzyme + sediment of secondary fermentation) gave the maximum wine yield of 0.892 and 0.865 ml/Kg of pulp (Table 12). Fig. 1 indicates the wine with various treatments using different inoculums.

This variation was attributed to inadequate ripening of fruits used for wine production. Based on the cost involved in the production of 865-892 ml wine/kg pulp the unit cost of a liter of papaya wine comes to around Rs.45/-.

Table: 11 Organoleptic evaluation of papaya wine using various yeast (Means \pm SD (Standard deviation) triplicate results).

| TREATMENT | Colour | Clarity | Body | Taste |
|--------------------|------------------|------------------|------------------|------------------|
| E ₁ PC | 2.75 \pm 0.029 | 2.76 \pm 0.031 | 2.81 \pm 0.029 | 3.36 \pm 0.139 |
| E ₁ PMD | 2.86 \pm 0.024 | 2.76 \pm 0.031 | 3.33 \pm 0.152 | 3.16 \pm 0.115 |
| E ₁ PMF | 2.55 \pm 0.037 | 2.35 \pm 0.021 | 3.17 \pm 0.012 | 3.07 \pm 0.094 |
| E ₁ SSF | 3.10 \pm 0.074 | 2.81 \pm 0.132 | 3.10 \pm 0.132 | 3.77 \pm 0.134 |
| E ₀ PC | 2.90 \pm 0.045 | 3.33 \pm 0.077 | 3.67 \pm 0.093 | 3.68 \pm 0.205 |
| E ₀ PMD | 3.54 \pm 0.037 | 3.17 \pm 0.012 | 3.71 \pm 0.133 | 3.51 \pm 0.162 |
| E ₀ PMF | 3.24 \pm 0.026 | 3.10 \pm 0.046 | 3.45 \pm 0.118 | 3.70 \pm 0.116 |
| E ₀ SSF | 3.25 \pm 0.025 | 3.46 \pm 0.135 | 3.27 \pm 0.017 | 3.61 \pm 0.079 |
| JPC | 4.00 \pm 0.090 | 3.38 \pm 0.052 | 3.43 \pm 0.232 | 3.80 \pm 0.092 |
| JPMD | 3.85 \pm 0.177 | 3.17 \pm 0.021 | 3.47 \pm 0.021 | 3.65 \pm 0.031 |
| JPMF | 3.86 \pm 0.025 | 3.10 \pm 0.081 | 3.82 \pm 0.008 | 4.31 \pm 0.228 |
| JSSF | 3.61 \pm 0.076 | 3.46 \pm 0.123 | 3.53 \pm 0.036 | 3.79 \pm 0.102 |

Table: 12 Effect of sources of inoculums on juice yield, wine yield and wine recovery of papaya.

| TREATMENT | Pulp wt(Kg) | Juice yield(ml) | Wine yield(ml)/kg | % recovery of wine based on pulp wt. |
|--------------------|-------------|-----------------|-------------------|--------------------------------------|
| E ₁ PC | 1 | 0.900 | 0.865 | 86.50 |
| E ₁ PMD | 1 | 0.858 | 0.820 | 82.00 |
| E ₁ PMF | 1 | 0.878 | 0.842 | 84.20 |
| E ₁ SSF | 1 | 0.895 | 0.875 | 87.50 |
| E ₀ PC | 1 | 0.875 | 0.846 | 84.60 |
| E ₀ PMD | 1 | 0.794 | 0.770 | 77.00 |
| E ₀ PMF | 1 | 0.825 | 0.800 | 80.00 |
| E ₀ SSF | 1 | 0.855 | 0.835 | 83.50 |
| JPC | 1 | 0.890 | 0.887 | 88.70 |
| JPMD | 1 | 0.890 | 0.885 | 88.50 |
| JPMF | 1 | 0.895 | 0.892 | 89.20 |

Fig. 6: Effect of inoculums (*Saccharomyces cerevisiae*) like pure culture, primary must (fresh and dry) and sediment of secondary fermentation on yield and quality changes of wine.

- *E₁PC : Enzyme (pulp) + Pure Cultures.
- *E₁PMD : Enzyme (pulp) + Primary Must Dry.
- *E₁PMF : Enzyme (pulp) + Primary Must Fresh.
- *E₁SSF : Enzyme (pulp) + Sediment Secondary Fermentation.
- *E₀PC : Without Enzyme (pulp) + Pure Cultures.
- *E₀PMD : Without Enzyme (pulp) + Primary Must Dry.
- *E₀PMF : Without Enzyme (pulp) + Primary Must Fresh.
- *E₀SSF : Enzyme (pulp) + Sediment Secondary Fermentation.
- *JPC : Enzyme (Juice) + Pure Cultures.
- *JPMD : Enzyme (Juice) + Primary Must Dry.
- *JPMF : Enzyme (Juice) + Primary Must Fresh.
- *JSSF : Enzyme (Juice) + Sediment Secondary Fermentation.

CONCLUSION

In this study all the inoculums was given good result for papaya wine making using clarified juice, non clarified juice and pulp. Among this the wine prepared from either the clarified or non clarified papaya juice is highly acceptable using the inoculums pure culture and sediment of secondary fermentation. It is quite possible to utilize papaya fruits successfully to make an acceptable quality of wine.

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