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Impact of Source of Yeast on the Physico-Chemical Properties of Rain tree (*Samanea saman*) Wine

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Abstract
The impact of yeast (Sacharomyces cerevisiae) strains isolated from Samanea saman pod, brewer's yeast, baker's yeast and control (without yeast inoculum) on the physico-chemical properties of Samanea saman wine was investigated. About 5% (v/v) of each yeast isolate was inoculated into four Samanea saman must samples ameliorated with sugar and yeast nutrient and fermented for twelve days. The results were analyzed by one- way Analysis of Variance (ANOVA) with mean separation using Least Significance Difference (LSD) t- test at ($p \leq 0.05$) and SPSS software of version 21. The pH, the total soluble solids, fermentative efficiency of the yeast isolates and alcohol contents were monitored during fermentation. The pH significantly ($p < 0.05$) decreased with sample inoculated with baker's yeast having the least pH (3.4) and all samples were significantly ($p < 0.05$) different. The total soluble solids of all samples significantly ($p<0.05$) decreased, with brewer's yeast retaining the least (6.27^{o}brix) followed by baker's yeast (6.3^{o}brix) and inherent yeast (7.3^{o}brix).There was no significant ($p > 0.05$) difference in the retained total soluble solids, fermentative efficiency and alcohol contents of all samples pitched with yeast strains, but they significantly (($p<0.05$) differed from control. The control retained the highest total soluble solids (11.0^{o}brix) . The sample with brewer's yeast was more fermentative efficient followed by those with baker's yeast, inherent yeast and control (69.94%,69.76%,65.11%,47.25%) respectively. The samples with brewer's yeast, inherent yeast, baker's yeast and without yeast inoculums produced wines with alcohol contents of 10.31%, 9.93%, 9.01% and 6.09% respectively. However, source of yeast affected the pH but not retained soluble solids, fermentative efficiency and alcohol contents of Samanea saman wine, hence any source of a particular yeast strain can be used for must fermentation to produce a quality wine. Inherent yeast can be used in absence of commercial yeast.

Keywords: *Source of yeast, Samanea saman, Rain tree, wine, fermentative efficiency, Physico-chemical properties.*

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I. Introduction

Samanea saman is a flowering tree in the pea or fabaceae family. Its common names are Saman, rain tree and monkey pod. The tree yields about 275kg of pods annually (Staples and Elevitcch,2006). In Federal University of Technology Owerri Nigeria, where it is planted as park trees, the pods are found litered on the tree site and sometimes eaten by children and animals because of its brownish, sticky and sweet flavoured pulp[1,2](Staples and Elevitch,2006 and Uzoukwu, et al.,2020) .Not withstanding its sugary taste, the fruits are not fully utilized in food industry such as alcohol production.

Though grape wine is perhaps the most common fruit alcoholic beverage, available literature shows that acceptable fruit wines have been produced from some tropical non-grape fruits such as pineapple, banana, paw-paw, apples and pears (Ifeanyi,2004) in combination with edible herbs and flowers by yeast fermentation (Akubor, et al.,2003).

Wine is an alcoholic beverage made by the fermentation of grape juice or any other fruit juices by the action of yeasts, mainly *Saccharomyces cerevisiae*. Fermentation of juice may be natural with inherent yeast or induced by added yeast cultures or their enzyme isolates. Yeasts play a major role during fermentation of fruit juices. They convert the fruit sugars into alcohol, CO_2 and other secondary products that affect the wine quality. The ability of producing alcohol depends on some characteristics of the yeast strain such as, alcohol tolerance, optimum pH and temperature, fermentative efficiency, etc. Some yeasts other than *Saccharomyces cerevisiae* species such as Candida, *Kluyveromyc*es and *Hanseniaspora* grow during the early stages of fermentation but their viability gradually decreases due to insufficient oxygen and increasing ethanol sensitivity[5]. It is therefore necessary to select yeast specie that can effectively ferment fruit juices. *Saccharomyces ceevisiae* is known for its high ethanol tolerance and alcohol yield. Specific yeast specie isolated from different sources may affect the physico-chemical properties of wine due to variable climatic conditions and fermentation performance[5] Sharmer, et al(2012). This study was conducted to evaluate how different sources of *Saccharomyces cerevisiae* may affect the pH, retained total soluble solids, fermentative efficiency and alcohol contents of Rain tree (*Samanea saman)* pod wine.

II. Materials And Methods

2.1 Source of materials

Ripe, fallen wholesome rain tree (*Samanea saman*) pods were picked from the tree sites around the School of Agriculture and Agricultural Technology (SAAT) of the Federal University of Technology, Owerri (FUTO) Nigeria.

2.2 Preparation of Rain Tree Pulp "Must" Samples

A six kilogram (6kg) portion of deseeded rain tree pulp was crushed with a manual corona grinding machine. The meal was mixed with 24 litres of portable water (1:4) and pasteurized at 70˚C for 10 minutes before cooling to 30˚C Shanker, et al (2006). The resulting mash was filtered with a clean muslin cloth to obtain a light, brown sugary liquid, which is the "Must". The total soluble sugar content (Brix value) of this initial pulp liquid extract was determined with a refractometer and recorded as 14.6 ^{o}brix. Then the sugar content of the must samples was ameliorated to 20.8˚Brix by the addition of 2kg of granulated sugar. Six grammes (0.1%) of sodium metabisulphite was added to the whole must (extracted pulp liquid) to destroy unwanted micro organisms. About 0.3% diammonium hydrogen phosphate ((NH4)2HPo4) was added as yeast nutrient (Pooja,2011).The mixture was allowed to stand for six hours Robinson(2003) at ambient temperature (28-30oC).From this basic "Must," the planned fermentation Must samples were prepared as indicated below:

Three litres (3L) was transferred to each of the four 5- litre plastic cans. Among these four samples, one sample was pitched with 5 %(v/v) starter of *Saccharomyces cerevisiae* isolated from the pulp cultures of rain tree pod ,brewer's yeast and baker's yeast The fourth fortified sample was not pitched with any yeast, bringing the Must samples to a total of four.

2.3 Yeast Strains

Three yeast strains isolated from the *Samanea saman* pod pulp, brewer's yeast and commercial baker's yeast were cultured Potato Dextrose Agar, identified as *Saccharomyces cerevisiae* with the standard identification manual and used for this study as starter cultures.

2.4 THE "MUST" FERMENTATION

Each of the "must" samples in three of the cans was inoculated with 5% (v/v) of a specific yeast strain as the starter culture leaving the "must" in the fourth can without any yeast addition as the control. All the four plastic cans containing the prepared must samples were left open for about six hours to encourage initial yeast starter(AOAO 2006). After 6 hours, the cans with contents were tightly fitted with fermentation locks and allowed to ferment.

The primary fermentation process lasted for seven days at ambient temperature (28^{o}C-30^{o}C). The first racking was done on the 7th day and the young turbid wine samples were returned to the fermenters under racks and secondary fermentation was allowed to continue until the 12th day. Some aliquots of each of the fermenting "must" were withdrawn during the primary and secondary fermentations for the analysis of some physiochemical properties.

At the end of the 12th day, they were again racked and passed through muslin filters and transferred to glass bottles where they were pasteurized at 70˚C for 10 minutes, cooled and stored in a freezer until needed for sensory evaluation.

***Plate: 1 Samanea saman* Must samples during the twelve days of fermentation**

2.5 DETERMINATION OF ALCOHOL CONTENT OF WINE SAMPLES

The alcohol content of the wine samples was determined using the method of Berry,(1987) .
Each alcohol content was estimated by dividing the drop in specific gravity by the constant 7.36.

$$\frac{SG_i - SG_f}{7.36} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..1$$

Where
ABV = Alcohol by Volume
SG =Specific gravity
SG =weight of sample ÷ Weight of water
SGi = Starting/initial specific gravity
SGf = Final specific gravity
7.36 = a constant

2.6 Determination of fermentation efficiency

The fermentation efficiency of the yeasts from different sources was determined by calculating the percentage of the difference in total soluble solids of must samples on 1st day and 12th day of fermentation.

2.7 Method of statistical analysis

The experimental design was Completely Randomized Design. The results were statistically analyzed by one-way Analysis of Variance (ANOVA) with mean separation using Least Significance Difference (LSD) t- test. The analysis was carried out using SAS Software, Version 21

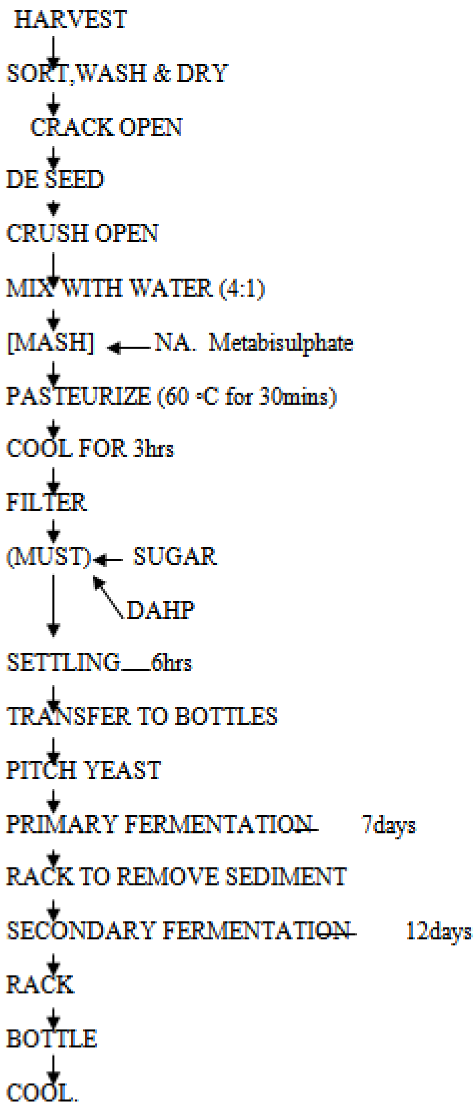

Fig. 1 Flow diagram of *Samanea saman* wine production

III. Results And Discussion

3.1 Effect of yeast source on the pH of *Samanea saman* must at different Fermentation Periods (Days)

The initial (first day) pH values of all the must samples ranged from 4.94 to 4.99 and these values gradually decreased in the samples to a range of 3.49 to 4.29 at the end of the fermentation showing significant ($p< 0.05$) differences between the samples (Table 1).The lowest pH value (3.49) at the end of twelve days fermentation was observed in the sample fermented with baker's yeast with both sugar and yeast nutrient added.

The sample fermented with brewer's yeast with sugar had a pH of 3.60 at the end of 12 days. The sample pitched with inherent yeast had the highest pH (4.29). This relatively higher pH could be because the inherent yeast was not as active as the conventional yeasts in utilizing the must sugars consequently at the 9th and 12th days the yeast population might still be lower than those of other samples for nutrient conversion. The significant ($p<0.05$) difference in pH among samples and decreased pH during the fermentation could be as a result of variable sources and the increased activity between yeast and sugar of the must which liberated acids in the medium during the twelve days of fermentation, thus increasing the acidity of wine samples and reducing its pH. This drop in wine pH as fermentation progressed conforms to Kamassah, et al.,(2013) that reported a pH drop from 4.3 to 3.82 after 98hours of fermenting local mango pulp.Nzabuherheza and Nyiramugwera (2014) observed a pH drop from 5.5 to 3.2 after twelve days of fermenting passion, mango and pineapple mixed fruit juices. Okafor, et al.,(2014) also reported a pH drop from 4.25 to 3.43 after eleven days of fermenting sour soup juice.

Table 1: Mean pH Values of Rain Tree (*Samanea saman*) pod must during the fermentation periods.

Wine Samples with	Fermentation Period (days) 1	3	6	9	12
Inherent yeast	4.9470 ±0.008c	4.7693 ±0.159a	4.5997 ±0.018a	4.4680 ±0.111a	4.2927 ±0.041a
Brewer'syeast	4.9893 ±0.003a	4.2000 ±0.685a	3.7303 ±0.024c	3.6897 ±0.017c	3.6017 ±0.037c
Baker's yeast	4.9763 ±0.003b	4.1507 ±0.717a	3.6000 ±0.020d	3.5670 ±0.005d	3.4870 ±0.049d
No yeast	4.9740 ±0.004b	4.2203 ±0.653a	3.8223 ±0.011b	3.7997 ±0.009b	3.8070 ±0.068b
LSD	0.0091	1.1280	0.0359	0.1064	0.0950

Note: Means with different superscripts on the same column are significantly ($p < 0.05$) different.

3.2 Effect of source of yeast on the Total Soluble Solids (°Brix) of *Samanea saman* must and fermentation efficiency at different fermentation period

ds. On the first day of must fermentation all the samples had brix values of about 20.84 (Table 2).In all the fermenting must samples, the brix values significantly ($p<0.05$) decreased with increase in fermentation period un till the 12th day which had brix values ranging from 6.27 to 11.00.At the 12th day, the must sample fermented with no pitched yeast, but had both sugar and nutrient, retained the highest total soluble solid (11.00°Brix) among all samples. This higher brix value could be attributed to the non-addition of starter-yeast culture since its fermentation was probably by wild yeast, there was a consequent lag-period and even at the 12th day, the yeast population could still be much lower than in other samples. This reason was supported by the fact that at each fermentation period, after the first day, the brix value of this same sample was highest among other samples with added yeast. This same reason was also supported by the fact that the least sugar utilization (or fermentation efficiency calculated as percentage total soluble solids utilized) value (47.24%) was observed in the same sample without yeast (Table 2).All samples inoculated or pitched with yeast had equal or greater than 60% fermentation performance or efficiency. Samples pitched with baker's and brewer's yeast had approximately 70% (69.77 % and 70.11 %) efficiency. This result implied that any of the yeast strains can be used for effective fermentation of the *Samanea saman* pulp, but when neither of these strains is available, then the yeast isolated from the pulp (inherent yeast) can be used with additional sugar and nutrient to achieve up to 65% fermentation performance. Fermentation efficiency depends on the ability of the yeast strain to respond to different stress conditions such as; high ethanol concentration, insufficient nutrient, etc,.Bauer and pretorius (2000) There was no significant ($p> 0.05$) difference in fermentation efficiencies of samples pitched with yeast isolated from brewer's, baker's yeast and inherent yeast but they significantly ($p< 0.05$) differed from sample without any added yeast isolate . Thus the fermentation efficiency was not influenced by source of yeast in this study but by presence of yeast isolate. The significant ($p<0.05$) decrease of total soluble solids during fermentation could be as a result of the utilization of must sugar by the yeast to produce alcohol and some metabolites. This could be why the sample without added yeast retained the highest total soluble solid content, probably because the sample did not have sufficient yeast to break down the sugars. This trend of decreasing soluble solids with fermentation period was observed in Kamassah, et al., (2013) where it decreased from 7.0 ^{0}Brix to 5.1 ^{0} and Nzeburuhereheza and Nyiramugwera (2014) . According to Helena et al.,(2015) a wine with specific gravity of 0.0990 and total soluble solids of 3.5 °Brix and below is a dry wine, while a wine with a specific gravity of 1.020 and a °Brix of 5.08 and above is a sweet or desert wine. Since the total soluble solids of 5.83 °Brix and above were recorded at the 12th day of fermentation, this therefore implies that all the *Samanea saman* wine samples produced in this research are sweet or desert wines.

Table 2

Mean Soluble Solids Values (°Brix) of Rain Tree (*Samanea oaman*) must Samples at Different Fermentation Periods

Samples with	Fermentation Period (days) 1	3	6	9	12
Inherent yeast	20.8333 ±0.0306^{a}	18.547 ±2.2236^{a}	14.200 ±1.6093^{ab}	10.7833 ±1.2887^{b}	7.2667 ±1.3013^{b}
Brewer's yeast	20.8467 ±0.0306^{a}	18.547 ±2.3205^{a}	13.200 ±1.0536^{b}	9.1000 ±1.4933^{bc}	6.2667 ±1.0017^{b}

Baker's yeast	20.8400 ±0.0400[a]	17.880 ±2.7895[a]	12.087 ±1.9261[b]	8.0500 ±1.5788[c]	6.3000 ±0.2000[b]
No yeast	20.8533 ±0.0231[a]	19.147 ±1.4968[a]	16.667 ±0.9866[a]	14.5500 ±0.8261[a]	11.0000 ±0.9539[a]
LSD	0.0595	4.2469	2.7258	2.5025	1.7978

Note: Means with different superscripts on the same column are significantly ($p < 0.05$) different.

3.3 Effect of Source of Yeast on the Alcohol Contents of *Samanea Saman* Must Samples at Different Fermentation Periods (Days)

Alcohol was not detected in any of the samples at the first day of fermentation (Table 3).On the third day of fermentation, the sample with no pitched yeast starter had the lowest level of alcohol (0.98%).On the 6^{th} day of fermentation, the sample with no added yeast continued to have the lowest alcohol value (4.01%) among all the samples and this position persisted until the final day (12^{th} day) of fermentation with an alcohol value of 6.09%.This result indicated that without addition of a starter culture, the must (with wild yeast) may not produce a wine product with more than 7.0% alcohol in twelve (12) days of fermentation. The alcohol data also indicated that brewer's yeast as starter culture, with sugar produced the highest alcohol levels (≥10.0%) during the 12 days of fermentation. It was encouraging that the *Samanea saman* must pitched with its inherent yeast, also produced a wine sample with 9.93% alcohol during 12 days fermentation. The must sample pitched with baker's yeast with added sugar and nutrient produced a wine with 9.0% alcohol. This result conformed with Thuy, et al .,(2011_b) which reported that *Saccharomyces cerevisiae* isolated from palm juice performed better than *Saccharomyces* cerevisiae isolated from commercial baker's yeast in fermentation of palm juice in terms of alcohol content which was 13.67% Vol.

However, the result suggested that if the level of alcohol is the critical quality factor needed in wines, the *samanea saman* must can be pitched with any of the yeast strains studied to produce a good wine (>9.0%) provided that sugar is added before the fermentation process.

TABLE 3: Mean Alcohol Content Values (%) of Rain Tree (*Samanea saman*) must Samples for Different Fermentation Periods

Samples with	**Fermentation Period (days)**				
	1	**3**	**6**	**9**	**12**
Inherent yeast	0.0200 ±0.0100[b]	1.623 ±1.764[a]	5.957 ±0.683[ab]	8.6233 ±1.0870[a]	9.9300 ±0.1386[ab]
Brewer's yeast	0.0300 ±0.0100[b]	1.500 ±1.558[a]	6.933 ±2.775[a]	10.0567 ±0.0451[a]	10.3133 ±0.1079[a]
Baker's yeast	0.0500 ±0.0100[a]	1.450 ±1.531[a]	8.047 ±0.507[a]	8.7533 ±0.1012[a]	9.0100[b] ±0.0755
No yeast	0.02667 ±0.0115[b]	0.980 ±1.194[a]	4.007 0.991[b]	5.5667 ±1.2503[b]	6.0933 ±0.9857[c]
LSD	0.0196	2.8719	2.8874	1.5632	0.9453

Note: Means with different superscripts on the same column are significantly ($p < 0.05$) different.

IV. Conclusion

The results suggest that different sources of Saccharomyces cerevisiae yeast may have affected the pH of the *Samanea saman* must and wine samples during and after twelve days of fermentation. The different source of yeast isolated from rain tree pod, brewer's and baker's yeasts did not have any impact on the total soluble solids, fermentation efficiency and alcohol contents of *Samanea saman* wine. We therefore conclude that the source of yeast did not affect the retained total soluble solids, fermentation efficiency and level of alcohol produced in all the rain tree (*Samanea saman*) wine samples after twelve days of fermentation but the presence of yeast starter culture influenced these physico- chemical properties of *Samanea saman* wine. However, Saccharomyces cerevisiae yeast strain can be isolated from any of the sources for effective fermentation of Samanea saman must to obtain a good quality wine with alcohol content of above 9% , hence inherent yeast can be used in the absence of commercial yeast to achieve a similar result.

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