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Wine from Water Melon Juice Using Palm Wine Yeast Isolate

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Abstract
A clear wine was produced from water melon juice using *saccahanomyce, cereuisiac* isolated from palm wine. *Saccahanomyce, cereuisiac* was found to have the essential/important features for wine production. It was employed in the production of wine at a regulated temperature $15^{o}C \pm 2^{o}C$. Method was employed for the wine production; the sensory evaluation of the wine was carried out with respect to brix level, pH, alcoholic content, colour, texture, flavor and taste. The result obtained revealed a brix level of 8.3%, pH of 3.50, alcoholic content of 9.86%.The colour was white, texture was sweet and flavor was generally acceptable. The statistical analysis carried out also showed that there was no difference in the values obtained from the text samples when compared with that of standard. Finally, it is suggested a good alternative to foreign wine that have dominated our local markets.
Keywords: alcoholic content, water melon, polyphenols, antioxydants

I. INTRODUCTION

Fermented food and beverage gives a significant proportion of all diets worldwide, producing a major contribution nutritionally and good flavor in the interest of our food consumption (Campbell, 1994).Wine is a beverage made of the fermented juice of any various kinds of grapes usually containing 10-15 percent alcohol by volume; sometimes it intoxicate or exhilarates. The colors vary depending on its type and are use for various purposes .Production of wine involves vinification, *oenology* of the *vinter* (Jancis,2003). Wine contains polyphenol, antioxidant and flavonoid, which protect the heart against cadiovascular disease such as heart failure (Streppel *et al*, 2009). Wines are classified according to color, relative sweetness, alcoholic and carbon dioxide content, the region where the grape are grown, and by country of origin (Jancis, 2003). Water melon (*Citrulluslanatus*) of the family *Cucumbitacea*is a tropical fruit which is round with green to grey colour, a red succulent flesh with black seed. It is grown abundantly in northern part of Nigeria. It consist of fiber which aid loss of weight, sugar and vitamin, can also be found in water melon as well. It is free of fat, cholesterol and low in sugar level, lowers blood pressure: and may even work to prevent risk of developing colon cancer (Daniel *et al*, 2006). Palm wine is a fermented sap of various palm trees especially *Palmyra, Silver date palm and Coconut palms*. Palm wine can be obtained from the young*inflorenscence* either male or female (Riffle, *et al*., 2003). Palm wine yeast is isolated for industrial utilization, is characterized with certain attribute necessary for ethanol production. Palm wine consist of many bacteria mainly lactic acid bacteria which include *Streptocoecus, Pediococcus, Leuconostoeand Lactobacillus* (Okagbue, 1998).Yeast is one of theeukaryotic type organism *Saccahanomycecereuisiae* is the important species of yeasts for vinification (*Boultoh et al*.,2001).Others pecies of yeast include *SchizoSaccharomyce ,Pombe, Debaryomyce,Hansemi and Zygosaccharomyces Rouxil* (Ogbulie, *et al.,* 2007). Imported wines are costly and so are only available for the privileged people who can afford it. It is supposed to be within the reach of a broader group of people, so production of wine from abundant Nigeria fruits will help contribute to saving foreign exchange, further enlarge our economy by providing employment for Nigerians and better help to prevent waste of these fruits and also give the broader population an acceptable and affordable alternative wine (Daniel, 2006).Water melon is thought to have originated in South Africa, where it is found growing wildly. Alphonse de Canoile claimed that water melon was an indigenous fruit to tropical Africa (Zohay, *et al*. 2000).Water melon contains about 88% of water and the remaining 12% consist of fiber, both sugar and vitamins and low in sodium as well (Johnson, 1993). (Daniel, *et al*., 2006). Ripeness is indicated by a fresh attractive bloom giving some appearance and a good colour that ranges from deep solid green to grey according to variety (Daniel, *et al*., 2006).The water melon juices are easily digestible and are completely absorbed for all its minerals, providing much needed nutrient to the body. The alkalinizing effect maintain the acid-alkaline balance in the body, neutralizing the toxic condition of the body resulting from excessive intake of acid forming foods (Rattry, 2012). Some of the world leading producer of wine include France, Germany, Italy, Spain, Australia, South Africa, Chile, China, U.S.A, Brazil, United State, Argentina, Hungary, Portugal, Canada, Uruguay, Russia, New Zealand and Russia (Jancis, 2006).Wine are classified into table wine, sparkling wine and fortified wine (Johnson, 1993).Table wine can be red, white or

rose. Red wine result when the crushed grape skin pulp and seeds of purple or red varieties are allowed to remain with juice during fermentation period. Pink/rose wine can be produced by removing the non-juice pumice from the juice during fermentation (Chris, 2010).Red wine contains more *polyphenol* than white wine and these are thought to be particularly protective against *cardiovascular* disease. A chemical in red wine called *resveratrol* has been shown to have both *cardioprotective and chemoprotective* effect in human bodies. The *resveratrol* is produced naturally by grape skins in response to fungal infection including exposure to yeast during fermentation. To benefit fully from resveratrol in red wine, it is recommended to be sipped slowly when drinking. Due to inactivation in the gut and liver, most of the *resveratrol* consumed while drinking red wine does not reach the blood circulation (Streppel *et al*., 2009). Red wine from France and Italy have been found to have the highest level of *procyanide* compounds thought to be responsible for heart benefits. It suppress the synthesis of peptide called *endothelin-1* that constrict blood vessel (Streppel *et al*., 2009).White wine can be made from pigmented grapes by removal of skins-pulp and seed before juice fermentation. (Daglia *et al*., 2007). Study found that both red and white is effective antibacterial agent against strains of *streptococcus*. In addition, issue of (Daglia *et al*., 2007) cancer epidermiology state that, moderate consumption of red wine may decrease the risk of lung cancer in men. In fortified wines, brandy is added to make the alcohol content higher (14 - 30 percent). These are less perishable and may be stable without pasteurisation. Wines are sparkling depending upon the amount of carbon dioxide they contain. The carbon dioxide may be formed naturally during the fermentation or may be added artificially. Sparkling wines are the one with bubbles, like champagne. Both table and sparkling wine tend to have alcohol content between 7 and 14. Table wine are the most natural, the alcohol concentration itself is not sufficient to preserve natural wine, they are pasteurized. The term light wine is also used to describe wine having alcohol content from 5-10% (Amerine *et al*., 1980).

II. Yeast for Wine Production

Yeast plays a central role in the fermentation of foods and beverages, mainly those with high carbohydrate content which can survive and grow under stress condition. Fermented beverages (burukutu, pito and palm wine) were selected in order to characterize the indigenous yeast flora (Jimoh *et al*., 2012). Microorganism reported in palm wine include both yeast and bacteria, the yeast are mainly *saccaromyces* and *candida*, a total of seventeen (17) yeast were isolated from samples of palm wine fermented from the sap, twelve of the yeast belong to saccaromyces, four were of *candida* species and one of endomycopsis species (Chandraskwar *et al*. 2012). Alan and Snow (1990), recognized wine yeast as mainly strain of *saccaromycecerevisiae* which was valued principally for their exceptional ethanol tolerance and resistance to sulphur dioxide. Akilo (1992) reported that yeast is unicellular, ova, nucleated non-motile chemosynthetic micro organism, which reproduce mainly by budding. It has the ability to efficiently and rapidly ferment juice containing 20-25% sugar without producing off flavor or aroma. A little of palm wine consist appropriately, 0.5-2.0g of protein, a major component of which is vitamin A, C and K helps consumer eye sight, it protect and improves the eye sight. Fermented palm wine should not be taken as it contain high quality of alcohol which is injurious to the organs of the body like liver, kidney and eye (Chandrasekhar*et al*., 2012). (Boulton *et al.,* 2001). (Yamamoto *et al*, 2002). (Masy *et al.,* 1991) Zoechlein *et al*. (1990), The most common preservative used in wine making is liquid sulphur dioxide, sodium or potassium metabisulphate and potassium sorbate. Sulphur dioxide acts, firstly as an antimicrobial agent and secondly as antioxidant. In the making of white wine, it can be added prior to fermentation and immediately after alcoholic fermentation is complete. If so, it will have the effect of oxygen scavenger to stop malactic fermentation and bacteria spoilage. For rose wine surphur dioxide may be used at high levels (100mg per liter) prior to fermentation to assist colour stabilization. However, small additions (20gm per liter) may be used to avoid bleaching red pigments. Further, addition (20mg per liter) to red wine may be necessary to overcome minor oxidation and prevent the growth of acetic acid bacteria. Without the use of surphur dioxide, wines can readily suffer bacterial spoilage no matter how hygienic the wine making practice may be (Jancis, 2003). Filtration of wine accomplish two objective, large particles that affect the visual appearance of the wine are removed and also in microbial stabilization. Clarification of the wine can take place naturally by putting the wine into refrigeration at 35^{o}F (2^{o}C) (Jancis, 2003).During bottling a final dose of suphite is added to help preserve the wine and prevent unwanted fermentation in the bottle (Dutoit *et al*., (2000), (Gibson *et al*., 2006).

The vast majority of wine faults are detected by the nose due to the distinctive aromas that they give off. However, the presence of some wine fault can be detected by visual and taste perceptions. Unusual breaks in the colour of wine could be a sign of excessive copper, iron or protein that were not removed during firing or filtering. Unusual colour for its varieties may be a sign of excessive or insufficient maceration or as well as poor temperature control during fermentation. Potential wine faults include burning acidic taste associated with volatile acidity that can make a wine seem out of balance (Gibson *et al*., 2006). The oxidation of wine is perhaps the most common of wine faults, as the presence of oxygen and a catalyst are the only requirements for the process to occur. Oxidation can occur throughout the wine making process. Anthiocyanins, catechins,

epicatechins and other phenols present in wine are those most easily oxidized which leads to flattening. In most cases compounds such as sulphur dioxide or erythorbic acid are added to wine by winemakers, to protect the wine from oxidation. Apart from phenolic oxidation, the ethanol present within wine can also be oxidised into other compounds responsible for flavour and aroma (Dutoit, 2005). Sulphur was used as an additive throughout the winemaking process, primarily to stop oxidation and also as antimicrobial agent. When managed properly in wine, its presence therefore is often undetected, however when used recklessly it can contribute to flavour and aroma taints which are very volatile and potent (Gibson, 2006)

III. Methodology

Material Collection

The water melon (Citrulluslanatus) and sugar were purchased from 'Wunti market' in Bauchi state. The palm wine from which the yeast was isolated was obtained from Angwan Angas, Yelwa Tudu, Bauchi State, northern Nigeria.

Preparation of the Media for Isolation of Yeast Strain

Potatoes dextrose agar was used as a medium for isolation. The medium was prepared according to the manufacturer's instruction and sterilized at 121^{o}C for 15minutes. The prepared medium was then dispensed aseptically into a sterile petri-dish and left on the bench to solidify slowly at room temperature.

Isolation Procedure of yeast cell

Palm wine was used as source of yeast cells. To obtain isolated colonies in discrete form, dilution of palm wine was made up to 10^{-1} to 10^{-3}using subsequently a serial dilution. A volume of each dilution was pipette into the surface of the potatoes dextrose agar earlier prepared and sterilized at 121^{o}C for 15minutes. Sample from palm wine was spread into the prepared agar using sterile bent glass rod and incubated at 27^{o}C for 48 hours. Microscopic colonies were observed for their morphological appearance. Suspected colonies were proved and stained with lactophenol (yeast indicator) using a sterile wire loop into the surface of the glass, the emulsion was then covered with cover slip and observed at x40 magnification under light microscope. The colony that gave positive result was sub-cultured by streaking on a media which was prepared with potatoes dextrose agar and then pure culture was streaked in a potatoes dextrose agar and stored.

Morphological Observation

The general morphology of the yeast and identification of the nucleus was carried out using Jack (2002) method which described the staining procedure: 40% ethanol solution, 0.1ml potassium hydroxide solution and 0.1 ml methylene were used. The smear of yeast strain was flooded with 40% ethanol after previously fixed by heating. The yeast nucleus was then hydrolysed by potassium hydroxide solution for one hour before staining with the addition of methylene blue stain. Observation was made under a lower power magnification. The criteria used in this morphological and physiological identification were based on the shape, size of yeast and presence of either mycelium or pseudo mycelium.

Innoculum Propagation

Water melon juice was used as a medium for the production of inoculum. The juice medium was obtained by washing the water melon that was purchased with soapy water; this is to remove any possible contaminants. It was sieved and the seed were removed, then the succulent flesh inside was sliced off, blended and sieved using cheese cloth. The water melon juice brix was standardized by raising the brix value to 24% using granulated sugar. The standardized water melon juice was analyzed for pH, brix level, and colour. All analysis was carried out and result presented in Tables 1 and 2. It was then pasteurized at 68^{o}C for 15minutes to reduce microbial load to a very low value and cooked at 68^{o}C for inoculation with yeast strains.

Fermentation

Saccharomyces cerevisiae initially isolated was used. The extracted juice was transferred into the fermenting tank and yeast isolated added. The fermenting juice was collected at 24hours interval for chemical analysis such as; Colour determination, brix level determination and Percentage alcoholic content. The juice was allowed to ferment for a period of 2 weeks.

Determination of pH

A highly sensitive single electrode pH meter : model pH s-25 was used, Standardized by using freshly prepared buffer solution according to the manufacturer's instructions.

Brix Determination

A refractometer was used for brix valve determination. The instrument was placed on the bench facing the natural light source from the window. A drop of the sample was placed on the lower prism and observed with the upper prism. The adjustment of the scale and mirror was made until the illumination was as bright as possible to obtain the dividing field as sharp as possible. The telescope was adjusted accordingly and the brix value for the sample was then made accordingly.

Colour Test

The color of the sample was determined by visual observation.

Percentage Alcoholic Content

The method described by Pearson (1993) was used. 50ml of produced wine with yeast strain was measured into a volumetric flask. The sample was then slowly poured into a 100ml volumetric flask. 45ml of the distillate was collected and made up to 50ml with distilled water. The specific gravity was determined using the specific gravity bottle. The corresponding alcoholic content was calculated using the formula below.

$$Specific\ Gravity = \frac{W_2 - W_1}{W_3 - W_1}\ x\ 100$$

Where W_1=Weight of empty bottle

W_2 = Weight of empty bottle + sample

W_3=Weight of empty bottle + sample + water

3.2.11 Racking and Fining

After two weeks of fermentation, the wine was racked by carefully transferred into another sterilized bottle. The wine was then clarified by adding 5g of gel.

Ageing

The wine was aged at refrigeration condition to mature at -2^{o}C + 15^{o}C for one month.

Racking and Bottling

The wine was racked again by transferring from one container to another to remove sodium pulp and bottled in sterilized bottles.

Pasteurization and Cooling

The matured wine was pasteurized at 68^{o}C for 15minutes, cooled, stored and ready for consumption.

Determination of Wine Stability

The freeze test for wine stability was adapted (Zocclalein *et al,* 1990), the method involved freezing or chilling the wine sample, at 6^{o}C for one week. After which wine sample was then examined for presence of crystals, if present, wine would be judged as 'unstable' and where absent the wine would be judged to be stable.

Sensory Evaluation

The wine was finally organoleptically analyzed to determine its receptiveness and to compare its taste with another stable wines. Wine tasting is by sensory examination and evaluation. The sweetness of the wine is determined by the amount of residual sugar in the wine after fermentation. Samples of the wine produced at a temperature 15^{o}C using a selected yeast strain was evaluated by taste panel of judges familiar with wine taste. The taste panel were given a precise instruction about the wine provided and are not allowed to discuss their observation with one another during evaluation session and the observation were converted to score and the students observation were scored within a given range (1-9).

IV. RESULTS

Table 1: Quality of Water Melon juice before and after Fermentation (Finished Wine)

Parameter	Water melon juice	Finished Wine
PH	5.3	3.5
Brix Level	5	8.3
Colour	Pink	White
Stability	Unstable	Stable
Alcohol content	-	9.86

Table 2: Analysis of Wine during Fermentation

Days	Brix level	pH	Temperature (^oC)	Alcohol content
1	24.00	5.30	15	0.00
2	21.50	5.20	15	0.54
3	19.50	5.15	15	0.97
4	18.10	5.00	15	1.39
5	17.20	4.94	15	1.62
6	16.10	4.85	15	2.49
7	15.30	4.80	15	2.90
8	14.20	4.72	15	3.52
9	13.50	4.65	15	3.91
10	12.70	4.58	15	4.55
11	11.80	4.45	15	4.72
12	10.20	3.90	15	5.27
13	9.60	3.78	15	7.86
14	8.30	3.50	15	9.86

V. Discussion

Wild yeast is not used for the primary fermentation, since it can give unpredictable result and problem. For fermentation to go to completion, cultured yeast is often used in the production of wine. Based on the morphological physiological identification, a yeast strain was identified to species level. The yeast isolated was *Saccaromycescerevisiae*. Colonies of *Saccaromycescerevisiae* grow rapidly and matured in three days, they are flat, smooth, moist, shining, and creamy in colour and elongated in shape (Boulton *et al.,* 2001).The yeast used in white wine production was pure culture of *Saccaromycescerevisiae* from palm wine. *Saccaromycescerevisiae* has an extensive history in the area of food processing. It is also known as Baker's or Brewer's yeast, the organism has also being used for the fermentation of alcoholic beverages (Anderson, 1992). *Saccaromycescerevisiae* is commonly used as industrial micro-organism, being present in fruits and vegetables. The public are in contact with this organism on a daily basis through both inhalation and ingestion; it is not considered a pathogenic microorganism (Dynamic, 1991). It is a normal inhabitant of soils and it is wide spread in nature. It is able to take up sugar and amino acid which enable the organism to survive for a longer period. *Saccaromyces*can also be isolated from fruit, grains and other materials with a high concentration of carbohydrate(Okagbue, 1998). The result obtained from the analysis of water melon juice is shown in Table 1 above. Various parameter such as pH, % brix (total soluble solid) and colour where also tabulated. The pH, % brix and colour gave values of 5.30, 5.0 and pink colour respectively. The analytical data of the fermented wine in Table 2 above shows a gradual decline in the brix level. Fermentation was arrested on the 14^{th} day at a brix level of 8.30. The pH also showed a gradual increase and the same gave a maximum value of 3.50, % alcoholic content increased from zero value on the 1^{st} day and attained a maximum value of 9.86%on the 14^{th} day at a temperature of 15^oC. This observation is in agreement with the report of (Jancis, 2003), which states that during primary fermentation, the yeast feed on sugar in the juice and multiply, producing carbon dioxide gas and alcohol. The recommended temperature for white wine is in the range of 15 to 18^oC. The result of sensory evaluation obtained statistically showed that wine produced at $15^oC\pm2^oC$ with *Saccaromycescerevisiae*isolated from palm wine has a good texture. The wine compared well with foreign wine samples used as a standard. The fact that the wine is generally acceptable suggests that *Saccaromycescerevisiae* can be use locally for wine production. Lastly, the result of analysis with respect to flavor gave a standard deviation of 1.02 compared to 1.24 of standard; the taste value gave a standard deviation of 0.71 compared to 1.02 of standard. Also the result obtained from colour assessment gave a value of 0.84 compared to 0.89 of the standard, the texture gave a standard deviation of 0.94 compared to 1.02 of standard and general acceptability gave a standard deviation of 0.90 compared to 1.02 of standard.

VI. Conclusion

A clear wine was successfully prepared from water melon juice. The result obtained from the sensory evaluation of water melon wine shows that, the wine was accepted by consumers .Thus water melon wine with respect to taste has a standard deviation of 0.71 compared to 1.02 of standard, texture has a standard deviation of 0.94 compared to 1.02 of standard, flavour has a standard deviation of 1.02 compared to 1.24 of standard. Also water melon wine gave a standard deviation of 0.84 with respect to colour compared to 0.89 of standard and standard deviation of 0.90 for general acceptability compared to 1.02 for standard wine.

Therefore, from the above quality parameter tested for by the consumer, water melon via yeast *Saccaromycescerevisiae* isolated from palm wine can be used to produce quality wine at an affordable price and

to reduce wastage due to lack of it preservation. It also suggests that, it can be used as an alternative tograpе which is commonly used for wine production.

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