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Full Length Research Article

COMPARATIVE QUALITY ASSESSMENT OF WINE PRODUCED FROM GREEN AND PURPLE GRAPES USING *SACCHAROMYCES CEREVISIAE* ISOLATED FROM FERMENTED MILK

*[1]Mohammed S.S.D., [1]Kwabo U.S., [2]Maiangwa J., [1]Nasir Z.J. and [1]Ibrahim M.I.

[1]Department of Biology, Microbiology and Biotechnology, Faculty of Natural and Applied Sciences, Nile University of Nigeria, FCT- Abuja
[2]Department of Microbiology, Faculty of Science, Kaduna State University, Kaduna, Nigeria

*Corresponding Author Email Address: sani.mohammed@nileuniversity.edu.ng Phone: +234(0)8035861774

ABSTRACT
This research aimed at the comparative quality assessment of wine produced from green and purple grapes using *saccharomyces cerevisiae* isolated from fermented milk. The yeast was isolated using the pour plate technique and identified using cultural morphology, microscopic appearance, the carbohydrate utilization (sugar) test and ethanol tolerance test. Green and purple grapes were placed in different conical flask. The flask were designated as GGW and PGW for each sample respectively. Each sample (1000 ml) in the flask was inoculated with 2 ml 1.0 x 10^6 CFU of *S. cerevisiae* / mL , mixed with 0.2 ml 10% sodium metabisulfite, fermented for 5 days at room temperature, degassed and stirred daily, pasteurized at 60 ° C to stop the fermentation process and evaporated in a rotary evaporator at 60 ° C to remove the alcohol. Changes in temperature, pH, titratable acidity, volatile acidity, alcohol content, specific gravity (physicochemical parameters) were determined at intervals using standard methods. The must and the produced wines were subjected to several analysis. The produced wines were subjected to a proximate analysis, microbial analysis (total bacteria, coliform and total yeast counts) and sensory evaluated using nine-point hedonic scale by 10 experts (employees and students) and a shelf life of the produced wine were also determined. The yeast isolate was microscopically suspected to have the same colony morphology as *Saccharomyces cerevisiae.* As it was observed that glucose, maltose, fructose, galactose and sucrose were used up by the yeast isolates: The yeast isolate had an ethanol tolerance of up to 14%. The physicochemical parameters were relatively stable throughout the wine production process. The temperature ranged from 27^0C - 28^0C. The pH rose after fermentation. The specific weight decreased in all produced wines. Results of the proximate composition of green and purple grape wines showed varied percentages. The microbial analysis showed that bacterial isolates were dominated by yeast isolates. The sensory evaluation showed that both green and purple grape wine had an overall acceptance. The shelf life results showed that purple wine got spoilt faster than green wines. This study has shown that locally isolated yeast strains can be used to produce wines from locally available fruits which can yield good nutritional and microbiological quality.

Keywords: Yeast, fruits, wine, sensory evaluation, shelf life.

INTRODUCTION

Wine is generally defined as the product of normal alcoholic fermentation of the juice of healthy ripe grapes. Grape wine is perhaps the economically most important fruit juice alcohol (Kelebek *et al.,* 2013), because of the commercialization of the product for industry, the process has received the most attention in research. Wine is one of the best-known products with a high added value made from fruit. It can also be used as a substrate for making vinegar, vinegar a by-product of wine fermentation. Very acceptable wines can be made from practically any fruit. Wine can be fermented using yeast, which is naturally found in grapes, and in other non-grape-producing countries, other fruits are usually looked at when making wine. There are some soft fruits from temperate and tropical regions whose pigment stability and taste profile are similar to those of any wine made from grapes, but suffer from the lack of intensive research and development for grape wine. Wine is a safe and healthy drink; it also provides calories and vitamins. At times when life was often stressful, it offered relaxation and relief from pain. There are enormous possibilities and potential for the use of microorganisms to meet the growing global demand for food through the efficient use of available natural food, feed and the conversion of waste materials. In addition, the role of yeast in winemaking is the most important element that distinguishes wine from fruit juice, and most industrial winemaking uses the commercial wine yeast strain *Saccharomyces cerevisiae* (Kelebek *et al.,* 2013). Grapes are fruits that are used to make wine and juice. Grapes, which have the botanical name *Vitis vinifera*, can be used for wine, brandy, or vinegar. Grape juice is made by crushing and blending grapes into a liquid. The juice is often sold in stores or fermented and made into wine, schnapps or vinegar. In the wine industry, grape juice, which contains 7.23 percent pulp, peels, stems and seeds, is often referred to as must in North America. Grapes, both varieties of Native American grapes, another type of European grape in California, sultanas (known there as seedless Thompson) are sometimes diverted from the vaisin or table market to produce white.

Fermented milk is the collective name for products such as yogurt, ymer, kefir, cultured buttermilk, Filmjlk (Scandinavian sour milk), cultured cream and Koumiss (a product made from horse milk). The generic name Fermented Milk is derived from the fact that the milk for the product is inoculated with a starter culture that converts part of the lactose into lactic acid. Depending on the type of lactic acid bacteria used, the conversion produces carbon dioxide, acetic acid, diacetyl, acetaldehyde and a few other substances that give the products their characteristic fresh taste and smell. The microorganisms used in making kefir and koumiss also produce ethyl alcohol. Fermented dairy products and beverages were among the first processed foods to be consumed by humans and have been used as a method of preserving food for centuries. (Marco *et al.,* 2017) Nowadays, fermented foods are generally defined as foods or beverages that are produced through controlled

microbial growth and enzymatic conversion of major and minor components of the food. In particular, fermented (or cultured) milk becomes more suitable when Bacteria are added to usually heat-treated animal milk and subsequent incubation to lower the pH with or without coagulation prepared. Pretreatment.

The most common examples of fermented milk are yogurt, cultured cream and buttermilk, and kefir, although there are many variations of these products based on historical practices, geography, and type of milk. Nonetheless, yogurt is generally defined as a cultured milk product made using *Streptococcus thermophilus and Lactobacillus delbrueckii subsp bulgaricus* (Rome, 2011). Decades of research suggest that consuming fermented foods, especially fermented dairy products, is linked to improved health outcomes. Although milk and milk products are included in almost all national nutritional guidelines, only a few expressly recommend fermented foods (Kneifel *et al.,* 2014). Products containing live bacteria when nutritional strategies for improving health are developed. (Panahi and Fernandez, 2017)

Saccharomyces cerevisiae is a eukaryotic microorganism. Microscopically it appears as a globe cell. The yeast forms one to four ascospores that are smooth and elliptical. Colonies appear smooth, raised and translucent (Martini and Martini, 1998). As with other plants, the naming of yeast is now regulated by the International Code for Botanical Nomenclature, and the accepted name for the main wine yeast is: *S. cerevisiae var.Elipsoideus*. Barnett *et al.* (2000) .*S. cerevisiae* is known for its role in food production. It is the critical component of the fermentation process that converts sugar into alcohol; an ingredient used in wine, beer, and distilled beverages. *S. cerevisiae* can come in two different forms: haploid and diploid. It is usually found in the diploid form (Laundry *et al.,* 2006). Unique strains of *S. cerevisiae* are involved in the fermentation of most fruits and vegetables, with high alcohol content, up to 20%, and acceptable taste and aroma (Jyoti and Kasipathy, 2010). Wine is made from fermented grapes or other fermented fruits. Due to their natural chemistry and natural yeast inoculation, grapes can ferment without adding acids, sugars, enzymes, water or other nutrients. Under the action of yeast, the sugar in the grapes is converted into alcohols (mainly ethanol) and carbon dioxide, making wine. In addition to its role as a popular drink due to its distinctive taste and aroma, wine can also act as a psychoactive drug, as wines are alcoholic beverages and can therefore have intoxicating effects. Growing grapes and making wine are a long-term commitment for a community, both financially and physically. Wine and its products, as well as related industries, diversify the local economy, creating jobs and new market opportunities. The research was aimed at the comparative quality assessment of wine produced from green and purple grapes using *saccharomyces cerevisiae* isolated from fermented milk.

MATERIALS AND METHODS

Collection of Samples

Fresh purple and green grape fruits were bought at the Farmers Market in Maitama, Abuja, Nigeria. The fruits were transported in clean plastic bags to the Microbiology Laboratory of the Nile University of Nigeria. The selected grapes were stored at refrigeration temperature (4^0C) (Haier Thermocool, Nigeria) for 24 hours before preparation and fermentationTwenty four (24) hour fermented milk was collected in sterile bottles from Ruggar-Fulani, Abuja, Nigeria and transported in a cold pack with an ice pack to the microbiology laboratory at Nile University of Nigeria, Abuja for the isolation of the yeast *(Saccharomyces cerevisiae*).

Fig 1: Map location of Maitama farmer's market

Isolation and Identification of Yeast from Fermented Milk

Sabouraud Dextrose Agar (SDA) (HIMEDIA) was prepared according to the manufacturer's instructions and supplemented with 50 mg / L chloramphenicol for selective enumeration of yeasts and molds. Serial dilution of fermented milk was performed and inoculated using pour plate techniques. A pure culture was produced on yeast-glucose agar plates. The microscopic examination of the isolate was carried out using the wet mount method according to Thais and Danilo (2006).

Carbohydrates Utilization Test on Yeast Isolate from Fermented Milk

One (1) % each of glucose, fructose, sucrose, lactose, xylose, mannitol, raffinose, galactose and maltose sugar were prepared using yeast fermentation broth and 10 ml volumes were added to clean test tubes. Clean Durham tubes were inserted into the tubes, all bubbles displaced, and then autoclaved at 1210 ° C for 15 minutes and allowed to cool. The sterile broth was inoculated with 0.2 ml of yeast culture broth and incubated at room temperature (27 ± 1^0C) for 72 hours and evidence of fermentation was observed through colour change and gas at the upper part of the Durham tubes in the broth.

Percentage Ethanol Tolerance Test of Yeast Isolates

The test was carried out according to Alobo and Offonry (2009). Where 2 %, 5 %, 8 %, 11 %, 14 %, 16 % and 19 % of ethanol in molten yeast glucose agar medium were prepared and poured in triplicates plates. The plates were inoculated with the pure culture of the yeast isolates and incubated at room temperature (27 ± 1^0C) for 48 hours.

Preparation of Substrate Blend (selected grapes)

The selected purple and green grape fruits were sorted, washed and sliced using a sterile shape knife to remove seed and homogenized by blending using a clean sterile commercial grind blender (Fadar plus FD989, China), to produce a must. Juice which was then pressed out and sieved with muslin clothe was labelled as grape juice (GJ), as described by Balogun and Towobola (2017).

Fermentation and Experimental Design
Pasteurization of Fruit Must
The prepared fruits must were pasteurized; the fruit must were treated with mild heat at 60^0C for 30 minutes. This was carefully carried out to safeguards the fruits must by destroying or inactivating organisms that contribute to spoilage, including vegetative bacteria but not bacterial spores (Butz, 2007).

Production of Grape Wines
The locally fabricated fermentation tank was washed and 1litre pasteurized fruit must was transferred aseptically to the fermentation tank, 0.2 mL of 10 % sodium metabisulphite was added and mixed well by stirring followed by the addition of 2 mL of 1.0 x108cells/mL of the yeast inoculum to the tank (using 0.5 McFarland standard) and while constantly shaking to disperse evenly in the tank. The tank was kept at 24±1^{0}C in a sterile laminar flow unit for fermentation up to 5 days (Butz, 2007).

Determination of Physicochemical Parameters

Determination of Alcohol Content during the Production of the Wine
One hundred (100) mL of the produced purple and green wine placed separately in two 100mL capacity graduated cylinder. These were refrigerated for 15 minutes until the temperature of the wines reached 15^{0}C. The alcohol meter was allowed to float freely on the sample and then the alcohol content was recorded. The reading was expressed as percentage (%) alcohol (Chim *et al.,* 2015).
Purified alcohol (L) =Volume of Alcohol (L) x Alcohol percentage (%)/100

Proximate Composition and Physicochemical Analysis of the Grape Juice and Wine
The proximate composition of the grape juice and the produced wine were analyzed using the Association of Office Analytical Chemist (AOAC) method and selected parameters (titratable acidity, pH and temperature) were determined using the Association of Official Analytical Chemist (AOAC) method with slight modifications as described by Balogu *et al.* (2016). The percentage promixate parameters analyzed include: moisture content, ash, protein, crude fibre, crude fatand carbohydrate as described by Moronkola *et al.* (2011)

Microbial Analysis of the Produced Grape Wines
Microbial quality of the purple and green grape wines were evaluated by inoculating the wine on PDA (yeast) and Nutrient Agar (bacteria) followed by Gram's staining and relevant biochemical assays (,catalase test, coagulase test, oxidase test, indole, MR-VR test, motility and sugar fermentation/utilization test) in accordance with the method ISO (International Standard Organization (Balogu and Towobola, 2017).

Sensory Evaluation Grape Wines
A total of 10 panelists (trained and untrained) from Nile University of Nigeria staff and students were selected to carry out sensory evaluation of the wine on a 9-point hedonic scale. Before the sensory assessment, the ISO standards for the selection, training (2 days) and monitoring of the examiner (ISO8886: 2012), the design of the test room (ISO8589: 2010) and the methodology for monitoring the performance of the sensory panel (ISO11132: 2009) followed. Each panelist was served approximately 50 ml test sample (18 2C) with 250 ml wine test glasses (ISO 3591: 1977); The result was ordered (ISO8587: 2006) and expressed according to the sensory vocabulary (ISO5492: 2008en) (Balogu and Towobola, 2017).

Shelf-life Study of the Grape Wines
The shelf life of the produced grape wines were determined by storing various samples for 3 weeks at 12 ±1° C and 16±1 ° C and performing a total number of viable yeasts, a total bacteria count at weekly intervals. The weekly counts were carried out as described under microbiological quality guide. The results were expressed as the percentage of viable cells with the original count being 100%.

RESULTS
Identification of Yeast Isolate

Morphological Identification of Yeast Isolate
The morphology of the vegetative cells of *Saccharomyces cerevisiae* was observed. Culturally, the yeast isolates had a creamy appearance (white-cream-colored colonies) and a leveled bump. microscopically the number of buds isolated varied, some were solitary, in pairs, if some had multiple buds (elongated) and appeared as smooth circular colonies, glabrous / yeast-like and immobile *Saccharomyces cerevisiae* reproduces by budding (Table 1).

Biochemical Identification of Yeast Isolate
The yeast showed variation of utilization of seven different sugars (Table 2). The fermented milk yeast strain was positive in maltose, fructose, galactose, sucrose, glucose but failed to grow in xylose and lactose after 72 h of incubation.

Ethanol tolerance test of Yeast Isolate
The result obtained revealed the alcohol tolerance test of the isolate revealed high ethanol tolerance up to 14%. Growth was observed in all plates, however the growth varied across the plates, 2%, 5%,8%, had the most growth (heavy growth), moderate growth was observed in 11%,14% while slight growth was observed in 16% and 19% plates respectively (Table 3).

Confirmation of Yeast Isolate
The result obtained after the various test carried out on the yeast isolate, confirmed the strain to be *Saccharomyces cerevisiae* (Table 3).

Proximate composition of selected grape Must prior to fermentation
The result obtained from proximate composition of green grape and purple grape juice are shown in (Table 4). The result showed that green grape juice had total carbohydrate of 7.26%, protein content of 0.35%, percentage moisture content of 91.74%, crude fat of 0.14%, ash content of 0.52% and crude fibre was not detected (ND). The result obtained showed that purple grape juice had total carbohydrate of 6.85%, crude protein content of 0.30%, moisture content of 92.28%, crude fat of 0.11%, ash content of 0.46% and crude fibre was not detected (ND).

Proximate Composition of Selected Grape Wine
The result obtained from proximate composition of green grape and purple grape juice are shown in (Table 5). The result showed that

green grape wine had percentage moisture content of 91.74% ash content of 0.52% protein content of 0.26%, crude fat of 0.145%, crude fibre of was not detected (ND) and total carbohydrate of 4.90%. The result obtained showed that purple grape wine had moisture content of 92.96%, ash content of 0.40%, crude protein content of 0.31%, crude fat of 0.16%, crude fibre was not detected (ND) and total carbohydrate of 6.17%.

Physicochemical Analysis of Grape Juice and Wines

Table 6 shows the result of the physicochemical composition of selected grape juice mixtures before and after fermentation. It was observed that before fermentation, the specific gravity and pH of green wine (Sample A) were 1.115 and 4.4, respectively, while the pH and specific gravity of purple wine (Sample B) were 1.120 and 4.3, respectively. On the other hand, the result showed that the specific gravity and pH of Sample A after fermentation were 1.002 and 5.0, respectively. The result obtained from Sample B showed that the specific gravity and pH were 1.003 and 5.1. The removal of alcohol changed the pH significantly from 4.4 and 4.3 to 5.0 and 5.2, respectively. The result showed that the total titratable acidity of the juice before dealcoholization was 13.3 for PGW and 14.1 for GGW (Table 6). The result obtained showed that the volatile acidity of the wines after fermentation was 5.3 and 5.7, respectively. The result obtained (Table 6) showed that the initial temperature of the selected grape wines before fermentation was 27^{o}C and 28^{o}C, respectively. The temperatures of samples A and B remained constant at 27^{o}C and 28^{o}C, respectively, after fermentation of the grapes. The result showed that the alcohol percentages obtained after the fermentation period were 10.5% and 10.2%, respectively (Table 6).

Microbial Analysis of Grapes Wine

The result obtained from the microbial analysis of the wine is shown in (Table 7) and shows the microbial analysis of the wine (bacterial count CFU / mL) before and after pasteurization. Sample A ranged from 8.5 x 10^2 CFU / mL to 9.9 x 10^6 CFU / mL, while Sample B ranged from 4.9 x 10^6 CFU / mL to 7.4 x 106 CFU / mL. After pasteurization, all of the plates had very few growths. Table shows the mycological analysis of wine (fungal count CFU / mL) before and after fermentation. Sample A was 4.5 x 10^6 CFU / mL while Sample B showed no growth on any of the plates. Very few growths were observed on any of the plates after pasteurization.

Determination of Total Colony Yeast Count

Fungal analysis of the wine samples shown in Table 8 revealed that the total number of viable yeasts for Sample A was 4.5 x 10^6 CFU / mL and 4.1 x 10^3 CFU / mL before pasteurization, while Sample B was 3.0 x 10^6 CFU / mL and 8.0 x 10^3 was CFU / mL. No count was recorded for purple wine (Sample A), while Sample B showed 6.0 x 10 ^{3} CFU / mL and 8.0 x 10 ^{6} CFU / mL after pasteurization. The fermentation recorded the lowest viable yeast count of 1.0 x 10^6 and the highest total viable yeast count of 8.0 x 10^6 CFU / mL.

Determination of Total Coliform Bacteria Count from the Produced Wine

Table 9 revealed the microbial analysis of the wine (coliform bacteria), the result obtained showed the presence of coliform bacteria in all test tubes by means of gas production and turbidity in all test tubes, however the amount of gas produced, and turbidity varied.

Sensory Evaluation of Produced Wines

Table 10 showed the sensory evaluation of the Green grape wine. Green grape wine had an overall acceptability which varied from neither like nor dislike (5 on the hedonic scale) to like very much (8 on the hedonic scale).Table 11 revealed the sensory evaluation of the Purple grape wine sample B. Purple grape wine had an overall acceptability which varied from neither like nor dislike (5 on the hedonic scale) to like very much (8 on the hedonic scale).

Shelf life of the Produced Wines

Table 12 showed the microbial analysis of the green grape wine and purple grape wine. Purple grape wine deteriorated faster than the green grape wine. The result obtained from the microbial analysis revealed that green grape wine and purple grape wine ranged from 1.1 x 10^6 CFU/mL -3.5 x 10^6 CFU/mL and 7.1 x 10^6 CFU/mL – 9.0 x 10^6 CFU/mL respectively.

Table 1: Morphological and Cultural Characteristics of Yeast Isolated from Fermented Milk

Parameters	Result
Elevation	Leveled/Flat
Shape	Circular
Budding	Positive
Pigmentation	Creamy White
Motility	Non-motile
Colony Nature	Smooth

Table 2: Carbohydrate Utilization Characteristics of Yeast Isolates from fermented milk

Sugars	Results
Glucose	positive
Maltose	positive
Fructose	positive
Galactose	positive
Sucrose	positive
Lactose	negative
Xylose	negative

Table 3: Percentage Ethanol Tolerance Test of Yeast Isolate

Fermentation isolates	Ethanol Concentration %						
	2	5	8	11	14	16	19
S1	+++	+++	+++	++	++	+	+

Key: +++ Heavy Growth, ++ Moderate Growth, + Slight Growth, S1 isolate code

Table 4: Proximate Composition of Selected Grapes Must

Parameters	Samples:	
	GGW	PGW
Moisture content (%)	94.33	92.28
Ash content (%)	0.52	0.46
Protein (%)	0.35	0.30
Crude fat (%)	0.14	0.11
Crude fibre (%)	ND	ND
Total carbohydrate (%)	7.26	6.85

Key: GGW: Green Grape Wine, PGW: Purple Grape Wine**,** ND: not detected

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Table 5: Proximate Composition of Grape Wines

Parameters (%)	Samples:	
	GGW	PGW
Moisture content	94.33	92.96
Ash content	0.38	0.40
Protein	0.26	0.31
Crude fat	0.13	0.16
Crude fibre	ND	ND
Total carbohydrate	4.90	6.17

Key: GGW: Green Grape Wine, PGW: Purple Grape Wine, ND: not detected

Table 6: Physicochemical Analysis of Grapes Wines

Parameters	Samples:	
	GGW	PGW
Initial temperature	27	28
Initial pH	4.4	4.3
Initial specific gravity	1.115	1.120
Final temperature	27	28
Final pH	5.0	5.2
Final specific gravity	1.0002	1.0003
Temperature after dealcoholisation	24	24
TTA before dealcoholisation	14.1	13.3
Volatile acidity after fermentation	5.7	5.3
Alcohol content	10.5	10.2

Key: TTA: Total titratable acidity, GGW: Green grape wine., PGW: Purple grape wine

Table 7: Bacteriological Analysis of the Produced Grape Wine

	Bacteria count in wine samples:	
Pasteurization	GGW (CFU/mL)	PGW (CFU/mL)
Before	$8.5x10^6$	$4.9x10^6$
After	11 (TFTC)	7 (TFTC)

GGW: green grape wine, PGW: purple grape wine., CFU/ML: colony forming unit, TFTC: Too few to count

Table 8: Mycological (fungal) Analysis of the Produced Grape Wine

	Fungal count in wine samples:	
Pasteurization	GGW (CFU/mL)	PGW (CFU/mL)
Before	$4.5x10^6$	$3.0x10^6$
After	8 (TFTC)	NGS

GGW: green grape wine, PGW: purple grape wine, NGS: no growth seen, CFU/ML: colony forming unit, TFTC: Too few to count

Table 9: Microbial (coliform) Analysis of Grapes Wine

	Samples:	
	GGW	PGW
Before Pasteurization	++	++
After Pasteurization	-	-

Key: GGW: green grape wine, PGW: purple grape wine, ++: very turbid and high gas produced
- : no turbidity and gas produced, CFU/ML: colony forming unit

Table 10: Sensory Evaluation of Green Grape Wine (GGW) Using Hedonic Scale

Parameters	1	2	3	4	5	6	7	8	9
Appearance	0	0	1	0	1	0	0	6	2
Texture	0	0	0	1	0	1	2	5	1
Aroma	0	0	0	2	1	2	1	3	1
Taste:									
Sweet	0	3	0	0	2	3	0	0	2
Sour	0	1	1	0	0	0	5	1	0
Bitter	0	1	0	0	1	1	0	0	1
Overall acceptability	0	1	0	0	1	2	2	4	0

Key: GGW: green grape wine, PGW: purple grape wine., Hedonic scale: 9=extremely like, 8=like very much, 7=moderately like, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=moderately dislike, 2=dislike very much, 1=slightly dislike

Table 11: Sensory Evaluation of Purple Grape Wine (PGW) Using Hedonic Scale

Parameters	1	2	3	4	5	6	7	8	9
Appearance	0	0	1	2	0	0	3	2	2
Texture	0	1	0	1	0	1	4	1	2
Aroma	0	1	0	3	0	1	2	2	0
Taste:									
Sweet	0	0	1	0	2	2	3	2	0
Sour	0	0	0	0	0	2	3	2	1
Bitter	1	0	0	0	0	0	1	0	1
Overall acceptability	0	0	0	0	1	2	3	4	0

Key: GGW: green grape wine, PGW: purple grape wine, Hedonic scale: 9=extremely like, 8=like very much, 7=moderately like, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=moderately dislike, 2=dislike very much, 1=slightly dislike

Table 12: Shelf Life Analysis of Grapes Wines

	Samples:	
WEEK	GGW	PGW
W1	1.1×10^6	7.1×10^6
W2	2.8×10^6	8.7×10^6
W3	3.5×10^6	9.0×10^6

KEY: GGW: green grape wine, PGW: purple grape wine, NGS: no growth seen, W1: week 1
W2: week 2, W3: week 3, CFU/ML: colony forming unit

DISCUSSION

Based on the morphological features (cultural and microscopic) and the budding pattern, it was determined that the isolates belong to the *Saccharomyces* genera, supported by their ability to utilize maltose, fructose, galactose, sucrose, glucose, the organism was identified as *Saccharomyces cerevisiae* in agreement with the report by Mohammed *et al.* (2017) and Bobai (2017) who reported that fermented milk contained various types of yeast which were predominately by *S. cerevisiae*. The same yeast species identified in this research was isolated in other fermented milk studies. However, other previous studies using traditional milk fermentations generally found greater biodiversity compared to the results of this study. For example, in *Amasi* made from cow's milk (Gadaga *et al.,* 2000) reported identifying 20 different species, with *S. cerevisiae, Candida lusitaniae, C. colliculosa, S. dairensis*

predominating in *Chal* made from camel milk (Yam *et al.,* 2015). It appears that the predominant species and biodiversity between different milk fermentations are different, probably due to differences in raw material and other physical factors (temperature, humidity, etc.).The yeast isolate showed a high ethanol tolerance of up to 14%. This implies that this yeast strain remains metabolically active in the fermentation medium and can tolerate up to 14% alcohol during the fermentation period. Bechem *et al., (*2007) found that high ethanol tolerance is a property that can be used for industrial applications. The proximate composition of the purple and green grape wine samples showed that the moisture content were between 91.74% and 92.28%. Purple grape wine had the highest moisture content of 92.28%. The composition immediately after fermentation did not maintain the same pattern, with green grape wine being 94.33% and purple grape wine being 92.28%, which, according to Okaka (2010), is responsible for its high perishability and short shelf life under ambient temperature. Grape fruit also contained total carbohydrates (7.26%) and (6.85%) for green and purple grape, invariably responsible for their high calorie count, suggesting the presence of an energy source for the yeast's metabolic activity. The protein content was 0.26% and 0.31%, for purple and green wine respectively. According to Okegbabile and Taiwo (2009), the low protein content of the grape wines reported in this study is a likely indication that there is no fear of over-accumulation of protein due to the consumption of the fruit. The total carbohydrate content after fermentation and wine making showed that the total carbohydrate content of wine made from green and purple grapes were 4.90% and 6.17%, respectively. This agrees with similar results by Balogu and Towobola (2017). Crude fat was detected in all samples before and after fermentation, with green grape 0.13% and purple wine 0.16, in contrast to the results of Balogu and Towobola (2017). Crude fiber was not detected in all samples before and after fermentation, this agrees with the report by Balogu *et al.* (2016).The removal of alcohol by rotary evaporation changed the temperature of the produced wines, as the expected temperature before dealcoholization was 27^0C for green grape wine and purple grape wine was at 28^0C. Rotary evaporation dealcholization changed the temperature from 27^0C and 28^0C in green and purple wines respectively to 24^0C in both. The temperature rises recorded during the fermentation to produce wines may be due to the catabolic reduction of sugar by yeast cells resulting in increased temperature. The heat produced during fermentation as an exothermic process leads to the temperature rising in the fermentation vessel. This is in line with the reports by Balogu and Towobola (2016). Malic and tartaric acids are the primary acids in grape wines and these acids have a direct impact on the growth and vitality of the yeast during fermentation (Bellman and Gallander, 1979). The result of this study showed pH values between 5.0 and 5.2, percentage titratable acidity between 13.3 and 14.1 and percentage volatile acidity between 5.7 and 5.3 after fermentation. In general, the lower the pH, the higher the acidity of the wine (Bellman and Gallander, 1979).Acetic acid is an organic acid with two carbon atoms that is produced in wine during or after fermentation. In this study it was observed that the post-fermentation specific gravity values steadily decreased in the range of 1.15 and 1.120 to 1.0002 and 1.003. The reduced volatile acidity may be due to the ethanol consumed during fermentation in the process, as reported by Gould *et al. (*1988). The percent alcohol content at the end of fermentation was measured as 10.5 and 10.2 for green and purple grapes, respectively. Similar report from Chilaka *et al*. (2010) show alcohol percentage between 10.14 and 12.8% during the fermentation of passion fruit, watermelon and pineapple fruits. However, according to Okunowo and Okotore (2005), this is comparable to moderate grape wine. High alcohol content are known to be important precursors for ester formation, which are associated with pleasant aromas (Clemente *et al.,* 2007).Microbial isolates were dominated by yeast isolates. The abundance of nutrients (sugar) in grapes make it a good breeding ground for microbial growth. This explains the apparently high prevalence of yeast among wine mixes. A similar report was made by Fleet (2007) and Lem *et al.* (2016) with high prevalence of yeast and bacteria from alcoholic wine. There was a rapid increase in the number of viable yeast cells during the first hours (72 hours) of fermentation and a decrease after wards. This increase in the number of yeast cells is due to the effective use of the available sugar component and the daily aeration of the fermentation must, which leads to rapid cell reproduction. This agrees with the results of Awe and Nnadozie (2015). The decrease in the number of yeast cells in the fermenting vessel could be due to the significant decrease in the sugar content as a result of the rapid and effective utilization of the sugar available in the must by the yeast cells, which leads to the fermentation of the must with a simultaneous increase in the alcohol content and the growth rate of the yeast, this is in conformity with the report of Awe (2011).The result of the microbial analysis showed that there were few or no microbial contamination of the wine after pasteurization. This shows the quality of the wine and implies that the wine was made under hygienic conditions and is safe for human consumption. A similar observation was reported by Adedeji and Oluwalana (2013). The fermentation was carried out under aseptic conditions in order to obtain a good fermentation yield and to ensure the stability of the entire fermentation process during the entire time. These precautions or measures could have been the reason why there were no impurities in the fermentation medium. The heat treatment (pasteurization) could also be another reason why there were elimination of contaminants in the produced wines. Thus the heat treatment was sufficient to destroy microbial contaminants in the wine. This is similar to the reports by Adedeji and Oluwalana (2013).The sensory evaluation rated the purple grape wine as preferable to the green grape wine. Purple grape wine received higher ratings on all parameters (appearance, texture, aroma, taste), and overall acceptance, where the mean ratings were the same. The average rating for appearance by the Panel for wine made from green grapes was higher than that of wine made from purple grapes. This could be due to the absence of anthocyanins that give the purple grapes their natural color. There was no difference in the taste of the two wines, as it was attributed to their comparable increase in alcohol content, as reported by Duarte *et al.* (2010). The result of the microbial analysis showed that the wine made from purple grapes deteriorates faster than the wine made from green grapes, which could be due to the properties of purple wines with high phenol content (Somer and Evan, 1997). Wines with a low phenol content are more prone to a loss of freshness, fruitiness and an increase in browning due to oxygen. (Vidal and Aagaard, 2008). According to Lopes *et al.* (2005), oxygen is one of the most important factors that determine the aging potential of bottled wine. Depending on the phenol content, one liter of wine can absorb 60,600 ml of oxygen, but the wine ingredients are oxidized and the character of the wine is changed or even lost (Singleton, 1987). In red wines, a little oxygen can improve the color stability and reduce the astringency due to the polymer formation. Oxygen in white wines reduces desirable sensory

properties and can therefore be an important factor in determining the shelf life of wines. According to Casey (1988), the eventual decline in wine quality is largely caused by the cumulative effects of the very gradual ingress of atmospheric oxygen as the permeability of traditional natural cork changes over time. The loss of cork elasticity reduces the sidewall force, which allows oxygen to penetrate through the glass cork interface. If the wine penetrates the cork, there is also evidence of changes in cork permeability, although no data is available on this. This situation is not limited to wines packed in glass bottles with cork stoppers, as oxygen can penetrate almost all packaged wines regardless of the type of closure or packaging material, albeit at different speed.

Conclusion

This study showed that fermented milk is an excellent habitat for *Saccharomyces cerevisiae* to be isolated. The results of the fermentation show that quality wine can be made from grapes. The study also provided insight into the efficiency of microbes (local yeast strains) and the role of *Saccharomyces cerevisiae* in the alcoholic fermentation of grapes as good and acceptable substrates for wine production. This study has shown that it is possible to produce quality wines from grapes, as the microbiological quality enhanced the physiological and sensory properties of the wines. The produced purple and green wines had general acceptability by the panelists but with the purple wine moderately liked compared to the green wine

REFERENCES

Association of Analytical Chemists (AOAC) (2000). Official Methods of Analysis of wine . *AOAC International USA*: AOAC International press. pp. 25-30.

Awe, S. (2011). Production and Microbiology of Pawpaw (Carica papaya L) Wine. *Current Research Journal of Biological Sciences* , 3(5): 443-447

Awe, S., & Nnadoze S. N. (2015). Production and microbiological Assessment of Date palm fruit and pawpaw (Phoenix dactylifera L.) Fruit Wine. *British Microbiology Research Journal,* 8(3), 480-488.

Ayogu, T.E.(2016). Evaluation of the performance of yeast isolate from Nigeria palm wine in wine production from pineapple fruits. *Bioresource Technology*: 69:189-90.

Balogu, T. V., & Towobola, O. (2017). Production and Quality Analysis of Wine from Honey and Coconut Milk Blend Using *Saccharomyces cerevisiae. Fermentation*, 3(16):2-9

Balogu, T. V., Abdulkadir, A., Ikegwu, M. T., Akpadolu, B., & Akpadolu, K. (2016). Production and Sensory Evaluation of Non-Alcoholic Wine from Sugarcane and Tiger Nut Blend Using Saccharomyces cerevisiae. *International Journal of Biosciences, Agriculture and Technology,* 7(2), 7.

Barnett, J.A., Payne, R.W. and Yarrow, D. (2000). Yeast Characteristics and Identification. 3rd Edn., *Cambridge University Press,* Cambridge, pp: 1139.

Bechem, E.E.T., Omoloko, C. Nwaga, D. and Titanji, V.P.K. (2007). Characterization of palm dehydrogenase. *African Journal of Biotechnology*, 6(14): 1715-1719.

Bellman, R.B. and Gallander, J.F. (2009). Wine deacidification in Chichester, *Advance in Food Reserach*., Academic press pp. 3.

Bobai, M., Mohammed, S.S.D., Emmanuel, S.D., and Ugboho, H. (2017). Production of Wine from Fermentation of Grape (Vitis vinifera) and Sweet Orange (Citrus seninsis) Juice using *Saccharomyces cerevisiae* Isolated from Palm Wine., *International Journal of Current Microbiology and Applied Sciences*, 6(1): 868-881.

Butz, E. (2007). Practical considerations for managing wine acidity. *Proceedings of the Spring*. Casey J. 1994. Is cork a good seal for wine? *Australian Grape grower and Winemaker* 372: 37, 39–41.

Chilaka, C. A., Uchechukwu, N., Obidiegwu, J. E., Akpor, O. B. (2010). Evaluation of the efficiency of yeast isolates from palm wine in diverse fruit wine production. *African Journal of Food Science*, 4(12): 764-774.

Clemente, J. M., Mingorance, C. L., Martíne, R., Heras, F. J., and Rodríguez, V. S. (2007). Influence of sequential yeast mixtures wine fermentation. *International Journal of Microbiology*, 301-308.

Duarte WF, Dias DR, Oliveira MJ, Teixeira JA, Silva JD, Schwan RF. Characterization of different fruit wines made from Cocoa, Cupuassu, Gabiroba, Jaboticaba and Umbu. *Food Science Technol*ogy 2010;30:1-9.

Fleet, G. H. (2003). Yeast interaction and wine flavour. *International Journal of Food Microbiology* 86: 11-22.

Gadaga T. H., Mutukumira A. N., Narvhus J. A. (2000). Enumeration and identification of yeasts isolated from Zimbabwean traditional fermented milk. *International Dairy Journal.* 10, 459–466. 10.1016/S0958-6946(00)00070-4.

International Standard Organization (ISO). (2017). *Sensory Analysis Standards*. 2017. Retrieved March 11, 2018, from https://www.iso.org

Jyoti, P. T., & Kasipachy, K. (2010*). Fermented Food and Beverages of the world. London:* CRC Press.

Kelebek, H., Selli, S., and Canbas, A. (2013). HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine. *Microchemistry Journal*., 91(2): 187-192.

Keller JB. Pineapple Wine: Directions for Pineapple Wine Baskets; 2010. Available from: http://www.ehow.com/way_5810589_directions pineapplewinebaskets.html#ixzz14iXAYA5s. [Last accessed on 2010 Nov 16].

Landry, C.R., Townsend, J.P., Hartl, D.L. and Cavalieri, D. (2006). "Ecological and evolutionary genomics of *Saccharomyces cerevisiae*". *Molecular Ecology*. 15; 559-575.

Lema, C., Garci-Jares, C., Orriolis, I. and Angulo, L. (2016). Contribution of Saccharomyces and non-Saccharomyces populations to the production of some components of Albarino wine aroma. *American Journal of Enology and Viticulture*. 47: 206-216.

Lopes P., Saucier C., Glories Y. (2005). Nondestructive colorimetric method to determine the oxygen diffusion rate through closures used in winemaking. *Journal Agricultural and Food Chemistry* 53: 6967–6973.

Marco, ML, Heeney D, Binda S. (2017). Health benefits of fermented foods: microbiota and beyond. *Current Opin Biotechnology* ;44:94–102.

Martini, A. (1993). "Origin and Domestication of the Wine Yeast *Saccharomyces cerevisiae"*.*Journal of Wine Research.* 4: 165-176.

Mohammed, S.S.D, Bamiteko, C., Abubakar, N.L., Wartu, J.R. and Aminu, A.H. (2017). Wine Production from Fermented Honey slurry and Coconut Milk Blend Using *Saccharomyces cerevisiae* Isolated from Palm Wine. *KASU Journal of Biochemistry and Biotechnology,* 1(2):24-34.

Moronkola, B.A., Olowu, R.A., Tovide, O.O. and Ayejuyo, O.O. (2011). Determination of proximate and mineral contents. *Scientific. Reviews and Chemical Communication*.1(1): 1 - 6

Okaka, J. C. (2010). Tropical Plant Perishable Processing, Storage and handling. *New Heaven,* 55-60.

Okegbile, E. O., & Taiwo, E. A. (2009). The Nutritional potential of black velvet. *Nigerian Journal of Nutritional Science,* 115-121.

Okunowo , W.O., and Okotore, R.S. (2005). The alcoholic fermentative efficiency of indigenous yeast strains of different origin. *African Journal of Biotechnology.* 4(11): 1290-1296.

Pretorius IS. And Hoj PB. (2005). Grape and wine biotechnology: Challenges, opportunities and potential benefits. *Austrialian Journal of Grape Wine Research*: 11:83-108.

Singleton V. 1987. Oxygen with phenols and related reactions in musts, wines, and model systems: observations and practical implications. *American Journal of Enology and Viticulture*:38: 69–77.

Somers T., Evans M. (1997). Spectral evaluation of young red wines: anthocyanin equilibria, total phenolics, free and molecular SO2 "chemical age". *Journal of the Science of Food and Agriculture :*28: 279–287.

Vidal S. and Aagaard O. (2008). Oxygen management during vinification and storage of Shiraz wine. *Australian Wine Industry Journal* : 23(5): 21–23.