

BACTERIOLOGICAL QUALITY AND SENSORY EVALUATION OF WINE PRODUCED FROM BLENDS OF DATE PALM FRUIT AND CUCUMBER JUICE USING *Saccharomyces cerevisiae*Mohammed, S.S.D^{1*}, Mohammed, I.A² and Balogu, T.V²

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ABSTRACT

Bacteriological quality and sensory evaluation of wine produced from blends of date palm fruit and cucumber juice using *Saccharomyces cerevisiae* was investigated. The yeast was isolated and identified using standard techniques. The *S. cerevisiae* inoculum was produced using standard procedures and used to ferment the blends of date and cucumber to produce wine. Five (5) samples of wine were produced from date and cucumber at various concentration of substrates, anaerobically (Aerobic fermentation were terminated after 6 days and the fermented ‘‘musts’’ were sieved to remove the shaft and debris of the crushed fruits. During the anaerobic phase of fermentation, the filtrates obtained after sieving the ‘‘musts’’ were transferred into anaerobic fermentation round bottom flask (s) and incubated at room temperature. An air trap were fixed to the fermenting jars. Fermentation were terminated after four (4) weeks). Physicochemical, microbiological and sensory evaluation (using 7 point hedonic scale by 20 panelist) of the produced wine were studied. The yeast was identified as *Saccharomyces cerevisiae*. The physicochemical analysis of the produced wines showed that percentage of alcoholic content of wine B had the highest at 16.6 %, as Wine A had 15.8 %, Wine D had 13.17 %, Wine C had 11.17% and Wine E had 10.18% of alcohol content after fermentation. The suspected bacteria isolate were *Corynebacterium* sp., *Neisseria* sp., *Corynebacterium* sp., *Micrococcus* sp., *Enterobacteriaceae* sp., *Lactobacillus* sp. and *Micrococcus* sp. and some fungi sp. were isolated from the wines before pasteurization for sensory evaluation. But no microorganisms were detected after pasteurization of the produced wines. The sample C (Date and Cucumber) of ratio 3:1 was significantly preferred (p<0.05) from other wine A, B, D and E in respect to the aroma, test and appearance and over all acceptability. The *S. cerevisiae* used in the production of the wines gave excellent results. Harnessing the date and cucumber for wine production will reduce post-harvest loss of the fruit (wastage), improve, contribute to the economy and reduce unemployment.

Keywords: Physicochemical, microbiological, sensory evaluation, wine, yeast

INTRODUCTION

Wine was derived from the Greek word ‘oines’ meaning wine while the science of wine is called ‘oenology’ (Okoro, 2007). It starts with the harvesting of grapes, separation of the juice before fermentation and concludes with the variety of storage and ageing steps. Apples, berries and black currants are sometime also fermented for wine production. Grape berries have a natural chemical balance which allows a completely fermentation without the addition of sugar, acid, enzymes or other nutrients (Okoro, 2007). It is a rich source of vitamins, many essential amino acids, minerals, fatty acid and others; however other fruits with same characteristics have been discovered and used effectively for wine production. During fermentation, microscopic single-celled organism called ‘yeast’ such as *Saccharomyces cerevisiae* digest sugar found in fruit juice, producing alcohol and carbon dioxide gas in the process. Wines are categorized using a number of different criteria, this include grape variety, region of origin, by colour, by name of the wine maker or viticulturist, or by production technique. However, three basic groups of wines are most easily distinguishable for the consumer: table wines, sparkling wines, fortified wines, fruit wine, dry wine sweet wine (Okoro, 2007). They may also be classified on the basis of the Countries of origins or fruit. From which they were obtained for example red table wine are made from black grapes while whites wine are made from black or white grapes. It is now known that it can be produced from other fruit such as mango, oranges, date fruit, pineapple, cucumber, Lemons bananas (Robinson, 2006; Amerine *et al.*, 2012). Wine has been produced and enjoyed by many people, from peasants to kings, for thousands of years. The consumption of red wine is known to have a remarkable protective effect against oxidative stress in blood plasmas. Most wines consumed in Nigeria are completely fermented, aged, bottled and imported ones. Imported products are costly now due to high duties paid on them. This had made imported wines too expensive to local consumers and for these reasons, there arose the need for more wine from other plants species.

Date Palm fruit (*Phoenix dactylifera* L.) originated in Mesopotamia (currently Iraq) and its cultivation spread to the Arabian Peninsula, North Africa and the Middle Eastern countries in ancient times about 5000 years ago (Kader and Hussein, 2009). Dates are very cheap fruits but rich in nutrition. The mineral contents in the dates have the potential to provide a good source of zinc, potassium, calcium, and sodium in the diet. (Mowunmi, 2013). Egypt places top among the top date fruits exporting countries around the world by economizing 1,373 metric tonnes averagely each year (UN Food and Agricultural Organization, 2016). Fruits of the date palm are a main source of staple food in

arid and semi-arid regions of North Africa, Middle East and South-Asian countries (Amina *et al.*, 2010). Dates have always played an important role in the economic and social lives of people of this area. These have abundant use as nutritional treat during Islamic holy month of Ramadan (Jamil *et al.*, 2012). Fresh dates compose of soft, easily consumable flesh and simple sugars like fructose and dextrose. When eaten, they replenish energy and revitalize the body instantly (Ragava *et al.*, 2017). They are deseeded and stuffed or chopped and used in a great variety of ways. They are mixed with cereals, in pudding, bread, cakes, cookies, ice cream or candy bars. Dates are also made into cubes, paste, spread, powder (date sugar), jam, jelly, juice, syrup, vinegar or alcohol. Date brittle, date arrack, a wine like drink were also prepared in Egypt from dates (Ragava *et al.*, 2017).

Dates are generally regarded as resistant to microbial spoilage. However some contaminants, especially some osmotolerant yeasts and moulds may survive longer times or even grow on the fruits (Siddig, 2012). Endophytic fungal species plays a major role in colonisation and survival of host plant. Date fruits also contains some anti-nutritional factors like tannins, phytates and oxalate contents. Consumption of tannin in large doses may cause bowel irritation, damage the liver, stomach and kidney irritation and gastrointestinal pain, chelate minerals and makes them unavailable to the body (Coe *et al.*, 2005). Phytate is a complex fibre content which cause mineral deficiency and pellagra (Coe *et al.*, 2005). Date extracts were contains stronger antibacterial activity against Gram positive bacteria than Gram negative bacteria and it has an observed notable antibacterial activity against *Staphylococcus saprophyticus* (Salah and Otiabi, 2013). Cucumber is a creeping vine that bears cylindrical fruits. It is scientifically known as Cucumis sativus it belongs to the gourd family cucurbitaceae (Abbey and other, 2017). Other vegetables which belong to this family include melon, squash, watermelon and pumpkins. It originated from the Asia continent. Cucumber plant can be cultivated in both temperate and tropical environment hence it is said to be a native of many regions of the world. There are several varieties of cucumber but the edible cucumber is classified under two groups the slicing and pickling cucumber (Abbey *et al.*, 2017). Cucumber fermentation relies on the process of naturally-occurring by LAB or in some cases inoculum from pure starter culture. The slicing cucumbers are longer and thinner when compared to the pickling cucumber. Cucumber contains nutrients vital for the body development. It also has several health benefits such as: rehydrating the body, health regulating the blood pressure, body weight management, cholesterol reduction, cancer prevention, bone health, diabetes cure and antioxidant activity (Abbey *et al.*, 2017). The microflora population of fresh cucumbers is dominated by Gram-negative aerobic bacteria. In general, LAB growth is primarily influenced by

various factors such as pH of the media, fermentation temperature, media composition (sugars and nutrients) and their mode of fermentation (Mussantto *et al.*, 2008). Fermentation is a viable technique in the development of new products with modified physicochemical and sensory qualities, especially flavour and nutritional components. Alcohol and acetic and lactic acid fermentation are important for quality in production. Of these, alcoholic fermentation is widely employed for the preparation of beverages in which alcohol is major constituent. Fermented beverages have been known to human kind from time immemorial. An alcoholic beverage is a drink that contains ethanol. These are divided into three general classes for taxation and regulation of production, namely, beers, wines, and spirits distilled beverages such as whisky, rum, gin, and vodka (Saranraj *et al.*, 2017). On the other hand fermentation is biotechnology in which desirable microorganisms are used in the production of value-added products of commercial importance. Fermentation occurs in nature in any sugar-containing mash from fruit, berries, honey, or sap tapped from palms. If left exposed in a warm atmosphere, available yeasts act on the sugar to convert it into alcohol and carbon dioxide (Saranraj *et al.*, 2017). The study was aimed at bacteriological quality and sensory evaluation of wine produced from blends of date palm fruit and cucumber juice using *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Study Area

The research was carried out at Applied Food Microbiology and Food Safety Laboratory of Ibrahim Badamasi Babangida University, Lapai, Niger State of Nigeria.

Collection of Samples

The Date fruit and cucumber used in this research work were purchased from local market at Lapai, Niger State and were deposited in sterile sampling bag. Both substrate (sample purchased) were transported down to Applied Microbiology and Food safety Laboratory, Ibrahim Badamasi Babangida University, Lapai and stored in the refrigerator at 4°C.

Media Preparation

The media: Nutrient Agar (NA), Potato Dextrose Agar (SDA) were prepared according to the manufacturers instructions.

Inoculum Preparation of Starter Culture

Pure culture of *Saccharomyces cerevisiae* used to ferment the fruit blends/mixtures was obtained from Applied Food Microbiology and Food Safety Laboratory of Ibrahim Badamasi Babangida University, Lapai, Niger State. The inoculum of *Saccharomyces cerevisiae* was prepared from 24 hours old culture and was serially diluted and turbidity was compared with 0.5 McFarland standard to get 10^8 cell/ml.

Preparation of Samples

The dried date fruit and fresh cucumber were weighed, washed, sliced, rewashed, with the removal of the seeds for date and then reweighed. The date fruit were diluted with sterile water to obtained softness of the date. Date fruit and cucumber were then minced and homogenised using a sterile blender and then filtered. The overall water added during the blending was 3000 ml distilled water to avoid friction in the blender. Exactly 3000 ml of distilled water was added to extract the "must" by filtering the juice with muslin cloth. After filtration, juice were then pasteurized at 60°C for 1hr and allowed to cool before 1000 ml was measured in to round bottom flasks of 2000 ml capacity in ratio of 1:1, 1:2, 1:3, 2:1, and 3:1 date and cucumber juice combination labelled as A, B, C, D and E respectively, all in duplicates.

Fermentation Process to Produce Wine

Standardized inoculum (10^8 cells/ml) of *Saccharomyces cerevisiae* were added to the "musts" in 2000 ml round bottom flask containing the date and cucumber juice blends, this was achieved by sprinkling it over the surface of the juice then stirred; the inoculated musts were covered with sterile muslin cloth and incubated

at room temperature ($28 \pm 2^\circ\text{C}$); and was aerated daily by stirring twice to encourage yeast multiplication. The physicochemical parameters such as temperature, pH, total titrable acidity (TTA) and specific gravity during fermentation were determined using standard techniques. Aerobic fermentation were terminated after 6 days and the fermented musts were sieved to remove the shaft and debris of the crushed fruits. During the anaerobic phase of fermentation, the filtrates obtained after sieving the musts were transferred into anaerobic fermentation jar and incubated at room temperature. An air trap were fixed to the fermenting jars. Fermentation were terminated after four (4) weeks; the wines were then stored at temperature of $28 \pm 2^\circ\text{C}$ to allow the yeast to flocculate for 21 days. The resulting wines were then aged for two months. The aged wines were then filtered using a sterile given set kit, decanted into sterile bottles and corked as described by Victoria *et al.* (2017).

Microbial Enumeration of the Produced Wines

Populations of bacteria in the wine were assessed by standard pour plate method on nutrient agar. The microbial load was counted and the bacterial and fungal growth were also identified under a high power microscope. Twenty five (25) ml of the wine sample was added to 225 ml of normal saline (1:9) for each sample respectively. Exactly one (1) ml was collected from each serially diluted sample solution. And were pour plated by pipetting 1ml of each sample into empty sterile petri dish and pouring 15ml of the Nutrient agar medium. The plates were rocked properly to achieve uniform distribution of both the media and sample after which the plates were allowed to solidify on a flat surface. The nutrient agar plates for enumeration of bacteria were incubated at 37°C for 24 hours while Sabouraud dextrose agar were used for the enumeration of fungi after serial dilution procedures (Tenfold serial dilution of wine made were aseptically pipette into 90 ml amount of sterile distilled water. 1ml of inoculum was transferred to the next test tube of distilled water (9ml) 10^{-5} dilution. The sample were diluted up to 10^{-6} of the wine, pour plated and were incubated at $28 \pm 2^\circ\text{C}$ for 72 hours. At the end of the incubation, the plates were retrieved and the number of colonies formed were counted as described by Awe and Nnadoze (2015). Colonies observed on the plates after incubation period were counter representative for bacteria and fungi were selected after random morphological screening for identification. Colonies were repeatedly subculture to purify by employing streaked plate method.

Identification and Characterization of Bacteria Isolates From Produced Wine

The identification and characterization of isolated organisms was done based on colonial morphology, cellular morphology and biochemical characterization tests.

Morphology of Bacterial Isolates from Produced Wine

Parameters, such as colour, shape, elevation surface, consistency edge and capacity were observed and recorded.

Cellular Morphology of Bacteria Isolates from Produced Wine

Microscopy of isolates were carried out using gram and spore staining for bacteria and lacto phenol cotton blue stain for fungal isolates.

Biochemical Characteristic of Bacterial Isolates From Wine

Gram Staining

Using sterile technique, smears of the fresh culture of 24 hours of the isolates were made by placing a drop of water on a clean slide with sterile cooled loop. The smear were air dried and heat fixed by passing the slides over flames. The slides were flooded with crystal violet for 1-2 min each. They were rinsed with tap water and Lugol's iodine was added and left to stand for 1 min. (acts as a mordant). The smears were then decolourised with until crystal violet failed to washed with water and then counter stain for 30 sec. The slides were washed with water and blot dried and further dried in air. The slides were then examined under an oil immersion lens (Oyeleke and Manga, 2008).

Catalase Test

Two drops of 3% hydrogen peroxide (H_2O_2) was placed on grease free slides using a rubber pipette. The test organisms were aseptically transferred onto the

drops on the slide using a sterile wire loop and were mixed properly. The smear on the glass slides were observed for gas bubbles as described by (Cheesbrough, 2000).

Coagulase Test

A small drop of distilled water was placed on clean glass slides. A sterile wire loop was used to pick the test organisms and was emulsified on the slides. Three drops of plasma was added and mixed properly (Cheesbrough, 2006).

Mannitol Salt Test

Exactly 2.8g of agar-agar and 0.08g of mannitol salt were dissolved in 250 ml of distilled water, and dispensed into a conical flask and autoclaved at 121°C for 15 minutes. The media was allowed to solidify on plates. The test organisms were streaked on the surface of the agar-agar and incubated at 37°C for 48 hours (Onyeagba, 2004).

Citrate Test

Twenty four 24g Simon citrate agar 24g was dissolved in 100/ml of distilled water, and dispensed into a conical flask and autoclaved at 121°C for 15 minutes. The media was allowed to solidify on plates. The test organisms were streaked on the surface of the citrate agar and inoculated at 37°C for 48 hours (Onyeagba, 2004).

Sugar Fermentation Test

One (1) % (i.e. 1g in 100ml) of peptone water was prepared and 9ml was dispensed into each test tube. 3-4 drops of phenol red was added and autoclaved at 121°C for 15 minutes. Double strand of a glucose sugar was prepared (2% of glucose). 1m of the sugar (glucose) was added to each test tube i.e after the prepared peptone water has cooled. A colony of organism was picked from the slant bottle and inoculated into the test tube containing the sugar, peptone water and an inverted Durham tube. Lactose, sucrose and fructose were also prepared (1%) following the same procedure. The test tubes were incubated 3 days after which each test tubes were observed for acid and gas production by comparing them to the control (Cheesbrough,2006).

Urease Test

Exactly 2.8g of Nutrient Agar was dissolve in 90 ml of distilled water, phenol red was introduced into the media, mixed and autoclaved at 121°C for 15 minutes. The urea was then added aseptically to the mixture of material agar and phenol red which was mixed properly and dispensed into test tubes and allowed to solidify in a slant position. The test organism were streaked on the entire slat surface and incubated at 37°C for 48 hours.

Sensory Evaluation of the Produced Date – Cucumber Wines

The sensory evaluation of the wines were assessed by twenty (20) panels drawn from staff and students of The Department of Microbiology of IBB University. Wine samples were assessed based on parameters such as appearance, texture, aroma, taste (sweet, sour and bitter) and the overall acceptability. The wine samples were assessed using a nine (9) point hedonic scale. Prior to sensory evaluation, ISO standards for selection, training (≥2 days) and monitoring of assessors (ISO 8586:2012), design of testing room (ISO 8589:2010), and methodology of monitoring performance of sensory panel (ISO 11132:2009) was followed. Each assessor was served with approximately 20 mL of test sample (18 ± 2°C) with 250 mL wine tasting glasses (ISO 3591:1977); results were ranked (ISO 8587:2006) and expressed in accordance with the sensory vocabulary (ISO 5492:2008) (Balogu and Towobola, 2017).

Statistical Analysis of Data

Data generated were subjected to analysis of variance (ANOVA). Mean separation and comparison was done using SPSS version 23.0. Significance was accepted at P < 0.05 and results were expressed as mean ± Standard deviation from the mean.

RESULTS

Assessment of the Date-Cucumber Wine samples showed that the temperature profile were relatively stable from week 0 to week 4 during fermentation. This was shown that Wine A (1:1) slightly varied from 29.8 °C - 30.8 °C, Wine B

(1:2) 30.8 °C - 31.0 °C, Wine C (3:1) 31.5 °C – 32.7 °C, Wine D (1:3) 29.9 °C – 31.7 °C and Wine E (2:1) 29.8 °C – 31.0 °C. Wine A had the lowest temperature of 29.8 °C – 30.8 °C and Wine C had the highest temperature (Fig1). The pH kinetic of Date-Cucumber wine showed maximum pH of (6.67) on Wine A followed by B (6.61), E (6.55), C (6.53) D (6.46) respectively on week 3 and steadily increased to pH (6.93) on week 6 (sample A) (Fig.2). There was a noticeable difference as the wine gradually reduce in quantity as the fermentation days of wine increases. The specific gravity of each wine samples decreased after fermentation. The gravity sample A range from (1.40-0.67), B (1.47-0.70), C(1.45-0.71), D(1.64-0.73),and E (1.75-0.72) (Fig.3). Fermentation velocity is the rate of fermentation. It tends to measure the rate or percentage sugar conversion to alcohol. Sample B had the best rate (0.770) and other (A, D, C and E) range from 0.5419-0.4126 (Fig4). The residual sugar mean sweetness in wine, it is usually measured in percentage (%). Basically, residual sugar or “RS” is the sugar form date-cucumber wines left over after fermentation. The more residual sugar remaining in the wine, the sweeter the wine. The residual sugar was stable from the first week and it starting reducing and sample C, D and E had the highest reduction factor (-95.828) and sample B and A (-72.313) had the lowest reduction rate or range (Fig.5). Alcoholic content of date-cucumber wine showed that sample B had the highest alcoholic content of 16.06 % followed by A (15.8 %) D (13.17 %), C (11.17 %) and E (10.18) (Fig.6). A steady progressive microbial growth curves of 7.8-8.3 were observed within 60 days of fermentation with sequential intervals week 0, week 2, week 4, week 6 and week 8, respectively (Fig.7). Morphological and biochemical characterization of bacterial isolates from date-cucumber wines are presented in table 1. The bacteria isolated include *Corynebacterium* sp, *Neisseria veinella*, *Microbacterium* sp., *Microbacterium* sp. *Enterobacteriaceae* sp. *Lactobacillus* sp, *Micrococcus* sp (Table1). Overall quality of date-cucumber wine were bars with different (P<0.05) where sample C and D were significantly preferred with rate of 1.5 and A, B, and E were less with preferred by seven points hedonic scale (Fig.8). The mean and standard deviation of the date-cucumber wines produced showed that wine C (3.1) and D (1.3) were significantly preferred. Post Hoc Duncan multiple range test found that factor that contribute to the acceptability were aroma, taste and appearance (Table2).

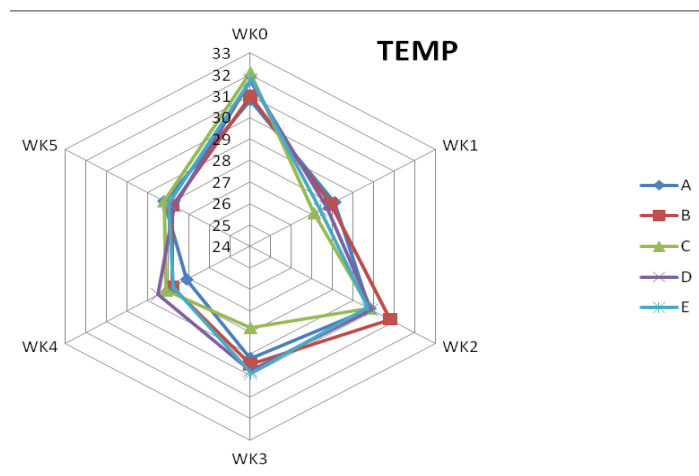


Figure 1 Temperature Profile of Wine Production during 8 weeks of fermentation

NB: Graph with different colour shows the different sample of Date-Cucumber Wine by ratio.

Wine A (1:1) =1000ml: 1000ml, Wine B (1:2) =500ml: 1000ml, Wine C (3:1) =500ml: 500ml

Wine D (1:3) =500ml: 1500ml, Wine E (2:1) =1000ml: 500ml

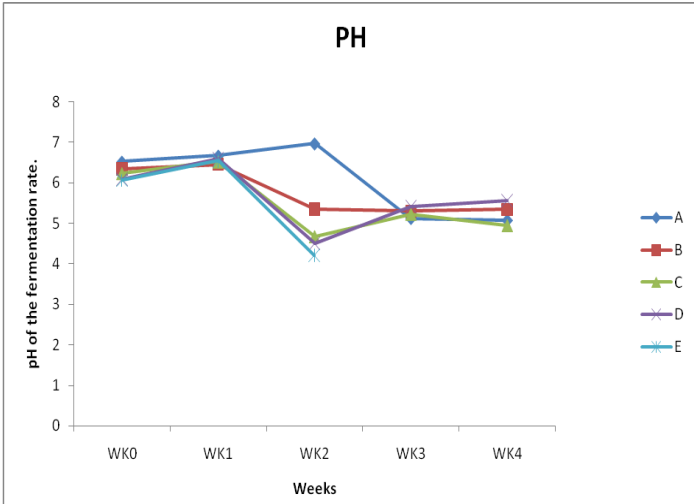


Figure 2 pH kinetic of Date – Cucumber Wine Produced after 60 days of fermentation
 NB: Graph with different colour shows the different sample of Date-Cucumber Wine by ratio.
 Wine A (1:1) =1000ml: 1000ml, Wine B (1:2) =500ml: 1000ml, Wine C (3:1) =500ml: 500ml
 Wine D (1:3) =500ml: 1500ml, Wine E (2:1) =1000ml: 500ml

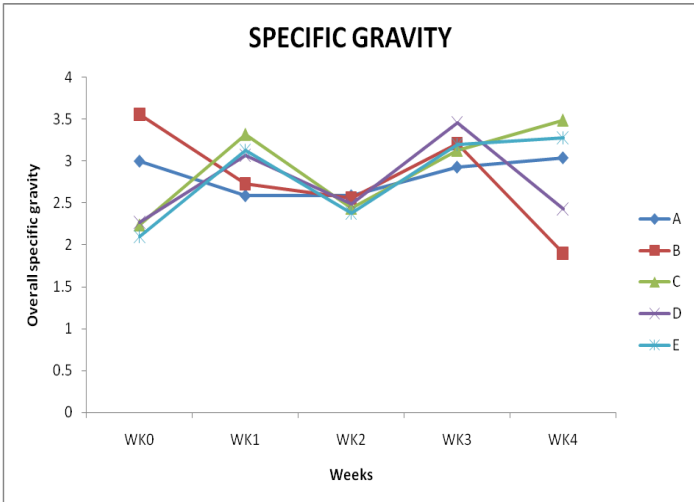


Figure 3 Specific gravity of Wine Production during 60 days of fermentation
 NB: Graph with different colour shows the different sample of Date-Cucumber Wine by ratio Wine A (1:1) =1000ml: 1000ml, Wine B (1:2) =500ml: 1000ml, Wine C (3:1) =500ml: 500ml
 Wine D (1:3) =500ml: 1500ml, Wine E (2:1) =1000ml: 500ml

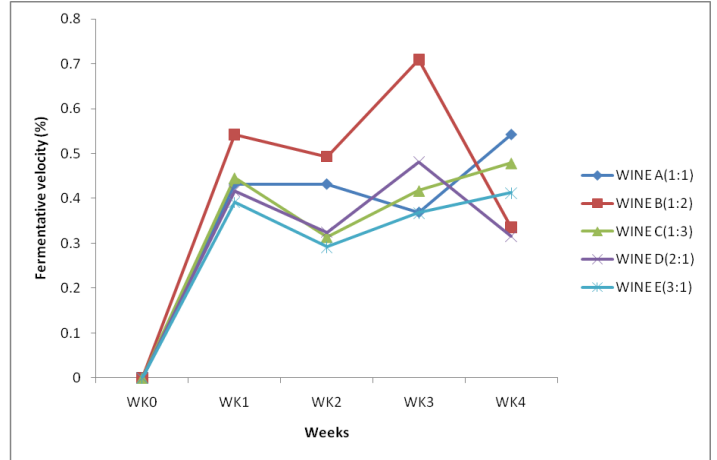


Figure 4 Fermentative Velocity of Date-Cucumber after 60 days Fermentation
 NB: Graph with different colour shows the different sample of Date-Cucumber Wine by ratio.
 Wine A (1:1) =1000ml: 1000ml, Wine B (1:2) =500ml: 1000ml, Wine C (3:1) =500ml: 500ml
 Wine D (1:3) =500ml: 1500ml, Wine E (2:1) =1000ml: 500ml

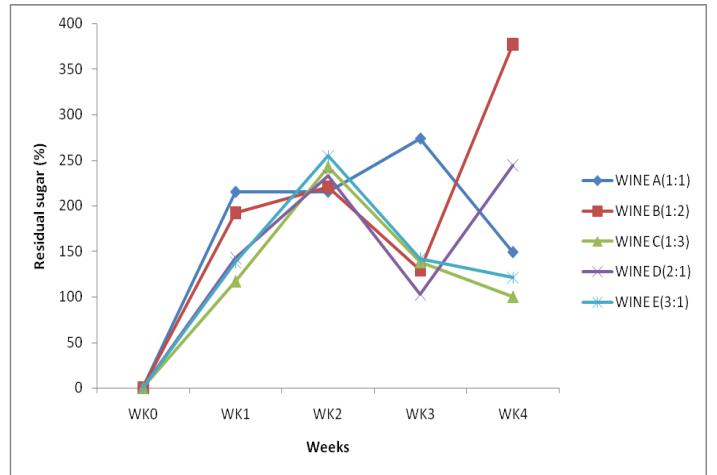


Figure 5 Residual Sugar of Date-Cucumber during the 60 days fermentation
 NB: Graph with different colour shows the different sample of Date-Cucumber Wine by ratio.
 Wine A (1:1) =1000ml: 1000ml, Wine B (1:2) =500ml: 1000ml, Wine C (3:1) =500ml: 500ml
 Wine D (1:3) =500ml: 1500ml, Wine E (2:1) =1000ml: 500ml

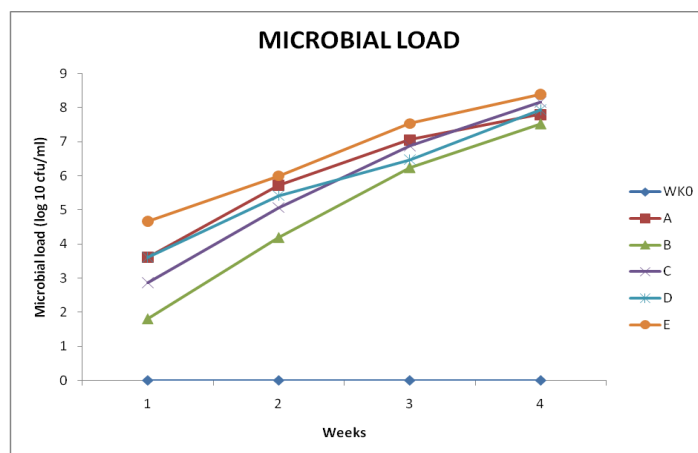
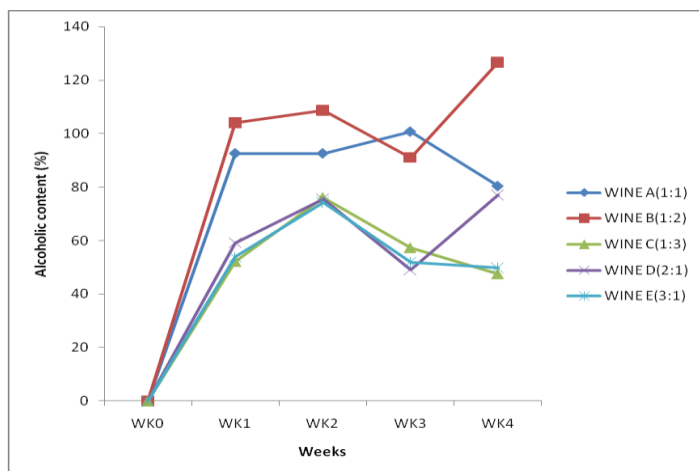


Figure 6 Alcoholic Content of Date-Cucumber Wine Produced after 60 days of Fermentation

Wine A (1:1) =1000ml: 1000ml, Wine B (1:2) =500ml: 1000ml, Wine C (3:1) =500ml: 500ml

Wine D (1:3) =500ml: 1500ml, Wine E (2:1) =1000ml: 500ml

Figure 7 Microbial growth curve of Wine Production at 60 days Fermentation

NB: Graph with different colour shows the different sample of Date-Cucumber Wine by ratio

Wine A (1:1)=1000ml:1000ml, WineB (1:2)=500ml:1000ml, Wine C (3:1) =500ml: 500ml

Wine D (1:3)=500ml:1500ml, Wine E (2:1) =1000ml: 500ml

Table 1 Morphological and Biochemical Characteristics of Bacterial Isolates from Date-Cucumber Wines before Pasteurization

Sample Code	Morphology	Shapes and Arrangement	Gram	Catalase	Coagulase	Citrate	Oxidase	Galactose	Lactose	Fructose	Probable Bacteria
A1	Circular irregular Off white flat Colony	Single bacillus	+	+	-	+	+	G	AG	G	<i>Corynebacterium</i> sp
A2	Circular irregular Milk raise colony	Clusters Bacillus	+	+	-	+	-	G	AG	AA	<i>Corynebacterium</i> sp
B1	Smooth regular Milk raise colony	Single Coccoi	-	-	+	-	-	AG	G	AG	<i>Neisseria, Veillonella</i>
B2	Smooth regular Milk dotted raise Colony	Single Coccoi	+	+	-	+	-	G	G	A	<i>Micrococussp</i>
C1	Circular regular Milk slightly raise	Single Coccoi	+	+	-	+	+	G	G	A	<i>Micrococcus</i> sp
C2	Round regular Mucoid slightly Raise	Single Bacillus	-	-	+	-	-	AG	G	A	<i>Enterobacteriaceace</i> sp.
D1	Smooth Regular milk flat colony	Clusters Bacillus	-	+	+	+	+	AG	AG	G	<i>Micrococcus varians</i>
D2	Smooth regular Dirty milk colony	Clusters Bacillus	+	+	-	-	-	A	G	AG	<i>Lactobacillus</i> sp
E1	Circular irregular Off whitecolony	Single Coccoi	+	+	+	+	-	AG	AG	AG	<i>Micrococcus</i> sp.
E2	Circular irregular dirtyMilk colony	Single Bacillus	+	+	+	+	-	A	AG AG		<i>Micrococcus</i> sp

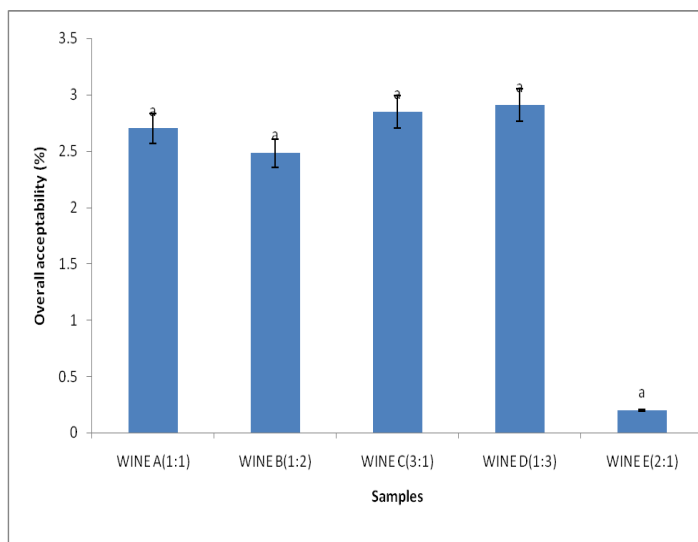


Figure 8 Overall acceptability of Date-Cucumber Wine Production after 60 days of fermentation

NB: Different bar chart represent each sample by ratio.

Wine A (1:1) =1000ml: 1000ml, Wine B (1:2) =500ml: 1000ml, Wine C (3:1) =500ml: 500ml

Wine D (1:3) =500ml: 1500ml, Wine E (2:1) =1000ml: 500ml

Table 2 Sensory Evaluation of the Produced Date –Cucumber Wines

Wine	Colour	Aroma	Taste	General Acceptability
Wine A (1:1)	B 3.20 ± 0.36	B 3.80 ± 0.29	B 3.00 ± 0.22	B 3.87 ± 0.31
Wine B (1:2)	A 1.20 ± 0.13	A 2.70 ± 0.39	C 3.40 ± 0.45	A 2.43 ± 0.27
Wine C (3:1)	A 1.50 ± 0.22	A 2.40 ± 0.40	A 2.90 ± 0.34	A 2.26 ± 0.19
Wine D (1:3)	A 1.50 ± 0.22	A 3.40 ± 0.37	A 2.00 ± 0.29	A 2.30 ± 0.18
Wine E (2:1)	A 1.90 ± 0.31	A 2.50 ± 0.26	A 2.40 ± 0.16	A 2.26 ± 0.18

NB: mean of sensory analysis have different alphabet down to the group are significantly different (P < 0.05), Wine A (1:1) =1000ml: 1000ml, Wine B (1:2) =500ml: 1000ml, Wine C (3:1) =500ml: 500ml, Wine D (1:3) =500ml: 1500ml, Wine E (2:1) =1000ml: 500ml

DISCUSSION

The identified *S.cerevisiae* used in the production of the wines performed excellently well, this could be as a result of its stability during the fermentation processes to produce the date-cucumber wines. This is in agreement with Mohammed et al. (2018) who characterized *S.cerevisiae* isolated from palm wine and used it to produce wine from blends of coconut milk and honey slurry and reported excellent results from the microbial and sensory evaluation of the produced wines. The physiochemical properties of Date-Cucumber wine revealed in this study include temperature, pH and specific gravity. In this study, the temperature of the wine produced varies and this could be as a result of microbial metabolism of viable nutrients to fabricate alcohol with resulting generation of heat. This result agrees with the report of Okafor (2007) and Robinson (2006). Based on this study, it is implicit that the pH of the wine throughout the period of fermentation ranged from 6.67-6.93, there was a significant difference among the samples. A comparable observation has been reported by Siddig (2013): in their study on Date wine, optimum pH value for quality wine production was 7.0 and should not exceed 7.0. Studies have shown that during fermentation: Low pH is inhibitory to the growth of spoilage organisms but creates a conducive environment for the growth of desirable organisms. Also, low pH and high acidity are known to give fermenting yeasts a modest advantage in the natural environment. In the case of specific gravity of the wine produced in the study,

there was an obvious difference as the wine gradually reduced as the fermentation days of the wine increased; this could be due to microbial progression: available nutrients, sugar and alcohol resulting in the production of acid. This result conforms to the report of Querol et al. (2003). The specific gravity of wine A ranges from (1.40-0.67), wine B (1.47-0.70), wine C (1.45-0.71), wine D (1.64-0.73) and wine E (1.75-0.72). These decreases were observed to be irrespective of the yeast strain used in the wine production. This result conforms to Nidhi et al. (2008). *Saccharomyces cerevisiae* has been reported to reduce specific gravity quality of fruit wines during fermentation (Okafor et al., 2007).

The fermentative capacity of different Date-Cucumber wine sample, on the week 4, wine B and A had the highest fermentative capacity of 76.9 and 73.2 respectively. While D, C and E had the lowest (44.1, 44.1 and 41.0), which is why sample B had the highest percent alcohol content. In increasing order, the alcoholic content of different Date-Cucumber wine where wine B (1:2) had the highest alcoholic concentration of 16.06, A (15.8), D (13.17) and wine C and E had the least alcohol content of 11.17 and 10.18 respectively. High alcohols are known to be important precursors for the formation of esters, which are associated with pleasant aromas (Clement-Jimenez et al., 2005).

The microbial analysis of Date-Cucumber wine revealed that there was no any mould and coliform growth at the dilution factors used, while the total viable count ranged between 6.7-7.4x10⁶log CFU/ml. This implies that the wine was produced under hygienic conditions and safe for human consumption. This agrees with the report of Frazier and Weshoff (1998). Therefore, the total viable count was within the acceptable limit according to Sri Lanka standard for all drinks/beverages and juice as reported by Ghana Standard Board (1995). Close observation on the predominant bacteria isolated from date-cucumber wines include: *Corynebacterium* sp, *Neisseria* sp, *Micrococcus* sp, *Enterobacteriaceae* sp, *Micrococcus* sp and *Lactobacillus* sp. The sensory scores of Date-Cucumber wine produced where wine C (1:3) ratio of Date-Cucumber were significantly preferred. The post Hoc Duncan multiple range test found that factors contributed to the acceptability of wine C (1:3) were aroma, taste and appearance and colour. Colour is an important sensory characteristic on which consumer preferences are dependent, this result obeys the rules with the result of Francis (1995) who reported that colour influences other sensory characteristics, which subsequently account for food acceptability, choice and preference. The result of sensory evaluation of the samples of wine produced from date-cucumber wine showed that wine C was rated highest in colour, taste, aroma and general acceptability followed by wine A, the least were wine B, C and D. There was a significant difference (P<0.05) among the wine samples with respect to flavor. The wine C was rated high generally thus can be prepared for commercial purposes to serve as a special wine with similar constituents as other already existing commercial wines as reported by Selli et al. (2013).

CONCLUSION

The present study has shown that acceptable fruit wines can be produced from Date fruit and Cucumber using *Saccharomyces cerevisiae* in a spontaneous fermentation. Therefore good wine can be obtained by controlled fermentation of a starter culture for 8 weeks using *Saccharomyces cerevisiae*. The statistical analysis showed that Sample C was significantly preferred at 5% confidence (P< 0.05). However, large-scale production of Date and Cucumber wines could be an alternative to extending the shelf-life of Date and Cucumber fruit and the level of post-harvest loss of the fruit.

Recommendations:

- Recommendations were made at the end of this study;
1. It was recommended that in the production of Date and Cucumber Wine, the fermentation temperature should be maintained at between 24°C to 27°C. The temperature should not exceed 29°C. Otherwise the growth of yeast cells will be stopped and encourage bacterial growth.
 2. The products (Date-Cucumber Wine) is recommended for commercial production in a large scale.

Conflict of interest: The authors of this research declare no conflict of interest.

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