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The effect of clarification methods on quality attributes of sugarcane-watermelon wine fermented by palm wine yeast (*Saccharomyces cerevisiae*)

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Clarification of wine is aimed at improving the quality of the product by removing haze. In this study, the effect of two clarification methods namely membrane filtration technique and the use of Keiselguhr diatomaceous earth powder on the physico-chemical and sensory attributes of wine produced using sugarcane (*Saccharum officinarum* L) and watermelon (*Citrullus vulgaris* L) juice blended in the ratio 1:1 (v/v) and fermented by *Saccharomyces cerevisiae* isolated from palm wine was determined. Sugarcane-watermelon wine not clarified was the control. Physicochemical analysis of the wine at 0 h indicates the following: sugar (10.73 °Brix), specific gravity (1.043 kg/m^3), pH (3.9), alcohol content (5.9%), titratable acidity (0.720 g/l), turbidity (94.32 NTU) and colour intensity (0.892 nm). During maturation of wine, the sugar content, specific gravity, pH, alcohol content, titratable acidity, turbidity and colour intensity of the samples clarified by membrane filtration/diatomaceous earth powder at 72 and 336 h were 6.5/9.9 and 7.2/10.8 °Brix, 1.026/1.040 and 1.029/1.043 kg/m^3, 3.7/3.8 and 3.0/3.13, 3.57/5.48 and 3.7/5.7%, 0.375/0.405 and 0.517/0.628 g/l, 29/32.6 and 15/20 NTU, and 0.649/0.873 and 0.642/0.628 nm, respectively. The cumulative sensory scores of wine clarified using Keiselguhr diatomaceous earth powder were slightly higher than the wine clarified by membrane filtration. Taking other parameters into consideration, the clarification of sugarcane-watermelon wine using membrane filtration is relatively better than Keiselguhr diatomaceous earth powder.

Key words: Alcoholic beverages, fruits and vegetables, fruit wine, fermentation.

INTRODUCTION

Wine is a popular beverage that contains alcohol. People of different social status drink wine to their delight (Ogbeide and Ele, 2015). Wine is produced by fermenting fruit juice preferably grape juice (Biri et al., 2015; Zainab et al., 2018). A single fruit or combination of different fruits depending on individual's choice is used for wine production (Saranraj et al., 2017; Velić et al., 2018). Production and consumption of wine is part of the history of man (Wurz, 2019). The type of fruit/vegetable and yeast strain(s) selected for wine production influences its sensory characteristics (Okemini and Dilim, 2017; Pino et al., 2019). The alcohol content of wine is within the range

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5-13%. Wine, also called fruit wine is an undistilled alcoholic beverage. A situation whereby grape is not used to produce wine, it is a convention to add the name of the fruit eventually used when the product is being referred to (Swami et al., 2014; Ire et al., 2020; Kantiyok et al., 2021).

Tropical fruit wine are prepared using fruits grown in subtropical and tropical regions of the world (Chakraborty et al., 2014). Watermelon red, cashew, banana, guava, pawpaw, orange, pineapple, mango and watermelon wines of acceptable quality were prepared by Djouldedarman et al. (2010), Awe and Olayinka (2011), Idise and Odum (2011), Nikhanj and Kocher (2015), Umeh et al. (2015), Patharkar et al. (2017), Qi et al. (2017), Ogodo et al. (2018), and Zainab et al. (2018), respectively. According to Ire et al. (2020), any kind of fruit is suitable for the production of wine. Vegetables such as fluted pumpkin (*Telfairia occidentalis*) leaves and cucumber (*Cucumis sativus* L.) have also been used to produce wine of acceptable quality (Ebana et al., 2019). Production of wine is possible using tigernut and sugarcane juices obtained from *Cyperus esculentus* and *Saccharum officinarum* L., respectively (Okemini and Dilim, 2017; Shah et al*.,* 2020).

Clarification is an important stage during wine production which takes place after fermentation. Acceptance or rejection of wine by consumers is influenced by sensorial assessment of the product based on sight, taste, and smell. The essence of wine clarification by filtration and/or fining is to remove cloudiness/turbidity of wine during production (Muñoz-Castells et al., 2022). After clarification of wine is completed, the final product becomes bright and clear. In order to remove dead or unreacted yeast, bacteria and grape debris during production of wine, it has to be filtered whereas the addition of fining agents is capable of removing soluble substances such as proteins, colouring phenols and polymerized tannins associated with a cloudy wine (Awe, 2018). Fining agents are placed in different groups referred as earths, animal proteins, plant proteins, wood charcoal, synthetic polymers and silicon dioxide based on their general nature (Kemp et al., 2022). A wide range of fining agents obtained from animal protein, vegetable protein and inorganic compounds is used in the wine industry with varying effects on the product (Chagas et al., 2012).

For more than 2,000 years, the medicinal use of wine has been established (Wurz, 2019). It is moderate for adults to consume 1 to 2 glasses of wine per day. The health benefits associated with wine consumption include longevity, increased cognitive performance and insulin sensitivity, reduced risk of stroke, prevention of cancer and cardiovascular diseases. A combination of food and wine helps digestion to take place. Intake of wine is healthy for the skin. Wine could also play a role to prevent blindness (Fehér et al., 2007; Wurz, 2019, Ire et al., 2020). However, too much consumption of wine could damage cellular processes responsible for bone tissue formation and the long-term effect is fractures occurring at high frequency (Oladipo et al., 2014).

Sugarcane (*S. officinarum* L.) is a tall perennial grass that grows abundantly in the tropics and warm temperate regions. It is cultivated mainly because it is the major raw material for production of sugar (Aina et al., 2015; Wada et al., 2017). The country of origin of sugarcane is yet to be substantiated. Available information suggests that *S. officinarum* and *Saccharum robustum* originated from New Guinea; *Saccharum barberi* from India; and *Saccharum sinense* from China (Brumbley et al., 2008). Sugarcane is a snack to many people who enjoy chewing the stem and swallow its juice (Aina et al., 2015; Williams et al., 2016). Sugarcane juice is one of the popular products of sugarcane (Singh et al., 2015; Pino et al., 2019). It is a good source of carbohydrates, minerals and amino acids (Williams et al., 2016; Arif et al., 2019). Sugarcane juice possess anticancer and antioxidant properties which confer health benefits to consumers. Regular consumption of sugarcane juice fights chronic diseases associated with old age (Hameed et al., 2016). Patients experiencing jaundice are advised to drink it. Sugarcane juice is associated with anti-inflammatory, diuretic, analgesic and hepatoprotective effects in humans. It also functions as a laxative, aphrodisiac, cooling, antiseptic, demulscent and tonic (Singh et al., 2015). Abundant nutrients mainly sugar, yeasts and bacteria especially *Leuconostoc* species present in sugarcane juice are responsible for easy spoilage of the juice (Bag et al., 2022).

Watermelon (*Citrullus vulgaris* L.) is a xerophytic tropical and subtropical fruit that grows abundantly in most African countries and South East Asia (Yusufu et al., 2018; Dube et al., 2020). North-East Africa is believed to be the origin of watermelon, reported more than 5,000 years ago (Mezue and Aghimien, 2016). According to Reetu and Tomar (2017), watermelon originated from Kalahari Desert. In Nigeria, watermelon is consumed as nectars, juice and fruit cocktails after the fruit has been fermented and blended (Kantiyok et al., 2021). The method of preparation will determine whether watermelon is going to be consumed as snack or appetizer (Ogodo et al., 2015; Zainab et al., 2018). During drought seasons, watermelon serves as a source of drinking water for people living in parts of Nigeria and Sudan (Dube et al., 2020). Watermelon is a good source of carotenoids such as β-carotene, lycopene, phytoene, phytofluene, neurospnene and lutein. It also contain carbohydrates, protein, fats, dietary fiber, vitamin A, B_1, B_6 and C as well as minerals such as potassium, iron, manganese and magnesium (Reetu and Tomar, 2017). Lycopne present in watermelon is associated with health benefits. Watermelon helps the body to fight arteriosclerosis, diabetes, hypertension, cancer, diabetes, arthritis, macular degeneration and some coronary heart diseases (Zainab et al., 2018; Asante et al., 2020).

Palm wine is the sap obtained from trees that grow abundantly in the tropical region belonging to the family Palmae (Agwuna et al., 2019). In Southern Nigeria, palm wine tapped from *Elaeis guineensis*, *Raphia hookeris* and *Raphia vinifera* is a milky alcoholic beverage consumed by the people (Ogodo et al., 2015). It is a potential source of yeast strains for producing industrially fermented products such as wine, bread, etc (Olowonibi, 2017; Zainab et al., 2018). Spontaneous fermentation of palm wine which contains sugars, amino acids, proteins, and vitamins create a favourable environment for yeast and bacteria to increase in large numbers (Onwumah et al., 2019; Kantiyok et al*.,* 2021).

Wine of acceptable quality was produced by Soibam et al. (2016) using a blend of sugarcane (*S. officinarum* L.) and watermelon juice (*C. vulgaris* L.). The researchers used *Saccharomyces cerevisiae* strain isolated from palm juice to ferment sugarcane-watermelon juice. However, the researchers did not evaluate the effect of using different fining agents on the quality of wine produced. The acceptability of wine by consumers could be affected if the product is not clarified using an appropriate clarifying agent/method (Verlić et al., 2019). Therefore, this study is aimed at evaluating the effect of membrane filtration and the use of Kieselguhr diatomaceous earth powder as clarification methods on the physico-chemical and sensory quality of sugarcane-watermelon wine fermented by palm wine yeast.

MATERIALS AND METHODS

Fresh and mature sugarcane and watermelon were purchased from Choba market along East- West Road, Obio-Akpor Local Government Area, Rivers State, Nigeria. About 1.5 L of fresh palm wine was obtained from palm wine tappers in Ozuoba also in Obio-Akpor Local Government Area, Rivers State using a sterile plastic jerry can that has a cork. The watermelon, sugarcane and palm wine were transported to Microbiology Laboratory, University of Port Harcourt, using clean big shopping bags within 2 h for laboratory analyses.

Extraction of watermelon juice

About 5 kg of watermelon was washed thoroughly using distilled water. A clean stainless steel knife sterilized with 70% ethanol was used to peel the watermelon. The fleshy part of watermelon which is reddish was deseeded and chopped into small pieces and transferred into sterilized electric blender (Sonic food processor 2203) for crushing. Thereafter, the slurry was filtered using a clean muslin cloth to obtain 2 L of watermelon juice.

Extraction of sugarcane juice

About 5 kg of sugarcane was washed thoroughly with distilled water and peeled using a clean sterilized stainless knife. Thereafter, the knife was used to chop the sugarcane into smaller pieces before transferring them quantitatively into electric blender (sonic food processor 2203) for crushing. The slurry containing insoluble sugarcane fibre was filtered using a clean muslin cloth to obtain 2 L of sugarcane juice.

Preparation of ‘must’

The ‘must’ was prepared using a mixture of sugarcane juice (2 L) and watermelon juice (2 L) which represent a ratio of 1:1 v/v. A total volume of 4 L of ‘must’ was achieved. The bowl containing the ‘must’ was carefully covered after 14.2 mL of 3.14% sodium metabisulphite ($Na_2S_2O_5$) was added to sterilize ‘must’ (Berry, 2000).

Preparation of yeast starter culture

Ten fold serial dilution of the palm wine was aseptically carried out using a sterile peptone water. A sterile pipette was used for each transfer from one dilution to the next. With the aid of inoculating loop sterilized in a Bunsen flame, a loopful of palm wine from dilution 10^{-4} was streaked on potato dextrose agar (PDA). The plate was covered and incubated at a temperature of 27°C for 72 h. The Petri-dishes were examined for growth of microorganisms.

Identification of the isolate

A speck of the fungal colony was mounted on a glass slide and viewed under the microscope for cellular characteristics. Standard morphological and physiological tests as described by Nwachukwu et al. (2006) and Ogbulie et al. (2007) for yeast identification were employed. The tests include morphology, surface characteristics, presence of pseudomycellium, ascospore formation, vegetative reproduction, growth in 10% NaCl + 50% yeast extract. The isolates were subcultured on potato dextrose broth incorporated with streptomycin to inhibit bacterial growth. The inoculated plates were incubated for 24 h and stored at 28°C.

Fermentation process

Aerobic phase of fermentation

Standard inoculum (*S. cerevisiae*) using MacFarland Standard which is equivalent to 1.5×10^8 CFU/mL was prepared and added into 1 mL. equivalent of sugarcane-watermelon juice. The fermentation broth was covered using a sterile cotton wool. Fermentation was allowed to proceed for 2 days at 30±2°C.

Anaerobic phase of fermentation

The fermenting vessel was made air tight by covering it with a lid and sealing the edge with paper tape to prevent contamination from undesirable microorganisms, ensure adequate nutrition, and growth of desirable yeasts as well as prevent excessive heat and oxidation. The fermentation process lasted for 14 days (Okoro, 2007). A constant alcohol content of 5.9% was reported.

Clarification of wine

Membrane filtration technique

The method described by Rosária et al. (2022) with some modification was adopted. Exactly 1 L of unfiltered wine sample was passed through membrane filtration apparatus. A special

porous membrane designed to trap microorganisms and sediments larger than 0.45 µm in size was used.

Application of Kieselguhr diatomaceous earth powder

A commercially available diatomaceous earth powder was dissolved in a 10 mL sterile water to form a cake layer of 800 g/m^2 on the surface of a filter paper. The wine was poured onto the pre-coated filter paper and allowed to flow into a collecting flask. Thereafter, 7 g/100 mL of the powder was re-added to the wine as a bodyfeed to trap the sediments and haze that may have been left unfiltered during the initial passage through the pre-coated filter paper (Devolli et al., 2017).

Aging

The wine was allowed to age in order to improve the flavour, palatability, appearance, clarity, and colour.

Wine pasteurization

The wine was pasteurized at 121°C for 15 min to eliminate the need for SO_2 addition at the time of bottling.

Bottling

The bottles were properly washed, dried and autoclaved at 121°C at 15 psi for 15 min. Aseptically, the bottles were filled with wine very close to a Bunsen burner with flame and screw capped. The bottled wine were refrigerated at 4°C for further maturation.

Physico-chemical analysis of the wine

Determination of pH

The pH of wine was monitored at 0, 72, and 336 h during clarification using pH meter (model PHS- 25C Precision pH/mV). About 10 mL of wine sample was poured inside a sterile beaker. Standard pH buffer 7.0 and 4.0 was used to standardize the pH meter. Afterwards, the glass electrode was immersed into the sample. The result was recorded after 2 min when the pH reading on the display was stable (Kantiyok et al., 2021).

Determination of specific gravity

The specific gravity (g/mL) was measured using the Triple Scale Hydrometer (Model HY110) for beer and wine. The value was taken from calibration on the stem. The specific gravity of the wine was determined at 0, 72, and 336 h.

Determination of total alcohol content

The total alcohol content of the wine was determined at 0, 72, and 336 h using the method described by Kantiyok et al. (2021). Exactly 100 mL of the sample was transferred to a graduated cylinder marked 100 mL l as the highest volume. The measuring cylinder and its content was kept inside a refrigerator for 15 min. When the temperature of the wine dropped to 15°C, alcohol meter was gently placed on the wine and allowed to float. The reading on the alcohol meter was noted and expressed as % alcohol (v/v). Using the following formula, purified alcohol content of the wine was calculated.

$$\text{Purified alcohol (L)} = \frac{\text{Volume of alcohol (L)} \times \text{Alcohol percentage}}{100}$$

Determination of titratable acidity

The wine sample was agitated to remove excess gas present (degassing). One millilitre (1 mL) phenolphthalein indicator was added to 200 mL of water to adjust the pH. To neutralize the water, 0.1 N NaOH was continuously added until a faint pink colour indicative of the end point was observed. Five millilitres of the degassed sample was pipetted into a 250 mL conical flask after which 100 mL of boiling water was poured inside the flask. The mixture was swirled to release CO_2. Titration of 0.1 N NaOH against the mixture inside the flask was carried out until a pale pink colour was observed (end point) and persisted for 30 s. Calculation of titratable acid was done using the following formula. The test was performed on the wine samples at 0, 72, and 336 h (Kantiyok et al., 2021).

$$\% \text{ Tartaric acid} = \frac{(\text{ml alkali}) \times (\text{normality of alkali}) \times 7.5}{\textit{Weight of sample (mls of sample)}}$$

Determination of total sugar content

Total sugars of the wine samples were determined using the method described by Dubois et al. (2022). One millilitre of the sample was pipetted into a test tube followed by 1 mL of 5% phenol. From a burette containing concentrated H_2SO_4, a total volume of 5 mL of the acid was added to the mixture inside the test tube. The concentrated sulphuric acid was added rapidly. To achieve good mixing, the stream of the acid was directed against the liquid surface rather than the side of the test tube. The mixture was shaken and allowed to stand for 10 min. A second shaking of the mixture inside the test tube was done before it was placed in a water bath for 20 min at 25 to 30°C. The optical density of the solution was read using a spectrophotometer at 490 nm. Preparation of the blank was carried out by substituting 1 mL of distilled water for the sample. A standard curve of glucose was used to estimate the total sugar content of the sample expressed as °Brix. The test was performed on the wine samples at 0, 72, and 336 h.

Determination of turbidity

The clarity of the wine was measured using a benchtop turbidity (2100P Turbidometer) nephelometery meter which measured the suspended particulates in the wine sample. The unit of measurement is nepholmetric turbidity units, usually abbreviated and referred to as NTUs. The principle of the test involves shinning infrared light on the wine sample and the light scattered by particles in the wine being measured. The wavelength of the scattered light was measured in nanometer and converted to NTUs. The turbidity of sample was determined at 0, 72, and 336 h.

Determination of colour intensity

The colour of the sample is characterized as absorptivity at 420 nm by a Helios-α spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA). According to Sudraud method described by Giosanu and Vijan (2013), colour intensity (I) is the sum of

Plate 1. Sugarcane juice.

Plate 2. Watermelon juice.

absorbances measured at 420 and 520 nm using the UV-1800 Spectrophotometer. The colour intensity (I.C.) was determined at 0, 72, and 336 h. Calculation of colour intensity involves a simple formula presented:

$c = a + b$

where 'c' is the colour intensity; 'a' is the absorbance at 420 nm; and 'b' is the absorbance at 520 nm.

Sensory evaluation

The method described by Okafor et al. (2014) with slight modifications was adopted. Sensory evaluation of the pasteurized wine samples was carried out by 10 semi-trained panelist who were undergraduate students in University of Port Harcourt. Each of the panelist was tasked to independently assign a sensory score for

Plate 3. Sugarcane-watermelon wine.

aroma, colour, clarity, appearance, and overall acceptability to each wine sample using a 9 point Hedonic scale (9 represents liked extremely and 1 is disliked extremely). The samples were presented to the panelist using a transparent glass cup coded with alphabets. Potable water was also provided for each panelist to rinse his or her mouth before evaluating the next sample.

Statistical analysis

Data obtained from the sensory report was analyzed using Microsoft excel and SPSS statistical software. Data source was compared using one way analysis of variance (ANOVA) and significant differences were accepted at $P<0.05$.

RESULTS

Yeast isolated from palm wine was moist, dull white, and smooth. Microscopic characteristics showed that the isolate was oval to round; large elipsidal budding yeast-like. *S. cerevisiae* count in sugarcane-watermelon wine slightly increased from 8.18 to 8.21 $\log_{10}$ CFU/ mL within 72 h, but declined to 7.15 $\log_{10}$ CFU/ mL at 336 h. Freshly prepared sugarcane and watermelon juice is depicted in Plates 1 and 2, respectively. Plate 3 shows a labeled sugarcane-watermelon wine ready for consumption.

Figure 1 shows the pH of clarified wine samples and the control during maturation. At 72 h, the pH of sugarcane-watermelon wine slightly reduced from initial value of 3.9 at 0 h to 3.8 and 3.7 for the product clarified using Kieselguhr diatomaceous earth powder and membrane filtration, respectively. At 336 h, the pH of clarified sugarcane-watermelon wine samples further reduced to 3.2. The control maintained the pH of 3.2 throughout the period of maturation.

Figure 2 shows the specific gravity of clarified wine samples and the control undergoing maturation. At 0 h, the specific gravity (SG) of sugarcane-watermelon wine

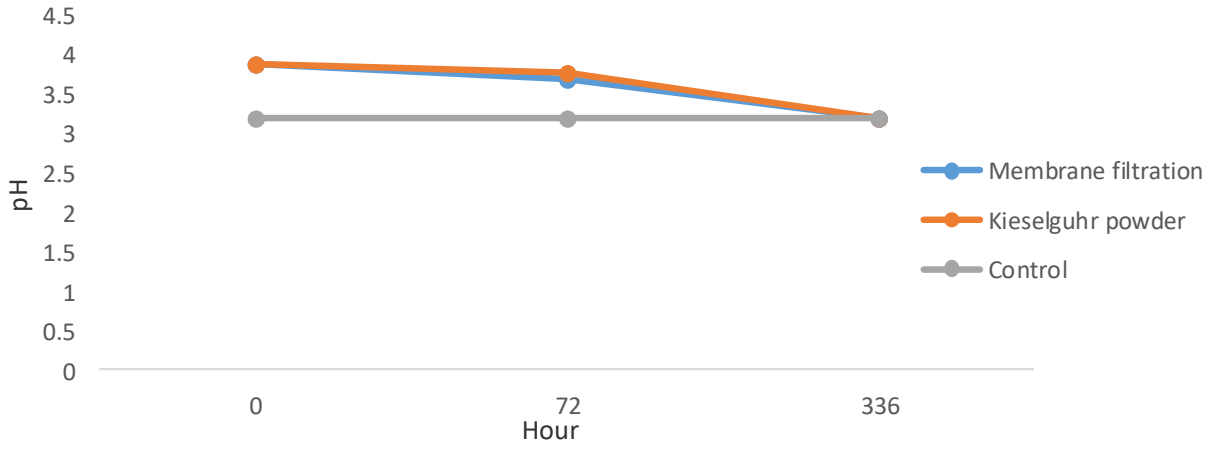

Figure 1. pH of clarified wine samples and the control undergoing maturation.

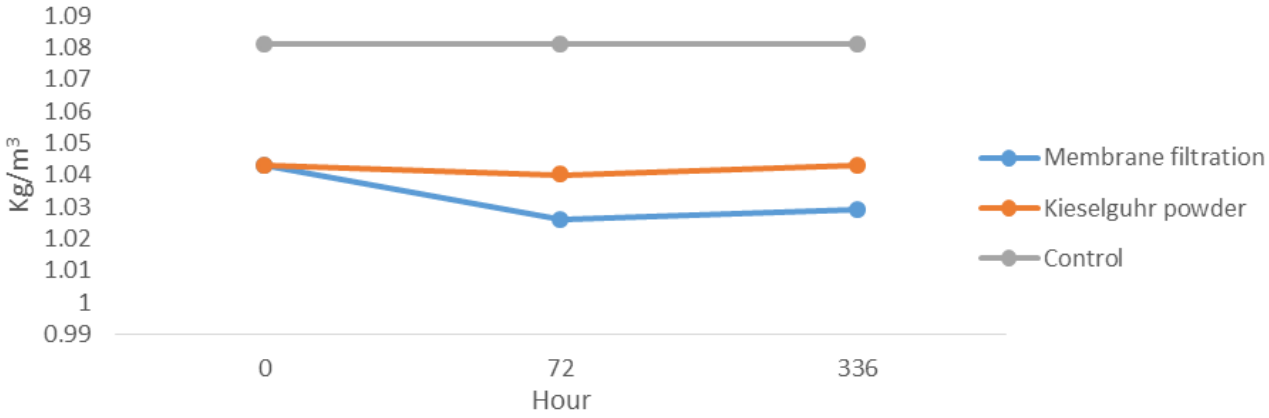

Figure 2. Specific gravity of clarified wine samples and the control undergoing maturation.

subjected to clarification and the control was 1.043 and 1.081 kg/m^3, respectively. During maturation, there was a slight reduction in SG of the wine samples with the exception of the control.

Figure 3 shows the sugar content of clarified wine samples and the control undergoing maturation. At 0 h, the sugar content of the clarified wines and the control was 10.73 and 19.52°, respectively. While the sugar content of the clarified wine samples reduced to 9.9 °Brix (Kieselguhr powder) and 6.5 °Brix (membrane filtration) at 72 h, the control remains unchanged. At 336 h, the sugar content of the samples slightly increased to 10.8 and 7.2 °Brix for wine clarified using Kieselguhr powder and membrane filtration, respectively.

Figure 4 shows the alcohol content of clarified wine samples and the control undergoing fermentation. There was a reduction in alcohol content of wine clarified using Kieselguhr powder and membrane filtration from 5.9% in both samples at 0 h to 5.48 and 3.47% at 72 h, respectively. At 336 h, there was a slight increase in alcohol content of wine clarified using Kieselguhr powder (5.7%) and membrane filtration (3.7%) whereas the control remain unchanged.

Depicted in Figure 5 is the titratable acidity (TA) of clarified wine samples and the control undergoing maturation. The result shows that TA of clarified wines at 0 h had the same value (0.72 g/l) which reduced to 0.375 (membrane filtration) and 0.405 g/l (Kieselguhr powder) at 72 h. At 336 h, the TA of the clarified wine increased to 0.517 and 0.628 g/l for wine samples clarified using membrane filtration and Kieselguhr powder. However, the specific gravity of the control was 0.9 g/l.

Figure 6 shows the colour intensity of clarified wine samples and the control undergoing maturation. At 0 h, the colour intensity of the clarified wine samples was the same (0.892), but the values reduced to 0.649 and 0.873 in wine samples clarified with membrane filtration and Kieselguhr powder, respectively. Further reduction in

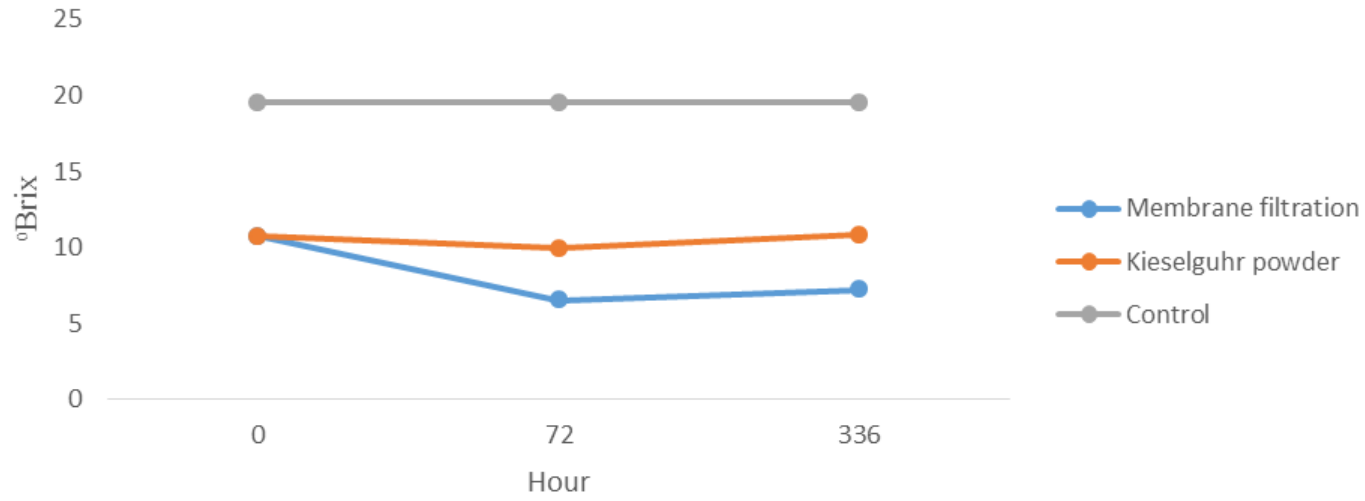

Figure 3. Sugar content of clarified wine samples and the control undergoing maturation.

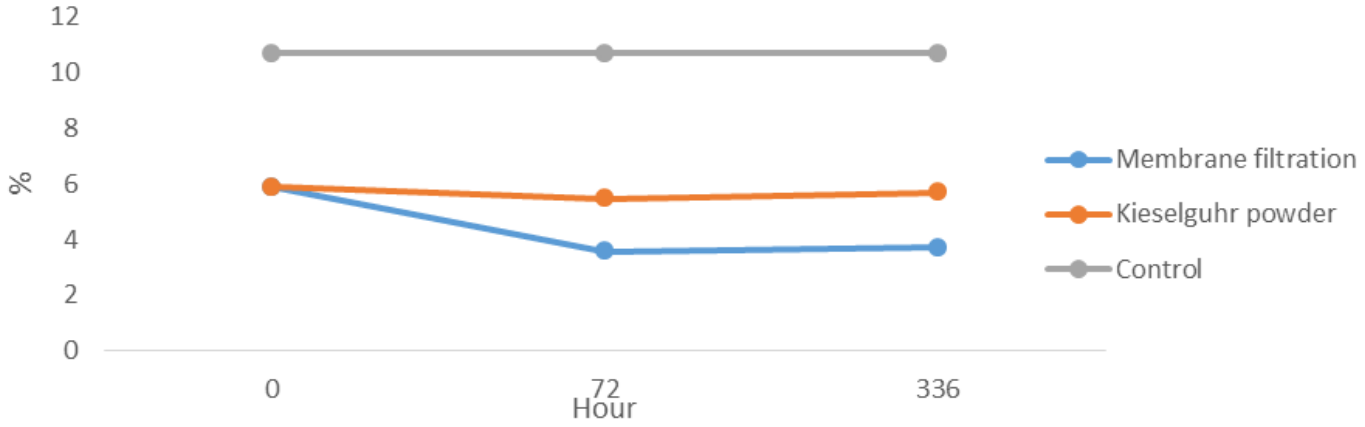

Figure 4. Alcohol content of clarified wine samples and the control undergoing maturation.

colour intensity to 0.628 was reported in both clarified wine samples. However, the values for the control was 0.33 throughout the period of maturation.

Figure 7 shows the turbidity of clarified wine samples and the control undergoing maturation. The result shows that turbidity of the control was constant (0.5 NTU) during the period of maturation whereas the samples clarified using membrane filtration and Keiselguhr diatomaceous earth powder which was 94.32 NTU at 0 h steadily reduced to 15 and 20 NTU at 336 h, respectively.

Table 1 shows the average score for each sensory attribute of clarified sugarcane-watermelon wine and the control evaluated by the panelist. The sensory report shows that the control was the most preferred wine followed by the wine clarified using membrane filtration and the least was wine clarified using Keiselguhr diatomaceous earth powder. There was significant difference ($p<0.05$) among the clarified wine samples including the control with regards to each of the sensory attribute.

DISCUSSION

The result obtained from this study shows that yeast isolated from palm wine was *S. cerevisiae*. It is in agreement with earlier studies which involved the use of *S. cerevisiae* isolated from fresh palm wine to ferment fruit juice into wine (Okoro, 2007; Ogodo et al., 2015; Okeke et al., 2015; Hafsat et al., 2015; Nwinyi and Hassan, 2021). A slight increase in population of *S. cerevisiae* during fermentation of watermelon-sugarcane 'must' is an indication that metabolizable nutrients in the medium were low. Secondly, the physico-chemical properties of the medium might not provide optimum growth conditions for *S. cerevisiae.*

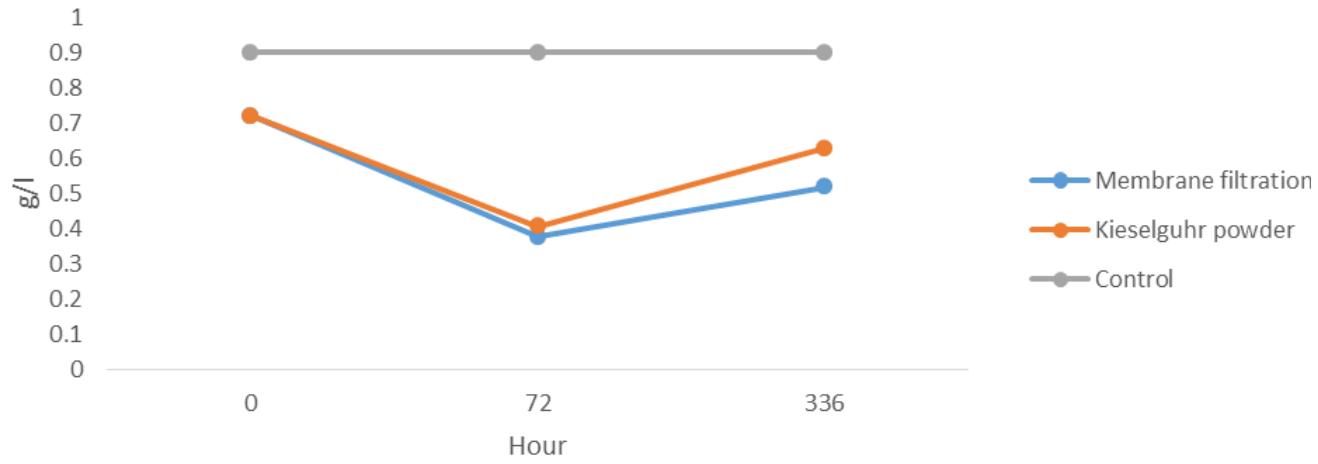

Figure 5. Titratable acidity of clarified wine samples and the control undergoing maturation.

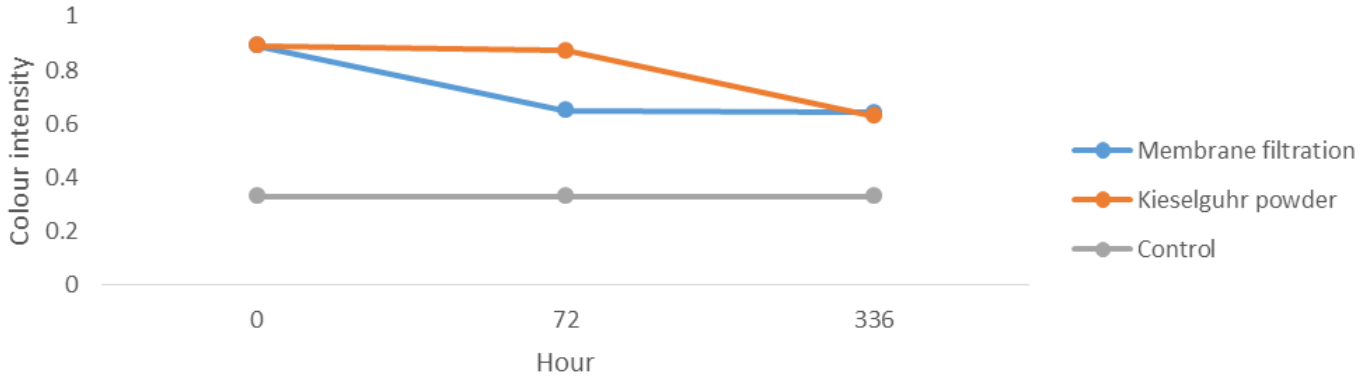

Figure 6. Colour intensity of clarified wine samples and the control undergoing maturation.

The reduction in pH of the clarified wine samples during maturation is in agreement with a related study carried out by Zainab et al. (2018) and Soibam et al. (2016). It could be attributed to formation of acetic acid by acetic acid bacteria in the clarified wine samples. The possible release of CO_2 which forms a weak acid in the wines is a contributory factor to reduction in pH reported in this study (Ire et al., 2020). At 0 h, the pH of the clarified wines was 3.9. Since the pH of the clarified wine samples was lower than 3.5 at the end of maturation, it is an indication that the product was high in acid content. The pH of wine samples clarified using Keiselguhr diatomaceous earth powder/membrane filtration at 72 and 336 h was 3.8/3.7 and 3.13/3.0, respectively. During the maturation period, the pH of the control was constant (3.2). Aging, clarifying or fining of wine is influenced by pH. Wine that has a pH below 3.5 is suitable for most fining and clearing agents of wine (Saranraj et al., 2017).

Initial reduction of titratable acidity of the wine samples clarified using Keiselguhr diatomaceous earth powder and membrane filtration between 0 and 72 h is in agreement with a related study by Okafor et al. (2014). Increase in titratable acidity of clarified wines between 72 and 336 h is a sign of maturation. The titratable acidity of the clarified wines and the control was within the range 0.5 - 1.0% recommended for good quality wines. According to Okafor et al. (2014), the titratable acidity of table wines is within the range 0.6 - 0.9%. So, the wine samples clarified using Keiselguhr diatomaceous earth powder and membrane filtration and the control are table wines. According to Ire et al. (2020), titratable acidity of wine influences its sensory attributes. Low pH and high titratable acidity are conditions that give competitive advantage to fermentative yeasts in their natural environment. Spoilage microorganisms present in wines are inhibited by low pH, but the condition encourage the growth of desirable organisms such as fermentative yeasts.

At 0 and 72 h, the specific gravity of sugarcane-watermelon wine clarified using membrane filtration/

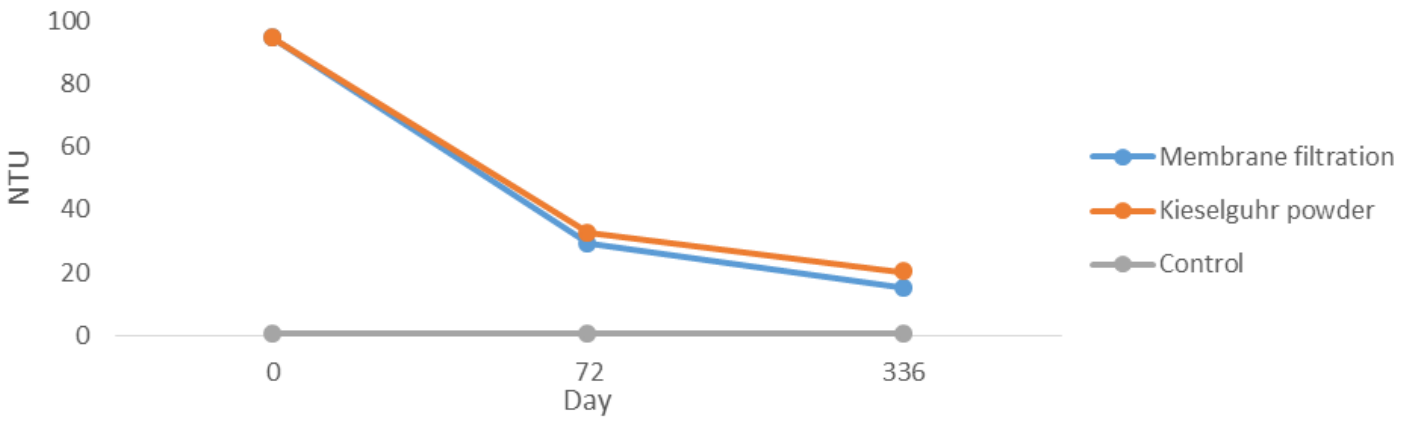

Figure 7. Turbidity of clarified wine samples and the control undergoing maturation.

Table 1. Average score of sensory attributes (Hedonic scale 1-9) of clarified sugarcane-watermelon wine and the control.

Attribute	Membrane filtration	Kieselguhr diatomaceous earth powder	Control
Clarity	7.2±0.63^{b}	6.1±1.20^{a}	8.3±0.67^{c}
Aroma	5.4±0.97^{a}	7.2±1.14^{b}	8.2±0.79^{c}
Appearance	7.1±1.10^{ab}	6.4±1.17^{a}	8.0±0.94^{b}
Colour	5.4±0.97^{a}	7.3±0.95^{b}	9.0±0.00^{c}
Overall acceptibility	7.3±0.95^{b}	6.3±0.95^{a}	9.0±0.00^{c}

Values show means of sensory scores of ten panelists ±SD. Values with different superscript across the row are significantly different (P<0.05). Hedonic scale: 9- like extremely; 8- like very much; 7- like moderately; 6- like slightly; 5- neither liked nor disliked; 4- disliked slightly; 3- disliked moderately; 2- disliked very much; 1- disliked extremely.

Keiselguhr diatomaceous earth powder was 1.043/1.026 and 1.043/1.040 kg/m^3, respectively. The reduction in specific gravity of the wines agrees with the findings by Okeke et al. (2015). In a related study, Zainab et al. (2018) reported that specific gravity of watermelon 'must' and watermelon wine was 1.075 and 1.020, respectively. This could be attributed to the activities of *S. cerevisiae* in the wine. At 336 h, the specific gravity of sugarcane-watermelon wine clarified using membrane filtration and Keiselguhr diatomaceous earth powder was 1.029 and 1.043 kg/m^3, respectively.

During maturation of wine, the reduction in sugar content of sugarcane-watermelon wine clarified using membrane filtration (10.73 to 6.3 °Brix) and Keiselguhr diatomaceous earth powder (10.73 to 9.9 °Brix) is in agreement with the report by Hafsat et al. (2015). This could be as a result of yeast utilizing sugar present in the medium for production of alcohol and other by-products of fermentation. This study shows that wines clarified by membrane filtration (7.2 °Brix) had a lower sugar content compared with wines clarified using Keiselguhr diatomaceous earth powder (10.8 °Brix). According to Nilar (2020), alcoholic beverage regarded as sweet wine contains residual sugar after fermentation within the range of 1 to 14%. During fermentation, sugar is a very important substrate for the production of ethanol, lactic acid and CO_2 (Saranraj et al., 2017). According to Okemini and Dilim (2017), insufficient quantity of sugar in fermenting 'must' is a challenge encountered during production of non-grape wine. In order to overcome the challenge, sugar is usually added to 'must' in the course of producing wine.

Findings from this study show that alcohol content of clarified sugarcane-watermelon wine reduced during maturation between 0 and 72 h. At 0 h, the alcohol content of the clarified wine was 5.9%. The alcohol content of wine clarified using membrane filtration and Keiselguhr diatomaceous earth powder at 72 h reduced to 3.57 and 5.48%, respectively. At 336 h, it was observed that alcohol content of wine clarified using membrane filtration (3.7%) was lower than wine clarified using Keiselguhr diatomaceous earth powder (5.7%). Throughout the period of maturation, the alcohol content of the control was constant (10.71%). According to Schmidtke et al. (2012), wine transported across a semi-permeable membrane or membrane is aimed at reducing its ethanol content. Since the alcohol content of clarified sugarcane-watermelon wine and the control was below the standard (7 - 24% v/v alcohol) stipulated in the Federal Alcohol Administration Act of the United States of

America (USA), the final product could be regarded as a dealcoholized wine. A slight increase in alcohol content during maturation of the clarified wines could be attributed to the release of various by-products of fermentation which include ethanol and alcohols. It suggests that fermentation was not completed. An increase in alcohol content during wine maturation agrees with the findings by Hasfat et al. (2015) and Soibam et al. (2016).

Findings from this study shows that colour intensity of sugarcane-watermelon wine clarified using membrane filtration and Keiselguhr diatomaceous earth powder steadily reduced during maturation. At 0 h, the colour intensity of clarified wines was 0.892 nm. The result for the wine clarified using membrane filtration/Keiselguhr diatomaceous earth powder at 72 and 336 h was 0.649/0.873 and 0.642/0.628 nm, respectively. The result is in agreement with the findings by Babincev et al. (2016) from a related study. On average, the colour intensity of sugarcane-watermelon wine clarified using Keiselguhr diatomaceous earth powder had a higher colour intensity than sugarcane-watermelon wine clarified by membrane filtration. Although the colour intensity of the control (0.330 nm) was constant during maturation, it was quite lower than sugarcane-watermelon wine clarified using membrane filtration and Keiselguhr diatomaceous earth powder.

During maturation of sugarcane-watermelon wine, the turbidity of unclarified wine was relatively stable (0.5 NTU) whereas the samples clarified using Keiselguhr diatomaceous earth powder and membrane filtration is within the range 15 - 94.32 NTU. According to Awe (2018), aging of wine without applying clarifying agent is a better approach to clarify wine. The turbidty of clarified wines at Day 0 was higher than the control sample. This result could be as a result of fining agent slurry added to the wine. The result is in agreement with the findings by Awe (2018). Among the two clarification methods sugarcane-watermelon wine samples were subjected to, findings from this study show that membrane filtration was more effective in reducing wine turbidty than the use of Keiselguhr diatomaceous earth powder. According to Awe (2018), filtration is not as effective as the use of fining agents which can only remove dead yeast cells and fruit fragments present in wine. The use of fining agent such as bentonite, kieselsol, casein, kaolin, albumin, gelatin and silicon dioxide is capable of removing soluble substances present in wine which include proteins, polymerized tannins, and colouring phenols.

The sensory report indicates that appearance, clarity and overall acceptability of sugarcane-watermelon wine samples clarified by membrane filtration were assigned a higher sensory scores than wine samples clarified using Keiselguhr diatomaceous earth powder. With regards to aroma and colour, wine samples clarified using Keiselguhr diatomaceous earth powder were assigned a higher sensory scores than sugarcane-watermelon wine samples clarified by membrane filtration. In a related study, Soibam et al. (2016) reported that wine produced using watermelon-sugarcane juice blended at 1:1 (v/v) had a good sensory rating with regards to colour, flavour and overall acceptability. It is worthy to note that all the sensory attributes of the control (sugarcane-watermelon wine without clarification) were assigned higher sensory scores than sugarcane-watermelon wine samples clarified using Keiselguhr diatomaceous earth powder or membrane filtration.

Conclusion

Winemakers who intend to use non-grape fruit(s) such as watermelon and other juices e.g. sugarcane juice to produce an acceptable wine in commercial quantity should evaluate the effect of clarification agent/method on the physicochemical and sensorial quality of the product. The acceptability of wine by consumers is influenced by the choice of fermentation substrate, yeast strain, among other factors. This study has proven that Keiselguhr diatomaceous earth powder or membrane filtration as clarification method/agent had some effect on the quality of sugarcane-watermelon wine fermented by *S. cerevisiae* isolated from palm wine.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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