Quality Improvement of Watermelon-*Clerodendrum volubile* Extract Wine Produced via Sequential Malolactic Fermentation by *Saccharomyces cerevisiae* and *Lactobacillus delbrueckii*

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Abstract: Herbal infusions medicinal benefits in wine and the impact of malolactic fermentation on wine quality is of high significance. The study aimed at improving the quality of watermelon wine with *Clerodendrum volubile* extract using *Saccharomyces cerevisiae* and *Lactobacillus delbrueckii* subsp. *bulgaricus. S. cerevisiae* and *L. delbrueckii* isolated from palm wine and yoghurt, respectively were used in this study. Fermentation must was prepared in various dilution ratios ranging from 95:5, 90:10 and 85:15 (watermelon to *C. volubile).* Static fermentation was carried out for 5 days with *S. cerevisiae* followed by malolactic fermentation with *L. delbrueckii* and then fermentation with *S. cerevisiae* for 23 days at room temperature. Physicochemical, phytochemical, mineral, and sensory properties were observed. Noticeable was pH decrease (5.21 - 3.33), increased titratable acidity (0.05-0.69 g/l), decreasing reducing sugar (0.59-0.011 mg/ml), temperature (30.5-24.2°C) and increasing total dissolved solids (19.7-48.9°B). Wine fermented with *S. cerevisiae* (D) had the highest phenolic content (481.68±0.37 mg/100 g), while vitamin C increased (20.2±0.73 - 29.28±0.70) with increase in *C. volubile* concentration. The Na^+ was most abundant (51.71 mg/100ml), while Ca^{2+} (5.23 mg/100ml) was improved. Watermelon wine (D and H) showed the least (1.38±0.5%) alcohol content while wine C and G recorded the highest. Organoleptic properties of wine E received the highest preference rating for flavour, colour and taste. Therefore, the nutritional and sensorial properties of Watermelon-*C. volubile* wine can be improved through sequential malolactic fermentation.

Key word: *Clerodendrum volubile*, *Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, watermelon, wine

INTRODUCTION

Wine, an alcoholic beverage, is made by fermenting fruit juices. During the wine making process, yeast turns the sugar in the fruit juices into alcohol, carbon dioxide, and organic acids, which then react to generate aldehydes, esters, and other chemical compounds that aid in the preservation of the wine (Aminu *et al.*, 2018). In recent years, several researchers have turned their attention to production processes involving fermentations with mixed yeast inocula in an attempt to improve the organoleptic characteristics of the resulting wines and to reduce the alcohol content. Hence, the use of non-*Saccharomyces* species as starters, along with various *S. cerevisiae* strains improved considerably various wine characteristics, such as: physicochemical properties, the composition and concentration of flavour, aroma of the final product and others (Dias *et al.*, 2020). Malolactic fermentation (MLF) is a natural process that occurs as a result of the metabolic activity of lactic acid bacteria (LAB). Malolactic fermentation plays three roles in wine making: it reduces wine acidity due to malic acid, stabilizes wine by removing a possible carbon source, and increases aroma and flavour modification (Virdis *et al.*, 2020). *Oenococcus*, *Leuconostoc*, and *Pediococcus*, are the only genera associated to wine (Holzapfel and Wood, 2014; Zheng *et al.*, 2020). Due to its high tolerance for low pH, high ethanol concentrations and scarcity of nutrients, '*O' oeni* is the main LAB of choice in winemaking. However, with increasing temperatures during growth and harvest, and a consequent rising pH trend for many wines, other LAB have the potential to become a valid alternative to *Oenococcus*, playing an important role in the modifications of wine aroma (Krieger-Weber *et al.*, 2020; López-Seijas *et al.*, 2020).

Currently, there is little or no information on the use of *L. delbrueckii* subsp. *bulgaricus* for malo -lactic fermentation (MLF). Wine production from grapes are commonly reported in the literature (Gavahian *et al.*, 2022), due to their natural chemical equilibrium, which aids in the fermentation process without the addition of sugars, acids, enzymes, or other nutrients. However, due to the climatic restrictions of grape, current research is directed towards producing similar and/or better wines from other tropical fruits sources. Fruits such as pawpaw, banana, cucumber and other fruits have been used as single or mixed fruit for wine production (Ogodo *et al.*, 2015). Watermelon (*Citrullus lanatus*) is a fruit which belongs to the family of Cucumbitacea, mostly grown in the northern part of Nigeria. It is nominally 60% flesh and about 90% of the flesh is juicy which contains 7 to 10 % (w/v) sugar. Thus, over 50 % of the watermelon is readily fermentable liquid (Ozcelik and Yavuz, 2016). Nutritional profile of watermelon is full array of nutrients, including carbohydrates, sugar, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids, amino acids According to Ozcelik and Yavuz (2016), a serving cup of watermelon contains 12.31 mg of vitamin C, 864.88 IU of vitamin A, 170.24 mg of potassium and 45.60 calories.

Clerodendrum volubile also known as white butterfly (Lamiaceae) is a shrub-like climbers' native to Africa. It is predominantly grown as ornamental plant in tropical West Africa including Ghana, Ivory Coast, Sierra Leone and Nigeria (Ajao *et al.*, 2018). *C. volubile* is usually 1–4.5 m long, stem terete or round, leaves oblong, commonly 1.5–15.5 cm long and 0.6–6 cm wide. It is commonly known as "Marugbo" or "Eweta" among the Ikale, Ilaje and Apoi people found in Southern-senatorial district of Ondo State, South West Nigeria. The leaf of is commonly consumed as vegetables sweet aroma and taste and have great nutritional value as well as herbal and medicinal value. According to Ogunwa *et al.* (2015), proximate analysis of *C. volubile* include vitamins, minerals and crude protein essential for healthy living. The anti-diabetic, anti-hyperlipidemic and anti-hypertension action of *C. volubile* have been demonstrated by Erukainure *et al.* (2016). In addition, the anti-inflammatory, anti-cancer, analgestic and anti-microbial activity of *C. volubile* extract have been previously demonstrated (Afolabi *et al.*, 2019; Okaiyeto *et al.*, 2021). Based on our previous knowledge, this will be the baseline research on the use of watermelone-*C. volubile* extract for wine production. Therefore, the current study aimed at evaluating the physichochemical, nutritional and organoleptic properties of water melon-*C. volubile* extract for wine production using sequencial fermentation with *S. cerevisiae* and *L. delbrueckii.*

MATERIALS AND METHODS

Sample collection: *Clerodendrum. volubile* was obtained at Moferere Market Ondo West Local Government of Ondo State, Nigeria, while watermelon was purchased from a native vendor at Tanke, Ilorin, Kwara state, Nigeria. Fresh palm wine was obtained from Asa Dam, Ilorin, Nigeria, while REV yoghurt fermented with *L. debdrueckii subsp bulgaricus* was purchased at Palms Mall, Ilorin, Kwara State, Nigeria.

Isolation and identification of yeast cell and Lactobacillus sp.: Yeast extract peptone dextrose (YPD) and lactobacillus De man Rogosa and Sharpen Agar (LMRS) were used as medium for isolation. The fermentation yeast and *Lactobacillus* were isolated from 25 ml palm wine and yoghurt, respectively using pour plate technique. The yeast and bacteria strain were identified according to the method of Franklin *et al.* (2019) which involves morphological and cultural characterization.

Sugar fermentation test: A colony of the isolate was inoculated into 10 ml phenol red fermentation broth containing sugars (lactose, maltose, mannitol, glucose, and fructose) and incubated at 27°C for 24 – 72 h. A positive sugar fermentation result was

indicated by a yellow colouration while the retention of initial red colour denotes negative fermentation.

Preparation of C. volubile extracts (infusion): The extraction process was conducted using the aqueous extraction method as described by Chawafambira, (2021). *C. volubile* leaves were sorted, washed thoroughly with tap water, oven dried at 60 °C and blended. Thereafter, 500 g of the powdered leaves were then boiled in 2 l sterile distilled water, strained using muslin cloth, and the liquid extract was stored at 4°C in a chiller until further use.

Pulp extraction and preparation of watermelon must: Ripe watermelon fruits were thoroughly washed and was disinfected using 70% ethanol to remove dirt, microorganisms and its vegetative form adhering to the surface of the watermelon. The watermelon was cut opened with a sterile knife, the pulp was cut into pieces and blended, then sieved with a muslin cloth to obtain the juice. Then 0.15 g of sodium metabisulfite ($Na_2S_2O_2$) was added to prevent fermentation of the must by autochtonous organisms as well as inhibit pathogenic microorganism (Chawafambira, 2021).

Fermentation of must to wine: Fermentation was carried out in a 2 l fermentation vessel. The wine was produced from mixtures of water melon and *C. volubile* extract at different ratios (90:5, 90:10, 85:15, 100: 0) and the sugar content was then adjusted by addition of 40 g sucrose. Aerobic fermentation was initialed by inoculation of 4 ml starter culture of *S. cerevisiae,* allowed for 5 days fermentation at 27°C, while anaerobic fermentation continued for 23 days by inoculation of 4 ml *L. delbrueckii.* After fermentation, the wine was chilled to 5°C, racked with minimum exposure to the air, and clarified. The fermented wine was then centrifuged at 10000 rpm for 10 min.

Proximate analysis of watermelon must and C. volubile extract: The moisture, ash, crude fibre, fat, crude protein and, total carbohydrate were determined according to Falade *et al.* (2014). The watermelon must was air dried for 7 days while the *C. volubile* leaves were blended to powder prior to analysis.

Determination of pH total dissolved solid (TDS) and total titratable acidity (TTA) of watermelon must and C. volubile extract wine: The pH and TDS of the wine samples were determined using a digital pH meter (Omega PHH44) and digital refractometer (ATAGO PAL-1) (AOAC, 2005). Total titratable acidity was determined in a 20:100 (wine:water) sample by titration with 0.1 M NaOH (Feldsine *et al.*, 2002).

Reducing sugar and specific gravity of watermelon must and C. volubile extract wine: The reaction of wine sample with dinitrosalicylic acid (DNS) in boiling water and further spectrophotometry was used for reducing sugar analysis (Miller, 1959). The specific gravity was determined using specific gravity bottle (Joseph *et al.*, 2019).

Mineral, phytochemical and alcohol content of watermelon must and C. volubile extract wine: The determination of Ca^{2+}; Na^{+}, Fe^{2+}, K^{+} and Mg^{2+} and total flavonoids were carried out as described by AOAC (2005). Total phenolic and vitamin C content were determined using spectrophotometric and titrimetric method, respectively (Ndawula *et al.*, 2004: Vijay and Rajendra 2014). Alcohol content was determined using the iodoform test technique (Tsegay *et al.*, 2018).

Sensory evaluation of the produced wine: Sensory evaluation of the watermelon-*C. volubile* wines was carried out by 8 panelists . This was done in order to ascertain its acceptability. The organoleptic parameters that were evaluated include taste, aroma, colour, clarity and overall acceptability using five-point hedonic scales. From the scale 5 indicates extremely like and 1 indicates extremely dislike (Wakil and Kazeem, 2012; Animu *et al.*, 2018).

RESULTS

The study was aimed at investigating the nutritional and health benefits of watermelon-*C. volubile* wine and the impact

of *L. delbrueckii* on the organoleptic properties of the resulting product.

The colonial, morphological and microscopic characterization of *S. cerevisiae* and *L. delbrueckii* is illustrated in Table 1. The yeast isolate present creamy, non-motile, oval shape and budding. On the other hand the LAB strain was creamy, Gram positive, rod and non-motile. Both isolates were able to ferment glucose, sucrose, maltose, fructose and lactose.

The findings showed that *C. volubile* contained high crude fiber, total ash, crude protein and crude lipid content (29.34%, 10.98%, 14.89% and 10.12%). However, watermelon must has higher carbohydrate and moisture content (50.36% and10.30%) in comparison with *C. volubile* extract Table 2.

There was a decrease in pH with increase in fermentation period from initial day to the 28th day (Table 3). The highest pH values were observed in samples before fermentation specifically in sample C (5.21), while the lowest pH values were obtained in wine with sequential fermentation with *S. cerevisiae* and *L. delbrueckii*, with the lowest (3.33) specifically recorded in wine sample E and F. The pH profile was recorded in order, before fermentation > 5 days fermentation with *S. cerevisiae* > 28 days fermentation with *S. cerevisiae* > 28 days fermentation with *S. cerevisiae* and *L. deibrueckii.* The titratable acidity increase with increase in fermentation from initial day to 28 days with the highest (0.69) obtained in wine sample E and F, while the lowest (0.05) was recorded in wine sample C. Overall, the titratable acidity of wine samples was higher in samples with sequential fermentation with *S. cerevisiae* and *L. delbrueckii* compared with samples fermented with only *S. cerevisiae.* The reducing sugar content of the wine samples decreased with increase in fermentation time. The highest reducing sugar (0.59 mg/ml) was observed in wine sample B before fermentation which reduce to (0.167 mg/ml) after 5 days fermentation with *S. serevisiae.* Reducing sugars were detected below minimum in the 28 days fermented wine samples. There was a decrease in temperature from 30.5°C in wine sample B and C before fermentation to 24.2°C in samples F, G and H of 28 days sequential fermentation with *S. cerevisiae* and *L. delbrueckii.* The co-fermentation wine samples experienced lower temperatures than fermentation with *S. cerevisiae* alone. The specific gravity decrease with increase in fermentation days. The highest specific gravity were recorded in wine samples before fermentation, with the highest (1.03 kg/m^3) from wine sample C. while the lowest specific gravity (0.97 kg/m^3) was observed in wine samples D (28 days fermentation with *S. cerevisiae* and H (28 days) fermentation with *S. cerevisiae* and *L. delbrueckii*).

The total phenol content ranged from 261.36 ± 0.46 unit in sample E to 481.68 ± 0.37 unit, with the highest obtained in wine produced from watermelon using *S. cerevisiae* (D) Table 4. It is worth to note that the phenol content increased with increase in concentration of the *C. volubile* extract concentration for the MLF (E-H). However, a decrease was observed with the A-C wine sample. The total flavonoids content ranged from 25.42 ± 0.95 unit in sample H to 48.94 ± 0.34 unit in sample C. In this case there was an increased with increase in *C. volubile* concentration for both fermentation with *S. cerevisiae* (A-C) and MLF (E-G), except for the D and H that recorded declination. Wine samples produced from watermelon alone contained the lowest flavonoid content for both types of fermentation. The vitamin C content increased with an increase in *C. volubile* with the highest (29.28 ± 0.70 units) obtained in sample (C) and the lowest (17.22) in wine from watermelon alone (H), fermented with *S. cerevisiae* and *L. delbrueckii.*

The mineral composition (calcium, potassium, magnesium sodium and iron) of the wine samples is shown in Table 5. The K^+ composition of all the eight wine shows slight difference in concentration, ranging

from 4.64± 0.014 (mg/100g) to 4.7 ± 0.00 (mg/100g), with the highest K^+ composition recorded in wine C and the lowest K^+ composition recorded wine E and F . The Ca^{2+} content raged from 3. 96 ± 0.00 (mg/100g) to 5.23± 0.012 (mg/100g) with the highest Ca^{2+} recorded in wine F and the lowest in wine C. The Mg^{2+}content ranged from 5.65 ± 0.001 (mg/100g) to 6.07 ± 0.01(mg/100g) with the highest Mg^{2+} recorded in wine G and the lowest in wine D. The Na^{2+} composition ranged from 51.71 ± 0.00 (mg/100g) to 55.21 ± 0.00 (mg/100g), with the highest Na^{2+} composition recorded in wine A and the lowest Na^{2+} composition recorded wine C. The Fe^{2+} composition shows slight difference in concentration, ranging from 0.08 ± 0.003 (mg/100g) to 0.23 ± 0.001(mg/100g) with the highest Fe^{2+} composition recorded in wine G and the lowest recorded in the control wine E and H.

The incorporation of *C. volubile* extract resulted in increased alcohol concentration as observed in wine A, B, C, E, F, G ranging from 2.74% to 2.76% with wine D and H having lower alcohol concentration of 1.38 %. Hence co-fermentation with *S. cerevisiae* and *L. delbrueckii* has no effect on alcohol concentration of the wine samples examined in the current study.

The findings of the sensory properties of the wine samples is as shown in table 6. The wine sample E was rated highest (4.5) in terms of flavour while the lowest rating (1.8) was observed with wine sample C. Overall, wine with lower concentrations of *C. volubile* (5 and 10) had higher flavour rating which reduced when the *C. volubile* concentration was increased to 15. Based on the taste evaluation, the wine sample E also had the highest rating (4.0), while the lowest rating (2.0) was observed in wine sample C. The taste rating reduced with increase in *C. volubile* concentration. Similarly, the wine sample E was rated highest (4.4) in terms of colour, while sample G was rated the lowest (1.8). Overall, the wine sample E with best combination of watermelon- *C. volubile* ratio (95:05) with sequential *S. cerevisiae* and *L. delbruekii* fermentation presented the best organoleptic properties in terms of flavour, colour and taste.

Table 1: Morphological and biochemical characterization of wine starter cultures

Test	*L. delbrueckii*	*S. cerevisiae*
Colony on culture media	Creamy, slimy, raised entire circular colony	Creamy, big raised, circular colony
Motility	Non motile	Non motile
Gram staining	Gram positive rod	NA
Budding	NA	Multiple budding
Lactophenol cotton blue stain	NA	Oval shape
Turbidity in broth	Slightly turbid	Slightly turbid
Growth on LMRS media	Positive	Nil
Glucose	Positive	Positive
Sucrose	Positive	Positive
Fructose	Positive	Positive
Maltose	Positive	Positive
Mannitol	Negative	Negative

Table 2: Proximate composition of *C. volubile* extract and watermelon pulp

Sample	Crude protein (%)	Crude lipid (%)	Moisture (%)	Crude fiber (%)	Carbohydrate (%)	Total ash (%)
C. volubile	14.89±0.03	10.12±0.8	10.14±0.35	29.34±1.35	39.16±0.09	10.98±0.71
Water melon	11.57±0.01	10.12±0.8	10.3±0.167	3.48±0.22	50.36±1.27	8.63±1.72

Table 3: Physicochemical properties of watermelon-*C. volubile* extract wine

Wine	Parameters					
	pH	TTA	RS (mg/ml)	TDS (°Brix)	TEMP (°C)	SG
	Before fermentation					
A	5.06±0.00	0.09±0.0	0.56±0.00	19.7±0.00	29.5±0.00	1.02±0.0
B	5.14±0.00	0.071±0.0	0.59±0.00	20.6±0.00	30.5±0.00	1.01±0.0
C	5.21±0.00	0.05 ±0.0	0.55±0.00	23.0±0.00	30.5±0.00	1.03±0.00
D	4.99±0.00	0.12±000	0.38±0.00	23.1±0.00	28±0.00	1.02±0.00
	After 5 days fermentation with *S. cerevisiae*					
A	3.73±0.00	0.42±0.00	0.192±0.00	18.6±0.00	26.7±0.01	0.99±0.00
B	3.80±0.00	0.44±0.00	0.1670.00	22.2±0.00	25.3±0.04	0.99±0.00
C	3.88±0.00	0.43±0.00	0.175±0.00	21.3±0.00	24.7±0.06	0.99±0.00
D	3.89±0.00	0.33±0.01	0.172±0.00	22.5±0.00	24.4±0.00	0.99±0.00
	After 28 days fermentation with *S. cerevisiae*					
A	3.39±0.037	0.56±0.00	0.09±0.00	46.5±0.00	26.6±0.00	0.98±0.00
B	3.43±0.06	0.54±0.00	0.11±0.00	44.2±0.00	26.4±0.00	0.98±0.00
C	3.57±0.053	0.55±0.00	0.08±0.00	48.3±0.02	24.2±0.00	0.98±0.00
D	3.86±0.00	0.54±0.01	0.09±0.00	46.3±0.01	27.3±0.00	0.97±0.00
	After 28 days fermentation with S. cerevisiae and *L. delbrueckii*					
E	3.33±0.00	0.69±0.00	0.08±0.00	48.7±0.00	24.2±0.00	0.98±0.00
F	3.33±0.00	0.69±0.00	0.11±0.00	45.4±0.00	24.0±0.00	0.98±0.00
G	3.44±0.00	0.60±0.00	0.09±0.00	47.8±0.00	24.2±0.00	0.98±0.00
H	3.69±0.00	0.58±0.02	0.11±0.00	48.9±0.00	25.9±0.00	0.97±0.00

Key: Watermelon to *C. volubile* dilution ratio TTA= total titrable acidity, RS= reducing sugar, TDS= total dissolved solid, TEMP= temperature SG= specific gravity. Values are means of triplicate readings ±SD. Blend formulation - Watermelon: *C. volubile*; A =95:05, B =90:10, C =85:15, D =100:00 (control), E =95:05, F =90:10, G =85:15, H =100:00 (control), A to D: wine fermented with *S. cerevisiae*, E to H: wine fermented with *S. cerevisiae* and *L. delbrueckii*

Table 4: Phytochemical properties of wine sample

Wine sample	Total phenol (%)	Total flavonoids (%)	Vit C (mg/100g)
A	372.3 ± 0.5	36.0 ± 0.14	20.2 ± 0.73
B	353.2 ± 1.83	44.33 ± 1.5	24.59 ± 1.30
C	346.69 ±0.3	48.94 ± 0.3	29.28 ± 0.70
D	481.7 ± 0.37	33.08 ± 0.3	19.98 ±0.27
E	261.5 ± 0.46	28.45 ± 0.6	17.9 ± 0.45
F	278.7± 0.37	37.13 ± 0.0	22.81 ± 0.21
G	328.6 ± 0.62	42.44 ± 1.7	24.59 ± 1.30
H	421.6 ± 0.54	25.42 ± 0.9	17.22 ± 0.04

Values are means of triplicate readings ±SD. Blend formulation. Watermelon: *C. volubile*: A =95:05, B =90:10, C =85:15, D =100:00 (control), E =95:05, F =90:10, G =85:15, H =100:00 (control), A to D: wine fermented with *S. cerevisiae*, E to H: wine fermented with *S. cerevisiae* and *L. delbrueckii*

Table 5: Mineral composition of water melon-*C. volubile* extract wine (mg/100g)

Wine	Minerals				
	K^+ (mg/100g)	Ca^{2+} (mg/100g)	Mg^{2+} (mg/100g)	Na^+ (mg/100g)	Fe^{2+} (mg/100g)
A	4.65 ± 0.0	4.22 ± 0.1	5.95± 0.00	55.21 ± 0.0	0.1 ± 0.0
B	4.65 ± 0.0	4.11 ± 0.0	5.955± 0.00	54.36 ± 0.3	0.22 ± 0.0
C	4.7 ± 0.0	3.96 ± 0.0	6.05 ± 0.01	51.71 ± 0.0	0.195 ±0.1
D	4.66± 0.0	4.94 ±0.1	5.65 ±0.001	52.11± 0.01	0.13± 0.0
E	4.64±0.02	4.01 ± 0.0	5.66 ± 0.01	52.06± 0.01	0.08 ± 0.01
F	4.64± 0.0	5.23± 0.0	5.92± 0.01	52.19 ± 0.0	0.11 ± 0.1
G	4.66 ± 0.0	4.23 ± 0.1	6.07 ± 0.01	52.13 ± 0.0	0.23 ± 0.1
H	4.65 ± 0.2	5.11± 0.0	5.79 ± 0.03	52.14± 0.0	0.08 ± 0.0

Key: Values are means of triplicate readings ±SD. Blend formulation - Watermelon: *C. volubile:* A =95:05, B =90:10, C =85:15, D =100:00 (control), E =95:05, F =90:10, G =85:15, H =100:00 (control), A to D: wine fermented with *S. cerevisiae*, E to H: wine fermented with *S. cerevisiae* and *L. delbrueckii*

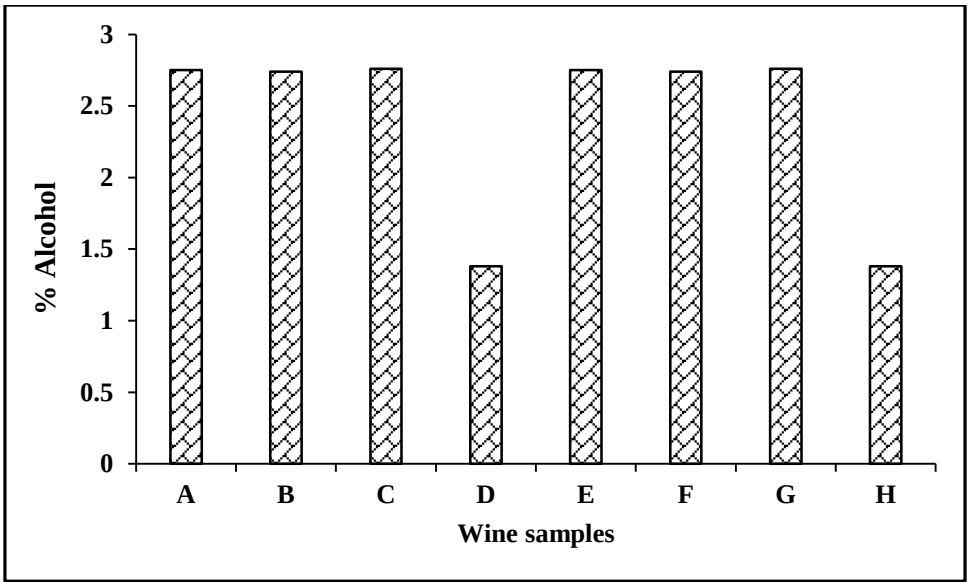

Figure: 1 Alcohol content of water melon-*C. volubile* extract wine

Key: Values are means of triplicate readings ±SD. Blend formulation - Watermelon: *C. volubile*: A =95:05, B =90:10, C =85:15, D =100:00 (control), E =95:05, F =90:10, G =85:15, H =100:00 (control), A to D: wine fermented with *S. cerevisiae*, E to H: wine fermented with *S. cerevisiae* and *L. delbrueckii*

Table 6: Mean scores of sensory evaluation of wine samples fermented with *S. cerevisiae* and *L. delbrueckii*

Parameters	Wine samples							
	A	B	C	D	E	F	G	H
Flavour	4.0	4.3	1.8	2.6	4.5	4.1	2.3	3.4
Taste	2.5	3.6	2.0	3.1	4.0	3.3	2.1	4.0
Colour	3.4	3.6	2.1	3.0	4.4	3.1	1.8	4.0

Key: Acceptability rating 1–5: 1 = very bad and 5 = very good. Values are means of triplicate readings ±SD. Blend formulation - Watermelon: *C. volubile*: A =95:05, B =90:10, C =85:15, D =100:00 (control), E =95:05, F =90:10, G =85:15, H =100:00 (control), A to D: wine fermented with *S. cerevisiae*, E to H: wine fermented with *S. cerevisiae* and *L. delbrueckii*

DISCUSSION

Clerodendrum volubile is consumed in some part of Nigeria due to its nutritional and health benefits. Several research articles have pointed out its medicinal and nutritional properties. Some species of lactic acid bacteria possess the ability to carry out malolactic fermentation, which is lacking in fermentation yeast, but which contributes greatly to the physicochemical and sensory parameters of the wine. This study clearly showed an improved watermelon wine produced with *C. volubile* via sequential fermentation with *S. cerevisiae* and *L. delbrueckii* subsp. *bulgaricus*. The isolates fermented different sugars as confirmed by (Amoroso *et al.* (1989) and Timmermans *et al.* (2022). The proximate content observed in this study, was in agreement with Ajao *et al.* (2018),who reported similar crude protein, lipid, carbohydrate and ash content, while Sadiq *et al.* (2021) reported similar proximate content with watermelon.

The pH is a desirable attribute during fruit fermentation because it creates a conducive environment favorable for the fermenting yeast while creating an undesirable environment for spoilage organisms. Several studies has confirmed this claim (Yusufu *et al.*, 2018). Aminu *et al.* (2018), reported acidity of watermelon in the range of 5.14 to 4.90. Acidity plays a vital role in the overall stability and characteristics of the wine. The inverse correlation between the pH and the

TTA shows that pH decrease lead to increase in acidity of watermelon juice-*C. volubile* wine. This is in agreement with the studies of Yusufu *et al.* (2018) who reported related findings in wine produced from watermelon and ginger. Also study carried out by (Awe and Nnadoze, 2015) and (Yusufu *et al.*, 2018) on date palm and watermelon with ginger extract respectively, shows comparable results.

The declination in specific gravity observed in this study have previously been reported by Soibam *et al.* (2016) and Chawafambira (2021) who separately reported reduction in specific gravity of wine produced from *U. kirkiana* juice and *Lippia javanica* and wine produced from watermelon and sugarcane. Prolong fermentation contributed to the reduction in reducing sugar in the wine samples. This is in agreement with Chawafambira (2021), who reported similar findings in wine produced from *U. kirkiana* juice and *Lippia javanica*. The *C. volubile* and malolactic fermentation contributed to the higher mineral composition of watermelon wine compared with the wine produced from watermelon and ginger (Yusufu *et al.*, 2018). Maarman (2014), reported increased minerals in wine fermented with co-culture of yeast and LAB. It is worth to note that the malolactic fermentation improved the calcium content of wine F compared with wine B (fermented with *S. cerevisiae* only). This could be that the dilution ratio is compatible with the natural requirement of *L. delbrueckii*.

Wine sample rich in phenolic compounds and flavonoids have impact on the overall taste and flavour of the wine. In this study, wine samples produced via sequential fermentation has lesser phenolic and flavonoids content which could be due to the activities of the *Lactobacillus* specie. However, the *C. volubile* concentrations has positive impact in improving the phytochemicals. Reports has shown that flavonoids has an antioxidizing power, which prevents browning and spoilage thus help protects wine from oxidation. Also, wine samples without *C. volubile* extract (wine D and H) has lesser vitamin C content, this indicates that *C. volubile* contributed to the vitamin C composition of the wine. The overall acceptability ratings decrease in the highest concentration of *C. volubile*. There were differences among the wine samples with respect to flavour, taste and colour, and similar findings was reported by Chawafambira (2021) in wine produced from *U. kirkiana* juice- *L. javanica* extract wine. However, wine 'E' was more preferred in terms of the sensory properties evaluated.

CONCLUSION

The study revealed that incorporation of *C. volubile* improved the nutritional and physicochemical properties of the watermelon wine. Sequential malolactic fermentation with *S. cerevisiae* and *L. delbrueckii* subsp *bulgaricus* improved the organoleptic properties of the wine. Therefore, production of wine and by extension other wines with incorporation of of *C. volubile* coupled with fermentation with *S. serevisiae* and *L. delbrueckii* should be encouraged based on its overall acceptability.

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