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PRODUCTION AND CHARACTERIZATION OF WINE FROM GOLDEN DELICIOUS APPLES USING *SACCHAROMYCES CEREVISIAE* ISOLATED FROM SELECTED NIGERIAN INDIGENOUS ALCOHOLIC BEVERAGES

Isaac Ayanniran Adesokan

Department of Science Laboratory Technology, The Polytechnic, Ibadan, UI Post Office, Oyo State, Nigeria.
Email: sokanisaac@yahoo.co.uk

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ABSTRACT

There are many health benefits associated with consumption of apples and fermented apple juice, such as apple cider and apple wine. As a result of several health claims associated with the consumption of apple wine, this study was conducted with a view of producing apple wine using *S. cerevisiae* isolated from indigenous Nigerian alcoholic beverages. The aim is to develop indigenous technology for the production of apple wine and, by extension, tropical fruit wines. During the fermentation of apple juice into wine, changes in pH, titratable acidity (TA), brix/acid ratio (B/A), ascorbic acid (AA), total soluble solids (TSS), reducing sugars (RS), total sugars (TS), alcohol content (AC) and yeast count (YC) were determined using standard methods. The apple wine produced was rated by taste panelists using a 9-point hedonic scale. The following result ranges were obtained: pH (3.49–4.02), TA (18.0–43.2mg/ml), B/A (0.221–0889), AA (4.90–74.51mg/ml), TSS (9.0–18.0 mg/g), RS (0.260–0.347 mg/ml), TS (0.023–0.07mg/ml), AC (0.0–10.2%) and YC (7.40–11.99 $\log_{10}$cfu/ml). The sensory evaluation scores are appearance (7.14–7.71), flavor (6.71–8.00), mouth feel (7.00–8.00), taste (7.00–8.00) and overall acceptability (7.04–7.89). The apple wine that was fermented with *Saccharomyces cerevisiae* PAW02 (sample A) received the highest scores for flavor (7.8), mouth feel (8.00), taste (8.00) and overall acceptability (7.82). This sample also had the highest alcohol content of 10.2%. Therefore, this yeast strain (*S. cerevisiae* PAW02) could be explored for industrial production of alcoholic beverages from apples (apple wine).

Contribution/Originality: In this study, S. cerevisiae were isolated from Nigerian traditional alcoholic beverages and were characterized using phenotypic and molecular methods. The S. cerevisiae were employed in the fermentation of apple juice for the first time to produce fruit wine. This is the first attempt to develop alcoholic beverages from apple using yeasts isolated from indigenous alcoholic beverages, such as palm wine, burukutu and agadagidi.

1. INTRODUCTION

Apples (*Malus domestica*) are a popular fruit because of their pleasant, subacid and aromatic nature. They contain a high level of sugars, vitamin C, minerals, proteins, pectin, calcium and phosphorus (Sukhvir & Kocher, 2019). Though apple is produced in temperate countries, it is consumed throughout the world, including Nigeria, because of its rich nutritional content and perceived health benefits, such as anti-cancer, anti-diabetes and anti-cholesterol properties (Wurz, 2019). Different useful products are produced from apples, including apple wine and apple cider. Also, wines in general are nutrient rich as they contain the nutrients of the fruits from which they are made (Sukhvir & Kocher, 2019).

Fermentation is a viable technique in the development of new products with modified physico-chemical and sensory qualities, especially flavor and nutritional components. Alcohol and acetic and lactic acid fermentation are important for quality in production (Saranraj, Sivasakthivelan, & Naveen, 2017). 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, spirits, and distilled beverages such as whisky, rum, gin and vodka (Saranraj et al., 2017).

Palm wine is an alcoholic beverage commonly consumed in southern Nigeria, Asia and South America. It is created from the sap of different types of palm trees (Elijah, Ojimelukwe, Ekong, & Asamudo, 2010). Palm wine can be consumed in a variety of flavors ranging from sweet (unfermented) to sour (fermented) and vinegary. It is produced by a succession of microorganisms, including yeasts such as Saccharomyces, Pichia, and Schizosaccharomyces (Adedayo & Ajiboye, 2011). Burukutu is an example of an African opaque beer that is commonly produced from sorghum, millet and maize and is consumed in Northern Nigeria. Its production involves different processes, such as malting, mashing, straining, souring, boiling and alcohol fermentation (Atter, Obiri-Danso, & Amoa-Awua, 2014). Agadagidi is a typically traditional African alcoholic beverage made from overripe bananas and plantains through fermentation. It is cloudy, effervescent, sweet-sour tasting, and is commonly consumed in South West Nigeria (Mogaji et al., 2021). The microorganisms responsible for the fermentation of these indigenous alcoholic beverages include yeasts, lactic acid and acetic acid bacteria, which contribute to the taste, flavor, enhancement of nutritional quality, alcoholic content and shelf life of the beverages (Adesokan, Sanni, & Marc-Andre, 2020; Oyewole & Isah, 2012).

In recent times, home wine production has been practiced with various fruits, such as apples, pears, strawberries, cherries, plums, pineapples and oranges (Isitua & Ibeh, 2010). Wine is a healthful beverage that has been used as a natural remedy for illnesses from early on and is said to aid recovery during convalescent periods (Okafor, 2007). Wine is a complex mixture, consisting of both organic and inorganic compounds, such as esters, high alcohols, fixed acids (malic, tartaric and citric acid), sugars, aldehydes, tannins, pectin, vitamins and minerals (Umeh, Udemezue, Okeke, & Agu, 2015).

Fermentation processes usually involve species of the yeast *Saccharomyces* whereby the sugars in the fruit juice are converted into alcohol and organic acid that later react to form aldehydes, esters, and other chemical components (Isitua & Ibeh, 2010). Fermentation can be spontaneous by the natural flora of the fruit or controlled by introducing an industrial strain of yeast to ferment the juice. However, most fermentation processes are uncontrolled with the risk of generating inconsistent products with an unappealing quality, a short shelf life, and safety concerns, such as spoilage and toxigenic fungi (Adekoya, Obadina, Phoku, Nwinyi, & Njobeh, 2017).

Wine is most commonly produced from grapes but is now also produced from other fruits including apples (Satav & Pethe, 2017). The processing of apple juice into a refreshing alcoholic beverage was first carried out in Eastern Mediterranean regions more than 2000 years ago (Alberti, Vieira, Drilleau, Wosiacki, & Nogueira, 2011). Nowadays, apple juice is fermented to produce apple wine or apple cider (Alberti et al., 2011). Apple wine and cider differ in terms of their ethanol content. While apple wine usually has an ethanol content above 7% (v/v), apple cider contains less than 7% (v/v) ethanol (Wang, Xu, Hu, & Zhao, 2004).

There has been a growing appreciation and understanding of the link between fruit and vegetable consumption and improved health. Research has shown that biologically active components in apple-based foods, particularly phytochemicals, have the potential to modulate many processes in the development of diseases, including cancer, cardiovascular disease, diabetes, pulmonary disorders, Alzheimer's disease and other degenerative diseases (Wurz, 2019). As a result of many health benefits associated with the consumption of apples and apple-based products, this study was conducted to produce and characterize apple wine using *S. cerevisiae* isolated from selected Nigerian indigenous alcoholic beverages. This is to encourage local production of apple wine in Nigeria using available raw materials and hence improve the well-being of people who consume apple wine. Moreover, fermented foods and alcoholic beverages, such as bread, wine and beer, produced on commercial levels are usually produced with yeasts

imported from developed countries. Therefore, developing yeast strains for the production of apple wine and other fermented foods will help conserve foreign exchange spent on importing active dry yeasts.

2. MATERIALS AND METHODS

2.1. Sample Collection

Samples of fermented alcoholic beverages (burukutu, palm wine and agadagidi) were obtained from local producers in Ibadan, Nigeria, immediately after production and transported in sterile containers to the laboratory for isolation of yeasts. Golden Delicious apples were obtained from a local market in Palampur, India, for immediate use in wine production.

2.2. Phenotypic and Molecular Identification of Yeasts

The samples of alcoholic beverages were serially diluted in sterile distilled water, and appropriate dilutions were spread plated on potato dextrose agar (PDA) supplemented with 3ug/ml of streptomycin. The yeasts that developed after 48 hours at an incubation temperature of 25°C were purified by repeated streaking on PDA. The pure cultures of yeasts were identified by their cultural, physiological and biochemical properties. The yeast classified as *Saccharomyces cerevisiae* was further confirmed by molecular method. The D1 and D2 domains of the large subunit ribosomal ribonucleic acids were amplified using primers NL 1 (52-GCA TATCAATAAGCGGAGGAAAAG) and NL 4 (52-GGTCCGTGTTTCAAGACGG). The polymerase chain reaction (PCR) was conducted, and amplified deoxyribonucleic acids were sequenced. The sequences obtained were compared with published sequences (Adesokan et al., 2020).

2.3. Extraction and Production of Apple Juice

The apple samples were washed twice with sterile distilled water and allowed to dry under hygienic conditions. The apple juice was extracted using a Binatone juice extractor, it was filtered using a clean muslin cloth and then pasteurized at 90°C for 30 minutes (Kanwar, 2016).

2.4. Fermentation of Apple Juice

The pasteurized apple juice was later inoculated with 1% yeast inoculums supplemented with diammonium hydrogen phosphate (DAHP) (300mg w/v), and fermentation was carried out at 30 ±2°C under static conditions. *S. cerevisiae* SC10, obtained from the Department of Microbiology, Chaudhary Sarwan Kumar Himachal Pradesh Agricultural University, Palampur, India, was used as the control. Samples were taken on a 24-hour basis for physical and chemical analysis and yeast counts (Umeh et al., 2015).

2.5. Fermentation Analysis

The following parameters were analyzed day by day during the course of fermentation: pH (determined with a Jenway 3030 pH meter), titratable acidity, total soluble solids (determined using a hand refraction meter, N-1a ATAGO, Tokyo), reducing sugars, total sugars, and alcohol content were determined according to standard procedures (AOAC, 2006). Ascorbic acid content was measured according to the method described by Weatherholtz and Holsing (1975). Additionally, 25 ml of metaphosphoric acid (5%) solution was mixed with 5 ml of apple wine and filtered using filter paper. Centrifugation was carried out on the filtrate obtained at 2000 rpm for 15 minutes. The filtrate was then transferred into a conical flask and titrated alongside with 2,6-dichlorophenolindophenol (0.025%) reagent. The experiment was carried out in duplicates, and average titer values were determined. The yeast population was evaluated by spreading 0.1 ml of apple wine on PDA at 24-hour intervals, and the plates were incubated at 28°C for 48 hours. The colony forming unit was expressed as logcfu/ml.

2.6. Sensory Evaluation

The sensory quality of the apple wine was evaluated after six days of fermentation by 20 panelists who are familiar with the product. The panelists were supplied with 30 ml apple wine samples and water to rinse their mouths in between samples. The samples were rated on a 9-point hedonic scale ranging from 1 (disliked extremely) to 9 (liked extremely). The parameters were assessed for flavor, taste, appearance, mouth feel, and overall acceptability (Kanwar, 2016).

2.7. Statistical Analysis

All the experiments were carried out in duplicate. The data obtained were analyzed using the one-way analysis of variance (ANOVA) and significance was accepted at the level of $\alpha_{0.05}$. Means were separated using Duncan's multiple range test.

3. RESULTS

The fluctuations in pH during the fermentation of the apple juice are reported in Table 1. There was a gradual reduction in the pH of the various samples from day 0 to day 6 of fermentation, and the pH ranged between 3.49 and 4.05. There was a gradual reduction in total soluble solids from day 0 to day 6 of fermentation, which ranged from 9 mg/ml to 18 mg/ml (see Table 2). The titratable acidity increased steadily throughout the fermentation period. On the first day, the total titratable acidity was 18 mg/ml, but this increased to 40.8 mg/ml in samples fermented with *Saccharomyces cerevisiae* palm wine 02, palm wine 24, burukutu 07 and agadagidi 08 (see Table 3).

On the first day of fermentation, the Brix/acid ratio was 1, but there was a gradual reduction from day 1 to day 6 of fermentation which ranged from 0.221 to 1 (see Table 4). There was a steady increase in the ascorbic acid content of the various samples from day 0 to day 6, which ranged from 4.90 for samples fermented with *Saccharomyces cerevisiae* PAW24 to 74.51 for samples fermented with *Saccharomyces cerevisiae* SC01 (see Table 5).

Table 6 shows the results of the reducing sugars in the apple juice fermented by selected yeast isolates. There was a steady increase in the reducing sugars from day 0 to day 6 of fermentation, which ranged from 0.260 mg/ml in samples fermented with *S. cerevisiae* PAW24 to 0.414 mg/ml for samples fermented with *S. cerevisiae* PAW02.

Table 1. Changes in pH during the fermentation of apple juice by indigenous *Saccharomyces cerevisiae* isolated from fermented alcoholic beverages.

Sample	Incubation periods (days)						
	0	1	2	3	4	5	6
A	4.01±0.02^{a}	3.76±0.05^{a}	3.73±0.05^{a}	3.69±0.05^{a}	3.66±0.05^{a}	3.60±0.05^{a}	3.57±0.05^{a}
B	4.02±0.01^{b}	3.78±0.06^{b}	3.85±0.06^{b}	3.82±0.06^{b}	3.79±0.06^{b}	3.74±0.06^{b}	3.68±0.06^{b}
C	3.99±0.05^{c}	3.77±0.06^{b}	3.86±0.06^{b}	3.81±0.06^{b}	3.75±0.06^{b}	3.68±0.05^{a}	3.66±0.06^{b}
D	4.05±0.03^{d}	3.81±0.07^{c}	3.82±0.05^{a}	3.80±0.06^{b}	3.74±0.06^{b}	3.70±0.06^{b}	3.67±0.06^{b}
E	4.00±0.01^{b}	3.74±0.06^{b}	3.78±0.05^{a}	3.74±0.05^{a}	3.70±0.05^{a}	3.70±0.06^{b}	3.63±0.06^{b}
F	4.02±0.01^{b}	3.80±0.07^{c}	3.62±0.05^{a}	3.61±0.05^{a}	3.60±0.05^{a}	3.59±0.05^{a}	3.49±0.05^{a}

Note: Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$.
Key: Sample A: Sample produced with *S. cerevisiae* PAW02, Sample B: Sample produced with *S. cerevisiae* PAW24, Sample C: Sample produced with *S. cerevisiae* BKT07, Sample D: Sample produced with *S. cerevisiae* BKT19, Sample E: Sample produced with *S. cerevisiae* AGG08, Sample F: Sample produced with *S. cerevisiae* SC01 (control).

The changes in total sugar are presented in Table 7. At day 0, the total sugar ranged between 0.023 and 0.024 mg/g, but by the sixth day of fermentation it had increased to 0.075 mg/g for samples fermented with *Saccharomyces cerevisiae* PAW24 and AGG08.

Table 2. Changes in titratable acidity during the fermentation of apple juice by indigenous *Saccharomyces cerevisiae* isolated from fermented alcoholic beverages.

Sample	Incubation periods (days) / Titratable acidity (mg/ml)						
	0	1	2	3	4	5	6
A	18.0±0.01^{a}	27.6±0.05^{a}	30.0±0.01^{a}	31.2±0.01^{a}	33.6±0.05^{a}	38.4±0.03^{a}	38.4±0.05^{c}
B	18.0±0.01^{a}	19.2±0.04^{b}	19.2±0.02^{b}	21.6±0.05^{b}	31.2±0.02^{b}	38.4±0.03^{a}	40.8±0.06^{b}
C	18.0±0.01^{a}	18.0±0.01^{c}	21.6±0.05^{c}	22.8±0.06^{c}	28.8±0.05^{c}	38.4±0.03^{a}	40.8±0.06^{b}
D	18.0±0.01^{a}	19.2±0.04^{b}	21.6±0.05^{c}	22.8±0.07^{d}	26.4±0.03^{d}	36.0±0.02^{b}	43.2±0.02^{a}
E	18.0±0.01^{a}	19.2±0.04^{b}	21.6±0.05^{c}	24.0±0.02^{ab}	31.2±0.02^{b}	36.0±0.02^{b}	40.8±0.06^{b}
F	18.0±0.01^{a}	19.2±0.04^{b}	21.6±0.05^{c}	26.4±0.04^{bc}	31.2±0.02^{b}	31.2±0.02^{c}	36.0±0.04^{d}

Note: Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$. Sample codes are as stated in Table 1.

Table 3. Changes in the Brix/acid ratio during the fermentation of apple juice by the indigenous *Saccharomyces cerevisiae* isolated from fermented alcoholic beverages.

Sample	Incubation periods (days)/Brix/acid Ratio						
	0	1	2	3	4	5	6
A	1	0.620	0.520	0.420	0.360	0.330	0.255
B	1	0.885	0.729	0.601	0.385	0.286	0.245
C	1	0.889	0.694	0.504	0.382	0.286	0.245
D	1	0.833	0.648	0.526	0.455	0.278	0.260
E	1	0.885	0.625	0.542	0.417	0.306	0.221
F	1	0.729	0.694	0.455	0.433	0.385	0.306

Note: Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$. Sample codes are as stated in Table 1.

Table 4. Changes in ascorbic acid content of apple juice during fermentation by indigenous *Saccharomyces cerevisiae* isolated from fermented alcoholic beverages.

Sample	Incubation periods (days)/ascorbic acid (mg/ml)						
	0	1	2	3	4	5	6
A	5.88±0.06^{a}	7.80±0.05^{a}	21.57±0.05^{a}	39.22±0.06^{a}	41.18±0.05^{a}	58.82±0.06^{a}	62.75±0.06^{a}
B	4.90±0.05^{b}	11.76±0.06^{b}	19.61±0.05^{a}	39.22±0.05^{b}	43.14±0.06^{b}	60.78±0.05^{b}	62.75±0.05^{b}
C	5.88±0.06^{a}	9.80±0.07^{c}	21.57±0.05^{a}	23.53±0.06^{a}	49.02±0.07^{c}	62.75±0.06^{a}	64.71±0.06^{a}
D	6.86±0.06^{a}	11.76±0.06^{b}	23.53±0.06^{b}	25.49±0.06^{a}	43.14±0.06^{b}	58.82±0.06^{a}	66.67±0.06^{a}
E	5.88±0.05^{b}	7.84±0.05^{a}	19.61±0.06^{b}	21.57±0.05^{b}	49.02±0.05^{a}	58.82±0.05^{b}	62.75±0.05^{b}
F	6.27±0.04^{c}	9.80±0.07^{d}	21.57±0.05^{a}	39.22±0.04^{c}	45.10±0.07^{d}	68.63±0.04^{c}	74.51±0.04^{c}

Note: Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$. Sample codes are as stated in Table 1.

Table 5. Changes in total soluble solids during fermentation of apple juice by indigenous *Saccharomyces cerevisiae* isolated from fermented alcoholic beverages.

Sample	Incubation periods (days)/total soluble solids (mg/g)						
	0	1	2	3	4	5	6
A	18.0±0.01^{a}	17.0±0.03^{a}	15.5±0.05^{a}	15.0±0.02^{a}	14.0±0.02^{a}	12.0±0.02^{a}	10.0±0.01^{b}
B	18.0±0.01^{a}	17.0±0.03^{a}	14.0±0.02^{b}	13.0±0.01^{b}	12.0±0.01^{b}	11.0±0.01^{b}	10.0±0.01^{b}
C	18.0±0.01^{a}	16.0±0.01^{b}	15.0±0.03^{c}	11.5±0.05^{c}	11.0±0.01^{b}	11.0±0.01^{b}	10.0±0.01^{b}
D	18.0±0.01^{a}	16.0±0.01^{b}	14.0±0.02^{b}	12.0±0.02^{a}	12.0±0.03^{c}	10.0±0.02^{a}	11.0±0.02^{a}
E	18.0±0.01^{a}	17.0±0.03^{a}	13.5±0.05^{a}	13.0±0.01^{b}	13.0±0.03^{c}	11.0±0.01^{b}	9.0±0.02^{a}
F	18.0±0.01^{a}	16.0±0.01^{b}	15.0±0.03^{c}	14.0±0.02^{a}	13.5±0.05^{d}	12.0±0.02^{a}	11.0±0.03^{c}

Note: Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$. Sample codes are as stated in Table 1.

Table 6. Changes in reducing sugars during the fermentation of apple juice by indigenous *Saccharomyces cerevisiae* isolated from fermented alcoholic beverages.

Sample	Incubation periods (days)/reducing sugars (mg/ml)						
	0	1	2	3	4	5	6
A	0.261±0.05^{a}	0.275±0.06^{a}	0.329±0.05^{a}	0.336±0.05^{a}	0.338±0.05^{a}	0.404±0.04^{a}	0.414±0.0
B	0.260±0.05^{a}	0.276±0.05^{b}	0.338±0.06^{b}	0.336±0.06^{b}	0.341±0.04^{b}	0.342±0.04^{a}	0.345±0.0
C	0.261±0.06^{b}	0.277±0.05^{b}	0.329±0.05^{a}	0.332±0.04^{c}	0.335±0.04^{b}	0.340±0.03^{b}	0.347±0.0
D	0.262±0.06^{b}	0.276±0.04^{c}	0.328±0.04^{c}	0.350±0.05^{a}	0.357±0.05^{a}	0.360±0.05^{c}	0.365±0.0
E	0.260±0.04^{c}	0.276±0.04^{c}	0.329±0.05^{a}	0.338±0.06^{b}	0.346±0.04^{d}	0.363±0.05^{c}	0.390±0.0
F	0.263±0.03^{d}	0.278±0.06^{a}	0.330±0.04^{c}	0.340±0.04^{c}	0.361±0.06^{c}	0.366±0.06^{b}	0.374±0.0

Note: Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$. Sample codes are as stated in Table 1.

The changes in alcoholic content are presented in Table 8, which shows that there was an increase in the alcoholic content of the fermented samples. The lowest alcoholic content detected after six days of fermentation was 9.0% for the samples fermented with *S. cerevisiae* BKT07 and AGG08, while the highest was recorded for the sample fermented with *S. cerevisiae* PAW02 (10.2%). The changes in yeast population are presented in Table 9. The lowest yeast count was recorded for the sample fermented with *S. cerevisiae* PAW02 (7.40 logcfu/ml), while the highest was recorded for the sample fermented with *S. cerevisiae* BKT07 (12.65 logcfu/ml) on the sixth day of fermentation.

The samples of apple wine were subjected to sensory analysis by 20 panelists. The samples were analyzed for appearance, flavor, mouth feel, taste, and overall acceptability (see Table 10). Samples B and C fermented with *S. cerevisiae* PAW24 and BKT07 were rated the best in terms of appearance with a value of 7.71, while sample E fermented with *S. cerevisiae* AGG08 was rated lowest with a value of 7.14. Sample B fermented with *S. cerevisiae* PAW24 was rated the best in terms of flavor with a value of 8.00, while sample F fermented with *S. cerevisiae* SC01 was rated lowest with a value of 6.71. Sample A fermented with *S. cerevisiae* PAW02 was rated the best in terms of mouth feel with a value of 8.00, while sample E fermented with *S. cerevisiae* AGG08 was rated lowest with a value of 7.00. Sample B fermented with *S. cerevisiae* PAW24 was rated the best in terms of taste with a value of 8.14, while samples E and F fermented with *S. cerevisiae* AGG08 and SC01 were rated lowest with a value of 7.00.

Table 7. Changes in total sugars during the fermentation of apple juice by indigenous *Saccharomyces cerevisiae* isolated from fermented alcoholic beverages.

S/N	Sample	Incubation periods (days)/total sugars (mg/ml)						
		0	1	2	3	4	5	6
1	A	0.024 ± 0.005^{a}	0.058 ± 0.001^{c}	0.059 ± 0.002^{b}	0.060 ± 0.001^{c}	0.065 ± 0.000^{d}	0.068 ± 0.003^{a}	0.070 ± 0.003^{c}
2	B	0.023 ± 0.001^{b}	0.058 ± 0.001^{c}	0.061 ± 0.001^{c}	0.065 ± 0.000^{a}	0.069 ± 0.003^{a}	0.070 ± 0.002^{b}	0.075 ± 0.002^{b}
3	C	0.023 ± 0.002^{c}	0.057 ± 0.002^{d}	0.060 ± 0.003^{d}	0.062 ± 0.002^{b}	0.065 ± 0.003^{a}	0.068 ± 0.003^{a}	0.070 ± 0.001^{a}
4	D	0.023 ± 0.001^{a}	0.057 ± 0.003^{e}	0.060 ± 0.004^{ab}	0.065 ± 0.002^{b}	0.068 ± 0.001^{b}	0.070 ± 0.003^{a}	0.072 ± 0.003^{c}
5	E	0.023 ± 0.002^{b}	0.057 ± 0.000^{a}	0.062 ± 0.003^{d}	0.065 ± 0.003^{d}	0.068 ± 0.002^{c}	0.072 ± 0.002^{b}	0.075 ± 0.002^{b}
6	F	0.023 ± 0.001^{a}	0.057 ± 0.003^{e}	0.061 ± 0.002^{b}	0.064 ± 0.001^{c}	0.067 ± 0.001^{c}	0.069 ± 0.003^{a}	0.072 ± 0.003^{c}

Note: Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$. Sample codes are as stated in Table 1.

Table 8. Changes in alcoholic content during the fermentation of apple juice by the indigenous *Saccharomyces cerevisiae* isolated from fermented alcoholic beverages.

Sample	Alcoholic content (%) at different incubation periods (days)						
	0	1	2	3	4	5	6
A	0.0	2.4 ± 0.02^{a}	8.0 ± 0.02^{b}	8.8 ± 0.05^{b}	8.9 ± 0.06^{a}	9.8 ± 0.07^{b}	10.2 ± 0.02^{a}
B	0.0	3.8 ± 0.05^{b}	8.4 ± 0.03^{c}	8.4 ± 0.03^{c}	8.5 ± 0.05^{b}	8.8 ± 0.07^{b}	10.0 ± 0.00^{b}
C	0.0	2.6 ± 0.05^{b}	7.0 ± 0.02^{b}	8.4 ± 0.03^{c}	8.5 ± 0.05^{b}	8.9 ± 0.06^{c}	9.0 ± 0.03^{c}
D	0.0	5.2 ± 0.02^{a}	8.0 ± 0.01^{a}	8.2 ± 0.02^{d}	8.5 ± 0.05^{b}	8.7 ± 0.05^{d}	9.2 ± 0.01^{d}
E	0.0	5.0 ± 0.01^{c}	6.8 ± 0.05^{d}	7.9 ± 0.06^{ab}	8.4 ± 0.03^{c}	8.6 ± 0.05^{d}	9.0 ± 0.01^{d}
F	0.0	3.8 ± 0.05^{b}	8.0 ± 0.01^{a}	8.3 ± 0.03^{c}	8.4 ± 0.03^{c}	8.6 ± 0.06^{c}	9.4 ± 0.02^{b}

Note: Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$. Sample codes are as stated in Table 1.

Table 9. Changes in yeast population during the fermentation of apple juice by indigenous *Saccharomyces cerevisiae*.

Sample	Yeast population (log_{10}cfu/ml) at different incubation periods (days)						
	0	1	2	3	4	5	6
A	7.40 ± 0.02^{a}	8.54 ± 0.06^{b}	10.29 ± 0.02^{a}	10.29 ± 0.01^{a}	10.63 ± 0.05^{a}	11.35 ± 0.03^{a}	11.20 ± 0.01^{a}
B	7.74 ± 0.05^{b}	8.01 ± 0.02^{a}	9.93 ± 0.07^{b}	9.96 ± 0.06^{b}	10.68 ± 0.06^{b}	11.15 ± 0.02^{b}	11.22 ± 0.02^{b}
C	7.78 ± 0.06^{c}	9.17 ± 0.03^{b}	9.69 ± 0.06^{c}	10.08 ± 0.03^{c}	10.66 ± 0.06^{b}	11.84 ± 0.06^{c}	12.65 ± 0.05^{c}
D	7.76 ± 0.05^{d}	8.70 ± 0.07^{c}	10.39 ± 0.03^{d}	10.55 ± 0.05^{bc}	11.37 ± 0.04^{c}	12.69 ± 0.05^{d}	12.61 ± 0.05^{c}
E	7.72 ± 0.05^{b}	8.51 ± 0.06^{b}	9.95 ± 0.06^{c}	10.85 ± 0.06^{bc}	11.70 ± 0.05^{d}	11.83 ± 0.06^{c}	11.88 ± 0.06^{d}
F	7.72 ± 0.06^{c}	10.16 ± 0.03^{b}	10.30 ± 0.02^{a}	10.36 ± 0.03^{d}	10.74 ± 0.05^{d}	11.74 ± 0.06^{c}	11.99 ± 0.07^{e}

Note: Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$. Sample codes are as stated in Table 1.

Table 10. Sensory evaluation of apple wine produced by indigenous *Saccharomyces cerevisiae*.

Sample codes	Sensory Parameters				
	Appearance	Flavor	Mouth feel	Taste	Overall acceptability
A	7.43^{b}	7.86^{b}	8.00^{b}	8.00^{b}	7.82^{b}
B	7.71^{a}	8.00^{a}	7.86^{a}	8.14^{a}	7.43^{b}
C	7.71^{c}	7.14^{c}	7.29^{a}	7.43^{b}	7.19^{a}
D	7.43^{b}	7.00^{c}	7.43^{a}	7.43^{b}	7.12^{a}
E	7.14^{d}	7.00^{c}	7.00^{b}	7.00^{c}	7.04^{c}
F	7.43^{b}	6.71^{b}	7.14^{b}	7.00^{c}	7.07^{c}

Note: Mean values of twenty replicates with different superscript down the column are significantly different at $\alpha_{0.05}$. Sample codes are as stated in Table 1.

4. DISCUSSION

The pH of the various samples fermented with different isolates was measured using a standardized pH meter. There was a general decrease in pH values of the fermenting cider up to the sixth day of fermentation. The pH of the samples was in the acidic range throughout the fermentation period. Chilaka, Uchechukwu, Obidiegwu, and Akpor (2010) reported that during the fermentation of fruit wines, the changes in pH did not follow any particular trend but was within the acidic range. The pH obtained for passion fruit wine ranged between 3.1 and 4.6; that of watermelon ranged between 3.4 and 4.8, and that of pineapple fruit wines ranged between 3.0 and 4.7.

A general increase in titratable acidity (TA) was recorded from the first to the sixth day of fermentation. TA is a measure of changes in the amount of the different acids (citric, malic, lactic or acetic) that may be present in the wine samples. Moreover, TA has a direct influence on the aroma and flavor of fermented products, and the amount present could be an indicator of shelf life (Berenguer et al., 2016). The TA reported for wine produced from pomegranates using three different strains of *S. cerevisiae* ranged between 5.66 ± 0.14 to 5.99 ± 0.06 g citric acid/L.

There was a gradual reduction in the Brix/acid ratio during the fermentation of apple juice for apple wine production as the fermentation period increases. Sukhvir and Kocher (2019) stated the overall range in respect of °Brix (6.2–9.6) and the Brix/acid ratio (16.31–24.61) for fresh and stored (4°C) Golden Delicious apple juice.

The ascorbic acid content of the various fermented apple wines was determined and ranged between 4.90 and 66.67 mg/g. Ascorbic acid has many health benefits, such as aiding the absorption of iron and helping to maintain capillaries, bones and teeth. It also has a strong antioxidant capacity (Erdurak-Kiliç et al., 2006). Cendrowski, Królak, and Kalisz (2021) reported that the final concentrations of ascorbic acid ranged from 61 to 155 mg/100ml for different variants of wines from rose fruits. Nwaichi, Chuku, and Oyibo (2015) found the ascorbic acid contents of some fruits, honey and red wine to range from < 0.0001 to 216.05 mg/100g, with guava having the highest value of 2166.05 ± 99.64, and red wine with the lowest value of < 0.43 ± 0.10 mg/100g.

As fermentation progresses from the first day to the last day, there was a broad decrease in the values of the total sugar content of apple wine. The values obtained ranged between 0.024 and 0.075 mg/g. A general decrease in total sugar (°Bx) was observed in apple wine (AW), apple-pine wine (APW) and apple-herb wine (AHW). The values reported are 8.0–24.0 for AW, 8.6–24.0 for APW, and 8.8–24.0 for AHW (Lee, Ong, Yu, Curran, & Liu, 2010).

A gradual increase in reducing sugars was recorded from the first day to the sixth day of fermentation. The lowest reducing sugar value (0.260 mg/g) was recorded for *Saccharomyces cerevisiae* PAW24 and *Saccharomyces cerevisiae* AGG08, while the highest value (0.414 mg/g) was recorded for *Saccharomyces cerevisiae* PAW02. Mugula, Narvhus, and Sørhaug (2003) reported that there was an increase in the sugars from 1.60 to 21.0 mg/g and reached a concentration of 22.0 mg/g in cereal gruel after 30 minutes. The observed difference in reducing sugar could be as a result of the difference in substrate and starter culture used for the fermentation. Moreover, Sharma, Singh, and Sawant (2012) reported a reducing sugar range between 1.48 and 16.42 g/l for white wines, and 2.83 and 5.86 g/l for red wines.

After 24 hours of fermentation, the lowest alcoholic content was recorded for sample PAW02 (2.4%), but by the sixth day of fermentation, the highest alcoholic content recorded was 10.2% for sample PAW24. Ogodo, Ugbogu, Ugbogu, and Ezeonu (2015) reported no significant difference in alcohol contents of fruit wines produced from mixed fruit juices. They reported the alcoholic content of the final wines to be 17.50 ± 0.02% (pawpaw and watermelon), 16.00 ± 0.02% (pawpaw and banana), 18.50 ± 0.02% (banana and watermelon wine) and 18.00 ± 0.02% (pawpaw, banana and watermelon).

The change in yeast population during the fermentation of apple cider wine was also recorded. The lowest yeast population recorded was 11.20 $\log_{10}$cfu/ml for the sample fermented with *S. cerevisiae* PAW02, while the highest yeast population was 12.6 logcfu/ml after six days of fermentation. Dierings, Braga, Silva, Wosiacki, and Nogueira (2013) reported the initial population of fermentative yeast in the apple wine made with commercial apples was 3.63 $\log_{10}$cfu/ml. When the wine was made with unclassified fruits, the yeast population was higher, reaching 3.96 $\log_{10}$cfu/ml. Nogueira, Mongruel, Simões, Waszczynskyj, and Wosiacki (2007) found higher values of 4.48 $\log_{10}$cfu/ml and 6.50 $\log_{10}$cfu/ml in commercial and unclassified apples, respectively.

The apple wines produced were tasted by 20 panelists who rated the alcoholic beverages on a 9-point Hedonic scale. The apple wine (sample A) fermented with *S. cerevisiae* PAW02 received the highest scores for flavor, mouth feel, taste, and overall acceptability. The apple wine (sample F) pitched with *S. cerevisiae* SC01 (control) received the lowest scores for appearance, flavor, taste and overall acceptability. A study by Cioch-Skoneczny, Satora, Skoneczny, and Klimczak (2021) revealed that wine samples fermented with *S. cerevisiae* MH020215 received the highest scores for organoleptic properties, while much lower values were reported for wine pitched with *Nakawazaea ishiwadae.*

5. CONCLUSION

It can be deduced from this study that the *S. cerevisiae* PAW02 strain produced wine with the most acceptable sensory properties, such as flavor (7.8), mouth feel (8.00), taste (8.00), and overall acceptability (7.82). This sample also had the highest alcohol content (10.2%) and therefore this strain could be explored for semi-industrial/cottage production of alcoholic beverages (wine) from Golden Delicious apples. However, further studies are needed to determine suitable methods of preserving *S. cerevisiae* PAW02 for easy distribution to small-scale producers, and analysis of volatile compounds present in the samples of wine produced should also be carried out.

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