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PRODUCTION OF WINE FROM PINEAPPLE AND WATERMELON JUICE USING *KLOECKERA APICULATA* ISOLATED FROM SPOILT WATERMELON AND PINEAPPLE FRUIT

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ABSTRACT

This study aimed to explore the potential of *Kloeckera apiculata* isolated from spoilt watermelon and pineapple fruit as a starter culture for producing wine from pineapple and watermelon juice. The research involved isolating, identifying, and characterizing *Kloeckera apiculata* from spoilt watermelon fruit using cultural, morphological, and biochemical identification methods, and the result was confirmed using an API test kit (API 20 C identification kit). Yeast population, pH, Total soluble solid, Total titratable acidity, and Alcohol content, were determined throughout the experiment. The wine was produced by submerged fermentation using pineapple and watermelon juice as substrates. The results showed that *K. apiculata* could effectively ferment both pineapple and watermelon juice as the watermelon wine fermentation started at a specific gravity of 1.07g/cm^3 and ended at 0.01g/cm^3 while pineapple started at 1.10g/cm^3 and ended at 1.03g/cm^3, the total soluble solid of watermelon wine was started at 17% and ended at 3.8% while that of pineapple wine started at 23.6% and ended at 8.2%, thus showing the gradual utilization of sugar by *Kloeckera apiculata* in both wines produced. The total titratable acidity and alcohol content increased daily as the sugar was been utilized while temperature and pH decreased gradually. The sensory evaluation of the wines indicated that they possessed unique flavor and aroma characteristics, with a preference for the pineapple wine. This study demonstrated the potential of *K.apiculata* as a starter culture for producing wine from underutilized fruits, which could contribute to the diversification of the wine industry.

Keywords: Production, Wine, Pineapple, Watermelon Juice, *Kloeckera apiculata*, fermentation, non-pathogenic

Introduction

Making wine is a common and historic practice that has long been present in human civilization (McGovern, 2007). It's not novel to utilize different fruits to make wine, and each one imparts a distinct flavor and personality to the finished product (Benucci *et al*., 2021). Tropical fruits like pineapple and watermelon have grown in popularity recently due to their distinct flavor and aroma. These fruits' juice can be used to make wine, however, there isn't much information on using particular yeast strains, like *Kloeckera apiculata*, to make wine from these fruits. In developing countries, 20-30% of fruits produced are wasted due to a lack of proper utilization, post-harvest and processing technology. Converting these fruits into value-added products like wine is a smart solution to this problem (Nwachukwu *et al*., 2006). There is an increased demand for wine production especially fruit wine because these fruits are available at certain seasons of the year and are highly perishable. Thus, wine production should be promoted for adding value to local fruits, imported wine reduction, job creation, income generation, and rural development. *Kloeckera apiculata* is a non-Saccharomyces yeast strain commonly found on damaged fruits and in the environment (Deak and Beuchat, 1996). Due to its capacity to ferment sugars into ethanol and generate diverse taste compounds, it has been extensively used in a variety of food and beverage sectors (Du Plessis and Von-Holy, 2010). *Kloeckera apiculata* is a good choice for making wine using pineapple and watermelon juice because it is known to generate beverages with fruity and floral flavors. *Kloeckera apiculata* has been used in different research to produce wine using various fruit juices(Valero *et al*., 2007; Sabate *et al*., 2002). The utilization of this yeast strain in wine production utilizing pineapple and watermelon juice is, however, the subject of few investigations. The purpose of this study is to ascertain whether *Kloeckera apiculata* can be used to make wine from pineapple and watermelon juice. The study's primary areas of interest include the fermentation process, sensory assessment, and chemical examination of the finished wine. The results of this study will advance our understanding of non-Saccharomyces yeast strains and how to use them to make wine.

Materials and Methods

The production of wine from pineapple and watermelon juice using *Kloeckera apiculata* isolated from spoilt watermelon and pineapple fruit involves several steps. This includes

Collection of Samples

Spoilt watermelon (*Citrulus vulgaris*) and pineapple (*Ananas comosus*) fruits were collected from Samaru market in the Zaria metropolis in Kaduna State, Nigeria. They were washed with potassium metabisulphite solution before isolating the yeasts used in fermentation.

Isolation of Yeast Strains

The fleshy part of the spoilt fruit sample was smashed and mixed in sterilized nine milliliters peptone water then a serial dilution was made up to 10^{-5}, and 0.1ml of the last dilution was cultured by spread plate method on malt yeast extract agar. The inoculated plates were incubated for 48h at 30ºC (Panneerselvam and Maragatham, 2011). Purified cultures were maintained on malt yeast extract agar slants and kept at

-4ºC for subsequent identification. The pure culture of the yeast isolates obtained was characterized using cultural, morphological, and biochemical tests and confirmed using an API test kit (API 20 C identification kit).

Purification of Cells Used for Fermentation of Fruit Juice for Wine Production.

The cells obtained were purified by inoculation of a single colony of the selected yeast strain into ten milliliters of yeast peptone dextrose (YPD) broth (1% yeast extract, 2% peptone, and 2% dextrose) and incubated overnight at 30°C. After this, 1 ml of the yeast culture was introduced into 200 ml YPD medium. The yeast cells were allowed to grow at a temperature of 30°C for three to four hours on a shaker. The yeast cell growth in the medium was determined using the hemocytometer then the cells were centrifuged at 5000 rpm for 5 min with a g-force of 2200. The pellets formed in the centrifuge tubes were re-suspended in 30 ml of distilled water. The starter was then inoculated at 3.0×10^8 cells per ml (Byaruagaba-Bazirake *et al*., 2013).

Cultural Conditions for Wine Production

One sound, healthy, and ripe pineapple and watermelon fruits were selected, washed, and aseptically peeled off using a knife. The juice was obtained by blending the sliced fruits in a sterilized blender and straining them to remove the pulp. The juice extracted was pasteurized at 85–90ºC for 5min (Panneerselvam and Maragatham, 2011). After cooling the juice, the pH of the fruit juice was adjusted to 4.5 by the addition of calcium carbonate (($CaCO_3$) food grade) and malic acid food grade. The required amount of sugar was added to adjust the final total soluble solids to 24ºBrix. Diammonium phosphate (DAP) was also added to the juice at a rate of 0.05% w/v before fermentation to supplement the nitrogen required in alcoholic fermentation (Andri *et al.*, 2012). Also, thiamine which is a very important source of nutrients for the growth of *Kloeckera specie* was added to the juice before fermentation. In the fermentation medium, bacteria activity was suppressed by pasteurizing the fruit juice before fermentation. Then the inoculum containing 3.0×10^8 cells/ml was then added to the cooled fruit juice (3.5 liters each) and left to ferment. After 6 days of fermentation, it was filtered through sterile muslin cloth and the filtrate was kept for secondary fermentation which lasted for 8 days making a total of 14 days of fermentation in transparent rubber containers with air-tight covers/seal which was used in place of a fermentor. Yeast population, pH, Total soluble solid, Total titratable acidity, and Alcohol content, were determined throughout the experiment. Also, the physicochemical analysis of the wine was determined by obtaining the Total Soluble Solids (TSS), Total Titratable Acidity (TTA), Ambient temperatures of wine (ºC), Alcoholic content, and pH of wine was ascertained.

Results

During the 14 days of fermentation, the following results were obtained

Table 1: Properties of Pineapple Wine from Pineapple Juice Produced Using *Kloeckera apiculata*

Time of fermentation (days)	Specific gravity (g/cm^3)	Total soluble solids (%)	Total titrable acidiDty (%)	Potential Alcohol content (%)	Alcohol content (%)	Temp.(°c)	pH
1	1.10	23.6	0.01	13.6	0	30	4.1
2	1.085	20.3	0.06	11.5	1.3	28	4.1
3	1.08	19.2	0.07	10.9	1.5	17	4.1
4	1.08	19.2	0.08	10.9	2.6	18	4.1
5	1.08	19.2	0.18	10.9	3.9	19	4
6	1.08	19.2	0.21	10.9	5.2	19	4
7	1.075	18.1	0.20	10.2	6.2	21	4
8	1.075	18.1	0.175	10.2	7.2	22	4
9	1.07	17.0	0.174	9.5	9.9	25	4
10	1.07	17.0	0.204	9.5	12.6	19	4
11	1.03	8.2	0.28	4.1	12.9	18	4
12	1.03	8.2	0.23	4.1	12.9	18	3.9
13	1.03	8.2	0.25	4.1	12.9	18	3.9

Table 2: Properties of Watermelon Wine from Watermelon Juice Produced Using *Kloeckera apiculata.*

Time of fermentation (Days)	Specific gravity (g/cm^3)	Total soluble solids (%)	Total titratable acidity (%)	Potential Alcohol content (%)	Actual Alcohol content (%)	Temp. (°C)	pH
1	1.07	17.0	0.01	9.5	0	28	3.8
2	1.04	10.4	0.05	5.4	4.1	27	3.7
3	1.035	9.3	0.09	4.8	5.4	24	3.7
4	1.03	8.2	0.095	4.1	5.4	25	3.7
5	1.03	8.2	0.103	4.1	5.4	25	3.7
6	1.03	8.2	0.12	4.1	5.4	25	3.6
7	1.03	8.2	0.15	4.1	5.4	25	3.6
8	1.03	8.2	0.17	4.1	5.4	24	3.6
9	1.03	8.2	0.2	4.1	5.4	24	3.6
10	1.03	8.2	0.26	4.1	5.4	23	3.6

11	1.02	6	0.3	5.4	6	20	3.6
12	1.02	6	0.37	2.7	6.8	20	3.5
13	1.01	3.8	0.5	1.4	7.5	17	3.5

Table 3: Sensory Analysis of Wines Produced from Pineapple and Watermelon Juices.

Parameter	**Standard pineapple wine**					**Pineapple wine produced**					**Watermelon wine produced.**				
	A	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
Color	-	-	-	2	8	-	-	1	3	6	-	1	2	3	4
Taste	-	-	3	2	5	-	-	1	2	7	-	1	-	3	6
Aroma	-	-	1	7	2	-	-	-	3	7	-	-	3	2	5
Overall acceptability	-	-	-	4	6	-	-	-	2	8	-	-	1	2	7

Key: **A**; dislike extremely, **B**; dislike very much, **C**; no preference, **D**; like slightly, **E**; like very much.

Discussion

Fruit wine production using Klockera apiculate is a relatively new and promising area of research. *Klockera apiculata* is a yeast species that is naturally found in a wide range of fruits and has been shown to be an efficient fermenter in fruit wine production (Lee *et al*., 2018). During the production of this pineapple and watermelon wine, the fermentation was carried out for 14 days during which the sugars available were used up. The fermentation in pineapple wine was started with a sugar level of 23.6° Brix at a specific gravity of 1.10g/cm^3 so that the complete utilization of the sugar will give an alcohol level of 13.6% alcohol in pineapple wine and the watermelon wine was started with a sugar level of 17°Brix at a specific gravity of 1.07g/cm^3 so that complete utilization of the sugar will give alcohol level of 9.5%. During the fermentation, (Fig 1) it was observed that as the days of fermentation increased the specific gravity decreased gradually in both wines (Table 1 and 2) and remained constant for a long period of time before decreasing further. This is because *Kloeckera apiculata* requires a long time for the uptake and release of specific amino acids during fermentation (Gump *et al*., 2010). The specific gravity decreased daily as the sugars were been utilized. The lowest specific gravity was observed on the last day in the pineapple fermentation and also in the watermelon fermentation and this was used as a marker to know the level of residual sugar remaining in the wine and the alcohol level of the wine. Specific gravity is a measurement of the density of a liquid compared to the density of water. In fruit wine production, specific gravity is an important indicator of the progress of fermentation. As the sugars in the fruit juice are converted to alcohol by the yeast, the specific gravity of the wine decreases. This is because alcohol has a lower density than sugar, resulting in a less dense liquid. The result obtained in this research was due to microbial metabolism of available sugar in the wine. This result agrees with the work of Uraih (2003), Awe (2011), and Huseyin *et al.* (2006) who observed a decrease in specific gravity as the days increased in different fruit wine production. The specific gravity values of both pineapple and watermelon wines were observed to differ, statistically, these differences were not observed to be significant across the days (p≤0.05). However, the decrease was almost equivalent in pineapple and watermelon wine which indicates the presence of an almost equal succession of microorganisms in both wines produced.

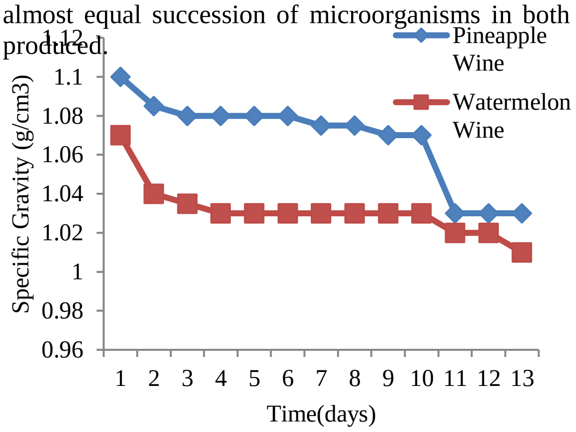

Fig 1: Specific gravity pattern during fermentation of watermelon and pineapple wine.

During the course of this research it was also observed that as the days increased, the total soluble solid decreased in both pineapple and watermelon wine (Fig 2). The total soluble solids of the pineapple wine was measured at 23.6°Brix to give a calculated alcohol level of 13.6% but the final alcohol level obtained was 12.9% while in watermelon wine the total soluble solid was measured at 17°Brix to give a calculated alcohol level of 9.5% but the actual alcohol level obtained at the end of the fermentation was 7.5% which means that not all the sugars were completely utilized in both wines.

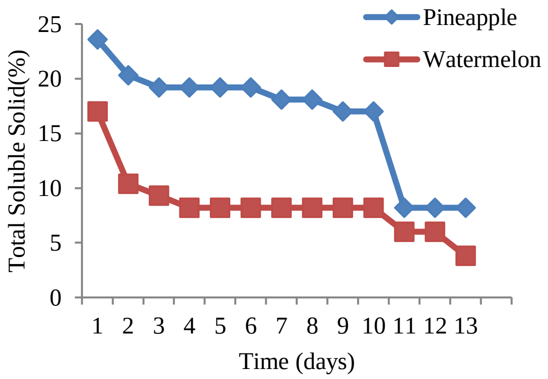

Fig 2: Pattern of Total soluble solid during fermentation of pineapple and watermelon wine

This was attributed to the fact that 90-95% of the total soluble solid is sugar and not all is converted to ethanol. Margalit (1997) stated that about 5% of the sugar is consumed to produce by-products (glycerol, succinic acid, lactic acid, 2,3-butanediol, and acetic acid), about 2.5% is consumed by the yeast as carbon source and about 0.5% are left over as unfermented residual sugar. In the pineapple wine produced, the total soluble solid decreased consistently which indicates active utilization of the sugars by the microorganisms and production of alcohol. Although, non-*Saccharomyces* yeasts are reported to lack the ability to complete fermentation due to their low ethanol tolerance but are effective in improving wine quality(Toro and Vazquez, 2002; Clemente-Jimenez *et al*., 2004). In watermelon wine, it was observed that in the first three days, the total soluble solid decreased because the population of yeast increased after which the total soluble solid remained constant till the tenth day of fermentation and further decreased. This agrees with the work of Chanprasartsuk *et al.*(2012) who used *Hanseniaspora uvarum* as a single culture and mixed culture and noticed that after the second day of fermentation, the total soluble solid remained stable throughout the fermentation period but yet there was a continuous increase in alcohol content. Also, this may be due to the slow utilization of the sugars by the yeast. The rate of decrease of total soluble solids was consistent in both wines which agrees with the fact that they contain an almost equal number of microorganisms.

They Total titratable acidity increased consistently as the days increased throughout the period of fermentation for both watermelon and pineapple wine due to the microbial succession that was evident with the concomitant production of intermediate acid from alcohol initially produced during yeast metabolism. It was further observed that as the titratable acid concentration increased daily, the pH of the wine decreased. This is attributed to yeast metabolism and also shows the fermentation during the fermentation stages which is crucial to wine production. Acidity plays a vital role in determining the wine quality by aiding the fermentation process and enhancing the overall characteristics and balance of the wine. A lack of acidity will mean poor fermentation. This agrees with the work of Awe *et al*. (2011) who stated that the total titratable acidity increased as the days increased. In this work, the total titratable acid concentration was observed to increase from initial concentration of 0.01% to a final concentration range of 0.5% for watermelon wine and 0.01% to 0.25% for pineapple wine. The lowest titratable acidity was observed in pineapple wine which means that the pineapple wine tastes more acidic than the watermelon wine because the titratable acidity relates more to the 'acid taste' of wine while the pH relates more to things like microbial stability and susceptibility to mold and bacterial spoilage. The titratable acidity is always less than the total acidity because not all of the hydrogen ions expected from the acids are found during the determination of titratable acidity. Despite the observed differences in titratable acidity concentration of the fruit wines, these differences statistically were not observed to be significant across the days ($p \leq 0.05$). This work is in agreement with that of Chikala *et al.* (2010) who stated that there was no significant difference between the values he obtained in presence of the tested yeast strains.

pH which is the logarithm of the concentration of free protons in the wine produced, and is usually expressed with a positive sign. The pH obtained for the final product which ranges between 4.1-3.5 for both watermelon and pineapple wines falls within the acidity level of sweet and dry wines. Usually, the acidity of wines lies between pH 3 and 7; higher acidity is sometimes encountered with fortified and sparkling wines. The decrease in pH value could be due to microbial succession from yeast to lactic acid bacteria resulting in the production of more acid as is evident in malolactic acid fermentation. In order to supplement the sugar content of the must, glucose was part of the additives. Reports have shown that the major problem associated with the use of tropical fruits in wine production is their low sugar content (Alobo *et al*., 2009) .

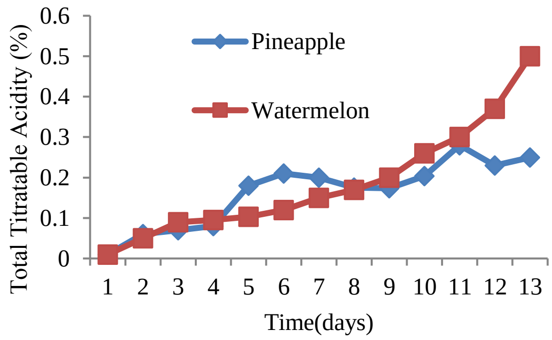

Fig 3: Pattern of Total titratable acidity during fermentation of pineapple and watermelon wine.

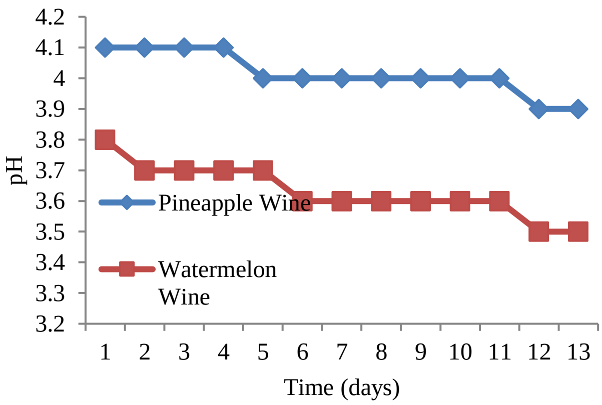

Fig 4: pH of pineapple and watermelon wine during fermentation

Remarkable amount of alcohol was produced from the fruit juice during fermentation with the test yeast strains. The alcohol content of both wines increased gradually as the days increased (Fig 5) indicating that the sugars were been utilized by the yeast, confirming the conversion of sugars into alcohol. As much as 80% of alcohol was produced during the aerobic fermentation stage which is consistent with previous submission that about 70% of fermentation activities occur during the aerobic fermentation phase. The final alcohol content of the pineapple wine was 12.9% while that of watermelon was7.5% which ranks among good table wine. As shown in Fig 5 above. Steady increase in alcohol content was observed in the fruit wines throughout the period of fermentation.

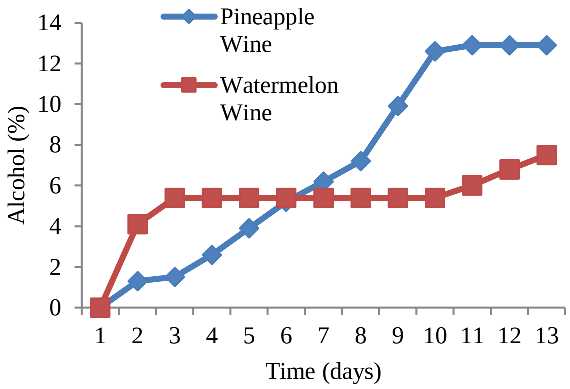

Fig 5: Alcohol level of pineapple and watermelon wine during fermentation

The highest alcohol level was observed in pineapple wine and lowest alcohol level was observed in watermelon wine this is attributed to the fact that pineapple wine contained higher amount of sugar compared to the watermelon wine. Although the values of alcohol content in the fruit wines were observed to be different during the different fermentation days, these differences were not observed to be significant statistically ($p \leq 0.05$). This trend is irrespective of the fruit wine. The variation in alcohol levels is usually due to difference in their optimal physico-chemical conditions such as temperature and pH. Alcoholic fermentation leads to a series of by products in addition to ethanol. The yeast produced glucanolytic and xylanolytic enzymatic activities which influenced alcoholic fermentation.

The temperature during the wine production did not follow any specific trend in both watermelon and pineapple wine (Fig 6) It has been reported that temperature affects the gene expression in yeast (Staci, 2003) The optimum growth temperature for *Kloeckera apiculata* is 17-25°C. However, the fermentation was carried out at 17-30°C thus favoring the growth of *Kloeckera apiculata* in total alcohol production. Temperature affects the sensitivity of the yeast to alcohol concentration, the growth rate of the microorganism, the rate of fermentation, viability, and the length of the lag phase. Temperature changes could be due to microbial metabolism of available nutrients to produce alcohol and other fermentation products with the resultant generation of heat. This is why the temperature was started at room temperature and then decreased using ice as the alcohol content of the wine increased since *Kloeckera apiculata* cannot survive in alcohol at high temperatures. This result agrees with the reports of Robinson *et al.* (2006) and Okafor *et al.* (2007).

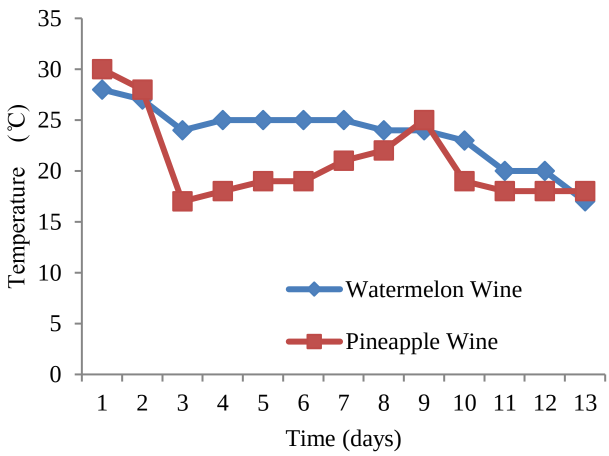

Fig 6: Temperature of watermelon and pineapple wine during fermentation

The result of the sensory analysis of the wine which is very important in determining the quality of the wine produced revolved around the taste, aroma, and color of the wine. From the result above in Table 3. It is observed that the color of the standard pineapple wine was mostly preferred followed by that of the pineapple wine produced. This is due to the fact that more sophisticated equipment was used in clearing the standard pineapple wine in the winery. This is because cell wall polysaccharides, principally mannoproteins which were released by yeast during autolysis combined with tannin and anthocyanine to impact the wine astringency and color. In the comparison of the taste and aroma of the wines, the pineapple and watermelon wines produced were preferred to the taste of the standard pineapple wine to which the wine produced was compared. This is because glycerol is a wine constituent that contributes to the sweetness, viscosity, and smoothness of the wine produced and it is produced only by *Kloeckera apiculata*. Secondary metabolites produced by *Kloeckera apiculata* augment the sensory qualities of the wine produced. *Kloeckera apiculata* have interesting enzymes such as beta-D-glucosidase and beta-D-xylosidase which are key enzymes to release aromatic compounds in winemaking. The reduction of acid in wine by bacteria is known as malolactic fermentation. It improves wine quality where employed. Malolactic fermentation impacts flavor and aroma of the wine and this was performed by the *Lactobacillus* and *Pediococcus* species present in the wine. Malolactic bacteria decreased acidity in the wines by transforming L-malic acid to L-lactic acid, enhanced wine flavor, and .complexity through the production of additional metabolites and increased stability of the wine by removal of residual nutrients and production of bacteriocin that prevent the growth of other bacteria in the wine. This is in agreement with the work done by Romano *et al*. (1997) who stated that wine makers and wine researchers have come to realize that also non-*Saccharomyces* yeasts contribute in significant measure to the flavor and quality of wine than previously thought.

Conclusion

This study demonstrates that *Kloeckera apiculata* isolated from spoilt watermelon and pineapple fruit has the potential to be used as a starter culture for producing wine from pineapple and watermelon juice. The yeast strain was identified using various cultural, morphological, and biochemical identification methods, and confirmed using an API 20 C identification kit. The results showed that *K. apiculata* was able to effectively ferment both pineapple and watermelon juice, with the sugar gradually being utilized over time, resulting in an increase in alcohol content and total titratable acidity. The sensory evaluation of the wines indicated that both pineapple and watermelon wines had unique flavor and aroma characteristics, with a preference for the pineapple wine. This study highlights the potential of using underutilized fruits for wine production, which could contribute to the diversification of the wine industry. Additionally, future studies could focus on optimizing the fermentation conditions for *K. apiculata* to further enhance the sensory characteristics of the resulting wines.

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