MICROBIOLOGICAL ANALYSIS OF WINE PRODUCED IN THE LABORATORY USING PINEAPPLE AND WATERMELON FRUITS FERMENTED BY *KLOECKERA APICULATA*

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ABSTRACT

This research focuses on the microbiological analysis of wine produced in a laboratory using pineapple and watermelon fruits fermented by *Kloeckera apiculata*. The study aimed at evaluating the microbial composition, including yeast, bacteria, and mold, and the physicochemical properties of the wine during the fermentation process. The experiment was conducted using two batches of fruit, pineapple, and watermelon, separately fermented using *Kloeckera apiculata*. Samples were collected at different stages of fermentation and analyzed for microbial composition, pH, alcohol content, and total acidity. The yeasts, mold, and bacteria present were enumerated and isolated by spread plate techniques and incubated at room temperatures for 24h. Resultant colonies were enumerated and identified by a standard protocol. The results showed that *Kloeckera apiculata* dominated the microbial population during the fermentation process. No mold was isolated due to the low level of oxygen present in the fermentor. Three bacteria species were identified and confirmed during the fermentation to be *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Pediococcus* species which are non-pathogenic bacteria and did not constitute any threat to health rather added a sour taste to the wine and also decreased the pH of the wine. The wine produced from pineapple had a higher alcohol content and lower acidity than the wine produced from watermelon. These findings demonstrate the potential of *Kloeckera apiculata* for the production of wine using tropical fruits and highlight the importance of microbiological analysis in the quality control of wine production.

KEYWORDS: *Kloeckera apiculate;* pineapple and watermelon; fermentation; wine; microbiological analysis; *Lactobacillus plantarum*

INTRODUCTION

Wine production is a significant global industry, and wine's microbiological makeup plays a key role in defining its quality and characteristics. In the fermentation process, various yeast and bacterial strains participate, and their interactions can influence the sensory qualities of the finished product (Fleet, 2008). There has been an increase in interest in recent years in exploring the use of non-grape fruits for wine production. Two commonly available tropical fruits-watermelon and pineapple-have been proven to have the potential to be used in the creation of wine (Sivakumar *et al*., 2017, Anthon *et al*., 2012). The amount of wine produced from non-grape fruits however is still insignificant. In many countries, fruit wines are in large demand with little or no attention paid to the microbiological standard or safety of the wine in question, as several contaminating sources around humans abound (Adegoke and Komolafe, 2008; Adegoke and Stenstrom, 2019). Wines especially of fruit origin are scares and in high demand for several reasons one of which is the seasonality of fruits and their limited quantity. A yeast called *Kloeckera apiculata* is frequently found on the fruit's surface and has been utilized to make a variety of fermented goods, including wine (Suzzi and Zapparoli, 2017). According to reports, K. apiculata produces wines with distinctive sensory qualities like fruity and flowery smells (Garde-Cerdán and Ancín-Azpilicueta, 2008). The microbiological and physicochemical characteristics of wine made from pineapple and watermelon utilizing *K. apiculata*, however, are not well understood due to the limited information available.

Therefore, this study aimed to investigate the microbial composition of wine produced in the laboratory using pineapple and watermelon fruits fermented by *K. apiculata*. The results of this study contribute to the understanding of the microbiological profile of the fermentation process using non-grape fruits and the potential use of *K. apiculata* for wine production.

MATERIALS AND METHODS

The fermented juice was analyzed for the presence of microorganisms using standard microbiological techniques (FDA, 2019). At every 24 h, a sample of the wine was aseptically withdrawn from each fermenter, and 1 ml of the wine sample was serially diluted in 0.1% peptone water. The yeasts, mold, and bacteria present were enumerated and isolated by spread inoculation of 0.1 ml of the 10-6 dilution onto plates of nutrient agar (NA) and incubated at 30°C for 24 h for bacterial growth. The potato dextrose agar (PDA) was incubated at 28°C for 72 h for the efficient growth of molds and yeast. Resultant colonies were enumerated with the aid of a colony counter, purified by streaking technique on freshly prepared nutrient agar (NA) and potato dextrose agar (PDA), and identified by a standard protocol (Ajay and Sanjay, 2010). Cultural and microscopic identification of bacteria isolates was carried out based on Growth on de Man Rogosa Sharpe agar, Gram staining reaction, spore formation test, temperature test, and motility test. Biochemical identification of bacteria isolates obtained was ascertained by catalase test, urease test, Methyl Red- Voges Proskauer Test (MR-VP Test), citrate test, nitrate reduction test, and indole test. The result obtained was confirmed using the sugar fermentation test, esculin test, sodium chloride test, and test for the production of ammonia from arginine. Sensory evaluation of the wine produced was obtained by

determining the organoleptic properties of the wine (Panneerselvam and Maragatham, 2011). The results obtained were analyzed using one-way ANOVA without replication at $p \leq 0.05$.

RESULTS AND DISCUSSION

Throughout the fermentation, quality control of the wine produced was carried out to determine the microbial standard of the wine produced. The results obtained include Table 1 showed the enumeration of the various microbial species within the Pineapple and Watermelon Wine Produced at Dilution Factor of 10^{-2} and 0.1 Volume of Inocula. There were large counts but they were mostly non-pathogenic organisms. Their characterization as depicted in Table 2 and Table 3 showed that they are mostly commensal, and in fact, probiotics.

This research investigated the Microbiological profile of wine produced in the laboratory using *Kloeckera apiculata*. The study explored the changes in the microbiological profile of the wine during the aerobic and anaerobic fermentation processes. The results showed that there was an increase in the total yeast count during both aerobic and anaerobic fermentation. In aerobic fermentation, the yeast count increased from 6.0 log CFU/mL on day 1 to 9.5 log CFU/mL on day 3. This increase in yeast count during aerobic fermentation is attributed to the presence of utilizable sugar and yeast nutrients, however daily aeration aided the rapid multiplication of yeast cells (Kiin-Kabar *et al.*, 2019). In the anaerobic fermentation, the yeast count increased significantly and then started reducing in the first week of anaerobic fermentation. This could be attributed to the fact that during this stage alcohol levels had increased in the fermenting medium and led to the death of yeasts and also depletion of nutrients had taken place which also lead to the death of lactic acid bacteria present in the medium (Navarro *et al*., 2020). These results indicate that *Kloeckera apiculata* is a robust yeast strain that can thrive in both aerobic and anaerobic conditions, leading to the growth of yeast populations during fermentation.

The study also found that the fermentation process was more efficient under anaerobic conditions, as evidenced by a higher total yeast count and a shorter fermentation period compared to aerobic conditions. This is attributed to the fact that *Kloeckera apiculata* is a facultative anaerobe, which means it can grow in both the presence and absence of oxygen Kacaniova *et al.*, 2015). During anaerobic growth, the yeast utilized intermediate product like acetaldehyde as hydrogen acceptors for alcohol production. Molds could not be isolated. This was attributed to the low level of oxygen as the fermenters were airtight and also it could as well be due to the enhanced level of acid produced by *Kloeckera apiculata* and *Lactobacillus* species. However, the quality and security of the wine produced can be significantly impacted by the presence of mold in a fermenter. Wine may develop unpleasant smells and odors from mold, as well as potentially dangerous substances like mycotoxins. Mold development may also be a sign of poor sanitation and control practices during the winemaking process. Nevertheless, mold contamination can happen when handling, fermenting, or storing fruit. This can cause odor and present a risk to consumer health. To avoid and manage mold growth, winemakers take extra precautions including using sterile equipment, regulating temperature and humidity, and adding sulfur dioxide to the fermenter. Furthermore, three bacteria species were identified and confirmed during the wine fermentation. They are found to be non-pathogenic bacteria and therefore they do not constitute any threat to human health. They are associated with fruits and locally fermented drinks this is in agreement with the work of Awe *et al.* (2011). They are known as Lactic Acid Bacteria.

Wine frequently contains lactic acid bacteria (LAB). Although, wine production benefits from the presence of lactic acid bacteria (LAB), including improved flavor and aroma, increased stability, and less chance of deterioration. But one of LAB's most important advantages in winemaking is the creation of lactic acid through malolactic fermentation (MLF). Malic acid is changed into lactic acid by LAB during malolactic fermentation, which can produce a wine that is smoother, less acidic, and has a better mouthfeel (Bartowsky,2009). The growth of hazardous microbes can also be slowed down by the creation of lactic acid, which lowers the likelihood of spoiling (De Vuyst andVandamme, 1994). Extra care is always needed against such bacterial contamination which can originate from myriads of sources (Adegoke and Komolafe, 2008; Adegoke and Stenstrom, 2019). LAB further creates appealing flavors and aromas in the wine, some strains are known to improve the fruity and flowery flavors of wine (Lerm, *et al* 2011). Due to the presence of LAB, sulfur compounds that can result in bad tastes and odors are less likely to occur (Tournier and Gomes, 2019). Furthermore, lactic acid bacteria can improve wine stability and can aid in lowering the possibility of deterioration brought on by oxidation or the development of undesirable bacteria or yeasts (Torija *et al*., 2010). This can be especially crucial for wines that are aged for a long time or weren't prepared with sulfur dioxide (Valero *et al*., 2019). Winemakers can therefore choose LAB strains with caution and manage MLF to maximize these benefits and produce high-quality wines.

Table 1: Microbiological Profile of Pineapple and Watermelon Wine Produced at Dilution Factor of 10^{-2} and 0.1 Volume of Inocula

Time (Days)	Number of colonies on PDA from Pineapple wine	Number of colonies on NA from Pineapple wine	Total yeast count (CFU/Ml) on PDA from Pineapple wine	Total bacterial count (CFU/Ml) on NA from Pineapple wine	Number of colonies on PDA from watermelon wine	Number of colonies on NA from watermelon wine	Total yeast count on PDA (CFU/ML) watermelon wine	Total bacterial count on NA(CFU/ML) watermelon wine
1	60	50	6	5	80	90	8	9
2	70	72	7	7.2	150	200	15	20
3	95	98	9.5	9.8	250	240	25	24
6	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
7	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
8	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
9	TNTC	TNTC	TNTC	TNTC	70	90	7	9
10	100	125	10	12.5	65	80	6.5	8
11	70	80	7	8	50	60	5	6
12	50	60	5	6	30	50	3	5
13	30	40	3	4	10	30	1	3

Key: **NA**; Nutrient Agar, **PDA**; Potatoes Dextrose Agar, **CFU/ML**; Colony Forming Unit per milliliters, **ML**; Milliliters.

Table 2: Cultural, Microscopic, and Biochemical Characteristics of Bacteria Isolates for Quality Control of Wine Produced.

Isolates code's	Growth on MRS GRM	SPR	MOT	Temp. Test			Biochemical Tests							Sugar Fermentation							Inference
				15°C	30°C	45°C	Cat	U	MR	VP	NR	CI	IN	Glu	Gla	Fru	Suc	Ara	Mal	Lac	
PWW1	Round whitish, convex, colonies with entire margin. +ve rods	-	-	+	+	-	-	+	-	-	+	-	-	+	+	+	+	-	+	+	*Lactobacillus plantarum*
WWW2	Round opaque, smooth, convex colonies with entire margins. +ve rods	-	-	+	+	-	-	-	-	-	-	+	-	+	-	+	-	+	+	-	*Lactobacillus brevis*
WWW3	Round, creamy colonies +ve spherical-cells	-	-	+	+	+	-	+	-	-	+	-	-	+	+	+	-	-	-	-	*Pediococcus specie*

Key: **MRS**; de Man Rogosa Sharpe agar, **GRM**; Gram's reaction test, **SPR**; Spore formation test, **MOT**; Motility test, **CAT**; Catalase test, **U**; Urease test, **MR**; Methyl red, **VP**; Voges-proskauer, **NR**; Nitrate reduction test, **CI**; Citrate test, **IN;** Indole test, **Glu**; Glucose, **Gla**; Galactose, **Fru**; Fructose, **Suc**; Sucrose, **Ara**; Arabinose, **Mal**; Maltose, **Lac**; Lactose.

Table 3: Confirmatory Test for Bacterial Isolates Obtained

Bacteria isolates	NH_3 from arginine	NaCl (%) 2	4	6.5	ARA	CEL	MAN	MAZ	MEL	RAF	RIB	SAL	LAC	MEZ	RHA	SOR	XYL	TRE	ESC	Inference
PWW 1	-	+	+	-	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+	*Lactobacillus plantarum*
WWW2	+	+	-	-	+	-	-	-	-	-	+	-	+	-	-	-	+	-	+	*Lactobacillus brevis*
WWW3	-	-	+	+	+	+	-	+	-	-	+	+	-	-	+	-	+	+	-	*Pediococcus specie*

Key: **ARA**; Arabinose, **CEL**; Cellobiose, **MAN**; Mannitol, **MAZ;** Mannose, **MEL**; Melebiose, **RAF**; Raffinose, **RIB;** Ribose, **SAL;** Salicin, **LAC;** Lactose, **MEZ**; Melezitos, **RHA**; Rhamnose, **SOR**;Sorbitol, **XYL**; Xylose, **TRE**; Trehalose, **ESC**; Esculin, **-**; negative, **+**; positive

Table 4: Bacteria Isolated, Characterized, and Identified from the wine produced.

The bacteria isolated, identified, and confirmed are stated below and are found to be non-pathogenic.

Sample Type	Nos of Bacteria Isolated	Specific Bacteria Isolated	Codes used for each Bacteria
Pineapple wine	1	*Lactobacillus plantarum*	PWW 1
Watermelon wine	2	*Lactobacillus brevis*; *Pediococcus* spp.	WWW 2 WWW 3

CONCLUSION

The microbiological profile of wine made in the laboratory from pineapple and watermelon fruits fermented by *Kloeckera apiculata* offers important new information about the microbial ecology of wine production. The results of this study point to *Kloeckera apiculata* as a promising candidate for use in wine fermentation because of its capacity to provide appealing flavors and smells. The presence of *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Pediococcus species* which are lactic acid bacteria could not be ignored though they are non-pathogenic but beneficial. They are also very important in the wine production process because of the advantages they pose as well as their capacity to prevent the growth of microorganisms that cause spoiling. The findings also imply that microbial makeup and wine quality can be greatly influenced.

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