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Research Article

Effects of Fermentation Time and pH on Physicochemical Parameters of Wine Must Produced from Hot Water Extract of *Rauvolfia vomitoria* Afzel Leaf using *Saccharomyces cerevisiae*

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ABSTRACT

The effects of fermentation time (Day 1 to Day 14) and pH (3.5, 4.5, 5.5, 6.5 and 7.5), on wine must physicochemical characteristics made by using hot water extract of *Rauvolfia vomitoria* Afzel leaf using *Saccharomyces cerevisiae* were studied. Yeast nutrient (Diazanium sulphate and magnesium salt) was added to the mixture and granulated sugar (sucrose) was used as the fermentation substrate. pH was measured with a pHep pocket-sized pH meter, using a portable refractometer, the total soluble solid (°Brix) was determined, and the amount of alcohol was determined using the potential alcohol difference approach. Results show that the alcohol content increased progressively and remained constant from day 6 (0.474 ± 0.000%) till day 14. Total soluble solids decreased, while from day 1 to day 14 of the must's fermentation, the pH increased and decreased. Similarly, the highest amount of alcohol (2.400 ± 0.000%) was produced at pH 5.5, while the lowest (0.592 ± 0.000%) was at pH 6.5 and pH 7.5. The study shows that the highest percentage of alcohols was obtained at pH 5.5 and at day 6 of fermentation. For the organism to produce the highest amount of alcohol, the fermentation period and pH of the fermenting media must be adjusted appropriately.

Keywords: Fermentation; Must; pH; *Rauvolfia vomitoria; Saccharomyces cerevisiae;* Wine

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INTRODUCTION

Wine can be defined as a complex microbial ecosystem, where different microorganisms interact in the function of different biotic and abiotic factors. Natural fermentation results in the creation of a complex and persistent microbiota that will determine the kinetics of the process and the finished product as a result of unanticipated interactions between bacteria and environmental conditions. Controlled multi-starter fermentation represents a microbial approach to achieve the dual purpose of having a less risky process and a distinctive final product (Comitini *et al.,* 2021). The fermentation of grape must or mash produces wine, which is regarded as a notable alcoholic beverage. Moderate wine drinking is healthy since it has protective benefits against neurological disorders, cancer, diabetes, and cardiovascular illnesses (Artero *et al.* 2015, Vecchio *et al.,* 2017). According to Snopek *et al.* (2018), Garrido and Borges, (2013), wine typically contains ethanol, sugars, acids, tannins, minerals, proteins, and other substances like organic acids, volatile substances, and phenolic substances.

Fermentation is a well-known natural process. It has been utilised by humans for hundreds of years to make alcoholic beverages like wine as well as other non-alcoholic items. A carbohydrate, such as starch or sugar, is converted by an organism into an alcohol or an acid during fermentation, which is a major metabolic activity. Wine is produced by the fermenting process from grape juice (must). This is a complex chemical reaction whereby the yeast interacts with the sugars (glucose and fructose) in the must to create ethanol and carbon dioxide. *Saccharomyces cerevisiae* strains, the most prevalent and commercially available yeast, and several lactic acid bacteria are routinely used in the fermentation procedures that produce wines. Their technological traits and fermentative tendency are widely known for enabling the production of products with consistent and standardised quality (Maicas, 2021). Through the inoculation of pure *S. cerevisiae* starter cultures, a practise of controlled fermentation that has gained popularity since the 1970s swiftly developed a predictable process with a dominating yeast population producing well-managed, certain, and consistent results (Ciani and Comitini, 2019).

pH is related to the hydrogen ions (H^+) content of a solution and is crucial for microbial stability, color, preservation, oxidation, tartrate stability, protein stability, and wine taste and astringency (Botezatu *et al.,* 2023). Alcoholic fermentation is influenced by a number of variables, including the addition of sulphur dioxide, the temperature at which the fermentation occurs, the pH of the must, the makeup of the fruit juice, the inoculation of particular yeasts, and the interactions of microorganisms, however, pH is thought to be a crucial factor (Bagheri *et al.,* 2020, Krieger-Weber *et al.,* 2020). For wine, a pH of about 3.6 is idea, whereas a pH of around 4.5 is preferable for yeast and lactic acid bacteria (Galli *et al.*, 2022). The growth of spoilage bacteria is inhibited below pH 3.6, however they can even thrive at pH 4.5 (Shankar *et al.*, 2021). A pH range of 3.3 to 3.6 is still suitable for the metabolism of wine yeasts and some lactic acid bacteria (Pradip and Archana, 2016, Capozzi *et al.,* 2021). Because the microorganisms involved grow slowly in low pH environments, fermentation can take longer to complete (Chidi *et al.*, 2018). A higher pH in wine promotes a faster rate of oxidation and encourages more microbiological deterioration. It is significantly more challenging to achieve the protection offered by potassium metabisulfite (KMBS), which serves as a wine preservative, at a higher wine pH. Wine's colour is a special phenomenon that is affected by pH and the stability of the chemical compound's flavonoid class member anthocyanins (Tang *et al.*, 2019).

pH directly affects the quality of wines because when it fall outside their optimal ranges, it has detrimental effects on the microbial and sensorial quality of the finished wines. To be more precise, wines with high pH levels are more prone to microbic contamination (Chidi *et al.*, 2018), and sulphur anhydride must be used at larger quantities to prevent microbial and oxidative concerns (Giacosa *et al.*, 2019). Thus, acidity is a key factor in crucial management choices including the risk of contamination (Comuzzo and Battistutta, 2019).

R. vomitoria often referred to as "poison-devil's pepper, serpent wood, or swizzle stick," Afzel is a wild plant in the Apocynaceae family. Based on scientific studies, the presence of carbohydrates, tannins, steroids/triterpenes, flavonoids, alkaloids, and cardiac glycosides has been linked to its powerful biological qualities, including antibacterial, antioxidant, anti-cancer, and anti-diabetic effects (Agbodjogbé *et al.*, 2022, Akpojotor and Ebomoyi, 2021, Chinonye *et al.*, 2021, Ekarika *et al.*, 2020, Bemis *et al.*, 2006). These characteristics makes it an important component of wine products that are simple to evaluate and can generate revenue for the general public. Several studies have been conducted and reported that look closely at the effects of pH and fermentation on the physicochemical characteristics of several fruits and plant juices (Ousaaid *et al.*, 2021, Huan *et al.*, 2021, Dennis-Eboh *et al.*, 2023). No research to date has documented the production of wine from hot water extract of *R. vomitoria* Afzel leaf using *Saccharomyces cerevisiae*. In this study, the effects of fermentation time and pH on wine must physicochemical characteristics made by using hot water extract of *R. vomitoria* leaf using *S. cerevisiae* were studied.

MATERIALS AND METHODS

Sample collection

R. vomitoria fresh leaves were collected at the Delta State University in Abraka, Delta State, Nigeria and the leaves identified in the Department of Plant Biology and Biotechnology Herbarium Unit, Faculty of Life Sciences, University of Benin, Benin City, Edo State, with the voucher number (UBH-R421).

Preparation of *R. vomitoria Afzel* Must

Fresh leaves of *R. vomitoria Afzel* were detached from the stem and rinsed with distilled water. Using an electric pressure pot cooker, *R. vomitoria Afzel* hot water leaf extract was obtained at a temperature of 100°C for 15 minutes. The extract was then filtered using muslin cloth that was folded twice and the juice was enhanced with 20g/liter of granulated sugar to change the soluble solids from 0.8 to 2.6°brix for the effect of the fermentation time study and to 3.6°brix for the effect of the pH study. Must was sterilized for 20 minutes at 121 °C in an autoclave. Before adding the starting culture, approximately 0.143g of sodium metabisulphite, a sulphur (IV) oxide salt, was incorporated into the extract to limit the development of microorganisms and non-domesticated yeast (Pradip and Archana, 2016, Ogodo *et al.*, 2018). The broth was fermented for 14 days at room temperature, with the following conditions: pH 5.0, 10% plant filtrate, 2% w/v of sugar, and 0.4% (v/v) of inoculum size to determine the ideal duration for fermentation. The effect of pH on fermentation parameters was examined by dividing the must into 300 ml aliquots in 500 ml flasks for pH modifications. Each requirement had a different pH, varying between 3.5, 4.5, 5.5, 6.5, and 7.5. The pH of the must was adjusted using citric and tartaric acid. Cotton wool plugs soaked in solution of sodium metabisulphite were placed on the lid of the flasks holding the must. After that, must was stored at room temperature until needed (Pradip and Archana, 2016, Ogodo *et al.*, 2018).

Preparation of Starter Culture

The method of Ogodo *et al.* (2018) was employed with slight modification. 200ml of *R. vomitoria Afzel* hot water extract was combined with 0.4% of commercial baker's yeast (*S. cerevisiae*) and vigorously agitated. Ammonium sulphate and magnesium sulphate, each weighing about 2g, were mixed with 100 ml of distilled water after being dissolved. After letting the combination sit for a while, the mixture was then incorporated into the must.

Fermentation of must to wine

A fermentation jar known as an aspirator was filled with the extract. To give the yeast population time to multiply, it was covered for roughly 15 to 20 minutes. To prevent oxidation, cotton wool plugs soaked in sodium metabisulphite solution were put on the lids of the flasks containing the must. At 24-hour intervals, the must was stirred, and aliquots were taken for evaluation. After 24 hours of inoculation, pH and total soluble solid (ToSS) of each fermenter was measured every 24 hours for 14 days in order to track the process of fermentation and investigate how pH affects the must's fermentation profile (Ogodo *et al.*, 2018).

Parameters studied

At the proper intervals throughout the fermentation, the pH and decrease in ToSS (°Brix) were measured. The wines were examined for a variety of physicochemical traits. Must and wine were measured for pH, using a pHep pocket-sized pH metre (HANNA instrument), which was precalibrated using pH buffers of 4.0 and 7.0. The ToSS was determined with the aid of a portable refractometer (ERMA INC. BRIX 0-32%) and the amount of alcohol was determined using the difference in potential alcohol method. The alcohol percentage was determined using the sugar levels of the must prior to fermentation and the must's ultimate sugar content (Jacobson, 2006, Tochukwu and Oyinloye, 2017).

RESULTS

The effect of fermentation time on pH, ToSS and alcohol quantity of wine produced from hot water extract of *Rauvolfia vomitoria* Afzel leaf is shown in Table 1. Alcohol production started at day 2 (0.138 ± 0.035) and increased with the increase of fermentation time till day 6 (0.474 ± 0.000) which had the maximum alcohol content with ToSS of 1.800 and pH 3.1.

The variation in pH, ToSS and AlcOH content of wine must produced from hot water extract of *Rauvolfia vomitoria* Afzel leaf with the change in pH (3.5, 4.5. 5.5, 6.5 and 7.5) for 14 days are indicated in table 2-6 respectively. At pH 3.5, maximum AlcOH content of 1.421 was recorded in day 12 and was unchanged till day 14 with ToSS of 1.000 and pH 3.3 (Table 2). At pH 4.5, maximum AlcOH was recorded in day 12 (1.421 ± 0.000) and was the same in day 13 and 14 with ToSS of 1.000 and pH 3.0 (Table 3). At pH 5.5, AlcOH content increased progressively from day 1 (0.118 ± 0.000) till day 6 (2.400 ± 0.000) which recorded the maximum AlcOH content and was unchanged till day 14 with ToSS of 1.200 and pH 3.1 (Table 4). At pH 6.5, maximum AlcOH content of 0.829 was recorded in day 12 with ToSS of 2.000 and pH 6.0 (Table 5). At pH 7.5, maximum AlcOH content was recorded in day 4 and was the same all through till day 14 with ToSS of 2.400 and pH 6.3 (Table 6).

Table 1. **Effect of fermentation time on pH, ToSS and alcohol content of wine produced from hot water extract of *R. vomitoria* leaf**

Fermentation time (Days)	pH	ToSS (°Brix)	AlcOH (%)
1	4.7 ± 0.000	2.600 ± 0.000	0.000 ± 0.000
2	4.4 ± 0.058	2.367 ± 0.058	0.138 ± 0.035
3	3.8 ± 0.000	2.200 ± 0.000	0.237 ± 0.000
4	3.6 ± 0.000	2.000 ± 0.000	0.355 ± 0.000
5	3.5 ± 0.000	2.000 ± 0.000	0.355 ± 0.000
6	3.5 ± 0.000	1.800 ± 0.000	0.474 ± 0.000
7	3.5 ± 0.000	1.800 ± 0.000	0.474 ± 0.000
8	3.7 ± 0.000	1.800 ± 0.000	0.474 ± 0.000
9	3.7 ± 0.000	1.800 ± 0.000	0.474 ± 0.000
10	3.6 ± 0.000	1.800 ± 0.000	0.474 ± 0.000
11	3.6 ± 0.000	1.800 ± 0.000	0.474 ± 0.000
12	3.6 ± 0.000	1.800 ± 0.000	0.474 ± 0.000
13	3.6 ± 0.000	1.800 ± 0.000	0.474 ± 0.000
14	3.8 ± 0.000	1.800 ± 0.000	0.474 ± 0.000

Triplicates values expressed as mean ± standard deviation. ToSS- total soluble solid and AlcOH- alcohol content.

Table 2. Effect of pH 3.5 on pH, ToSS and AlcOH content of wine produced from hot water extract of *Rauvolfia vomitoria* Afzel leaf

Fermentation time (Days)	pH	ToSS (°Brix)	AlcOH (%)
1	4.7 ± 0.000	3.200 ± 0.000	0.118 ± 0.000
2	3.7 ± 0.000	2.800 ± 0.000	0.355 ± 0.000
3	3.7 ± 0.000	2.200 ± 0.000	0.710 ± 0.000
4	3.7 ± 0.000	2.000 ± 0.000	0.829 ± 0.000
5	3.7 ± 0.000	2.000 ± 0.000	0.829 ± 0.000
6	3.6 ± 0.000	1.400 ± 0.000	1.184 ± 0.000
7	3.6 ± 0.000	1.400 ± 0.000	1.184 ± 0.000
8	3.5 ± 0.000	1.400 ± 0.000	1.184 ± 0.000
9	3.6 ± 0.058	1.400 ± 0.000	1.184 ± 0.000
10	3.1 ± 0.000	1.200 ± 0.000	1.302 ± 0.000
11	3.6 ± 0.000	1.400 ± 0.000	1.184 ± 0.000
12	3.3 ± 0.000	1.000 ± 0.000	1.421 ± 0.000
13	3.3 ± 0.000	1.000 ± 0.000	1.421 ± 0.000
14	3.3 ± 0.000	1.000 ± 0.000	1.421 ± 0.000

Triplicates values expressed as mean ± standard deviation. ToSS- total soluble solid and AlcOH- alcohol content.

Table 3. Effect of pH 4.5 on pH, ToSS and AlcOH content of wine produced from hot water extract of *Rauvolfia vomitoria* Afzel leaf

Fermentation time (Days)	pH	ToSS (°Brix)	AlcOH (%)
1	4.5 ± 0.000	3.200 ± 0.000	0.118 ± 0.000
2	3.6 ± 0.058	2.433 ± 0.058	0.572 ± 0.034
3	3.3 ± 0.000	1.800 ± 0.000	0.947 ± 0.000
4	3.5 ± 0.058	1.400 ± 0.000	1.223 ± 0.068
5	3.5 ± 0.000	1.400 ± 0.000	1.302 ± 0.000
6	3.2 ± 0.000	1.200 ± 0.000	1.302 ± 0.000
7	3.2 ± 0.000	1.200 ± 0.000	1.302 ± 0.000
8	3.2 ± 0.000	1.200 ± 0.000	1.302 ± 0.000
9	3.4 ± 0.000	1.200 ± 0.000	1.302 ± 0.000
10	3.0 ± 0.058	1.200 ± 0.000	1.302 ± 0.000
11	3.2 ± 0.000	1.200 ± 0.000	1.302 ± 0.000
12	3.0 ± 0.000	1.000 ± 0.000	1.421 ± 0.000
13	3.0 ± 0.000	1.000 ± 0.000	1.421 ± 0.000
14	3.0 ± 0.000	1.000 ± 0.000	1.421 ± 0.000

Triplicates values expressed as mean ± standard deviation. ToSS- total soluble solid and AlcOH- alcohol content

Table 4. Effect of pH 5.5 on pH, ToSS and AlcOH content of wine produced from hot water extract of *Rauvolfia vomitoria* Afzel leaf

Fermentation time (Days)	pH	ToSS (°Brix)	AlcOH (%)
1	3.6 ± 0.058	3.400 ± 0.000	0.118 ± 0.000
2	3.4 ± 0.000	3.200 ± 0.000	0.237 ± 0.035
3	3.4 ± 0.000	2.000 ± 0.000	1.600 ± 0.000
4	3.4 ± 0.000	1.600 ± 0.000	2.000 ± 0.000
5	3.4 ± 0.000	1.400 ± 0.000	2.200 ± 0.000
6	3.1 ± 0.000	1.200 ± 0.000	2.400 ± 0.000
7	3.1 ± 0.000	1.200 ± 0.000	2.400 ± 0.000
8	3.2 ± 0.000	1.200 ± 0.000	2.400 ± 0.000
9	3.3 ± 0.000	1.200 ± 0.000	2.400 ± 0.000
10	3.1 ± 0.000	1.200 ± 0.000	2.400 ± 0.000
11	3.2 ± 0.000	1.200 ± 0.000	2.400 ± 0.000
12	3.0 ± 0.000	1.200 ± 0.000	2.400 ± 0.000
13	3.0 ± 0.000	1.200 ± 0.000	2.400 ± 0.000
14	3.0 ± 0.000	1.200 ± 0.000	2.400 ± 0.000

Triplicates values expressed as mean ± standard deviation. ToSS- total soluble solid and AlcOH- alcohol content.

Table 5. Effect of pH 6.5 on pH, ToSS and AlcOH content of wine produced from hot water extract of *Rauvolfia vomitoria* Afzel leaf

Fermentation time (Days)	pH	TOSS (°Brix)	ALC (%)
1	5.5 ± 0.000	3.200 ± 0.000	0.118 ± 0.000
2	5.7 ± 0.058	3.000 ± 0.000	0.237 ± 0.000
3	5.6 ± 0.058	2.800 ± 0.000	0.355 ± 0.000
4	5.2 ± 0.000	2.600 ± 0.000	0.474 ± 0.000
5	5.2 ± 0.000	2.600 ± 0.000	0.474 ± 0.000
6	5.7 ± 0.000	2.400 ± 0.000	0.592 ± 0.000
7	5.7 ± 0.116	2.400 ± 0.000	0.592 ± 0.000
8	5.9 ± 0.000	2.400 ± 0.000	0.592 ± 0.000
9	5.9 ± 0.058	2.400 ± 0.000	0.592 ± 0.000
10	5.1 ± 0.000	2.400 ± 0.000	0.592 ± 0.000
11	5.8 ± 0.000	2.200 ± 0.000	0.710 ± 0.000
12	6.0 ± 0.000	2.000 ± 0.000	0.829 ± 0.000
13	6.0 ± 0.000	2.000 ± 0.000	0.829 ± 0.000
14	6.1 ± 0.000	2.000 ± 0.000	0.829 ± 0.000

Triplicates values expressed as mean ± standard deviation. ToSS- total soluble solid and AlcOH- alcohol content.

Table 6. Effect of pH 7.5 on pH, ToSS and AlcOH content of wine produced from hot water extract of *Rauvolfia vomitoria* Afzel leaf

Fermentation time (Days)	pH	ToSS (°Brix)	AlcOH (%)
1	6.9 ± 0.058	3.200 ± 0.000	0.118 ± 0.000
2	6.3 ± 0.000	3.133 ± 0.116	0.158 ± 0.069
3	6.3 ± 0.000	2.800 ± 0.000	0.355 ± 0.000
4	6.3 ± 0.000	2.400 ± 0.000	0.592 ± 0.000
5	6.3 ± 0.000	2.400 ± 0.000	0.592 ± 0.000
6	6.3 ± 0.000	2.400 ± 0.000	0.592 ± 0.000
7	6.3 ± 0.000	2.400 ± 0.000	0.592 ± 0.000
8	6.3 ± 0.000	2.400 ± 0.000	0.592 ± 0.000
9	6.3 ± 0.000	2.400 ± 0.000	0.592 ± 0.000
10	6.0 ± 0.058	2.400 ± 0.000	0.592 ± 0.000
11	6.4 ± 0.058	2.400 ± 0.000	0.592 ± 0.000
12	6.6 ± 0.000	2.400 ± 0.000	0.592 ± 0.000
13	6.6 ± 0.000	2.400 ± 0.000	0.592 ± 0.000
14	6.7 ± 0.000	2.400 ± 0.000	0.592 ± 0.000

Triplicates values expressed as mean ± standard deviation. ToSS- total soluble solid and AlcOH- alcohol content

Figure 1 shows the variation in physicochemical properties of *R. vomitoria* Afzel must after fermentation at various pH. pH 5.5 had the highest alcohol content (2.400%) while pH 4.5 and pH 3.5 had equal amount of alcohol (1.421%) followed by pH 6.5, which had alcohol content of 0.829% and pH 7.5 had the least alcohol content (0.592%). The total soluble solid followed the trend: pH 3.5 = pH 4.5 < pH 5.5 < pH 6.5 < pH 7.5.

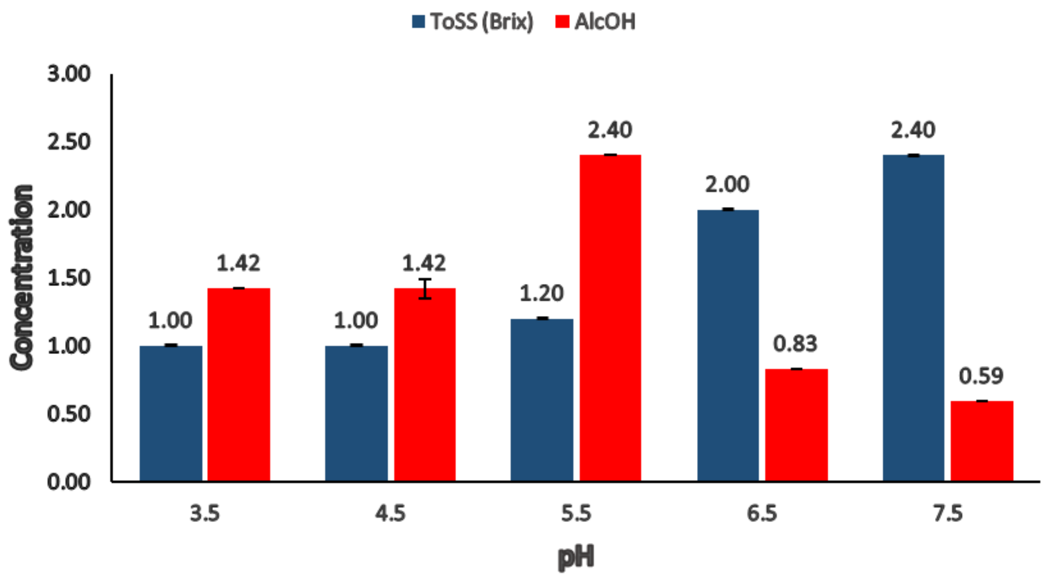

Figure 1. **Physicochemical properties of *R. vomitoria* Afzel must after fermentation at various pH. Triplicates values expressed as mean ± standard deviation. ToSS- total soluble solid and AlcOH- alcohol content**

DISCUSSION

The effects of fermentation time and pH on physicochemical parameters of wine must produced from hot water extract of *Rauvolfia vomitoria* Afzel leaf using *Saccharomyces cerevisiae* were studied. The results showed that while alcohol content increased, total soluble solids reduced as fermentation time increased. The highest amount of alcohol was recorded at day 6 of fermentation. This corroborates the work of Muhammad *et al.* (2014). They investigated the relationship between fermentation time and pH of molasses solution and found that with the increase of fermentation time from 40 to 56 hours, more alcohol was produced. ToSS was documented to drop as the time of fermentation extended (Worku *et al.*, 2019). ToSS is a reliable indicator of the amount of sugar present; this establishes the potential level of alcohol that may arise from fermentation through yeast consumption of sugar (Aude, 2020). Alcohol dehydrogenase in the yeast converted the sugar in the must into alcohol (Iersel *et al.*, 1999). According to Iersel *et al.* (2000), sugar was converted to alcohol molecules such as ethanol, butanol, propanol, isobutanol, and isoamyl alcohol. This shows that more alcoholic compounds were produced the longer the fermentation process lasted. This is because the yeast has enough time to produce more alcohol dehydrogenase in the sample, which then causes the sugar to be fermented into alcohol (Okamura *et al.*, 2001, Rajko and Janez, 1999). The yeasts were able to utilize sugar completely at day 6, this accounts for the constant value recorded from day 6 till day 14 of the fermentation period.

The ability of plasma membrane-bound protein to function, including enzymes and transport proteins, depends critically on the pH level (Narendranath and Power, 2005). According to Hossain *et al.* (2017), Mengesha *et al.* (2022), pH has a significant impact on both cell growth and alcohol output. According to Rezael *et al.* (2009), yeast can tolerate a pH range of 3.5 to 6.5. The alcohol content was maximal at pH 5.5 and decreased above this value. Our result is similar with the work of Hashem *et al.* (2013). According to their findings, cultures cultivated at pH 5.0 and pH 5.5 produced the highest levels of ethanol concentration, which increased obviously at this value and reduced somewhat above it. Ohgren *et al.* (2006) in their study observed a similar outcome in the simultaneous saccharification and fermentation of maize Stover, wherein the maximum concentration of ethanol was achieved at a pH of 5.5. The highest amount of ethanol was also produced from banana peels at pH 5.5 (Shilpa *et al.*, 2013). The highest amount of ethanol produced from grape fruit waste by *Saccharomyces cerevisiae* at pH 5.4 was also reported by Jannani *et al.* (2013). Dennis-Eboh *et al.* (2023) investigated how pH affected the physicochemical characteristics of wine must produced from hot water extract of broom-cluster fig (*F. capensis*) leaf and found that pH 4.5 provided the highest amount of alcohol. According to Zohri and Mostafa's (2000) analysis of Dates in Saudi Arabia on an Industrial Scale, the optimal pH for ethanol production was 3.5, followed by 4.5 using *S. cerevisiae*. It is obvious that the acidic environment is favourable for the activity; therefore, we may conclude that the *S. cerevisiae* yeast is an acidophilus microbe. In addition, the acidic pH is advantageous for the invertase enzyme's breakdown of sucrose into fermentable sugars. Meanwhile, the alcohol content produced at pH 7.5, a slightly basic pH was decreased than the alcohol produced in acid medium. The fact that enzymes are more active in moderately acidic media may be the cause of the decrease in alcohol production. According to Ogbonda and Kiin-Kabari (2013), higher levels of ethanol were generated in more acidic (lower pH) conditions, and as acidity increased (higher pH), the amounts of ethanol produced decreased with the least amount being produced at pH 7.0. Previous researches by Jin *et al.* (2019), Zentou *et al.* (2017), Aiyejagbara *et al.* (2016) supports the finding that ethanol decreases when pH trends towards neutral. As the fermentation time increases, the total soluble solid; an estimate of the sugar content was reduced in all the pH being set (pH 3.5, 4.5, 5.5, 6.5 and 7.5). This showed that yeast was able to convert the amount of sugar and nutrients in the must into alcohol. This has been reported by Worku *et al.* (2019), Okamura *et al.* (2001) that total soluble solid reduced when fermentation duration increased.

CONCLUSION

This study established the feasibility of making wine using *Saccharomyces cerevisiae* to ferment a hot water extract of *Rauvolfia vomitoria* Afzel leaves, which has the potential to improve the nutritional and economic benefits of the plant as well as introduce new applications for it. Wine produced from hot water extract of the leaf of *Rauvolfia vomitoria* Afzel was affected by the duration of the fermentation process and the wine's pH. It was discovered that pH 5.5 and 4.5 had the lowest and equal total soluble solid concentration. However, pH 5.5 is recommended since it recorded the highest alcohol content. Extension of fermentation time resulted in an increase in alcohol production and a decrease in total soluble solid, notably in the first six days. Thus, day 6 was taken as the optimum fermentation time.

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