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RESEARCH PAPER

INFLUENCE OF YEAST STRAINS ON THE PHYSICOCHEMICAL CHARACTERISTICS OF WATERMELON WINE

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ABSTRACT

The research was conducted to determine the physicochemical characteristics of watermelon wine developed from four different *Saccharomyces cerevisiae* ***strains (Lalvin EC 1118, Lalvin QA 23, Redstar rouge and Redstar premier classique). The physicochemical characteristics of the treatments determined included pH, titratable acidity, °Brix, and ethanol content. The data obtained were subjected to two-way analysis of variance. The mean pH, titratable acidity, apparent °Brix and ethanol during fermentation ranged between 3.629-3.848, 1.090-1.85%, 17.69-8.13°Brix, and 1.530-7.545ABV% respectively. It was noticed that the fermentation time significantly influenced the physicochemical parameters examined irrespective of the yeast strain used. However, the physicochemical characteristics findings showed that all the yeast strains used were suitable for watermelon wine production.***

Keywords: *Sacchromyces cerevisiae*; fermentation time; ethanol; titratable acidity

INTRODUCTION

Watermelon (*Citrullus lanatus*) from the family of Cucurbitacea harbours quite several bioactive compounds besides vitamins A and C, which are available in most fruits (Georgieva *et al*., 2015). It has been asserted that about 6.8% of the total area in the world dedicated to fruit production is for watermelon production (Goreta *et al*., 2005). In Ghana, cultivation of watermelon is concentrated around the Southern belt (Greater Accra, Ashanti region, Volta region, Western region, Central region and Eastern region) and in the Upper East and Upper West regions (Lamptey, 2013). Watermelon is well known for its rich source of lycopene and "thirst quenching" ability (Fish and Davis, 2003; Perkins-veazie *et al*., 2001). Earlier clinical studies have reported antioxidant activity of lycopene as a free scavenging property on biological systems (DeSteffani *et al*., 2000; Gann *et al*., 1999). The nutritional profile of watermelon reported by Charoensiri *et al*. (2009) revealed that appreciable levels of carbohydrates, sodium, vitamins, minerals, fatty acids, and amino acids are in different varieties of watermelon.

The watermelon fruit is about 60% flesh, and approximately 90% of the flesh is juice that contains 7-10% (w/v) sugars; thus, more than 50% of watermelon is readily fermentable (Tabiri *et al*., 2016). A study by Wang *et al*. (2018) compared fermented and unfermented watermelon juices and reported that the fermented juice showed intense antioxidant activity. Similarly, blended watermelon juice and ginger extract fermented into wine revealed improved scavenging activity, hence watermelon is a promising raw material for the fruit wine industry (Yusufu *et al*., 2018). Though several fruits have been used for winemaking, coupled with health benefits, watermelon has not attracted much attention when it comes to wine production from non-grape juice. There is also scanty literature on optimised production parameters for fermenting watermelon juice into wine (Fish *et al*., 2009).

Wine production involves the use of yeast to metabolise the carbohydrate (sugars) in the juice into an alcoholic beverage (wine) with desirable characteristics (Rainieri and Pretorius, 2000). It is one of the oldest technologies in human era that relies on the spontaneous fermentation of sugar in grape juice by naturally occurring yeasts (Moreno-Arribas and Polo, 2005). However, the spontaneous fermentation often results in sluggish fermentation processes, rendering it a complex challenge to produce wine on a commercial scale (Zhao and Bai 2009). The spontaneous fermentation can also result in undesirable characteristics, particularly complex organoleptic properties (Samoticha *et al*., 2019). However, as technology in oenology advanced, inactive dry yeast strains with unique characteristics are cultured and now selectively used (Samoticha *et al*., 2019). Hence, several commercial yeast strains with varietal characteristics are being used extensively in winemaking (Pozo-Bayón *et al*., 2009). The selected yeast strains for fruit winemaking are the *Saccharomyces cerevisiae* (Sun *et al*., 2014).

Grape is the most preferred raw material for winemaking due to its natural chemical balance; however, non-grapefruit wines have been developed (Pretorius and Høj, 2005). However, in developing a variety of wines at the cellar, studies have reported fruit winemaking to have different wines in cellars and salvage menace in postharvest losses. Hence, one of the widely known non-grapefruit wines is the apple fruit wine (cider) (Saranraj *et al*., 2017), probably due to its high sugar concentration and better fruity flavour. However, watermelon oenology is still under-researched in Ghana, even though watermelon has been used to produce other products such as watermelon fruit juice and jam. The metabolic activities of fermentative species (yeast strains) depend

much on the physicochemical properties (particularly pH, acidity) of the juice since a change in these properties affects the metabolism of sugar into wine (Velić *et al*., 2018). However, regardless of the changes in the physicochemical attributes in juice and other external factors (e.g., temperature) during fermentation, yeast strains should ably adapt to complete their metabolic activities (Bauer and Pretorius, 2000).

It is well-known that the physicochemical properties of juice remain the key factors that influence yeasts activity during fermentation. Therefore, profiling physicochemical characteristics changes during fermentation will provide a systematic approach in selecting a suitable yeast strain for the production of fruit wine from watermelon. Though research has extensively dealt into oenology, scanty research is done in profiling fermentation characteristics of yeasts strains in non-grapefruit oenology. Therefore, the objective of this study was to develop wine from watermelon juice by exploring different commercial yeast strains and comparing their fermentation characteristics using physicochemical properties as indicators during fermentation.

MATERIALS AND METHODS

Experimental Design

Fresh whole watermelon fruits of the Charleston grey variety were purchased from the Tamale Central market in the Northern Region of Ghana. A 4x7 factorial experimental design was used for the research. The factors include yeast type (four strains) and fermentation time (seven days). The four yeast strains used for the study were Lalvin EC 1118, Lalvin QA 23, Redstar premier classique and Redstar premier rouge.

Juice Extraction

The protocol for juice extraction was done according to Darman *et al*. (2010) with slight modification. First, all containers and equipment were sterilized with 1% sodium metabisulphite, and then fruits processed under aseptic conditions. The watermelon fruits were washed under running potable water and then rinsed with 1% sodium metabisulphite. The washed watermelon fruits were cut into pieces separating the red flesh from the rind and seeds. The red flesh (without the seeds) was extracted by manually squeezing out the juice, and clear juice obtained by filtration with a sterilized filter cloth. Approximately 20 L of the juice was extracted for the experiment, thus, 5 L of juice was used for each treatment. The clear juice (must) was then transferred to the fermenting drum. Approximately 1 g of tartaric acid and 1 g of citric acids were added to a litre of clear juice to adjust its pH from 5.15 to 3.5; the must was also chaptalized with table sugar to increase the °Brix from 6 to 20. With the protocol followed, there was no adjustment of pH with tartaric and citric acids, and the must chaptalization was done with sugar cane juice instead of table sugar. Sulfiting was employed with a pill of Campden tablet dissolved in 3 mL of distilled water and then added to the 5 L of the clear juice for sterilisation. The set-up was then left on the laboratory bench for 24 hrs at room temperature (25 **°C) before** being pitched with the yeasts.

Yeasts Conditioning, and Watermelon Must Pitching and Fermentation

The modified protocol of Darman *et al*. (2010), coupled with the manufacturer's protocol, was followed for the starter culture conditioning. Each yeast strain was first rehydrated by making a slurry from 30 mL of the must and 3 g of yeast and then allowed to stand for about 10 minutes before pitching with the fermentable must. The fermentation

proceeded for 7 days at room temperature (25°C) in an airtight high-density polyethylene (HDPE) plastic fermenter. Samples were aseptically siphoned at 24 hrs intervals for physicochemical properties determination. Prior to the sampling, fermenters were gently shaken for two minutes to ensure uniformity of the samples.

Wine Clarification/fining

After the seven days, the fermentation process was terminated by flocculating with a yeast cell agent (Bentonite). The bentonite slurry was prepared by mixing 3 tablespoons of the sample with 30 mL of warm water (45 **°C) in a saucepan** per the manufacturer's protocol. Averagely, 2 tablespoons of the bentonite slurry were added to 5 L of the wine and stirred upon addition of the slurry. According to the manufacturer's protocol, the lees was allowed to settle for about 12 hrs before the wine sample was siphoned. The wine was racked twice at 12 hrs intervals before bottling and maturation.

Physicochemical properties Determination

The physicochemical characteristics of the wines determined include pH, titratable acidity, total soluble sugar, and ethanol content. All analyses were done in triplicates.

pH Determination

The pH of the wine samples was determined according to AOAC (2005). Buffer solutions of pH 4, 7 and 9 were used to calibrate the pH meter (Model: PH-9901 Brand: PmoYoKo; Country of origin: Taiwan). For each sample, approximately 30 mL of the wine was measured into a clean polyethylene terephthalate (PET) cup, its temperature adjusted to about 25–26 °C, and the pH was determined. The pH was determined by inserting the electrode of the pH meter into the sample and the readings recorded.

Titratable Acidity Content Determination

The titratable acidity of the wine samples was determined according to AOAC (2005). For each sample, 10 mL of the wine was pipetted into a clean dried conical flask, then three drops of 1% phenolphthalein were added. The wine sample was then titrated against 0.1 M NaOH with a faint pink colour change as end point. The titre value for NaOH was recorded as V_2. The titratable acidity expressed as tartaric acid was calculated using the formula;

$$\text{Tartaric acid g/100ml} = \frac{V_1 \text{ X } 75 \text{ X } M \text{ X } 100}{V_2 \text{ X } 1000}$$

where, V_1 = volume of the wine sample used

V_2 = volume of the NaOH

M = molarity of the NaOH used

75, 100 and 1000 are constants

Determination of Total Soluble Sugar (TSS) Content

The total soluble sugar (TSS) content was measured using a handheld refractometer (Model: RHW80 ATC; Brand: Oumefar; Country of origin: China). The instrument was calibrated with distilled water. Three (3) drops of the wine sample was placed on the sensitive prism and gently closed with the prism lid. A period of about 30 was allowed for temperature adjustment of the sample with the internal temperature of the refractometer. The result was read from the °Brix scale under natural light (AOAC, 2005).

Determination of Ethanol Content

The ethanol content of the wine was determined using a handheld Alcohol refractometer (Model: RHW80 ATC; Brand: Oumefar; Country of origin: China) calibrated purposely for the measurement of alcohol content in beverages such as wines. First, the instrument was calibrated with distilled water. Then few drops of the wine sample were placed on the sensitive prism, gently closed

with the prism lid, and allowed for about 30 for temperature adjustment of the sample with the internal temperature of the refractometer. The result was read from the alcohol scale under natural light (AOAC, 2005).

Statistical Analysis

The obtained data was subjected to the two-way analysis of variance using GenStat software version eighteenth with the Tukey pairwise means separation comparison done at 95% confidence level.

RESULTS AND DISCUSSION

Effect of pH and Titratable acidity on Fermentation of Watermelon Wine

Changes in pH and titratable acidity during watermelon wine fermentation have been shown in Figure 1. The pH ranges were 3.657 - 3.853 (Lalvin EC 1118), 3.643 - 3.853 (Redstar premier rouge), 3.620 - 3.853 (Lalvin QA 23) and 3.597 - 3.830 (Redstar premier classique) during the fermentation period. Redstar premier classique recorded the minor pH range, while the highest range of pH was observed in the Lalvin EC 1118, however, no significant differences were shown among the treatments. The pH of the treatments was within the standard of pH of wine (3.5 to 4.0) affirmed by Chilaka *et al*. (2010), as reported in passion fruit, watermelon and pineapple wine which ranged from 3.0 to 4.8. The pH values increased for all the treatments throughout the fermentation period. Similarly, a study by Satav and Pethe (2016) also reported increase in pH values with fermentation time for Banana wine production. The increased in pH as observed in this study indicates the abundance of malic acid in the watermelon must. The absence of malic acid increases pH during wine fermentation regardless of the added tartaric acid since *S. cerevisiae* strains cannot metabolise the malic acid during wine fermentation (Husnik *et al*., 2006; Redzepovic *et al*., 2003). The instability of pH across the fermentation period could result from other yeast competitors actively involved in the fermentation process, which got off as the fermentation progressed due to accumulation of ethanol (Romano *et al*., 2003). Furthermore, pH (3.5) of the must was suitable enough for the fermentative agents to escape pH stress, as it is reported by Liu *et al*. (2015) that pH less than 3.0 could lead to stress which in turn prolongs the lag phase thereby causing delay in the metabolic activities of yeast strains. On the contrary, other researches have indicated pH decreased as fermentation progressed (Ajit *et al*., 2018; Yusufu *et al*., 2018; Lowor *et al*., 2016). The decrease in pH could be influenced by the evolution of acetic acid bacteria during ethanol fermentation (Lin *et al*., 2012).

On the other hand, titratable acidity increased gradually from the initial fermentation stage to the end. All the treatments except in Lalvin EC 1118 decreased steadily after 72 hrs and slightly increased above the initial fermentation hours as the fermentation progressed to the end. All treatments were significantly different ($p=0.001$) during the fermentation period. In terms of the fermentation hours, at 24 hrs of fermentation, mean titratable acid (1.090%) was significantly different ($p=0.001$) from the rest of the fermentation days. There was no significant difference between 48 (1.695%) and 96 (1.752) hrs of fermentation. Also, fermentation hours of 96 (1.752%) to 168 (1.792%) showed similarities in their mean titratable acidity as well. In addition, the mean titratable acidity values recorded during fermentation at 72 (1.850%), 120 (1.792%), 144 (1.833%) and 168 (1.825%) hrs were not significantly different.

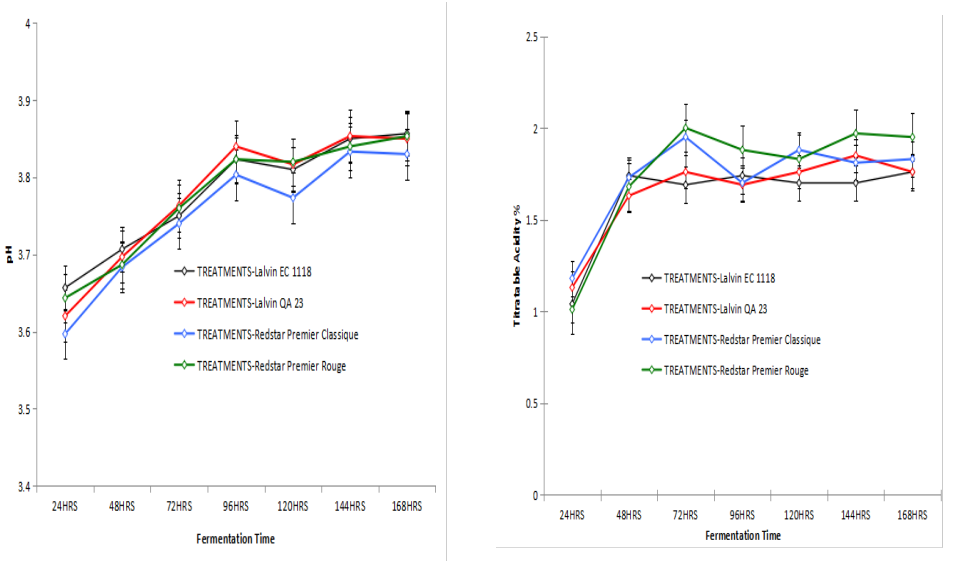

Figure 1: pH (a) and titratable acidity (b) of the watermelon wine during fermentation. *There were significant difference ($p<0.05$) between treatments for pH and titratable acidity across fermentation time. Bars represent Standard error.

The mean titratable acidity values recorded, 1.624% (Lalvin EC 1118) and 1.654% (Lalvin QA 23), did not differ from each other statistically. Similarly, the percentage means of sample Redstar premier rouge (1.760%) and Redstar premier classique (1.726%) were not statistically different. However, the wine sample's titratable acidity content was higher than the recorded range (0.015 to 0.060) reported by Zainab *et al*. (2018) in watermelon wine during fermentation. The increment recorded in the titratable acidities during this study could be linked to evolution of organic lactic, acetic, and succinic acids during conversion of sugars into ethanol (Kasture and Kadam, 2018). In general, when titratable acidity increases, pH, on the other hand, decreases. However, that was not the trend in this study. The increasing trend in pH which could be attributed to low buffer capacity of the juice and titratable acidity was also observed in a study by Kasture and Kadam (2018) on sapota wine fermentation.

Utilization of Sugar and ethanol evolution in Watermelon juice during Fermentation

Changes in dissolved solids or sugar content (°Brix) of the watermelon juice monitored throughout the fermentation period showed a significant reduction in values during fermentation among the treatments. The sugar concentration decreased as the fermentation days progressed regardless of the yeast strain, which invariably accounted to produce ethanol. However, sugar utilization was significantly different ($p=0.001$) among the yeast strains (Figure 2). The mean apparent °Brix range was 17.69°B to 8.13°B from the start of fermentation (24 hrs) to the end (168 hrs), respectively. From this result, Redstar premier classique sample recorded a maximum efficient reduction in the sugar content, followed by Lalvin QA 23 during the fermentation. In contrast, Lalvin EC 1118 and Redstar premier rouge recorded the least sugar utilization. This reduction trend in the sugar concentration during the fermentation, irrespective of the utilization efficiency of the

yeast strains was an indication of ethanol evolution. On the other hand, the mean apparent °Brix at 24 hrs (17.69), 48 hrs (10.01), 72 hrs (9.00) and 96 hrs (8.92) of fermentation showed significant differences (p=0.0001). However, no significant difference was exhibited in the mean apparent °Brix at 120 hrs (8.27), 144 hrs (8.20) and 168 hrs (8.13) of fermentation. The rapid reduction of sugar observed within the early hours (within 48 hrs) of fermentation in all the treatments recorded was similar to reports by Bhatane and Pawar (2013) during fermentation of sapota must into wine. This vigorous reduction in sugar at the initial stage could result from the active stage of the yeasts with sufficient nutrients present in the limpid at the initial stage.

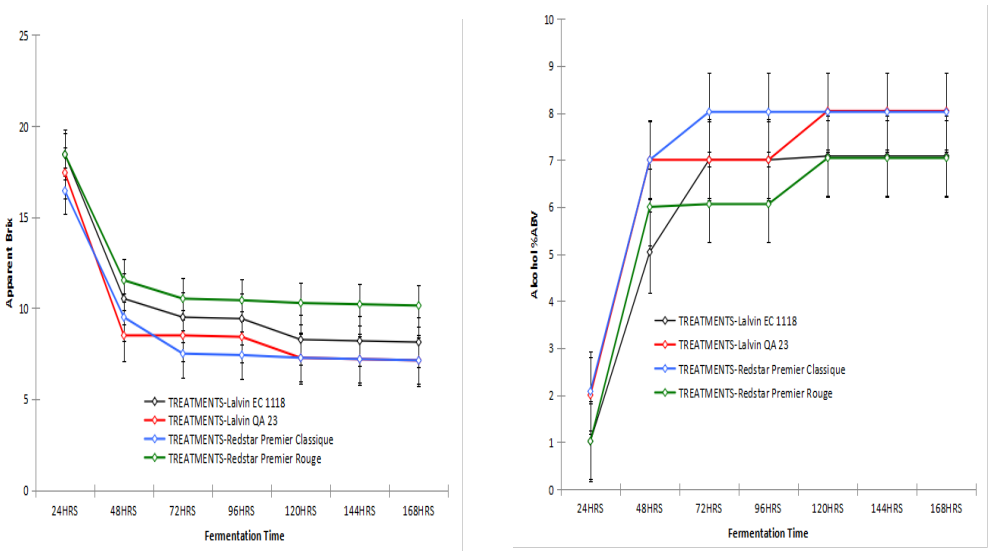

Figure 2: Utilization of sugar (apparent °Brix) (a) and ethanol content (b) during watermelon must fermentation. *There were significant differences (p<0.05) between treatments in °Brix and ethanol concentrations within yeasts and fermentation time. Bars represent standard error

The mean ethanol concentrations ranged from 1.530% to 7.545% between the treatments. As the fermentation hours progressed, ethanol concentration was significantly different (p=0.001) from each day, mainly from the early hours (24 and 48) of fermentation. The ethanol production trend directly correlated to the utilization efficiency of the sugar in the must. The maximum ethanol produced every 24 hrs interval was observed in Redstar premier classique, followed by Lalvin QA 23, Lalvin EC 111 and Redstar premier rouge. The maximum ethanol content in the Redstar premier classique yeast strain sample indicated that the yeast strain had good potential for efficient utilization of sugar (Figure 2) than the other yeast strains used. This observation confirmed that the usage of different yeast strains during fermentation contributes considerably to variations in higher alcohol profiles and concentrations in wine (Swiegers *et al*., 2005).

The rate of ethanol produced per every 24 hrs interval during the fermentation, the duration where the maximum rate of ethanol produced was between 24 and 48 hrs after that increased gradually as the fermentation progressed to the end (fig 2). The mean ethanol percentages on fermentation day 3 (7.020%) and day 4 (7.020%) were the same. Also, the mean ethanol percentages at fermentation hours of

120 (7.545%), 144 (7.545%) and 168 (7.545%) did not show any significant difference. This increasing trend in ethanol content as fermentation time progressed affirmed reports of other studies during fruit wine fermentation as well (Sharma *et al*., 2018; Jagtap and Bapat, 2015; Many *et al*., 2014; Swami *et al*., 2014; Bhatane and Pawar, 2013; Darman *et al*., 2010)

CONCLUSION

It is evidenced from the results that all the yeast strains (Lalvin EC 1118, Lalvin QA 23, Redstar star rouge and Redstar premier classique) selected for this study exhibited similar pattern of the physicochemical parameters examined. Although the Redstar premier classique produced the most desirable results, followed by Lalvin EC 1118 yeast strains, it is possible to use any of these yeast strains to produce wine from watermelon juice. Thus, transforming watermelon into wine could help salvage the perennial postharvest losses that plunges watermelon during the bumper.

AUTHORS' CONTRIBUTIONS

Linda Dari conceived the study and participated in all phases of the research. Dominic N. Najoin conducted the experiments, then collected and analysed the data.

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