



## **Performance Evaluation of Non-Saccharomyces in Wine Production**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Author DNO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author JOW managed the analyses of the study. Author AA performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

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### **ABSTRACT**

Wines with different tastes, aromas, colours and acceptability produced through the fermentative action of yeasts are consumed worldwide. Several studies have centred on the use of *Saccharomyces* sp. as the choice yeast for fermentation due to its desirable characteristics; however, in recent years, researches are being focused on alternatives to *Saccharomyces* sp. This study however was aimed at carrying out a performance evaluation of wine produced by the fermentative action of non-*Saccharomyces* (*Meyerozyma guilliermondii* and *Pichia guilliermondii*). The wines were produced using a mixture of pineapple juice and blended banana pulp as substrate. The fermentation process comprised of two set ups: one was fermented by *Meyerozyma guilliermondii* strain 1621 and the other was fermented by *Pichia guilliermondii* strain PAX-PAT 18S. An organoleptic (sensory) evaluation of both wines produced was carried out to ascertain the quality of the wines in comparison to a commercial wine. This was done using a 9-point hedonic scale rating. The wines were evaluated for their organoleptic characteristics including aroma, colour, clarity, taste and overall acceptability. The wine fermented by *Meyerozyma guilliermondii* had mean values of 7.3, 7.0, 6.4, 3.8 and 6.1 for aroma, colour, clarity, taste and overall acceptability, respectively. The wine fermented by *Pichia guilliermondii* had mean values of 6.2, 7.1, 5.5, 4.7 and 5.9 for aroma, colour, clarity, taste and overall acceptability, respectively. The

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commercial wine had mean values of 4.7, 6.6, 7.4, 7.4 and 6.5 for aroma, colour, clarity, taste and overall acceptability, respectively. Statistically, there was significant difference between the aroma of the wines ( $P \leq 0.05$ ) while there were no significant differences in colour, clarity, taste and overall acceptability of the wines. This study shows that acceptable wines can be produced using non-*Saccharomyces* yeasts like *Meyerozyma guilliermondii* and *Pichia guilliermondii*.

**Keywords:** Wine production; pineapple and banana substrates; fermentation; non-*Saccharomyces* species (*Meyerozyma guilliermondii* and *Pichia guilliermondii*).

## 1. INTRODUCTION

Wine is an alcoholic beverage produced from juices of a variety of fruits by the fermentative action of microorganisms, either spontaneously or seeding with a particular strain mainly of yeast species to adopt a particular quality of wine. In the European Union, wine is legally defined as the fermented juice of grapes. Wine can be made from virtually many plant materials that can be fermented [1]. Fruits such as banana, cucumber, grape and pineapple are used as substrates in wine production [2,3,4]. Wine making involves the use of yeast to ferment the “must” of a chosen fruit or fruits for a number of days, depending on the objective of the wine maker. Yeasts play a key role in wine production as fermenters. These yeasts can arbitrarily be divided into two categories: *Saccharomyces* and non-*Saccharomyces*. Non-*Saccharomyces* are also known as wild yeasts because they are naturally occurring yeasts in the air and are mostly found in grape vines, grape clusters and berry surfaces or other fruits used in wine making [5,6,7].

Over the decades, the dry yeast market based on *Saccharomyces cerevisiae* has allowed alcoholic fermentation to begin faster than the normal spontaneous methods, reducing the production times but non-*Saccharomyces* species have been inhibited by *Saccharomyces cerevisiae* at the industrial level, despite being the predominant species in grapes before fermentation begins [8]. However, some non-*Saccharomyces* yeasts have been reported to improve certain parameters of wine quality [9-18]. As a result of this, manufacturers are now commercializing strains of non-*Saccharomyces* from *Pichia*, *Torulasporea*, *Lachancea* and *Schizosaccharomyces* [19]. Although *S. cerevisiae* have been the choice yeasts for wine production, screening of yeasts such as *M. guilliermondii* and *P. guilliermondii*, have been reported to have the ability to breakdown sugars in a mixture of banana and pineapple substrates to produce wine [20].

*Meyerozyma guilliermondii* is often used for wine colour improvement which is because of its high hydroxyl cinnamate decarboxylase enzymatic activity [21]. This enzymatic activity allows the production of pyrano anthocyanin adducts which condensates with grape anthocyanins. *Meyerozyma guilliermondii* can increase hydroxycinnamate decarboxylase enzymatic activity up to 90% and this aids in producing wines that have up to 11-times higher concentrations of vinylphenolic pyranoanthocyanin adducts, which produces the most stable colour formed in wine making [21]. *Pichia kluyveri* produces higher levels of esters, like 2-phenylethyl acetate, by 20%, or ethyl octanoate by 10% compared to *Saccharomyces cerevisiae*. The total terpene concentration has been reported to increase by 20% and this contributed to an increase in the grape typicity [22].

Sensory evaluation is a measure taken to ascertain if a new product (food, wine) is liked by the appropriate consumers and to ensure quality assurance. This is done mostly when there is a launching or development of a new product in the market and there has been many rating scales developed for measuring the degree of likelihood of the product [23]. These scales include the labeled hedonic scale known as the LIM scale and the LAM (labeled affective magnitude) scale [24,25,26]. The nine (9) - point hedonic scale has been the most used over the years which comprises of a series of nine (9) verbal categories ranging from 1- extremely dislike to 9- extremely like.

The sensory analysis is obtained by an objective description of its attributes based on the aroma and perceived taste of the wine. Aroma is an important attribute that influences the quality of wines and it can describe the differences between lots of wines [27,28]. The aroma of wine is as a result of the biological and technological sequence such as grape destemming, crushing and pressing technology that also has an influence on the fermentation.

Knowledge about the identity of wines from specific viticultural areas is of increasing concern in the highly competitive wine industry and many studies have examined the sensory characteristics and typicality of wines. Sensory studies have contributed to better understanding of the sensory characteristics of varietal wines from emerging wine areas, such as local red wine varieties from Brazil or Cabernet Sauvignon from China [29,30]. Organoleptic evaluation of wines are determined not only by the origin of the substrate (fruit) and viticultural practices such as ripeness of the fruit at harvest, but also by the wine making process, such as the microorganisms used for the fermentation [31].

Numerous methods have been developed to define the aromatic characteristics of wines such as flavour profile, texture profile, quantitative descriptive analysis or sensory spectrum. A generalized method known as Conventional Descriptive Analysis (CDA) was derived from these methods [32]. To improve the knowledge of organoleptic characteristics of wine, this study was aimed at determining the performance evaluation of non- *Saccharomyces* (*Meyerozyma guilliermondii* and *Pichia guilliermondii*) in wine production.

## 2. MATERIALS AND METHODS

### 2.1 Sensory Evaluation by Panelists

Participants were made up of semi-trained panelists who comprised of staff and students of the Departments of Microbiology and Food Science & Technology, Rivers State University, Port Harcourt, Nigeria. The panelists were persons who had prior exposure to alcoholic beverages [11]. The panel was made up of fifteen (15) men and five (5) women who were trained on visual identification of the wines which include the colour and clarity of the wines. They were also trained for orthonasal olfactory identification of the aroma and retronasal olfaction of taste of wines.

### 2.2 Evaluated Wines

Three (3) wines were evaluated for their organoleptic characteristics. The wines contained alcohol content below 7% volume per volume (v/v). The wines were stored in bottles at a temperature of 17°C. The wines were given codes to eliminate bias during the evaluation. Two of the wines evaluated were produced by the fermentative action of *Meyerozyma guilliermondii* (coded "wine F1") and *Pichia*

*guilliermondii* (coded as "wine F3") in the laboratory, while the third was a commercially obtained wine which was coded as "wine F2".

The fermentation process for the laboratory produced wines was a period of 28 days followed by a series of racking, clarification and aging process (which was for 2 months). The wine fermented by *M. guilliermondii* and *P. guilliermondii* had a pH of 3.3 and 3.5 respectively.

The wines were served in sterile transparent cups and potable water was provided for mouth rinsing between each tasting during the evaluation session.

### 2.3 Evaluation Form

Panelists were trained on use of the evaluation form. The same form was completed for each wine evaluated. The form consisted of nine (9)-point hedonic scale ratings from 1 to 9 with 1 denoting extremely dislike and 9 denoting extremely like. The descriptors, "aroma evaluation", "colour evaluation", "clarity evaluation", "taste evaluation" and overall acceptability evaluation" were accessed.

### 2.4 Statistical Analysis

The results obtained from the evaluation of the wines were analyzed using Analysis of Variance (ANOVA).

## 3. RESULTS

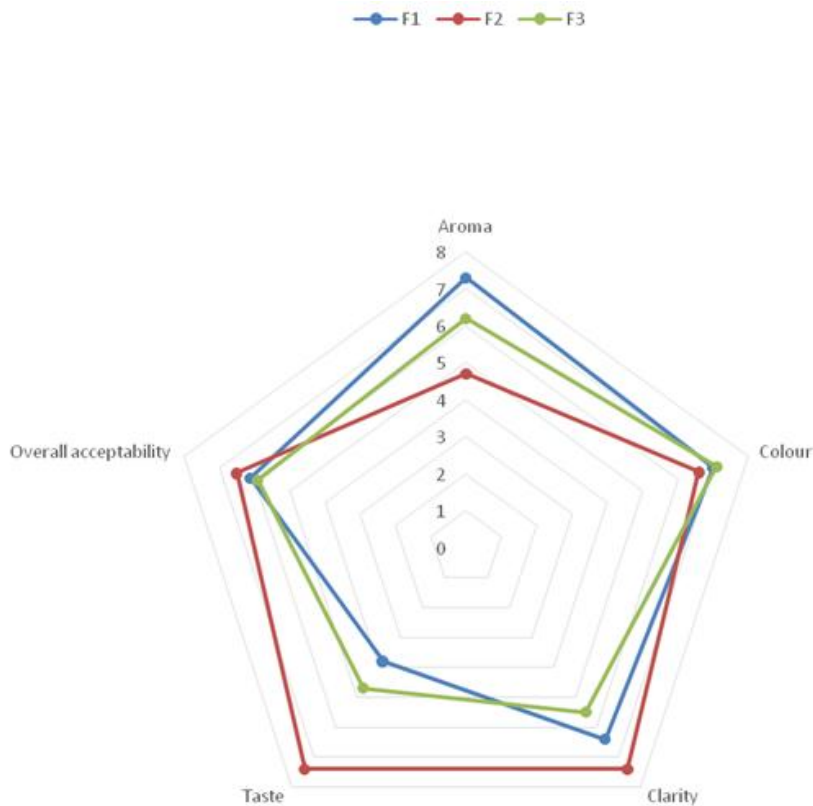
Fig. 1. shows the results obtained from the sensory evaluation of the wines produced and a commercial fruit wine which was used as a basis for comparison. The wines were evaluated for their organoleptic characteristics (aroma, colour, clarity, taste and overall acceptability).

Table 1 shows the results obtained from the sensory evaluation of the wines produced and a commercial fruit wine which was used as a basis for comparison. Statistically, there was significant difference between the aroma of the three (3) wines ( $P \leq 0.05$ ). There was no significant difference between the colours of the three (3) wines ( $P \leq 0.05$ ). There was a significant difference between the clarity of the wine produced by fermentative action of *P. guilliermondii* and the commercial fruit wine but there was however no significant difference between the wine produced by the action of *M. guilliermondii* and the commercial fruit wine; similarly there was no significant difference

between the wines produced from the two yeasts. There was no significant difference between the taste of the wine fermented by *M. guilliermondii* and the wine fermented by *P. guilliermondii* but there was significant difference between taste of the wine produced from fermentation by *M. guilliermondii*, *P. guilliermondii* and the commercial fruit wine ( $P \leq 0.05$ ). For overall acceptability, there was a significant difference between the wine produced by the fermentative action of *P. guilliermondii* and the commercial fruit wine but there was no significant difference between the laboratory-produced wines and the commercial fruit wine.

#### 4. DISCUSSION

Organoleptic (sensory) evaluation of the produced wines was carried out to ascertain their quality using a 9-point hedonic scale rating. In this study, a commercial wine was purchased and used for comparison with the wines produced in the laboratory. The wines were given codes: F1 (wine fermented by *M. guilliermondii*), F2 (commercial fruit wine) and F3 (wine fermented by *P. guilliermondii*). The wines were evaluated for their organoleptic characteristics including aroma, colour, clarity, taste and overall acceptability.



**Fig. 1. Sensory evaluation of the wines produced**

Key: Wine F1: Wine fermented by *Meyerozyma guilliermondii*  
 Wine F2: Commercial fruit wine that was used as a basis for comparison  
 Wine F3: Wine fermented by *Pichia guilliermondii*

**Table 1. Mean and standard deviation of sensory evaluation values of the wines**

Wines	Aroma	Colour	Clarity	Taste	Overall acceptability
F1	7.25±1.02 <sup>c</sup>	6.95±1.4 <sup>a</sup>	6.35±1.90 <sup>ab</sup>	3.80±2.38 <sup>a</sup>	6.110±0.83 <sup>ab</sup>
F2	4.70±0.865 <sup>a</sup>	6.60±1.31 <sup>a</sup>	7.35±1.2 <sup>b</sup>	7.40±1.14 <sup>b</sup>	6.535±0.52 <sup>b</sup>
F3	6.20±1.436 <sup>b</sup>	7.10±1.02 <sup>a</sup>	5.50±2.140 <sup>a</sup>	4.70±0.1.42 <sup>a</sup>	5.900±0.70 <sup>a</sup>

Mean with same alphabets across the group show no significant difference ( $P < 0.05$ )

The wine fermented by *M. guilliermondii* had the highest value of 7.3 for the aroma compared to the wine fermented by *P. guilliermondii* and the commercial fruit wine. Statistically, there was significant difference between the aromas of the three (3) wines ( $P \leq 0.05$ ). This is similar to the findings of [33], who reported a mean value of 5.6 for the aroma of their sweetened wine produced by the fermentative action of *S. cerevisiae* on water melon using a seven (7) point hedonic scale rating. The aroma of the wines fermented by *M. guilliermondii* and *P. guilliermondii* were preferred when compared to the commercial wine. This has been reported by [34] who stated that wines produced by non-*Saccharomyces* (*M. guilliermondii*) had fresh, floral and very good aroma. In combination with *Saccharomyces* yeasts, they have been found to add pleasant, fruity aroma, as well as enhance freshness of the wines [35].

The wine fermented by *P. guilliermondii* had the highest mean value of 7.1 for colour evaluation, compared to the wine fermented by *M. guilliermondii* and the commercial fruit wine. Statistically, there was no significant difference between colours of the three (3) wines ( $P \leq 0.05$ ). This is also similar to the findings of [33], which recorded a mean value of 5.8 for the colour of their sweetened wine using *S. cerevisiae*. The acceptability of the colour of the wines fermented by *M. guilliermondii* and *P. guilliermondii* compared to the commercial wine is in consonance with the report by [21] who stated that non-*Saccharomyces* yeast especially *M. guilliermondii* is capable of improving the colour of wines as a result of high hydroxycinnamate decarboxylase enzymatic activity which allows pyranoanthocyanin adducts (colour) production.

The commercial fruit wine had the highest mean value of 7.4 for the clarity compared to the wines fermented by *M. guilliermondii* and *P. guilliermondii*. Statistically, there was a significant difference between the clarity of the wine fermented by *P. guilliermondii* and the commercial fruit wine but there was no significant difference between the wine fermented by *M. guilliermondii* and *P. guilliermondii*; *M. guilliermondii* and the commercial fruit wine. This is similar to the findings of [33], which recorded a mean value of 5.9 for the clarity of their wine by the fermentative action of *S. cerevisiae*.

For the taste of the wines, the commercial fruit wine had the highest mean value of 7.4 compared to the wines fermented by *P.*

*guilliermondii* and *M. guilliermondii*. Statistically, there was no significant difference between the taste of the wine fermented by *M. guilliermondii* and the wine fermented by *P. guilliermondii* but there was significant difference between the tastes of the laboratory produced wines with commercial wine ( $P \leq 0.05$ ). This report is similar to the findings of [33], who recorded a mean value of 6.1 for the taste of their sweetened wine fermented by *S. cerevisiae*. The taste of the laboratory produced wines may be due to the fact that non-*Saccharomyces* (*Pichia* sp.) have been found to produce  $\beta$ -glucosidase in wine conferring properties such as low pH of 2.5-3.8 [36].

The commercial fruit wine had the highest mean value of 6.2 for the overall acceptability compared to the wines fermented by *M. guilliermondii* and *P. guilliermondii*. Statistically, there was a significant difference between the overall acceptability of the wine fermented by *P. guilliermondii* and the commercial fruit wine but there was no significant difference between the wine fermented by *M. guilliermondii* and *P. guilliermondii*; *M. guilliermondii* and the commercial fruit wine fermented by *S. cerevisiae*. This is similar to the findings of [33], which recorded a mean value of 6.1 for the overall acceptability of their sweetened wine fermented by *S. cerevisiae* using a seven (7) point hedonic scale rating. The overall acceptability of the commercial wine was higher than the laboratory produced wines largely because of the taste of the wines. This may be as a result of the duration of the aging of the laboratory produced wines (2 months). Aging of wine has been found to improve the quality of already-made wines as the activities of microorganisms persisting from the fermentation process increases [37]. According to Dharmadhikari [38], wine aging improves the flavour and taste of produced wine over time.

## 5. CONCLUSION

This study demonstrated the use of semi-trained panellists to evaluate the sensory properties of wine using a 9-point hedonic scale rating. The method provided a descriptive sensory evaluation of the intensities for the aroma, colour, clarity, taste and overall acceptability of the wines in comparison to a commercial wine. The wines fermented by *M. guilliermondii* and *P. guilliermondii* showed similarities in the parameters evaluated. On the other hand, there were some differences between the wines

produced and the commercial wine. The aroma and colour of the wines fermented by *M. guilliermondii* and *P. guilliermondii* was preferred to the commercial wine while the taste and clarity of the commercial wine was preferred to the wines fermented by *M. guilliermondii* and *P. guilliermondii*. This research carried out reveals that acceptable wines can be produced by the fermentative action of non-*Saccharomyces* yeasts.

## CONSENT

Participant consent was collected and preserved by the author.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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