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Characterization of Yeast Strains for Wine Production

Effect of Fermentation Variables on Quality of Wine Produced

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Abstract

Sixteen yeast strains isolated from grapefruit (*Citrus paradis*), orange (*Citrus sinensis*) and pineapple (*Ananas comosus*) were characterized using standard microbiological procedures. The species were identified as *Saccharomyces uvarum*, *S. cerevisiae*, *S. carlbergensis*, and *S. ellipsoideus*. Their abilities for wine production were tested by using sugar and ethanol tolerance tests. The best biochemically active strain, *S. ellipsoideus*, was used along with commercially available baker's yeast (*S. cerevisiae*) to produce wine from grapefruit, orange, and pineapple juices. After fermentation for 14 d with *S. cerevisiae* and 21 d with *S. ellipsoideus*, wines produced were compared with Baron de Valls (standard). The highest (10.47% [v/v]) and lowest (7.68% [v/v]) alcohol concentrations with corresponding residual sugar concentrations of 1.88% (w/v) and 7.7% (w/v) were produced from orange after fermentation with *S. cerevisiae* and *S. ellipsoideus*, respectively. *S. ellipsoideus* was found to be the best yeast strain producing wine with the highest acceptable score of 7.41 from orange. The study revealed the possibility of producing wine from our locally available fruits using simple, cheap, and adaptable technology with biochemically characterized yeast strains.

Index Entries: Yeast strains; fermentation; wine production.

Introduction

Wine and other alcoholic drinks are important in fulfilling social obligations such as marriage, christening, and burial ceremonies *(1)*. In Cameroon, conferences, rallies, marriages, as well as traditional and

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social gatherings are graced by a reception, and wine has become an integral part of it. Because many people have learned of its ability to prevent cardiovascular disease because of its high content of resveratrol *(2)*, and the fact that it eases digestion, the demand for wine has drastically increased. The wines available on the Cameroon market are relatively expensive. This could be attributed to the fact that it is considered ostentatious or because the raw material for wine making, *Vitis vinefera*, or the wine itself is imported. This underscores the need to look for alternatively cheap, easily available fruits and a relatively inexpensive method to produce wine of comparable quality with those in the Cameroon market.

Yeasts are the most important and extensively used microbes in the wine industry *(3)*. Depending on growth conditions, yeast can be aerobic when cultured for the cells themselves or facultatively anaerobic when cultured for their products *(3,4)*. They are found on the surface of fruits, vegetative parts of plants, and in association with insects. Nester et al. *(5)* showed that as many as 10 million yeast cells can be found on the surface of a single fruit with *Saccharomyces cerevisiae* var ellipsoideus constituting about 1%.

The presence of a mitochondrion allows yeast cells to oxidize sugars aerobically completely to CO_2 and H_2O via the citric acid cycle as the yeast grows *(4)*. In the must, yeast live in an aerobic environment, and anaerobic conditions set in when available O_2 has been utilized to start fermentation *(6)*. When anaerobic conditions set in, the citric acid cycle is shut down in favor of fermentation to produce ethanol with the evolution of CO_2. This makes yeast cells very important to brewers.

Several authors *(7–10)* have documented the potential of yeasts in the production of wine and alcohol-related beverages. In their study in Ghana, Sefa-Dedeh et al. *(10)* reported on the ability of different yeasts in the traditional brewing of Pito, a local alcoholic beverage. They isolated and characterized yeasts belonging to the genera *Saccharomyces*, *Candida*, *Kloeckera*, *Hansela*, and *Schizosaccharomyces*.

In the present study, our objectives were to isolate yeast from grapefruit (*Citrus paradis*), orange (*Citrus sinensis*) and pineapple (*Ananas comosus*); identify and characterize the yeast; and use the best biochemically active strain along with baker's yeast to produce wine from these fruits. We report herein on wine produced from these fruits and the effect of different fermentation variables on the quality of the wine.

Materials and Methods

Choice of Fruits

C. paradis, *C. sinensis*, and *A. comosus* were bought from a fruit retailer in Buea, Cameroon. These fruits were chosen because they are juicy, the juices are readily accessible, and the fruits are available throughout the year.

Isolation and Identification of Yeast Isolates

The fruits were washed with distilled water, surface sterilized with 70% alcohol, sliced, and exposed in the field for 48 h to serve as bait. Juice was aseptically extracted from the fruits and diluted to 10^{-8} with sterile distilled water. Yeasts were isolated by spread plating 0.1 mL of suitable dilutions of each sample on malt extract agar (Oxoid, England) and potato dextrose agar (Difco, Detroit, MI) acidified with tartaric acid as previously reported *(10)*. Inoculation was at 30°C for 48 h, and pure cultures were obtained using the conventional streaking method. Purified cultures were routinely maintained on malt extract slants and kept at –4°C. The isolates were subjected to various physiologic and biochemical tests, including sugar fermentation; assimilation of carbon and nitrogen compounds; urease testing; growth at 25, 30, 37, and 50°C; growth in the presence of actidione; and gelatin liquefaction. Identification was based on an established scheme *(11)*.

Processing of Must and Yeast for Fermentation

Following extraction of juice from the fruits, they were left overnight at room temperature for the pulp to settle. The supernatant (must) was collected. The sugar content of the musts was adjusted to 23% by adding table sugar *(12)*, giving a final concentration of 23% (w/v) sugar in 3 L of must (chaptalization). A double-fold chaptalization was then carried out on each fruit to obtain 6 L of each must. The isolated and confirmed yeasts used as starter cultures for fermentation were checked for biochemical activity by subjecting them to sugar and ethanol tolerance tests at different concentrations as previously reported *(9)*. The chaptalized musts were then dispensed into fermentation jars labeled O_b, O_i, G_b, G_i, P_b, and P_i. Sodium metabisulfite (3 g) was used to prevent growth of contaminants. The musts were pasteurized at 60°C for 30 min and allowed to cool at room temperature. After cooling, they were pitched with the best biochemically active strain and commercially available *S. cerevisiae* (baker's yeast) according to the labels on the jars. O, G, and P represented orange, grapefruit, and pineapple respectively, while subscripts b and i represented must pitched with baker's and isolated yeasts, respectively. Immediately after pitching, the fermentors were sealed with air-trap devices and allowed to ferment at room temperature anaerobically. Cessation of CO_2 production was used as an indicator for the end of fermentation *(12)*.

Analysis of Must Before and During Fermentation

Samples were collected at intervals from all the fermentors and analyzed for pH, titratable acidity, and yeast and bacteria counts. They were collected after 2, 4, 7, 14, and 21 d *(13)*.

End Processes in Production

Measurement of ethanol and residual sugar content, racking, clarification, pasteurization, aging, and storage constituted the end processes of production.

Ethanol and residual sugar were measured with a hydrometer. The readings were obtained in specific gravity, and actual percentage of concentration was referred from standard tables. Racking was done 24 h after cessation of CO_2 production. The "new" wines were siphoned off the lees into sterile bottles. This was done three times at 72-h intervals. After racking, the wines were filtered through silica gel using a Buchner filter and a vacuum pump. The wines were then transferred into sterile bottles and corked, pasteurized at 60°C for 30 min, and allowed to age in the dark to avoid deleterious effects of sunlight.

Sensory Evaluation of Produced Wine

A taste panel was set and panelists compared wines produced with Baron de valls, which served as the standard. Wines were compared for color, flavor, taste, and general acceptability as previously reported *(7)*. Parameters were scored on a scale of 1 to 10, based on individual appreciations.

Statistical Analysis

Analysis of variance was conducted using the Microsoft excel package to deduce if there was any significant difference at $p = 0.05$ between the parameters for wines produced and the standard.

Results

The yeast isolates were characterized on the basis of their morphologic, physiologic, and biochemical properties. Sixteen isolates identified as *Saccharomyces uvarum (4)*, *S. cerevisiae (3)*, *S. carlbergensis (3)*, and *S. ellipsoideus (5)* were obtained.

Table 1 summarizes the percentage (w/v) of sugars tolerated by the isolates. At 35% (w/v) sugar concentration, all isolates grew profusely. *S. ellipsoideus* tolerated the highest amount of sugar, 55% (w/v). All isolates did not grow at 60% (w/v) sugar concentration.

The percentage (v/v) of ethanol tolerated by the isolates was as shown in Table 2. All isolates grew well at 6% (v/v) ethanol, with *S. cerevisiae* and *S. ellipsoideus* tolerating the highest concentration of 12% (v/v). However, *S. ellipsoideus* grew better than *S. cerevisiae* at 12%. No growth was observed at 15 and 20% (v/v) ethanol.

Analysis in Course of Fermentation

Fermentation lasted 14 d when *S. cerevisiae* was used and 21 d when *S. ellipsoideus* was used to pitch musts.

Table 1
Sugar Tolerance of Yeast Isolates

Isolate[a]	Sugar concentration (% [w/v])[b] 35	40	45	50	55	60
P	+++	++	+	−	−	−
Q	+++	+++	++	+	−	−
R	+++	++	+	−	−	−
S	+++	+++	+++	+	+	−

[a]P, *S. uvarum*; Q, *S. cerevisiae*; R, *S. carlsbergensis*; S, *S. ellipsoideus*.
[b]+++, Crowded growth; ++, moderate growth; +, scanty growth; –, no growth.

Table 2
Ethanol Tolerance of Yeast Isolates

Isolate[a]	Ethanol concentration (% [v/v])[b] 6	8	10	12	15	20
P	++	+	−	−	−	−
Q	+++	++	++	+	−	−
R	+++	+	+	−	−	−
S	+++	+++	++	++	−	−

[a]P, *S. uvarum*; Q, *S. cerevisiae*; R, *S. carlsbergensis*; S, *S. ellipsoideus*.
[b]+++, Crowded growth; ++, moderate growth; +, scanty growth; –, no growth.

pH

Figure 1 shows a plot of the pH of the musts before and during fermentation. The must from pineapple had the highest pH of 4.71, falling to 3.02 and 2.99 when pitched with *S. cerevisiae* and *S. ellipsoideus*, respectively. The pH in orange must fell from 3.5 to 3.04 and 2.85 while that of grapefruit dropped from 3.28 to 2.67 and 1.71 when they were respectively pitched with *S. cerevisiae* and *S. ellipsoideus*.

Titratable Acidity (TA)

The initial titratable acidity was 0.04, 0.05, and 0.21 for grapefruit, orange, and pineapple musts, respectively. After fermentation for 2 d, the titratable acidity rose to 0.08 and 0.15 in grapefruit must pitched with *S. cerevisiae* and *S. ellipsoideus*, respectively, and then fell to 0.05 when fermentation stopped (Fig. 2). The titratable acidity for orange must rose to 0.23 in 4 d and 0.17 in 7 d after pitching with *S. cerevisiae* and *S. ellipsoideus*, respectively, and respectively fell to 0.11 and 0.08. In pineapple must, the titratable acidity reduced to 0.06 when pitched with *S. cerevisiae*; it rose to 0.33 in 4 d before falling to 0.07 by the end of fermentation when pitched with *S. ellipsoideus*.

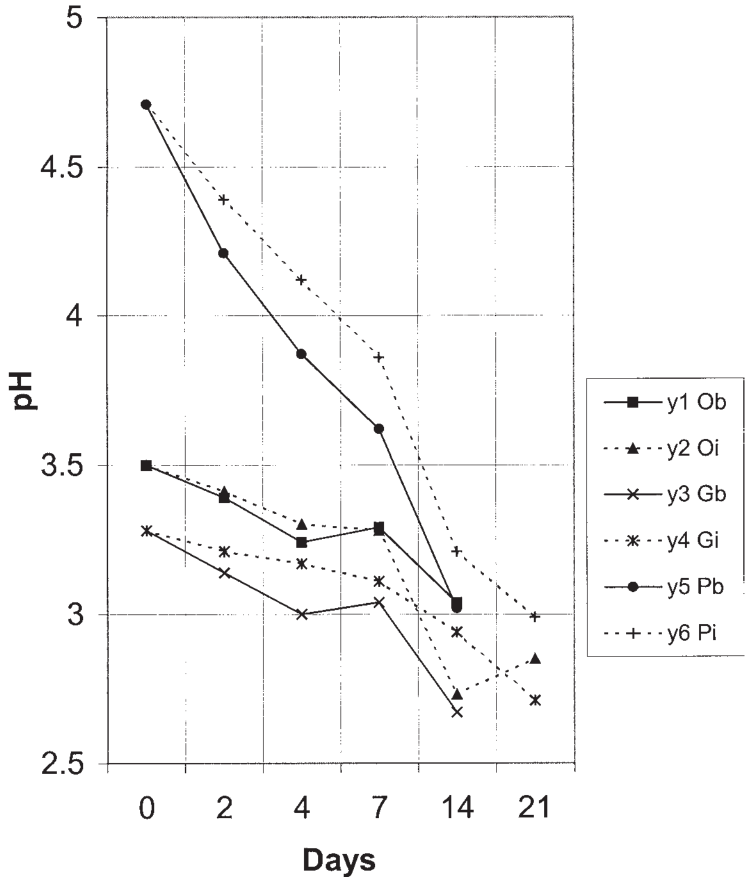


Fig. 1. Variation in pH in course of fermentation. y1, Orange wine (baker's yeast); y2, orange wine (*S. ellipsoideus*); y3, grapefruit wine (baker's yeast); y4, grapefruit wine (*S. ellipsoideus*); y5, pineapple wine (baker's yeast); y6, pineapple wine (*S. ellipsoideus*); ( ), yeast used in pitching.

Yeast Count

All yeast counts peaked after 4 d (Fig. 3), with the highest count ($\log_{10}$ = 8.08 colony-forming units [CFU]/mL) recorded in grapefruit must pitched with *S. cerevisiae* and lowest count ($\log_{10}$ = 7.92 CFU/mL) in orange must pitched with *S. ellipsoideus*. At the end of fermentation, the counts reduced with the lowest count ($\log_{10}$ = 7.73 CFU/mL) observed in pineapple must pitched with *S. ellipsoideus*, and the highest count ($\log_{10}$ = 7.97 CFU/mL) in grapefruit must pitched with *S. cerevisiae*. The values of yeast counts reduced to zero at 10^{-4} dilution after filtration and pasteurization.

Bacteria Count

The highest count ($\log_{10}$ = 8.28 CFU/mL) was observed in orange must pitched with *S. ellipsoideus* whereas the least count ($\log_{10}$ = 8.14 CFU/mL) was observed in grapefruit must pitched with *S. cerevisiae* (Fig. 4). When

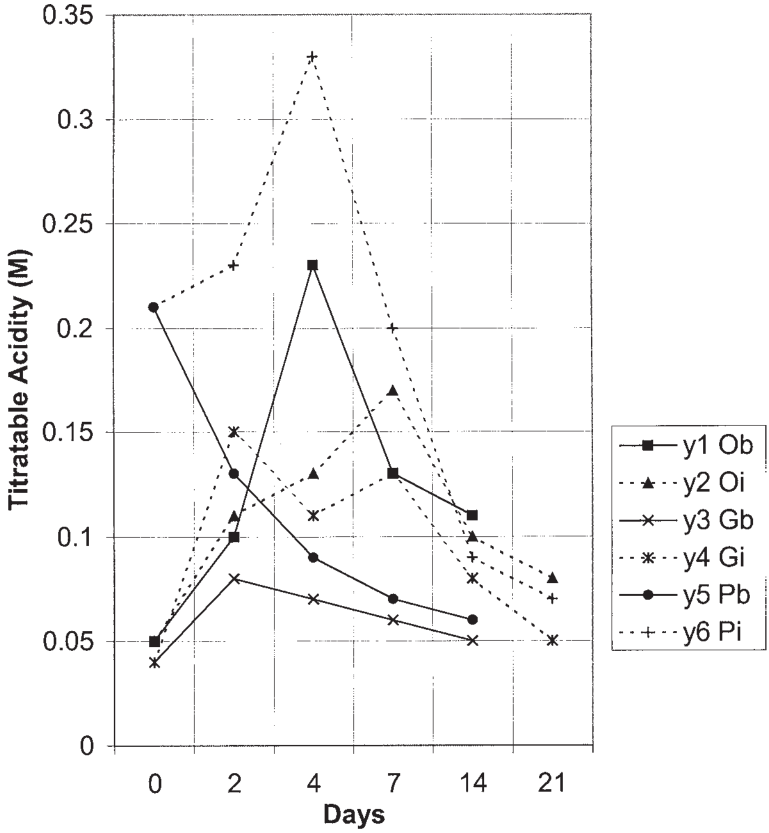


Fig. 2. Variation in titratable acidity in course of fermentation. y1, Orange wine (baker's yeast); y2, orange wine (*S. ellipsoideus*); y3, grapefruit wine (baker's yeast); y4, grapefruit wine (*S. ellipsoideus*); y5, pineapple wine (baker's yeast); y6, pineapple wine (*S. ellipsoideus*); ( ), yeast used in pitching.

fermentation stopped, the counts reduced and the least count ($\log_{10}$ = 7.23 CFU/mL) was observed in grapefruit must pitched with *S. cerevisiae* whereas the highest count ($\log_{10}$ = 7.83 CFU/mL) was observed in pineapple must pitched with *S. ellipsoideus*. The values of bacteria counts reduced to zero at 10^{-4} dilution after filtration and pasteurization.

Analysis After Fermentation

Residual Sugar and Ethanol Concentration

After production of CO_2 ceased, the residual sugar and ethanol concentrations were measured and recorded (Table 3). The highest amount of ethanol (10.47% [v/v]) and least residual sugar (1.88% [w/v]) were found in wine from orange must fermented by *S. cerevisiae* whereas the least percentage of ethanol (7.91% [v/v]) and highest residual sugar (7.22% [w/v]) was found in pineapple wine pitched with *S. ellipsoideus*.

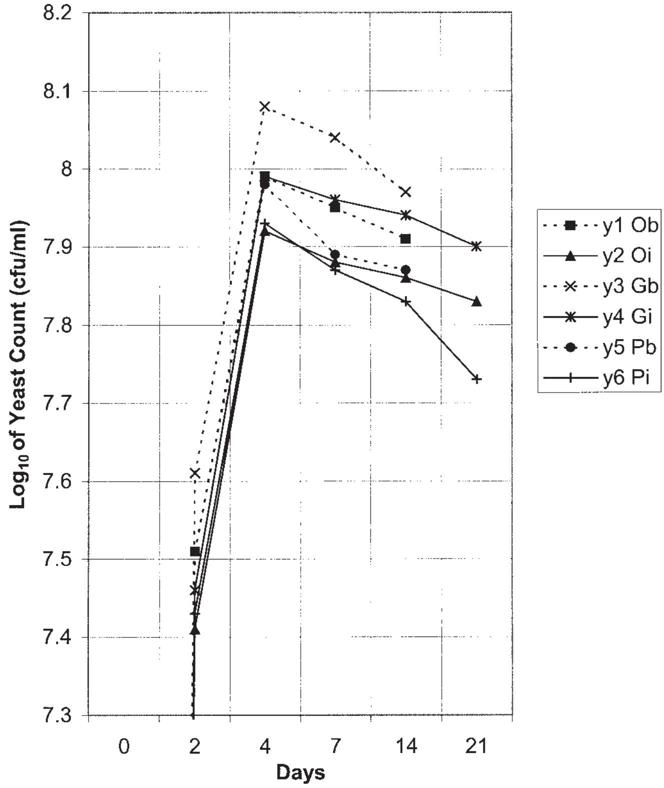
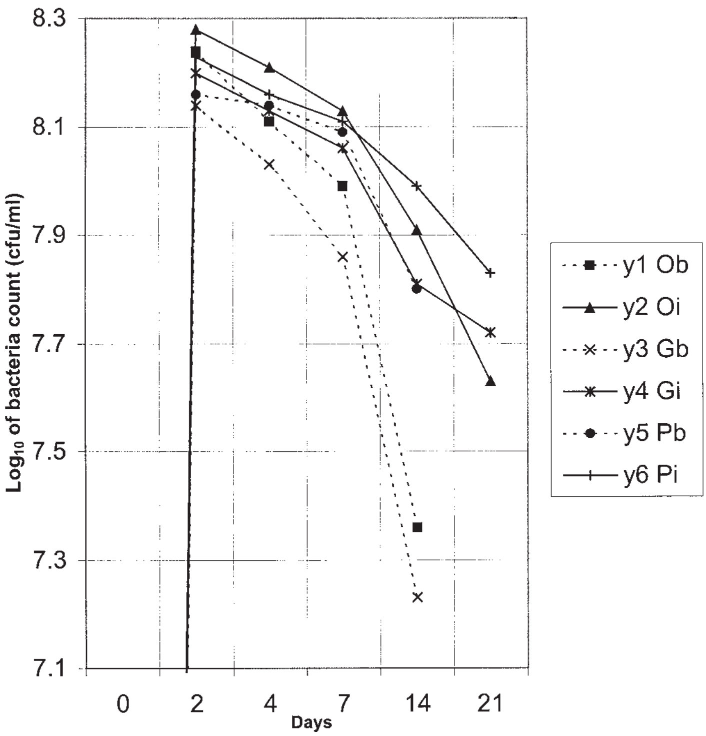

8.2
8.1
8
7.9
7.8
7.7
7.6
7.5
7.4
7.3
Log10 of Yeast Count (cfu/ml)
0
2
4
7
14
21
Days
y1 Ob
y2 Oi
y3 Gb
y4 Gi
y5 Pb
y6 Pi

Fig. 3.


8.3
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Log10 of bacteria count (cfu/ml)
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Days
y1 Ob
y2 Oi
y3 Gb
y4 Gi
y5 Pb
y6 Pi

Fig. 4.

Table 3
Residual Sugar and Ethanol Concentrations of Wines

Type of wine[a]	Residual sugar (%)	Final ethanol concentration (%)
O_b	1.88	10.47
O_i	7.7	7.68
G_b	4.1	9.41
G_i	5.3	8.83
P_b	6.5	8.26
P_i	7.22	7.91

[a]O, Orange; G, grape; P, pineapple; subscript b, baker's yeast; subscript i, isolated yeast.

Table 4
Sensory Evaluation of Wines Produced[a]

Wine sample	Color	Flavor	Taste	General acceptability
Baron de Valls (standard)	8x	7.16x	7.83x	8.16x
O_b	6.5yx	7.08xx	6.75yx	7.08xx
O_i	5.5yx	6.75xx	7.16xx	7.41xx
G_b	6yx	6.5xx	6.16yx	6.83xx
G_i	6.58yx	6.66xx	7.16xx	7.16xx
P_b	6yx	6.16xx	6.25yx	6.16yx
P_i	7.16xx	6.5xx	6.83yx	6.66yx

[a]O, Orange; G, grapefruit; P, pineapple; subscript b, baker's yeast; subscript i, isolated yeast; x, no significant difference at $p = 0.05$; y, significant difference at $p = 0.05$.

Sensory Evaluation of Produced Wine

Duly filtered and pasteurized wines were compared with a standard, Baron de valls (white wine). The mean of the results obtained is given in Table 4. The most accepted wine was orange wine, from orange must pitched with *S. ellipsoideus*, with a mean score of 7.41. The least accepted was from pineapple must pitched with *S. cerevisiae*, with a mean score of 6.16.

Fig. 3. *(previous page)* Variation in yeast count in course of fermentation. y1, Orange wine (baker's yeast); y2, orange wine (*S. ellipsoideus*); y3, grapefruit wine (baker's yeast); y4, grapefruit wine (*S. ellipsoideus*); y5, pineapple wine (baker's yeast); y6, pineapple wine (*S. ellipsoideus*); ( ), yeast used in pitching.

Fig. 4. *(previous page)* Variation in bacteria count in course of fermentation. y1, Orange wine (baker's yeast); y2, orange wine (*S. ellipsoideus*); y3, grapefruit wine (baker's yeast); y4, grapefruit wine (*S. ellipsoideus*); y5, pineapple wine (baker's yeast); y6, pineapple wine (*S. ellipsoideus*); ( ), yeast used in pitching.

Discussion

Sixteen yeast isolates were identified. The species isolated included *S. uvarum*, *S. cerevisiae*, *S. carlbergensis*, and *S. ellipsoideus*. At varying ethanol concentrations, no growth was observed at 15 and 20% (v/v) (Table 2). This finding is in line with studies by Kirsch and Szajani *(14)* demonstrating that yeast will not grow at 20% (v/v) ethanol concentrations. *S. ellipsoideus* proved to be the best isolate biochemically because it tolerated a 12% (v/v) ethanol concentration and 55% (w/v) sugar. It was then used to ferment must from orange, grapefruit, and pineapple along with baker's yeast (*S. cerevisiae*).

The decrease in the growth of yeast observed until the end of fermentation is thought to be owing to undesirable byproducts from the yeast, as suggested earlier *(15)*. Brown et al. *(16)* demonstrated that alcohol inhibits RNA and protein synthesis in yeast. A decrease in pH was also noted. This could be attributed to the fact that the fruits used in our study have malic acid *(12)*. Phaff and Amerine *(17)* demonstrated that a fall in pH indicates the production of acids during fermentation. The same reasons could be advanced to explain why the titratable acidity increased and eventually fell.

The drop in bacteria counts observed could be attributed to the fact that the pH and alcohol produced inhibited bacterial growth. Lowest counts were observed in grapefruit and orange wines pitched with *S. cerevisiae*. These wines recorded the lowest pH (2.67) and highest ethanol concentration (10.47 [v/v]), respectively. This result is in line with the findings of Eghafona et al. *(8)*, who showed that a high concentration of alcohol, a denaturating agent, is deleterious to microbes. In their study, the normal flora of pineapple when allowed to ferment pineapple must was reduced owing to the production of alcohol. The highest yield of alcohol (10.47% [v/v]) in the produced wines was obtained from orange must pitched with *S. cerevisiae* whereas *S. ellipsoideus* produced the least ethanol concentration (7.68% [v/v]) from orange must (Table 3). These wines had the least and most residual sugar concentrations of 1.88% (w/v) and 7.7% (w/v), respectively. However, studies by Sanni and Onilude *(18)* as cited by Eghafona et al. *(8)* indicated that ethanol tolerance is not directly linked to ethanol production. *S. ellipsoideus* tolerated 12% (v/v) ethanol but was incapable of producing this amount of ethanol; the highest amount it produced from grapefruit must was 8.83% (v/v).

From the results of the sensory panel (Table 4), all wines were significantly different from the standard in color, except wine from pineapple pitched with *S. ellipsoideus* ($p = 0.183$). This color was achieved because part of the lees was used as a precoat on silica gel but without a clarifying agent. The vacuum pump we used for filtration was relatively weak, and therefore filtration stopped rapidly. However, Steinkraus *(19)* stated that filtration should be combined with clarifying agents such as bentonite in order to obtain good resolutions. Since we did not use any clarifying agent, a crystal clear wine comparable with the standard was not obtained.

There was no significant difference at $p = 0.05$ between flavor of the wines produced and the standard; all p values were above 0.05: $p = 0.861$, $p = 0.380$, $p = 0.279$, $p = 0.478$, $p = 0.419$, and $p = 0.172$ for wines produced from orange, grapefruit, and pineapple pitched with baker's yeast and the best isolate, respectively. This shows that these fruits have the potential for being used to produce wine of a quality comparable with those available in the Cameroon market. When these wines were tasted, the highest mean score (7.16) was obtained from orange and grapefruit wine fermented by *S. ellipsoideus*. These wines were not significantly different in taste from the standard, with p values of 0.199 and 0.256, respectively. All other wines with relatively high mean scores (>6) were significantly different from the standard, with p values <0.05. This confirms *S. ellipsoideus* as a better wine yeast, as reported by others *(12,20)*. These wines were also readily accepted with mean scores of 7.41 and 7.16, respectively, for orange and grapefruit wine that was fermented with *S. ellipsoideus*. The wines from some fruits fermented by *S. cerevisiae* were also readily accepted ($p = 1.110$ and 0.104, respectively). Wines from pineapple, however, with relatively high acceptable scores were statistically significantly different from the standard ($p =$ 0.002 and 0.006, respectively) for must pitched with baker's yeast and our isolate. From the study, we can conclude that oranges could serve as a better fruit for wine production and that *S. ellipsoideus* is a better yeast strain for wine production than *S. cerevisiae*.

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