Aptitude of three yeast strains isolated from palm wine for citrus wine production

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Abstract

Strains Pichia kudriavzevii AR 2-32-2, Pichia manshurica RE 2-2 and Saccharomyces cerevisiae RA 1-2 isolated from palm wine were evaluated for the production of citrus wine. For this purpose, auxanography and fermentation tests from six sugars and growth kinetics were realized. None of three strains ferment lactose and S. cerevisiae was the only strains able to assimilate five sugars and fermentate efficiently glucose, fructose and sucrose. Moreover, S. cerevisiae and P. kudriavzevii showed better growth kinetic with doubling times of 3.3h and 2.88h respectively. The ability of the strains to produce citrus wine was assessed and physico-chemical parameters measured showed a decrease of total sugar content from 3.58 to 0.99%, 3.58 to 0.95% and 3.58 to 2.11% for wines produced with P. kudriavzevii, S. cerevisiae and P. manshurica respectively. For reducing sugars a decrease from 3.33 to 0.78%, 3.33 to 0.57% and 3.33 to 1.31% was observed. Concerning volatile compounds detected in wines, 12 compounds among which six aroma compounds were detected after 96h of fermentation. Sensory analysis conducted for wines produced revealed that citrus wine produced with P. manshurica RE 2-2, characterized by a sweet taste and a honey smell, was more appreciated.

Keywords: *yeast, fermentation, citrus, wine*

I. INTRODUCTION

Fruits are an excellent source of sugar, vitamins, minerals and aroma. Also, they constitute a potential substrate for production of fruit wine by fermentative processes [1]. The nutritional role of wine is important since its average contribution to total energy intake is estimated to be 10 to 20% in adult males [2]. Wine is made principally from grapes. However, several studies have investigated the suitability of other fruits as substrates for the purpose of wine production ([3], [4], [5]). In Côte d'Ivoire, there is abundance of tropical fruit such as citrus which are highly perishable and difficult to keep for considerable length of time which cause post-harvest losses of excess fruits and then, bring a loss of earnings for producers and resellers. High rate wastage of these fruits especially at their peak of production during their season necessitates the need for alternative preservation food forms towards an enhanced utilization of these fruits. The production of wines from common fruits could help reduce the level of post-harvest losses and increase variety of wines ([6], [7]). Clemente-Jimenez et al. [8] studies revealed that the fermentation of fruit juices using yeast from different sources creates variety in flavour and varying levels of alcoholic contents in wines. Thus, research of yeasts from unexplored or under-explored ecological niche as palm wine could allow to produce interesting citrus wine. Palm wine is traditional beverage resulting of palm sap fermentation by environmental microorganisms [9]. Uzochukwu et al. [10] and Wellala et al. [11] studies shown that yeasts isolated from palm wine have interesting potential for flavours production. This study is therefore aimed at investigating the potential of three yeast strains isolated from palm wine in the production of fermented citrus wine.

II. MATERIAL AND METHODS

A. Citrus juice preparation for fermentation

Materiel used in this study is citrus (*Citrus sinensis*, variety Pineapple). Citrus were purchased from local markets in the municipality of Abobo (Abidjan, Côte d'Ivoire). These fruits were thoroughly washed with clean water before peeling and deseeding. Then, fruit pulps were extracted by using a pulper and then filtered through a strainer. The resulting juice was sterilized with a 0.2µm Millipore filter (Nalgene, France).

B. Preparation of starter culture

Three yeast strains of culture collection from biotechnology and food microbiology lab of University of Nangui Abrogoua (Abidjan, Côte d'Ivoire) were used for this investigation. They were isolated from palm wine of Côte d'Ivoire and identified by PCR-RFLP molecular techniques from the rDNA region 5.8S and sequencing from the D1/D2 region. The isolated strains were identified as *Pichia kudriavzevii* AR 2-32-2, *Pichia manshurica* RE 2-2 and *Saccharomyces cerevisiae* RA 1-2. These strains were subculture onto petri dishes containing Sabouraud Chloramphenicol Agar at 30°C for 48 hours.

C. Auxanography and fermentation tests

Tests was performed using carbohydrates: D-glucose, D-galactose, D-fructose, D-lactose, D-maltose and D-sucrose. A colony of each yeasts was used to inoculate 9 ml of YP medium (1% yeast extract and 1% (w/v) bacteriological peptone) in a 30 mL tube and supplemented with each carbohydrate (2% final concentration) to be tested. A Durham tube was included in the culture medium that was then incubated at 30 °C for between 48 h and three weeks, depending on the strains. The presence of cloud in tubes indicated assimilation ability and gas in the Durham tubes indicated fermentative growth with CO2 production. The experiments were carried out in duplicate.

D. Production conditions of citrus wine

Citrus wines production from the three yeast strains were carried out in 1l coloured flasks. Culture medium was composed of 500 mL of sterile fresh citrus juice. The medium was inoculated with 105 CFU / mL of yeasts from a preculture prepared beforehand in 300 mL of sterile fresh citrus juice. Incubation was performed at 30°C for four days. At different times (T = 0h, T = 2h, T = 4h, T = 6h, T = 24h, T = 48h, T = 72h and T = 96h), 5 ml aliquots of each sample were taken for microbial counts and other analyses. The experiments were performed in duplicate.

E. Determination of growth kinetics parameters

Successive dilutions were realized from the aliquots taken during the citrus wines production. These dilutions were then spread onto Sabouraud agar with Chloramphenicol and incubated at 30 °C for 48h. Plates with valid accounts (colonies number between 15 and 150) have been enumerated in order to calculate yeast loads which were used for plotting the growth curve. According to Eqs. (1 and 2), kinetic parameters such as growth rate and doubling time were calculate.

$$\mu_{max} = \frac{(LnN1 - LnN0)}{(T_1 - T_2)} \quad (1)$$

$$g = \frac{Ln2}{\mu_{max}} \quad (2)$$

μ_{max}: maximal growth rate (h)
N_0: cell concentration at the beginning of the exponential growth phase (CFU/ml)
T_0: time of beginning of the exponential growth phase (h)
N_1: cell concentration at the end of the exponential growth phase (CFU/ml)
T_1: time at the end of the exponential growth phase (h)
g: generation time or doubling time (h).

F. Determination of total and reducing sugars

To evaluate total sugars, the phenol–sulfuric acid method was used [12]. Briefly, 0.5 ml of distilled water and 0.5 ml of 5% phenol (w/v) were added to 150 µL of sample. After shaking, 1 ml of concentrated H2SO4 (97%) was added. The mixture was left to stand for 30 min and absorbance was read at 490 nm with a spectrophotometer (Rayleigh, China) against a control (distilled water).

The quantitative estimation of reducing sugar of the wine was determined using the method described by Bernfeld [13]. 300 µL of 3.5-Dinitrosalicyclic acid (DNS) was added to 150 µL of supernatant of sample in a test tube and the mixture heated in boiling water for 5 minutes. The test tube was cooled rapidly in tap water and the volume adjusted to 2 mL with distilled water. A blank containing 150 µL of distilled and 300 µL of DNS was prepared. The optical density of the sample was read against the blank in the spectrophotometer or 540 nm absorbance. The concentration of reducing sugar in the supernatant was estimated from the glucose standard curve.

G. Analysis of volatile compounds by LLE-GC/MS

Volatile compounds were extracted by the liquid-liquid extraction (LLE) method described by Solis-Solis et al. [14]. In a bottle, 10 mL of fruits juice and 20 µl of n-octan as surrogate standard (IS) with 1.5 mL of CH2Cl2 were vigorously shaken for 5 min using a Vortex mixer in order to extract the free volatile fraction. The mixture was centrifuged at 4000 rpm for 5 min (this extraction was done twice). Watery phase was removed and the organic phase (CH2Cl2 + volatile compounds) was adjust from 1 ml to 0.1 ml in a dry flow of nitrogen and then injected (1 µl of extract) in a GC/MS system. For GC/MS conditions, analysis was performed by chromatograph (PerkinElmer, Connecticut, USA) coupled with a mass spectrometer (PerkinElmer, Connecticut, USA), operating in electronic impact ionization (EI) mode. Electron energy was set at 70 eV and ion source at 230ºC. Separations were carried out using a DB-5MS fused silica capillary column (30 m x 0.25 mm i.e., film thickness 0.5 µm). Helium was employed as the carrier gas with flow rate of 1.5 ml/min at constant flow. 1 µL of extract sample was injected in split less mode at 250°C using the following program: 60°C (2 min), then 6°C/min ramp to 240°C. The identification of volatile compounds is based on the comparison of their spectra and relative abundances using the NIST mass spectral research program (NIST/EPA/NIH database version 2.0 of May 19, 2011). The identity of the compounds was also confirmed by comparing the calculated linear retention index (LRI) with those in the NIST/EPA/NIH database.

H. Sensory analysis

A sensory evaluation test was done to determine the quality and consumer acceptance of the wines. The samples were coded. Then, the tasters were given an evaluation form. They were asked to taste one sample at a time, and record their responses

allowing time between samples so that the tasters can record their opinion. In this test, a 20-member trained panel was selected to evaluate the quality of wines obtained after 48 and 96 hours of fermentation. Five taste attributes (alcoholic, sweet, sour, sour, bitter) and five odour attributes (palm wine, orange, beer, honey and other) were used to characterize the beverages. A general assessment of the beverages produced was also conducted by the panellists using a scale of four categories.

I. Data analysis

The one-way ANOVA associated with Tukey HSD test was performed using XLSTAT 2018.5.5.52459 software to compare the means between the different beverages. The differences are considered significant for values of $P < 0.05$.

III. RESULTS

A. Carbohydrates assimilation and fermentation

None of the strains tested assimilated lactose and only *S. cerevisiae* RA 1-2 metabolized the remaining sugars. *P. kudriavzevii* AR 2-32-2 and *P. manshurica* RE 2-2 did not assimilate galactose. Regarding the fermentation profile, *S. cerevisiae* RA 1-2 showed excellent fermentation ability compared to the other species tested for glucose, fructose and sucrose (Table 1). *P. manshurica* RE 2-2 fermented the least carbohydrate. It fermented only sucrose and glucose with moderate intensity.

B. Maximum growth rate and doubling time

The three yeast strains tested showed a standard growth curve with a lower growth observed in *P. manshurica* RE 2-2 (Fig. 1). *S. cerevisiae* RA 1-2 strain had the highest growth rate (0.24 h-1) and the lowest doubling time (2.88 h). In contrast, the *P. manshurica* RE 2-2 strain showed the lowest growth rate (0.15 h-1) and the highest generation time of 4.62 h (Table 2).

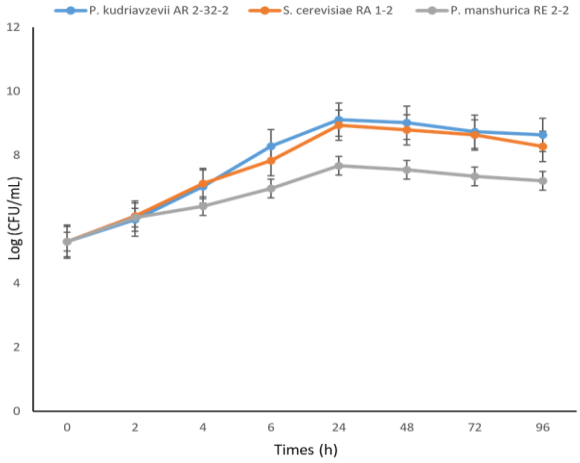


Fig. 1: Growth kinetic of yeasts during citrus wine production

TABLE 1: Sugars assimilation and fermentation profile of the three yeast strains

Strains	Galactose		Fructose		Sucrose		Glucose		Maltose		Lactose	
	F	A	F	A	F	A	F	A	F	A	F	A
P.kudriavzevii **AR 2-32-2**	-	-	+++	x	++	x	+++	x	+	x	-	-
S.cerevisiae **RA 1-2**	-	x	+++	x	+++	x	+++	x	-	x	-	-
P.manshurica **RE 2-2**	-	-	-	x	++	x	++	x	-	x	-	-

+++ = intensive fermentation, ++ = moderate fermentation, + = weak fermentation, - = no response, x = growth, F: fermentation, A: assimilation

TABLE 2: Growth kinetics parameters of yeasts tested

Strains	μ_{max} (h^{-1})	g (h)
***P. kudriavzevii* AR 2-32-2**	0,24	2,88
***S. cerevisiae* RA 1-2**	0,21	3,30
***P. manshurica* RE 2-2**	0,15	4,62

C. Total sugar content and reducing sugars

Total and reducing sugar content of the control did not change during the 96 hours of fermentation (3.58% for total sugars and 3.33% for reducing sugars). Citrus wine produced with *P. manshurica* RE 2-2 showed the highest sugar content of 2.97% and the total and reducing sugars of wine obtained was similar from 48h to 96h (Table 3a). In contrast, citrus wine obtained from *S. cerevisiae* RA 1-2 showed the lowest sugar content (1.85%) after 24 hours of fermentation and the reducing sugar content of wine obtained varied considerably from 3.33% to 0.57% after 96 hours of fermentation (Table 3b). During the 96 h of fermentation, the total sugar content of the wine produced from *P. kudriavzevii* AR 2-32-2 was significantly different but the reducing sugars content did not vary.

A total of 12 volatile organic compounds (VOCs) were identified, including three higher alcohols, one pentane, one organic acid, five esters, one terpene and one monoacylglyceride. Table 4 presents the different types of compounds that were detected after 96 hours of fermentation in each wine and the control. Isoamyl alcohol and (S)-2-methyl-1-butanol were detected in wines obtained from P. kudriavzevii AR 2-32-2 and S. cerevisiae RA 1-2 as in the control. Excepted these compounds, 3-phenylpentane, ethyl tridecanoate, D-limonene and 2-mono-linoline were detected in wine obtained with P. kudriavzevii AR 2-32-2 and ethyl oleate, ethyl palmitate and ethyl 9-hexadecenoate were detected in wine obtained with S. cerevisiae RA 1-2. For wine obtained with P. manshurica RE 2-2, only three VOCs were detected: phenylethanol, trimethoxybenzoic acid and 1-propylpentyl acrylate. Six of the 12 VOCs were aroma compounds. There were isoamyl alcohol, (S)-2-methyl-1-butanol, phenylethanol, ethyl oleate, ethyl palmitate and D-limonene.

TABLE 3a: Total sugar content (%) of citrus wines produced by the three strains at different time intervals

Strains	0 h	24 h	48 h	72 h	96 h
Control	$3,58 \pm 0,03^{a}$	$3,58 \pm 0,03^{a}$	$3,58 \pm 0,03^{a}$	$3,58 \pm 0,03^{a}$	$3,58 \pm 0,03^{a}$
***P. kudriavzevii* AR 2-32-2**	$3,58 \pm 0,03^{a}$	$2,36 \pm 0,18^{b}$	$1,86 \pm 0,06^{c}$	$1,40 \pm 0,08^{d}$	$0,99 \pm 0,15^{e}$
***S. cerevisiae* RA 1-2**	$3,58 \pm 0,03^{a}$	$1,85 \pm 0,12^{b}$	$1,37 \pm 0,23^{c}$	$1,15 \pm 0,16^{cd}$	$0,95 \pm 0,03^{d}$
***P. manshurica* RE 2-2**	$3,58 \pm 0,03^{a}$	$2,97 \pm 0,80^{a}$	$2,52 \pm 0,09^{b}$	$2,33 \pm 0,06^{b}$	$2,11 \pm 0,05^{b}$

The values are the means of three independent trials with cius wines samples ± standard deviations. On the same line, mean values with the same letter are not significantly different ($p > 0.05$). Control is fresh sterile citrus juice.

TABLE 3b: Reducing sugar content (%) of citrus wines produced by the three strains at different time intervals

Strains	0 h	24 h	48 h	72 h	96 h
Control	$3,33 \pm 0,10^{a}$	$3,33 \pm 0,10^{a}$	$3,33 \pm 0,10^{a}$	$3,33 \pm 0,10^{a}$	$3,33 \pm 0,10^{a}$
***P. kudriavzevii* AR 2-32-2**	$3,33 \pm 0,10^{a}$	$1,76 \pm 0,16^{b}$	$1,36 \pm 0,16^{b}$	$0,98 \pm 0,06^{c}$	$0,78 \pm 0,10^{c}$
***S. cerevisiae* RA 1-2**	$3,33 \pm 0,10^{a}$	$1,28 \pm 0,19^{b}$	$0,90 \pm 0,01^{bc}$	$0,75 \pm 0,26^{c}$	$0,57 \pm 0,05^{c}$
***P. manshurica* RE 2-2**	$3,33 \pm 0,10^{a}$	$2,15 \pm 0,10^{b}$	$1,72 \pm 0,17^{c}$	$1,54 \pm 0,45^{c}$	$1,31 \pm 0,10^{c}$

The values are the means of three independent trials with citrus wines samples ± standard deviations. On the same line, mean values with the same letter are not significantly different ($p > 0.05$). Control is fresh sterile citrus juice.

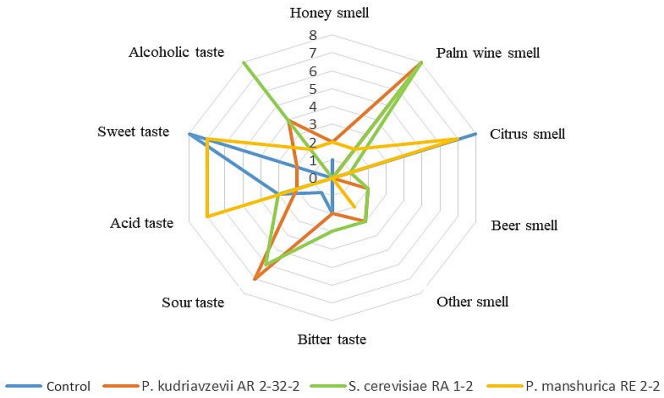
TABLE 4: Volatile compounds detected in citrus wines after 96h of culture

Volatile compounds	Control	*P. kudriavzevii* AR 2-32-2	*S. cerevisiae* RA 1-2	*P. manshurica* RE 2-2
Alcool Isoamylique	+	+	+	-
(S)-2-méthyl-butan-1-ol	+	+	+	-
Phenyléthanol	-	-	-	+
3-Phénylpentane*	-	+	-	-
Acide triméthoxybenzoique*	-	-	-	+
Éthyl 9-hexadecenoate*	-	-	+	-
Éthyl oleate	+	-	+	-
Éthyl palmitate	+	-	+	-
Éthyl tridécanoate*	-	+	-	-
1-propylpentyl acrylate*	-	-	-	+
D-Limonène	+	+	-	-
2-mono-linoléine*	-	+	-	-

+: detected ; -: not detected; *: aroma compound

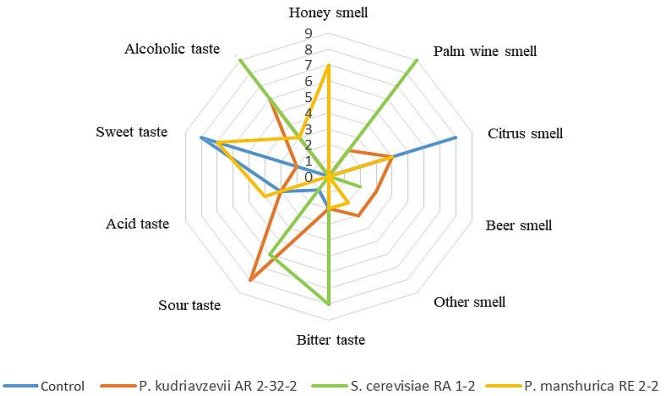
E. Sensory characteristics of wines

Sensory analysis showed that the fermented drink with *P. manshurica* RE 2-2 was sweet as well as the control. This wine was also acidic and slightly alcoholic. However, the acidic taste of this wine decreased at 96 hours of fermentation (Fig. 2b). The taste of wines produced from *P. kudriavzevii* AR 2-32-2 and *S. cerevisiae* RA 1-2 were different from that of the control and the drink produced with *P. manshurica* RE 2-2. It was sour and alcoholic at 48 hours of fermentation but much more bitter than sour at 96 hours of fermentation for wine obtained with *S. cerevisiae* RA 1-2 is (Figs. 2a and 2b). In addition, the panellists indicated that wine produced from *S. cerevisiae* RA 1-2 was more alcoholic than that produced from *P. kudriavzevii* AR 2-32-2 at 48h and 96h of fermentation. Concerning odour, wines produced from *P. kudriavzevii* AR 2-32-2 and S. cerevisiae RA 1-2 were similar after 48 hours of fermentation but different from the control. Panellists assigned this smell to that of palm wine. At 96 hours of fermentation, the smell of wine obtained with *S. cerevisiae* RA 1-2 was stronger than at 48 hours of fermentation while that of wine obtained with *P. kudriavzevii* AR 2-32-2 could not be accurately determined by the panellists. This smell closed to 44.44% of that of citrus, to 22.22% of that of palm wine and the remaining 33.34% were assigned to an undefined odour. Wine produced with *P. manshurica* RE 2-2 had a honey smell.



Numbers on the diagram represent the scale of appreciation

Fig. 2a: Radar diagram of the description of the wines (taste and smell) obtained after 48h of fermentation



Numbers on the diagram represent the scale of appreciation

Fig. 2b: Radar diagram of the description of the wines (taste and smell) obtained after 96h of fermentation

F. Wine appreciation

During the sensory analysis, the orange wines and the control were subjected to a general assessment of their organoleptic quality. The results showed that wine produced by *P. manshurica* RE 2-2 was most appreciated both at 48h and 96h of fermentation. Indeed, 50% of the panellists appreciated much more wine produced with *P. manshurica* RE 2-2 after 48 hours of fermentation and 60% after 96 hours of fermentation. These wines presented positive assessment of 90% and 100% respectively at 48h and 96h of fermentation. Only 10% of the panellists were indifferent to the taste of the wines collected after 48 hours of fermentation (Table 5a). For wines obtained with *P. kudriavzevii* AR 2-32-2, only wine obtained at 48h of fermentation was appreciated by the panellists (80%) but that of 96 h of fermentation was not appreciated. Indeed, 70% of panellists were indifferent to the organoleptic quality of the wine while 30% expressed their disgust (Table 4b). The wine produced from *S. cerevisiae* RA 1-2 was not appreciated by the panellists in general. After 48 hours of fermentation, 80% of the panellists expressed unfavourable opinion on the organoleptic quality of this wine while 20% appreciated it. Wine produced from *S. cerevisiae* RA 1-2 after 96 hours of fermentation not received a positive rating by all panellists: 70% were indifferent while 30% did not appreciate (Table 5b).

TABLE 5a: General assessment (%) of the organoleptic quality of citrus wines at 48h

Parameters	**Control**	***P. kudriavzevii* AR 2-32-2**	***S. cerevisiae* RA 1-2**	***P. manshurica* RE 2-2**
Not liked	0	10	40	0
Indifferent	20	10	40	10
Slightly liked	80	40	10	40
Really liked	0	40	10	50

Values in the table represent percentages of appreciations

TABLE 5b: General assessment (%) of the organoleptic quality of citrus wines at 96h

Parameters	**Control**	***P. kudriavzevii* AR 2-32-2**	***S. cerevisiae* RA 1-2**	***P. manshurica* RE 2-2**
Not liked	0	30	50	0
Indifferent	20	70	50	0
Slightly liked	80	0	0	40
Really liked	0	0	0	60

Values in the table represent percentages of appreciations

Iv. DISCUSSION

Yeasts are important in fermentation process and in metabolites production. In order to study their contribution to the production of fermented beverages, three yeast species (*Pichia kudriavzevii* AR 2-32-2, *Pichia manshurica* RE 2-2 and *Saccharomyces cerevisiae* RA 1-2) were studied. These yeasts have been tested for their fermentation capacity and their ability to assimilate lactose, galactose, fructose, glucose, maltose and sucrose. All three yeast species have fermented and assimilated sucrose and glucose. However, they used differently the remaining sugars. This confirms the phenotypic variability between yeast species. Similar sugar assimilation and fermentation profiles to those of the species tested in this study were observed in Khorjuvenkar's [15] works for the same yeast species as well as different profiles. The study of sugars utilization by yeast during the production of citrus wines was useful in order to predict yeasts ability to use sugars from citrus juice (mainly consisted of 34.13% sucrose, 17.94% fructose and 15.71% glucose for fresh juice).

The growth of the three yeast species during the fermentation of citrus juice that varied from one species to another with maximum growth rates ranging from 0.21h-1 to 0.24h-1. *P. kudriavzevii* AR 2-32-2 showed better growth with the lowest generation time which was 2.88h, indicating that this strain produces much more biomass than the other two yeasts. The doubling time of *S. cerevisiae* determined was 3.3h. This result is not in accordance to that of Boekhout and Robert [16] who showed a doubling time between 1.25h and 2h for *S. cerevisiae*. However, Alloue-Boraud et al. [17] found a doubling time of 2h 09s when *S. cerevisiae* yeast is grown on YPD and 5h 09s when *S. cerevisiae* is grown on barley malt extract. According to these authors, the composition of the medium, in particular the sugar content in the medium, would explain the variation in doubling time. Sensory analysis of wines after 48 and 96 hours of fermentation revealed that the beverage produced

from *P. manshurica* RE 2-2 had a sweet taste unlike the others. The total sugar content of the drink obtained with *P. manshurica* RE 2-2 (2.52% and 2.11% respectively at 48h and 96h fermentation) was closer to that of the control (3.58%) than that of the other two wines at 48h and 96h fermentation. Thus, the contents of sucrose, glucose and fructose were higher in the wine of *P. manshurica* RE 2-2 produced at 48h and 96h of fermentation, explaining the sweet taste in this wine. The total and reducing sugar contents in wines during production from the three yeasts studied varied respectively from 3.58% to 0.95% and from 3.33% to 0.57% suggesting that the sugars were metabolized by the yeasts to produce ethanol and secondary metabolites including volatile compounds. After 96 hours of fermentation, 12 volatile compounds were detected and identified. Among these compounds, Isoamyl alcohol, (S)-2-methyl-butan-1-ol, phenylethanol, ethyl 9-hexadecenoate and ethyl palmitate have been identified as compounds resulting from yeast metabolism by Rita et al. [18] and Ravasio et al. [19]. Isoamyl alcohol and (S)-2-methyl-1-butanol were not found in wine from *P. manshurica* RE 2-2 unlike the wines produced from the others strains and in the control. This could be explained by the degradation of these compounds by *P. manshurica* RE 2-2 during its metabolism. Moreover, six VOCs among the 12 VOCs detected were identified as aromas. One of these compounds, phenylethanol, has been known to produce a honey flavour [20]. This compound which was detected in wine from *P. manshurica* RE 2-2 could have contributed to the honey taste mentioned by the panellists. Beverages produced with *P. kudriavzevii* AR 2-32-2 and S. cerevisiae RA 1-2 was more alcoholic. This would be explained by the presence of a significant amount of alcohol as isoamyl alcohol which was detected only in these beverages. According to Holt et al. [20], this alcohol produces an alcohol flavour at a level above the detection limit. The citrus smell noticed by the panellists in the control and in wine from *P. kudriavzevii* AR 2-32-2 could be due to the presence of D-limonene, whose typical aroma is that of citrus [21]. In addition, the panellists gave a general assessment of the three wines produced and less appreciated the wines produced with *P. kudriavzevii* AR 2-32-2 and *S. cerevisiae* RA 1-2. This could be explained by the presence in wines of undesirable compounds (with unpleasant aromas). Another explanation is that the compounds identified would be produced at too high level which would impacted negatively wine aroma as mentioned by Belda et al. [22].

V. CONCLUSION

The objective of this work was to study the potential of three yeast species (*Pichia kudriavzevii* AR 2-32-2, *Pichia manshurica* RE 2-2 and *Saccharomyces cerevisiae* RA 1-2) isolated from palm wine in the production of citrus wine. None of the three strains tested were able to ferment lactose. *S. cerevisiae* RA 1-2 showed excellent fermentation ability for glucose, fructose and sucrose compared to the other two species studied. The microbial kinetics performed showed better growth of *P. kudriavzevii* AR 2-32-2 with the lowest generation time (2.88h). In contrast, *P. manshurica* RE 2-2 showed low growth and higher generation time. The drink produced *with S. cerevisiae* RA 1-2 had a low content of total and reducing sugars unlike that obtained with *Pichia manshurica* RE 2-2. Wine appreciated by panellists was that produced with *P. manshurica* RE 2-2 which had a sweet taste and a honey smell. *P. manshurica* RE 2-2 could be a good candidate in order to use it as stater for beverage production but detailed investigations will be required.

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