Evaluation of fermentation kinetics and ethanol yields of watermelon juice using two types of yeast

ABSTRACT

In Senegal, watermelon production has increased significantly in recent years. However, this increase is accompanied by post-harvest losses estimated at around 30%, due to a lack of value-added processing, which is mainly consumed fresh. The aim of the study was to compare the fermentation performance of two types of yeast during the alcoholic fermentation of watermelon juice (*Citruluslanatus*): a commercial strain and a strain used in the fermentation of barley-based beer. Three types of fermentation were carried out at room temperature for 07 days, with physico-chemical parameters were monitored. Comparisons were made on ethanol yield, sugar consumption, pH and total aciditychanges. Results showed significant differences between strains in terms of substrate to alcohol conversion. L_{saff} showed a higher conversion efficiency(0.69g.l.h) and a faster and higher sugar consumption (2.11g.l.h) than L_{org}. pH andacidity values during fermentation varied significantly between strains during fermentation. No alcohol was producedduring the control fermentation, despite the consumption of sugar, indicating the presence of a non-alcohol-producing native flora. These results highlight the influence of yeast strain selection on the physicochemical parameters of watermelonbasedalcoholic fermentation, demonstrating the fruit's potential as a viable substrate for value-added fermentation products.

KEYWORDS: watermelon- alcohol fermentation- yeasts- fermentation kinetics

I. INTRODUCTION

Ethanol is a metabolite that can be produced from biomass via a biochemical process, and is designed for use in various fields.The interest in ethanol production from its strategic importance as anenergy resource.Its use covers a wide range of industrial activities:

production of spirits, chemical intermediates (cosmetics, pharmaceuticals, carbonated alcohol), solvents, detergents, disinfectants, organic acids and can be a substitute for fossil energy sources (gas, petrol and diesel). It can also enablethe production of ethylene, ethyl, acrylate, acetaldehydealdehydes, ketones, carboxylic acid).This resource's production requires fermentable raw materials (fruit, cereals, lignocellulosic biomass, etc.) as well as process steps to convert this biomass into ethanol(Bakaï et al., 2024; Fossi et al., 2009; Hedible et al., 2018a; Toure et al., 2019). Fermentation is a technology that allowsconversion oforganic matter into a variety of metabolites that are used in a number of fields. Alcoholic fermentation is generating considerable interest, not only to promote local agricultural products, but also to diversify and develop fermented beverages from various substrates.

In Senegal, literature on the artisanal production of fermented beverages whose local names also « Boumakaye », « Bessoudioury », « Mbite », « Niéniébane », « Poukh » are rare or disparate. Millet, honey, fermented plant extracts (roast and palm wines, marula) and medicinal plants (*Abrusmelanospermus* and *Bosciasenegalensis*)are the principal raw materials used (Cisse, 2020). There is no local data on the use of watermelon as a matrix for artisanal production of fermented beverages, nor in the food industry. However, the cultivation of this fruit has been booming for over a decade and can be grown all year round.

In 2006, Senegal produced 225,930 tons of fresh watermelon, compared with 1,492,961 tons in 2023, of which 87% were marketed as such, with 11% consumed by households and 2% processed for sale(FAO, 2024 ; DAPSA, 2023). Post-harvest losses are around 30% and the high water content makes the fruit highly perishable(Niane et al., 2021). In order to reduce these losses, it is essential to find ways to create value. A watermelon is composedof approximately 60% flesh, of which 90% is juice that contains 7 to 10% (w/v) sugars. Thus, over 50% of watermelonisreadily fermentable liquid(Fish et al., 2009). Although it is consumed directly, or processed (drink, jam, nectar, bread made from puree and juice...), there

is a lack of valorizationand diversity in the processing of this fruit(Darman et al., 2010; Sadji et al., 2018; Soibam et al., 2016).

The aim of this study was to compare the fermentation performance of two types of yeasts one used in the production of barley beer and the other a commercial strain, for alcohol production from watermelon juice.

II. MATERIALS AND METHODS

1. Plantmaterials

Watermelons used in this study were produced locally. Ripe fruits were purchased in a market in Dakar (Senegal).

2. Biologicalmaterials

Two types of alcoholic fermentation yeasts were used in this study: commercial yeasts fromLesaffrebrand named L_{sacc} and brewer's yeasts used in barley-based beer fermentation named L_{org}. The latter were kept in Petri dishes containing Chloramphenicol Glucose Agar (CGA) culture medium at 4°C.

3. Yeast inocula

Starter cultures were prepared according to a method adapted from Gbohaida et al. (2016) by inoculating L_{org}yeast into a sterilized Erlenmeyer flask (500 ml)containing 250 ml watermelon must, then incubating at 30°C for 6 hours.L_{sacc} yeast was inoculated under the same conditions, but incubated at 30°C for 1h.

4. Watermelonjuice fermentation

Fruits were washed and peeled to extract the pulp using stainless steel knives. The seeds were removed and the flesh was transformed into juice with a juice extractor. The obtained extract was filtered through gauze to obtain a clear liquid. Three types of trials werecarried out :

- First batch served as control trial with natural fermented: juice at room temperature without pasteurization or yeast addition: P_T
- Second batch:the mustwas pasteurized at 80°C for 20min, then cooled tobelow 30°C.It was inoculated with $L_{org}$and incubated at room temperature P_{org}
- Third batch:the must was pasteurized at 80°C for 20min and inoculated with L_{sacc}: P_{sacc}

Following parameters were monitored over time:pH, brix, total acidity, alcohol content.

5. Analyticalmethods of fermentation parameters

a. Estimation of pH

pH of the must was determined by reading with pH meter*Crison*. The electrode of pH meter was inserted into the sample and the readings recorded.

b. Total acidity

Total acidity was estimated by potentiometric titration using a pH meter in accordance with method OIV-MA-AS313-01: R2009. It wasexpressed as tartaricacid in g/l.

c. Kineticmodels

In microbial biotechnology, several mathematical models are employed to describe the dynamics of growth, consumption and product kinetics. We used the Monod model to study substrate consumption and ethanol production.

Sugars content

According to Silva et al. (2007),sugar concentration has a linear relationship with brix as shown by the following equation :

Sugars (g/L) = 10.13 * (ºBrix) + 1.445 **(1)**

Brix degree is the soluble matter content and is determined using a portable refractometer.

Sugar consumption rate was calculated according to the formula proposed by Swain et al. (2007) :

$$Q_s = \frac{\text{consumed substrate(g/l)}}{\text{time (h)}} (\mathbf{2})$$

100

101 **Alcohol content**

102 During fermentation, ethanol concentration noted A was determined using the method
103 ofHedible et al. (2018a) ; Hedible et al. (2018b); Parente et al. (2014). The following equation
104 was used to determine the ethanol concentration in g/L of the sample from the alcoholmeter
105 measurement.

106 A (g/L) = $\rho_{alcohol}$ *n*10**(3)**

107 $\rho_{alcohol}$: specific gravity of ethanol 0.7895g/ml

108 A: concentration of ethanol in g/L

109 n: concentration of ethanol obtained with the alcoholmeter in %

110 Sugar-to-alcohol conversion yield and hourly ethanol productivity were calculated by the
111 following equation(Swain et al., 2007 ; Fontan et al., 2011 ; Hedible et al., 2018b;Parente et
112 al., 2014; Rorke and Kana, 2017):

$$Y_{P/S} = \frac{\text{mass of formed product (ethanol)}}{\text{mass of consumed substrate (glucose)}} (\mathbf{4})$$

113

$$Q_p = \frac{\text{mass of formed product (ethanol g/l)}}{\text{fermentation time (h)}} (\mathbf{5})$$

114

115 **6. Statistical analyses**

116 All experimentswerecarried out in triplicate and resultswereexpressed as mean ± standard
117 deviation. XLSTAT software (v2013.4.08) was used to perform statistical evaluationof the
118 different parameters. Data were subjected to an analysis of variance (ANOVA) ($p < 0.05$)
119 followed by the Fisher test for multiple comparisons.

III. RESULTS AND DISCUSSION

1. Evolution of monitored parameters

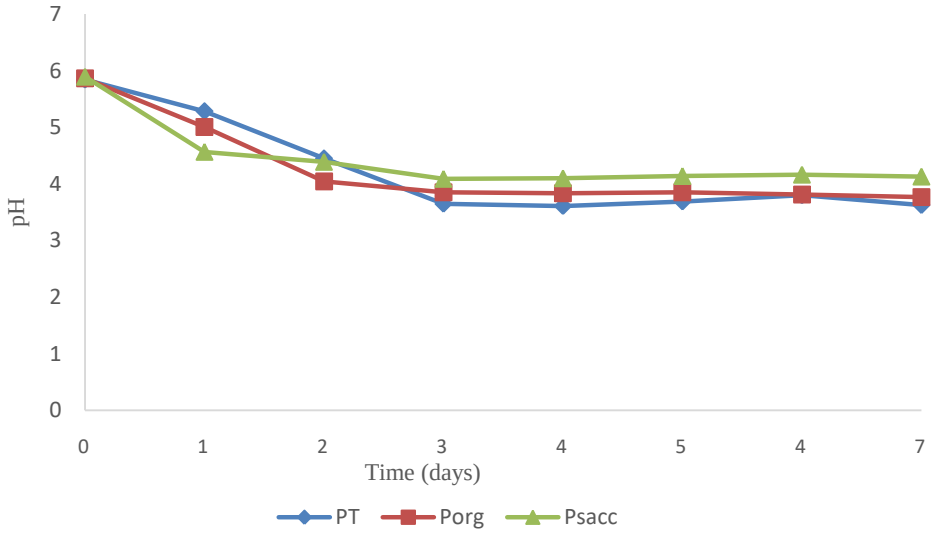

Figure 1: Evolution of pH during watermelon fermentation.
(P_T: control; P_{sacc}: fermentation with commercial yeast; P_{org}: fermentation with yeasts used in barley-based beer)

The pH variation was not significant between the three fermentations over time (figure 1). However, the final pH values revealed a substantial difference: P_T (3.62±0.06), P_{org} (3.76±0.05) and P_{sacc} (4.13±0.06). At the beginning of fermentation, the decrease was progressive from 24 hours until stabilization from the third day until the end of fermentation. This trend was found in the work of Biri et al. (2015)and these low values around 3may be explained by the formation of organic acids by microorganisms as yeasts can secrete acidic compounds into the environmentthus lowering the pH(Gbohaida et al., 2016). An acidic pH inhibits spoilage microorganisms while simultaneously promoting yeast growth, giving them a competitive advantage in the environment (Biri et al., 2015; Ojo and Eniola, 2019; Soibam et al., 2016).

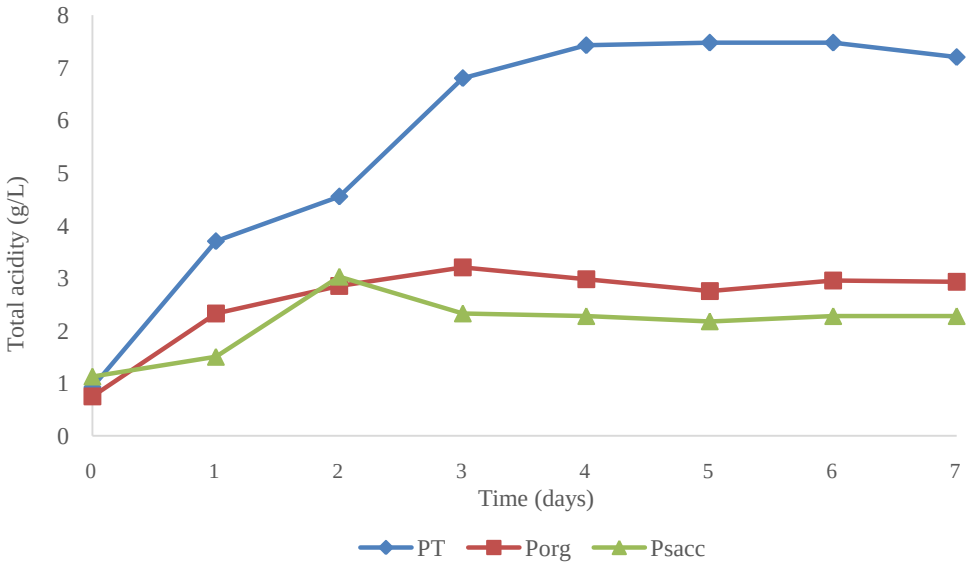

Figure 2: Variation of total acidity during watermelon fermentation.
(P_T: control; P_{sacc}: fermentation with commercial yeast; P_{org}: fermentation with yeasts used in barley-based beer)

The pH evolved in parallel with must acidity, which increased one day after inoculation and stabilized from the third day. The acidity of the musts indicated a significant difference ($P<0.0001$) between those inoculated and the P_T control (unpasteurized and uninoculated), which had higher acidity values compared to the others ($P_{sacc}$and P_{org}) during fermentation(figure 2). In fact, the total acidity obtained at the end for P_T is 7.2 g/l (0.08), whereas$P_{sacc}$and P_{org}values are 2.93 g/l (0.07) and 2.28 g/l (0.04), respectively. These inoculated musts values are lower than those found in the studies of Darman et al., (2010) and Kantiyok et al., (2021) but higher than those in the work of Najoin and Dari (2023). The control remains highwhich could be attributed to the diversity of microorganisms present in the P_T must, which has notbeen pasteurized and non-inoculated. The increase in total titratable acidity could also be due to the formation of carbon dioxide in the reaction medium. Acidity plays an essential role in the alcohol production process by facilitating fermentation and improving the overall characteristics and balance of the final product, while a lack of acidity results in poor fermentation (Hedible et al., 2018b; Ojo and Eniola, 2019).

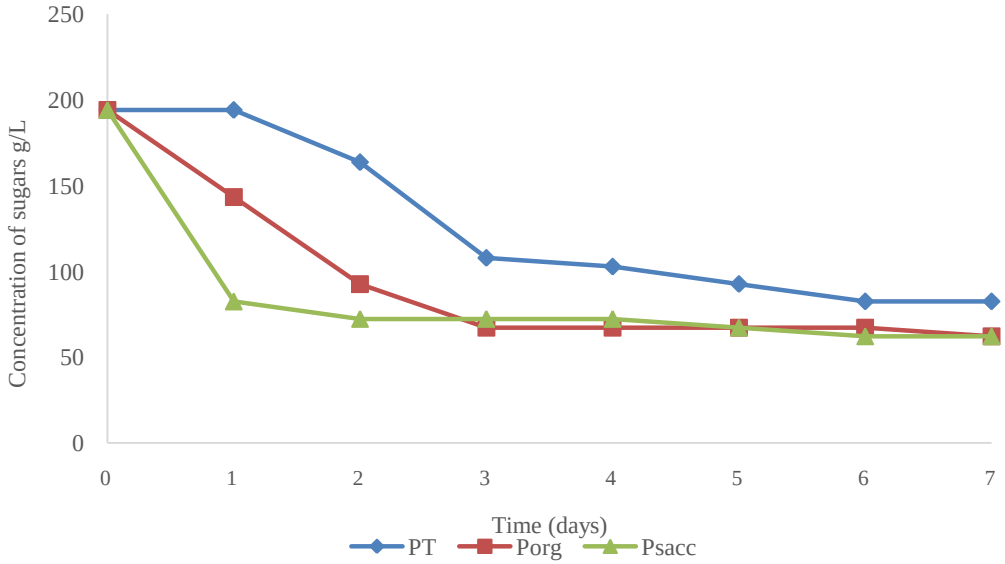

Figure 3: Sugar content during watermelon fermentation.
(P_T: control; P_{sacc}: fermentation with commercial yeast; P_{org}: fermentation with yeasts used in barley-based beer)

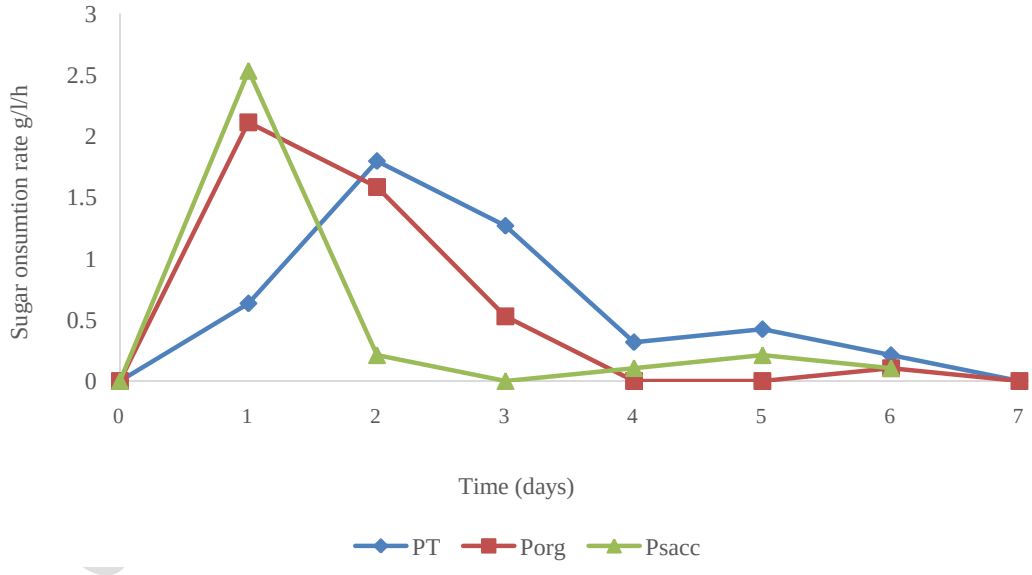

Figure 4: Rate of sugar consumption at time tduring watermelon fermentation.
(P_T: control; P_{sacc}: fermentation with commercial yeast; P_{org}: fermentation with yeasts used in barley-based beer)

Sugar consumption follows the same pattern over time for all three trials, confirming that native flora is present in the control sample (figure 3). The inoculated batches P_{org} and P_{sacc}had a significant difference in sugar content compared to the control sample over time

(P<0.0001). The P_T flora began consuming the substrate 24 hours after the start of incubation, and the consumption rate slowed down from the 3rd day, going from 1.79 g/l/h to 1.26 g/l/h and to 0.31 g/l/h on the 4th day, before stabilizing until the end. As for the $L_{org}$and L_{sacc} yeasts, they consume the substrate rapidly from the first day, with L_{sacc} having the highest rate 2.53 g/l/h, followed by L_{org} 2.11 g/l/h (figure 4). The speed slowed down and stabilized until the end and this phenomenon could correspond to the depletion of fermentable sugars in the medium or the saturation of the medium with secondary metabolites that may inhibit yeast growth or slow down their fermentation activity. The adaptation of the strains to the medium could explain the difference in sugar consumption rate between L_{org} and L_{sacc}(Hedible et al., 2018a; Hedible et al., 2018b; Toure et al., 2019).The final sugar concentrations in the different musts were 82.48 g/l for P_T and 62.25 g/l for P_{org} and P_{sacc}. The final values for the inoculated watermelon juices correspond to those obtained by Fontan et al. (2011).

2. Impact of yeasts on ethanol production

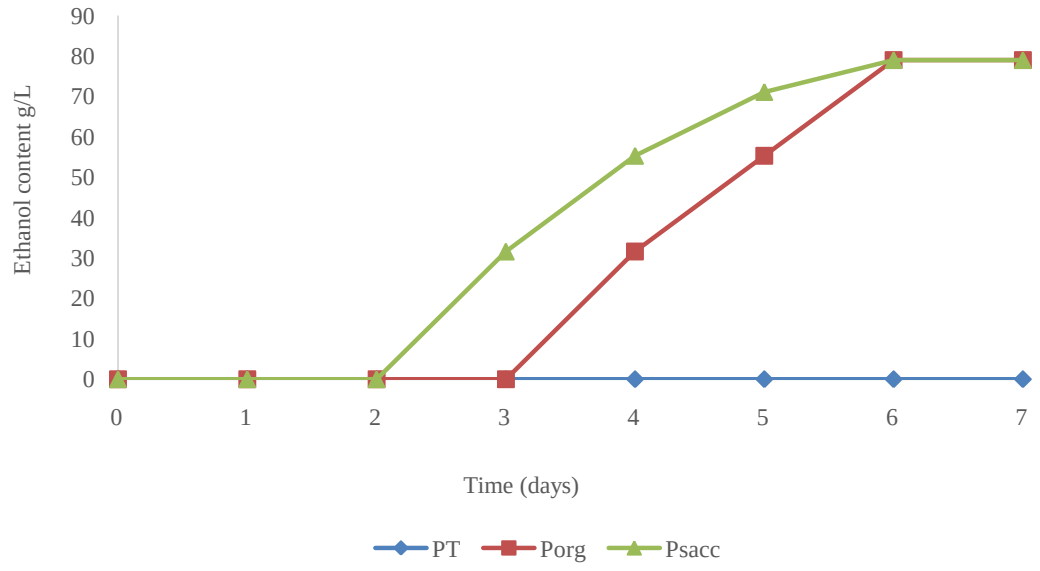

Figure 5: Evolution of ethanol content during watermelon fermentation
(P_T: control; P_{sacc}: fermentation with commercial yeast; P_{org}: fermentation with yeasts used in barley-based beer)

194 In figure 5, the lack of alcohol production in the control sample could be explained by an
195 absence of yeast cell growth, which would mean that there were no native yeasts present in
196 unpasteurized watermelon juice or that they were insufficient in number compared to other
197 flora present. Indeed, among the flora analyzed in the studies of Dania and Imadu (2023),
198 yeasts (*Saccharomyces cerevisiae*) were found in sliced watermelon sold, unlike in the work
199 of Abdulkareem and Odeh (2021) where no yeast was isolated.
200 Alcohol production was detected only in the P_{sacc} and P_{org} fermentations and was significantly
201 different between the two samples during fermentation ($P<0.011$). In fact, L_{sacc} began ethanol
202 production first (2 days later) and production stopped after 6 days. L_{org} started on the 3rd
203 dayand reached its maximum concentration on the 6th day. Alcohol yields, i.e., the conversion
204 of sugars to alcohol, are 0.62 g ethanol/g sugar and 0.59 g ethanol/g sugar for L_{sacc} and L_{org},
205 respectively. These values are higher than those of the experiments of Fish et al. (2009) ;
206 Hedible et al. (2018a) ; Hedible et al.(2018b) on watermelon alcohol production, those of the
207 studies of Silva et al. (2007) who worked on cashew apple juice, and those ofRorke and Kana
208 (2017) who used sorghum leaves as a substrate. Bakaï et al. (2024) demonstrated among the
209 seven strains used in their study, ethanol yield varied considerably depending on the genetics
210 of the strains and nitrogen availability.
211 The hourly ethanol production rates obtained from $L_{org}$and L_{sacc} are 0.65 g/l/h and 0.98 g/l/h,
212 respectively, and are higher than those obtained from watermelon fermentation by Abdel-
213 Hady et al. (2014); fermentation of *Madhucalatifolia* L. flowers carried out by Swain et al.
214 (2007). In performance studies of four commercial yeast strains used in cashew apple
215 fermentation byHedible et al. (2018a), two had higher productivity, one had lower
216 productivity than the strains in our study, and the third had a lower value than L_{sacc}but
217 exceeded L_{org}. The final alcohol concentrations obtained were 78.95 g/l, exceeding those

reported by Abdel-Hady et al. (2014) ; Bassey et al. (2022) ; Darman et al. (2010), but equal to the values obtained in the work of Fontan et al. (2011).

The variations observed in the kinetic parameters calculated for several studies could be related to several factors such as the type of yeast, temperature, nature of the substrate, and biochemical reactions during fermentation, as well as the methods of analysis (Afolalu et al., 2021; Bakaï et al., 2024; Fontan et al., 2011).

IV. CONCLUSION

This study tested three watermelon fermentation trials, one of which was spontaneous and the other two controlled with different yeasts. The ferments used showed interesting kinetic parameters, particularly for the commercial yeast L_{sacc}, with higher ethanol yield and faster consumption of sugars reflecting its adaptation to fermentations conditions. These results are very important for future modeling and simulation studies of large-scale bioethanol production from watermelon. The choice of yeast type is very important and influences fermentation kinetics, ethanol yield, and physicochemical parameters. Watermelon is a fruit that is a source of easily fermentable sugars and represents a raw material available in large quantities and has so far been unexploited for the production of ethanol-based biofuels. This fermentation process is environmentally friendly, as it does not release toxic gases. The ethanol obtained can be used as fuel or serve as an additional source of income for producers. This research highlights the potential for producing bioethanol from various underutilized raw materials such as watermelon.

COMPETING INTERESTS: The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS: All authors contributed to the work and to the preparation of the manuscript.

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