



Detection of Invertase Activity Produced by *Saccharomyces cerevisiae* as Source of Sucrose Degradation in Sugarcane Juice

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Authors' contributions

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

Article Information

DOI: 10.9734/JABB/2019/v22i330114

Editor(s):

(1) Dr. Maria Serrano, Department of Applied Biology, EPSO, University Miguel Hernández, Ctra Beniel km 3.2,
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Complete Peer review History: <http://www.sdiarticle4.com/review-history/51905>

Original Research Article

Received 02 August 2019
Accepted 07 October 2019
Published 16 October 2019

ABSTRACT

The decline in purity of sugarcane juice used for the production of sucrose leads to huge losses in food processing chains. However, this sugar is much consumed by the population. Knowledge of the degradation origin of sucrose in the process of transformation is therefore necessary. It was more precisely a matter of assessing the quality of four sugarcane (*Saccharum officinarum*) juice of different varieties (R 570, R 590, Co 997 and SP 711406) by determining their physicochemical parameters and by highlighting the presence of *Saccharomyces cerevisiae* and invertase activity produced. These analyses were conducted in the Agrovalorization laboratory of the UFR Agroforesterie; University Jean Lorougnon Guédé, between November 2018 and February 2019. Thus, all juices are acidic (pH 5.05 ± 0.13) with average dry matter and sugar contents of 16.70 ± 1.04 and 59.03 ± 5.02 , respectively, with favorable conditions for yeasts proliferation. In addition, *S. cerevisiae* was found in three juices (R 590, Co 997 and SP 711406) with a specific invertase activity of $0.42 \cdot 10^{-2}$ IU / mg protein. This activity would be the basis of the degradation of sucrose in

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crude sugarcane juice. On the other hand, the juice resulting from the variety R 570 contained germ deprived of invertase activity ($1.57 \cdot 10^{-5}$ IU / mg of proteins). Its use would be a good source of crude materials for optimizing sugar production.

Keywords: *Sugarcane; Saccharomyces cerevisiae; sucrose degradation; invertase.*

1. INTRODUCTION

Sugarcane stems contain a sweet juice from which sucrose or crystallizable sugar is extracted. Their industrial extraction, from certain plants, makes it possible to obtain large quantities of sucrose and starch intended mainly for human consumption. These raw materials are increasingly considered as basic materials for the chemical industry in agrosources [1]. To cover the needs of the population, the production of sugar requires a follow-up of the extraction process, which starts from the sugarcane cutting to the storage of the finished product. The sugar production consists essentially for separating pure sucrose from different materials make up sugarcane juice. The extraction of this juice by crushing the sugarcane and its concentration constitute the two main stages [2]. Nadia & Khwaja [3] stated the performance of a sugar industry depends both on the technological quality of the sugarcane, particularly the purity and quality of technological process. However, in recent years, sugar industries are facing a drop in purity or a loss of sucrose at different juices used from grinding station (extraction) to treatment plant and evaporation. Despite the industrial means and methods used (physical, biochemical), the results are still unsatisfactory. In addition, studies have shown that as soon as sugarcane is harvested, bacteria cause acidification of the product by transforming sugars and storage substances (starch, cellulose) [4]. This acidification favors a biological and chemical phenomenon in the presence of invertases widely distributed in the biosphere and mainly isolated from sugarcane (*S. officinarum*) and micro-organisms such as *Saccharomyces cerevisiae* [5,6]. It is therefore imperative to determine if the loss of sucrose is due to the invertase activity of the yeast *S. cerevisiae* present in the juices and also to determine the characteristics of the juices of different varieties of sugarcane used. The objective of this study is to characterize sugarcane juice (*S. officinarum*) from Zuénoula (Côte d'Ivoire) in order to determine the origin of the decrease in sucrose production. Specifically, we will determine the physicochemical parameters of juice, check the presence of *S. cerevisiae* in

each sugarcane juice and highlight their invertase activity.

2. MATERIALS AND METHODS

Sugarcane juice extract ready for characterization is obtained from 500 g of fibers of 4 varieties of sugarcane (R 590, Co 997, SP 711406 and R 579) from Zuénoula. These fibers were introduced into the hydraulic press after removal of the leaves; cane stalks, cutting and reduction of sugarcane slices using a grinder (EFFCO) and filtration on a filter paper.

pH measurement is made from extract using a pH meter (pHs-38w). Dry matter content or brix degree is determined according to the method described by ICUMSA [7] using the refractometer thermostated at 20°C. Measurement of the sugar content of a solution (polarization) is carried out according to the method described by ICUMSA [7] using the polarimeter (POLASER-S/R.E.I.) thermostated at 20°C. Purity is the amount of sucrose in 100 g of dry matter [8].

For the detection of *Saccharomyces cerevisiae* in juices, isolation techniques followed by the identification of *S. cerevisiae* were carried out using different sugarcane juices. The isolation and purification of the different colonies were performed by streaking in solid medium on the surface of the agar (Sabouraud dextrose) at 30°C incubation. The macroscopic and microscopic observations of the cultures were made after 48 h and 72 h of incubation, respectively. Isolated strains from juices were identified with that of *Saccharomyces cerevisiae* using the method described by Barnett et al. [9].

For the measurement of the invertase activity of each purified strain, the crude enzyme extract was obtained according to the method described by Tek et al. [10] followed by sonication for 15 min and centrifugation at 12000 rpm at 4°C for 30 min. The reducing sugars were determined according to the method described by Bernfeld [11] in the presence of a reaction medium consisting of 0.1 ml of enzymatic crude extract and 0.2 ml of sucrose prepared in citrate phosphate buffer (0.1 M - pH 6). This medium is incubated at 37°C. for 30 min. Reaction was

stopped by adding 0.3 ml of dinitrosalicylic acid reagent (DNS). The new medium was then homogenized and heated on a steam bath for 5 min and then cooled during 10 min at room temperature (25°C). Absorbance was measured with a spectrophotometer (Gilson) at 540 nm. This absorbance was then converted into micromoles of reducing sugars by means of a calibration line obtained using a glucose solution (2 mg/ml). Specific activity was expressed in μmole of reducing sugars released per min and per mg of protein or IU/mg of protein assayed according to the method of Lowry et al. [12].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Physicochemical characteristics from juices

Physicochemical characteristics of sugarcane juices vary according to the variety grown. pH varies between 4.9 and 5.2 with an average value of 5.05 ± 0.13 . Thus, pH values are respectively 4.9 for R 570, 5.2 for Co 997, 5.1 for SP 711406 and 5 for R 579. Brix values of the R 570, R 579, Co 997 and SP 711406 varieties are respectively 16.77%, 15.24%, 17.15% and 17.65%. Results reveal different dry matter contents by variety with a maximum of 17.65% for SP 711406. This explains a purity of 89.57% in this variety (Table 1).

3.1.2 Detection of *Saccharomyces cerevisiae* from juices

For the detection of *Saccharomyces cerevisiae* the cultivation of microorganisms from sugarcane

juice on Sabouraud dextrose medium revealed the presence of 3 yeast strains (yellow_1, white_2 and pink_3) (Fig. 1). Strain 1 had a smooth (curved and round) shape of bright yellowish cream color with no pigment and odor felt in Petri dish (Fig. 1A). The strain 2 had a smooth (curved and round) shape of bright whitish cream color with absence of pigment and odor felt (Fig. 1B, 1C and 1D). As for the strain 3, it had a round shape with a relatively pink cream color, an absence of pigment and an odor felt (Fig. 1B). The crude juice of R 570 consisted only of the yellow strain (Fig. 1A). The yellow and white strains proliferated in juices of SP 711406 and R 579 varieties (Fig. 1C and 1D). As for Co 997, it presented the three yeast strains (Fig. 1B).

Microscopic observation of the appearance of colonies of the white strain (Fig. 2A) revealed a similarity with *S. cerevisiae* reference (Fig. 2B). Thus confirming its presence in the juice of the three of sugarcane varieties (Co 997, R 579 and SP 711406).

3.1.3 Demonstration of invertase activity of isolated strains

For the demonstration of invertase activity of isolated strains, the brick-red coloring in the tubes revealed its presence in crude enzyme extracts of white strain (Fig. 3A). However, an absence of coloration is observed respectively in the yellow and pink strains (Fig. 3B and 3C). The white strain had the highest invertase activity ($0.42 \cdot 10^{-2}$ IU/mg relative to the others (yellow and pink) confirming the presence of *S. cerevisiae* in these juices (Table 2).

Table 1. Physicochemical characteristics from juices of four sugarcane varieties

Sugarcane varieties	pH	Brix (%)	Polarisation (%)	Purity (%)
R 570	4.90	16.77	55.23	85.48
Co 997	5.20	17.15	61.53	87.41
SP 711406	5.10	17.65	64.88	89.57
R 579	5.00	15.24	54.49	87.92
Average	5.05 ± 0.13	16.70 ± 1.04	59.03 ± 5.02	87.60 ± 1.68

Table 2. Invertase activities of isolated strains from sugarcane juice

Yeast strain	Enzymatic extract (ml)	Protein (mg)	Relative activity ($\mu\text{mol}/\text{min}$ ou UI)	Specific activity (UI/mg)
White strain (WS)*	0.1	76.47	0.32	$0.42 \cdot 10^{-2}$
Yellow strain (YS)	0.1	255	$0.36 \cdot 10^{-2}$	$1.57 \cdot 10^{-5}$
Pink strain (PS)	0.1	80	$0.18 \cdot 10^{-2}$	$0.25 \cdot 10^{-4}$

* Strong Activity from white strain

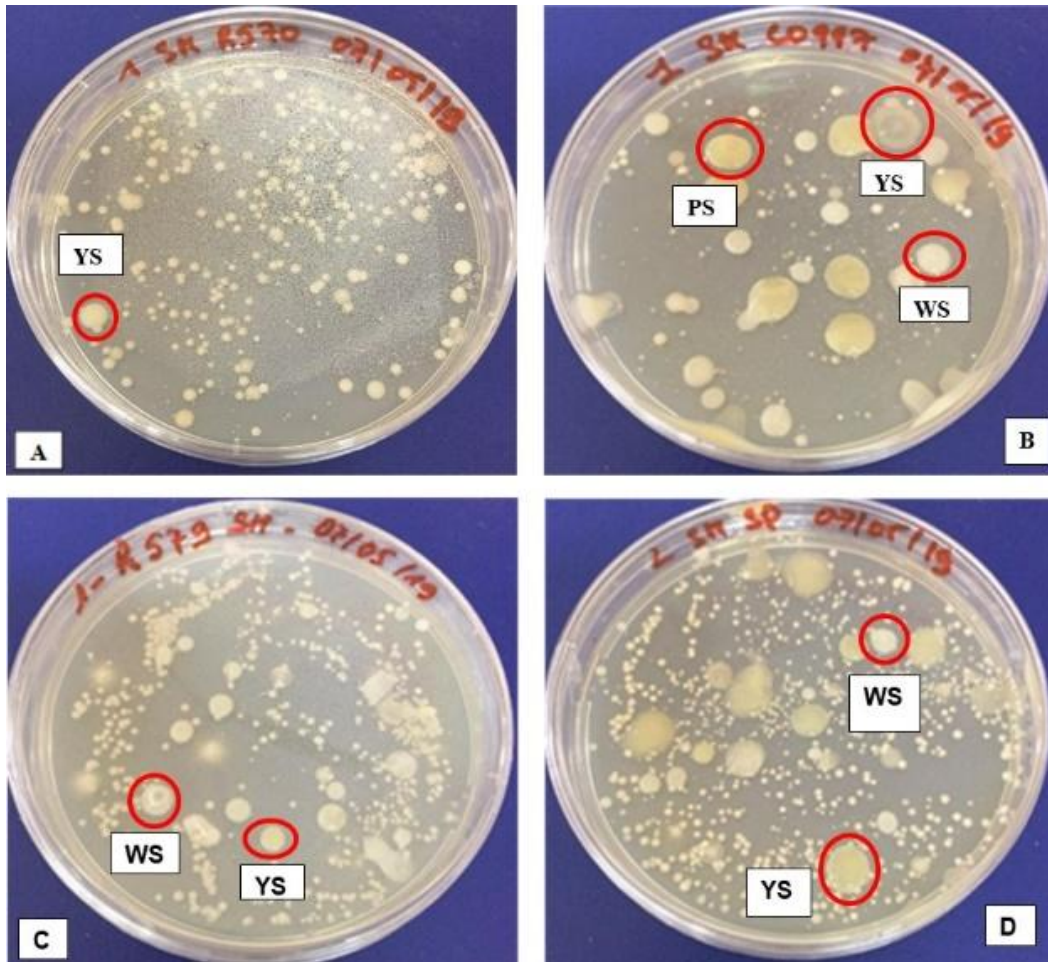


Fig. 1. Cultural aspects of microorganisms from juice of the four sugarcane varieties
A: R 570 variety; B: Co 997 variety; C: R 579 variety; D: SP 711406 variety, WS: white strain;
YS: yellow strain, PS: Pink strain

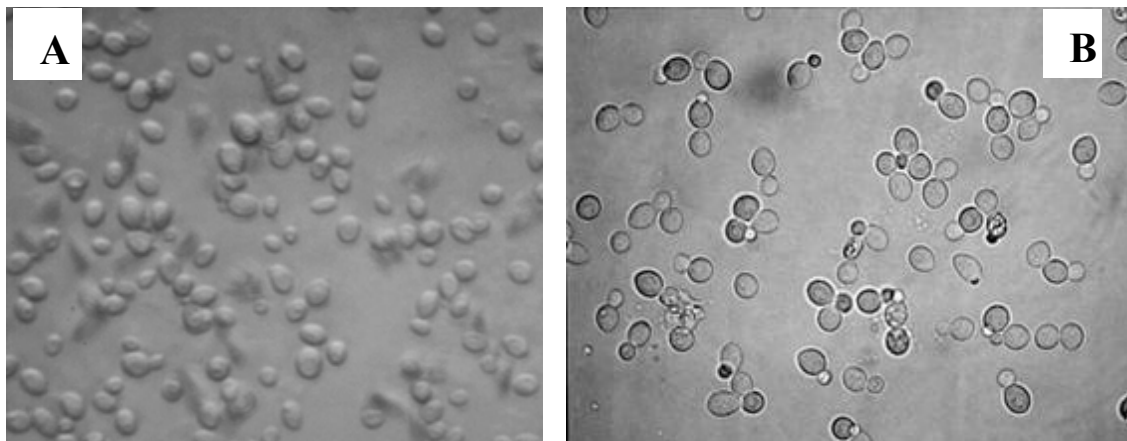


Fig. 2. Microscopic appearance of *S. cerevisiae* yeasts
A: White strain isolated from sugarcane juice; B: *S. cerevisiae* strain reference

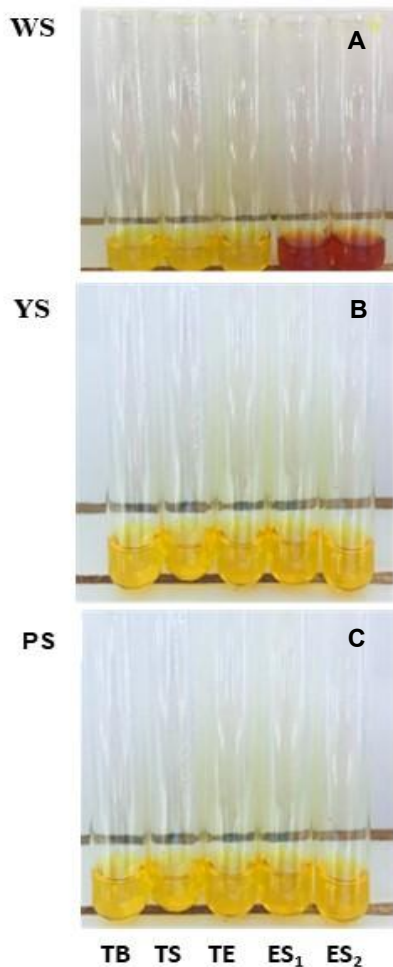


Fig. 3. Demonstration of the invertase activity in the crude extracts from the strains

WS: Reaction medium of the white strain; YS: Reaction medium of the yellow strain and PS: Reaction medium of the pink strain. From the left to the right the tubes are represented by TB: control containing neither sucrose nor enzymatic extract; TS: substrate control; TE: enzymatic extract control; ES₁: Tests 1 and 2 containing the substrate and the enzyme extract

3.2 Discussion

The acidity ($4.9 \leq \text{pH} \leq 5.2$) of the juices and the high sucrose content could constitute a favorable environment for the proliferation of certain molds and yeasts [13,14]. Most bacteria do not develop on this medium, which allows us to evaluate the technological quality of juices, confirming those obtained by Schmidt et al. [15], according to which the average pH of sugarcane was 5.6, but the accentuated and variable acidity of varieties would be influenced by the peculiarity of

the cultivated varieties as mentioned by Plancher [16]. Chriki et al. [17] found an average pH value (6.5 ± 0.24) on some sugarcane varieties, while the average brix value of 16.4% on other varieties quantity of free water available in sugarcane juice that could promote the development of certain microorganisms as highlighted by Monroe [18]. Their multiplication could increase the rate of inversion [19]. In addition, Chriki et al. [17] evaluated brix of some sugarcane varieties different from ours and noted an average value of 21.38 ± 2.38 . This difference could be due to the nature of the juices as reported by Plancher [16], but also to edaphic conditions [20]. Sugarcane had a good sucrose content, when it was freshly cut. An average purity of 87.60 ± 1.68 is noted. This assertion is confirmed by the results of Touré [2] obtaining an average of 88.15%, after an analysis from eleven varieties. Raivire [20] also obtained an average value of 89.40% purity in some varieties of sugarcane juice. On the other hand, Péné & Kéhé [1] got an average of 79.9% purity of twelve varieties of sugarcane. This variability of purity may be a function of soil types, varieties, age, and growing conditions [21]. According to Caro [22], the contact of soil and straw would favor yeasts contamination. According to this author, these microorganisms penetrate through the wounded stem part. A study showed that microorganisms grew 6 cm per hour in the sugarcane stem [22]. To know the origin of this contamination, it must be emphasized that after cutting the sugarcane are cleared leaves. Thus, the tissues would be invaded by various yeasts that are naturally found in the atmosphere and on the bark of the sugarcane [18]. Kim et al. [23] extracted the enzymes from *Pisum sativum* in a medium also containing ammonium sulfate as extraction reagent. The highlighting of the invertase activity by color observation was used by Benmedjahed & Bengrine [24]. Indeed, the invertases or β -Fructofuranosidases (EC 3.2.1.26) belong to the family of GH32 glycoside hydrolases which catalyze the hydrolysis of sucrose to give an equimolar mixture of monosaccharide D-glucose and D-fructose called invert sugar. According to these authors, in an alkaline medium, in the presence of heat and in the presence of reducing sugars, 3,5 dinitrosalicylic acid is reduced to 3-amino-5-nitrosalicylic acid conferring the yellow-orange color. This method has also been used to test the activity of invertase produced by microorganisms (respectively *Aspergillus caespitosus*, *P. sativum* and *S. cerevisiae*) [23,10]. It is therefore likely that the enzyme contained in the extract is an

invertase. Similarly, physicochemical parameters such as pH, brix and purity of juice studied could promote invertase activity. Kim et al. [23] obtained a specific invertase activity of 0.03 IU/mg from *P. sativum* under the same operating conditions as ours. However, this of *S. cerevisiae* of studied sugarcane juice is $0.42 \cdot 10^{-2}$ IU / mg. Tek et al. [10] obtained a higher specific activity ($17.8 \text{ IU} \cdot \text{mg}^{-1}$) of the invertase produced by *S. cerevisiae*. In addition, invertase is widely used in food industries (confectionery) for the production of high fructose sugar syrup from sucrose [25].

4. CONCLUSION

The acidity of the juice as well as the high sucrose content therefore constitute a favorable environment for the proliferation of *S. cerevisiae*, responsible for the invertase activity in the juice of the three varieties (R 579, SP 711406 and Co 997) of sugarcane. This activity is therefore at the origin of the decrease in the production of sucrose.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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