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Biofuel Potentiality of Pineapple Peelings in the Presence of the Yeasts *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*

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Abstract: In pineapple industries and processing units, peelings constitute waste piled up in landfills and therefore cause real problems for environment. The present study aims to develop this available and neglected bioresource, through the study of the kinetics of its conversion into bioethanol by fermentation with a view to its use as a biofuel. To do this, the pineapple peelings juice was converted into bioethanol by fermentation in the presence of the yeasts: *S. cerevisiae* and *S. carlsbergensis*. Monitoring of fermentation kinetic parameters such as Brix degree, pH, titratable acidity and density, shows a great variability of these parameters during the fermentation process in bioreactors. The distillation of the musts at the end of fermentation made it possible to obtain ethanol levels (% v/v at 20°C) between (2.77 ± 0.03%) and (28.69 ± 0.03%). The best ethanolic bioconversion performance was recorded with the yeast *S. carlsbergensis* on the must enriched with urea (CON_2H_4) followed by the strain (S3) of the yeast S. cerevisiae. Analysis of the results shows that the alcoholic degrees of the different distillates depend on the type of microorganism as well as whether or not growth factor added to the fermentation musts. It appears that the addition of a selected strain especially in the presence of growth factor promotes the kinetics of the alcoholic fermentation process, thus leading to a better yield of ethanol production. Production of ethanol from agricultural and biodegradable waste would also provide a viable solution to environmental problems creating a sink for waste and renewable energy production as well.

Keywords: Bioethanol, Pineapple Peelings, Fermentation Kinetics, Yeasts, Urea

1. Introduction

Nowadays, crude oil reserves and refining capacities are limited. In addition, the worrying degradation of the environment offers excellent prospects for bioethanol [10]. Bioethanol has a number of advantages over conventional fuel. It comes from renewable resources that are crops [28]. Although second-generation bioethanol obtained from lignocellulosic biomass is recognized as a promising alternative source of energy, ineffective pretreatment pathways and the high cost of enzymatic hydrolysis are the main causes hindering the commercialization of bioethanol

[1]. Accordingly, attempts to produce bioethanol are often oriented towards inedible agroforestry resources rich in sugars [16]. Fruits and by-products of the fruit processing industry are obtained each year in significant quantities all over the world [21]. Pineapple is a very perishable product because of its high water content, rich in fermentable sugars and generates 40 to 50% of waste during processing [23]. The government of Benin, in its development policy for the agricultural sub-sector, has for several years oriented its strategy towards the development of the pineapple sector through the establishment of processing plants in different production areas of this fruit in Benin. Unfortunately this activity's by-products are not recycled and are often piled up in illegal dumps located in the vicinity of processing centers [3]. Fruit waste like pineapple peelings containing fermentable sugars should no longer be abandoned in our environment. But they should be converted into useful products like bioethanol [14]. Nadzirah and al. [18] found that sucrose was the most important sugar present in pineapple waste extract. Indeed, the possibilities of energy recovery from biomass by biotechnological processes represent a solution of choice for the use of agricultural products with low commercial value, crop residues and agro-industrial waste [26]. The ethanol production from agricultural and biodegradable waste also provides a viable solution to environmental problems creating a sink for waste and renewable energy production [22]. The new strategies' setting up for the bioconversion of this waste into bioethanol would undoubtedly contribute to improving the income of producers and to solving environmental and public health problems [11]. Likewise, the promotion of biofuels obtained from neglected biomass contributes to reducing countries energy dependency. It therefore creates new agricultural sectors and could offer new promising niches for farmers, especially in developing countries [2].

In addition, the interest in producing bioethanol stems from the fact that it is a strategic energy substance whose use also covers a wide range of industrial activities (detergents, solvents, disinfectants, pharmaceuticals and cosmetics, etc.). The results of this study will generate interest in investing in the field. Despite its direct use as a fuel in specialized engines, it is also now possible to blend bioethanol with standard gasoline, at a reasonable rate (5-10%) without having to change the conventional engine. Ethyl Tertio Buthyl Ether (ETBE), another type of fuel synthesizable from 49% bioethanol and 51% isobuthylene can also be incorporated in gasoline at a proportion of 15% [8]. In this perspective, the present work proposes to evaluate the biofuel potentiality of pineapple peelings in the presence of the yeasts *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*.

2. Material and Methods

2.1. Vegetable Material Sampling and Juice Extraction

Pineapple peels were collected from pineapple sellers in Abomey-Calavi in southern Benin. They were processed and the collected juices were kept in the freezer at 4±1°C while waiting to be used.

2.2. Microorganisms and Inoculum Preparation

Four strains of yeast (non-pathogenic to humans) including three lyophilized (S1, S2 and S3) of the genus *Saccharomyces cerevisiae* produced and marketed by the Chinese company Ryan Wu/Angel Yeast Co., Ltd and *Saccharomyces carlsbergensis* (S0) obtained from Benin's Brewery Society (SBB), have been used as biological material or ferments.

S0: *Saccharomyces carlsbergensis*
S1: *Angel brand super alcohol active dry yeast*
S2: *Angel super alcohol active dry yeast*
S3: *Angel brand Thermal-tolerant alcohol active dry yeast*

The inoculum was prepared by introducing 1.0 g of each yeast strain in 9mL of buffered peptone water [17].

2.3. Nutrient Additive

The nutrient additive used is Urea [$CO(NH_2)_2$], from KEM LIGHT Laboratories PVT. LTO.

2.4. Fermentation Process

Pre-fermentation was carried out in an aerobic and aseptic medium by leaving the inoculum and 1/10 (v/v) under agitation for 30min. The mixture obtained was added to the rest of the sterile must. Urea was used at a concentration of 4g/L. A control was carried out without nutrients. The batch method was adopted according to the protocols followed by Kouwanou et al. [15]. Each fermenter was kept hermetically sealed and alcoholic fermentation of the musts was carried out for about eight (08) days. Periodic sampling followed by analysis made it possible to monitor the variation of physico-chemical parameters such as pH, titratable acidity (expressed for the must in % acetic acid), density and Brix degree during the fermentation.

2.5. Alcoholic Distillation

At the end of fermentation, the ethanol contained in the wines was extracted at the top of the column by fractional distillation at a temperature of 79°C at the top of the column [11].

2.6. Analytic Methods

The pH of musts was measured with HANNA pH meter previously calibrated with buffer solutions of pH 4.0 and 7.0 [29]. Total acidity (g acetic acid/L) was determined by titrimetric method with sodium hydroxide (0.1 N) with phenolphthalein as colored indicator according to AOAC [7]. The relative density at 20°C of juice was determined according to the method described by Novidzro [19]. Total soluble dry matter content (Brix degree) was determined during fermentation using a PAL 3-ATAGO portable digital refractometer. The alcoholic degree (% v/v) of the various distillates was quantified according to pycnometric method recommended by Sidney [25].

2.7. Optimization Parameters

Fermentation optimization parameters determined are essentially fermentation duration (t), limit attenuation (AL), final ethanol content (Pexp), ethanol productivity (Qp), Ethanol yield (Yp/s), rate of yield improvement (TAR) and production yield's efficiency (Ey). These parameters are determined according to the following formulas:

$$AL = \frac{Brix_{initial} - Brix_{final}}{Brix_{initial}} \times 100 \quad (1)$$

$$Q_p = \frac{P_{exp}(g.L^{-1})}{Duration(h)} \quad (2)$$

$$Y_{p/s} = \frac{Ethanol\ mass}{Consumed\ sugar's\ mass} \quad (3)$$

$$Ey = Y\ p/s\ /0.54 \quad (4)$$

$$TAR = \frac{DAN - DASN}{DAN} \times 100 \quad (5)$$

With:

DAN= Alcoholic Degree with Nutrient and DASN= Alcoholic Degree without Nutrient [12].

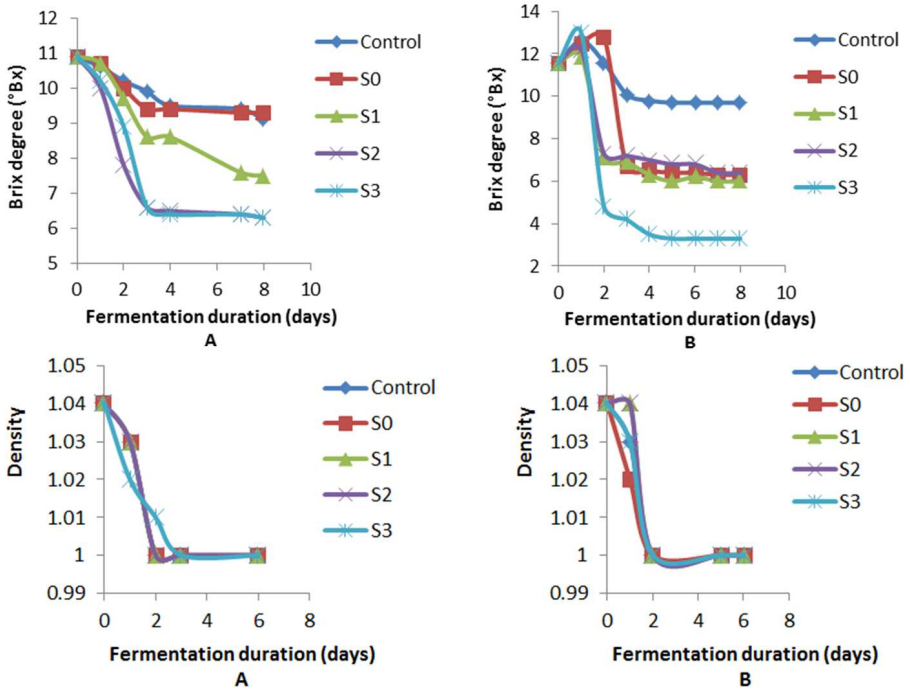
2.8. Statistical Processing of Results

Repeated trials were used to calculate the mean and standard deviation associated with each measure based on Microsoft Excel 2013. Analysis of variance (ANOVA) for appreciating the difference significance between some averages was made with the Minitab 16.0 software. The method employed to discriminate averages is that of the smallest significant difference at the probability threshold P < 5%.

3. Results and Discussion

3.1. Kinetic Study of Alcoholic Fermentation

Figure 1 shows the evolution of the brix degree and of the density of our medium as a function of time. A decrease in Brix degree is observed during fermentation for the four strains used, as well as for the control without added ferment. This decrease is more pronounced for strains S1, S2 and S3 than for strain S0 and the control. The addition of ferment therefore has a significant influence on the fermentation, especially with strains S1, S2 and S3. It is observed that the maximum time for the conversion of sugars from pineapple waste into bioethanol is 4 days of fermentation. Hajar and al. had achieved this conversion in 72 hours of fermentation [13]. However, Aidoo found that the maximum time for the conversion of pineapple peels from Ghanaian farms by co-culture in the presence of Saccharomyces cerevisiae var [4]. Ellipsoideus was greater than 14 days. It must be recognised that the pre-treatment of the biomass and the operating conditions are determining factors in optimising the bioethanol conversion time. Similarly, our study showed that fermentation in the presence of the selected strains was more efficient than spontaneous fermentation. The decrease in Brix degree leads in parallel to a decrease in density during fermentation, especially in the S3 strain followed by the S0 strain. This is indicative of the loss of mass of the residues in the form of CO_2 [20].

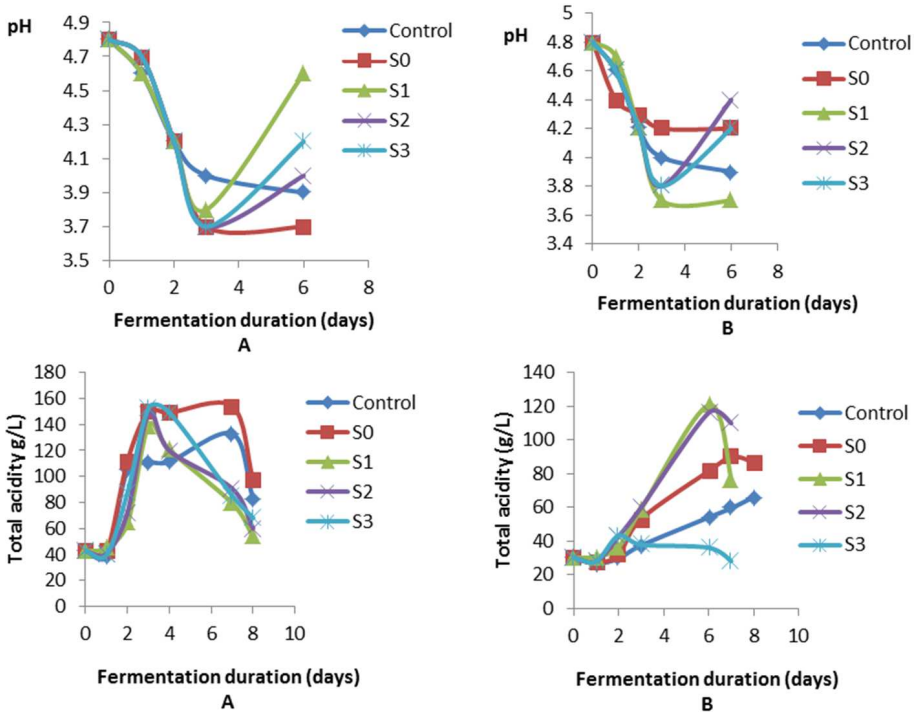


A: Non-enriched must; B: Enriched must

Figure 1. *Variation of Brix degree and density of musts during fermentation.*

Figure 2 shows the evolution of pH and titratable acidity during fermentation. During the stop phase of fermentation, the pH gradually increases. The trend observed for acidity is consistent with the variation

observed for pH during fermentation. The pH is inversely proportional to the acidity of the medium. This can be observed in all curves, especially with the S3 strain. Akin, in his work on alcoholic fermentation, obtained the same evolution of the pH: a decrease at the beginning of fermentation concomitant with the growth of the biomass and the consumption of nitrogen, then an increase at the end of fermentation with the production of ethanol [5]. He attributed this evolution of pH to two phenomena (nitrogen assimilation and the effect of ethanol on dissociations) that are mainly responsible for the evolution of pH during must fermentation. Similarly, Vaitheki and Deepa had noticed the decrease in pH (6 to 5.3) during the alcoholic fermentation process carried out for 72 hours on pineapple rind hydrolysates obtained by thermal pre-treatment in Tamilnadu, India [27].



A: Non-enriched must; B: Enriched must

Figure 2. *Variation of pH and titratable acidity of musts during.*

3.2. Urea Effect on Fermentation Process

The presence of nitrogen in urea helped optimize ethanol production. The addition of urea increased the dry matter level at the start of fermentation, which is indicated by the increase in Brix at the start of fermentation. A remarkable reduction in the Brix is noted from the second day on all the samples. This addition resulted in a greater and faster decrease in Brix compared to that of unenriched wort. The nutrient supply therefore has a significant influence on the fermentation kinetics, especially with the strain S3.

3.3. Effect of Urea on Ethanol Content

The results from the quantification of ethanol reported in Table 1 confirm the beneficial effect of nutrient supply on ethanol production. The alcoholic degrees obtained showed significantly different values ($P < 0.05$) depending on the strain of yeast used. Urea-enriched musts distillates exhibited the highest ethanol levels compared to unenriched musts. Enrichment is therefore a determining factor in the growth of microorganisms in yeast strains during the production of bioethanol by ethanolic fermentation. The presence of nutrients for yeasts in a culture medium has a proven positive influence on ethanol production [20]. The fermentation carried out in the presence of the yeast Saccharomyces cerevisea on the hydrolyzate of pineapple peelings with hydrochloric acid (2M) revealed an ethanol rate of (11.44 ± 0.29% v/v) according to the work of Alvarenga and al. [6]. Orji and al. obtained an ethanol content of 24.9% with an acid hydrolyzate enriched with urea (1.5g/L) [22].

Table 1. *Alcoholic degree of distillates.*

Fermentation condition	Ethanol content (% v/v)				
	Control	S0	S1	S2	S3
Ferment only	2.77 ± 0.02^{a}	21.23 ± 0.05^{c}	7.59 ± 0.02^{b}	2.84 ± 0.02^{a}	9.46 ± 0.01^{b}
Ferment+nutrient	2.77 ± 0.02^{a}	28.69 ± 0.03^{e}	14.13 ± 0.04^{b}	17.01 ± 0.02^{c}	23.78 ± 0.02^{d}

In the same line, the values not sharing any letter are significantly different (P < 5%) according to ANOVA and Tukey's multiple comparison tests.

3.4. Fermentation Optimization Parameters

3.4.1. Fermentation Duration (t: h)

Analysis of the results obtained shows that the four microbial strains used have variable fermentation times, depending on the presence or absence of the growth factor (Table 2). In fact, the enriched musts presented shorter fermentation times, around 48 hours. In the opinion of several authors, 72 hours of fermentation or sometimes even less is sufficient to have the maximum amount of ethanol in the pineapple waste's wine [13].

Table 2. *Fermentation duration.*

Fermentation condition	Control (h)	S0 (h)	S1 (h)	S2 (h)	S3 (h)
Ferment only	96 ± 2^{a}	72 ± 2^{b}	72 ± 2^{b}	72 ± 2^{b}	72 ± 2^{b}
Ferment+nutrient	96 ± 2^{a}	72 ± 2^{b}	48 ± 2^{c}	48 ± 2^{c}	48 ± 2^{c}

In the same line, the values not sharing any letter are significantly different (P < 5%) according to ANOVA and Tukey's multiple comparison tests

3.4.2. Limit Attenuation (AL: %)

The limit attenuation values obtained are between 16.38% and 72.41% (Figure 3). Analysis of these values reveals that the fermentation took place under better conditions for culture media initially having pH values required for good alcoholic fermentation. A comparison of these results shows that the S3 strain was found to be more efficient in terms of sugar consumption. We also note that the fermentation carried out in the presence of yeast strains was more efficient than the spontaneous one (control). Alvarenga and al. obtained efficacy of the yeast *Saccharomyces cerevisea* of (59.94 ± 0.18%) [6].

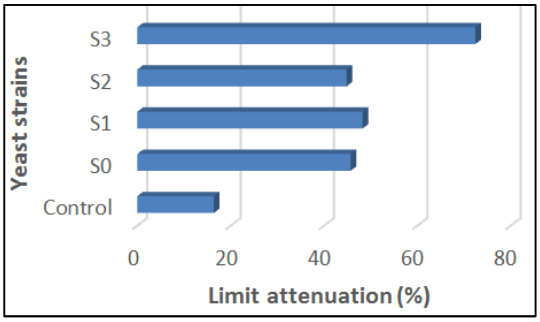


Figure 3. *Selected ferments effect on ethanol production.*

3.4.3. Rate of Yields Improvement (TAR: %)

Figure 4 shows the improvement in ethanol production yields of different yeast strains in the presence of urea, a yeast growth factor, during ethanol fermentation from pineapple peelings. Rates of 26.00% – 83.36% were observed. The TAR values obtained are all positive. This shows a marked improvement in the yield of the yeast strains used. This observation could be due to the depletion of sugar in pineapple peelings. All the strains used as ferments have seen their performance improved by the addition of nutritive substance to the musts. The results obtained here are in perfect agreement with the work carried out by certain authors indicating that alcoholic fermentation takes place under better conditions when the pH of the reaction medium is between 4-5 and by incubating it at 30°C [15]. In this interval, productivities and ethanol yields are maximum. This pH range makes it possible to avoid contamination or parasitic reactions due to the presence of other microorganisms such as bacteria [9].

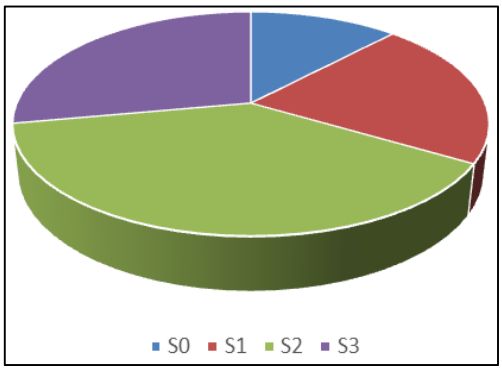


Figure 4. *Nutrient effect on ethanol production.*

3.4.4. Final Ethanol Contents (Pexp: g/L)

The final ethanol contents are (21.91±0.20) g/L to (226.94±2.73) g/L (Table 3). These values reflect the alcoholic degrees previously obtained. The final ethanol contents were significantly different (P=0.000) depending on the yeast strain, the addition of urea or not. The production of ethanol from the banana peel by the SSF method during 48 h of fermentation at 30°C with stirring of the fermentation medium, presented an ethanol content of 25.08 g/L with an attenuation of 78.04% followed by a yield of 398 g/kg [24]. This testifies to the polysaccharide richness of the banana peel.

Table 3. *Ethanol's final content (g/L).*

Fermentation condition	Control	S0	S1	S2	S3
Ferment only	21.91 ± 0.20^{d}	167.93 ± 2.4^{a}	60.04 ± 0.67^{c}	22.39 ± 0.18^{d}	74.8 ± 0.45^{b}
Ferment+nutrient	21.91 ± 0.20^{e}	226.94 ± 2.73^{a}	111.77 ± 1.15^{d}	134.55 ± 1.87^{c}	188.10 ± 1.89^{b}

In the same column, the values not sharing any letter are significantly different (P < 5%) according to ANOVA and Tukey's multiple comparison tests.

3.4.5. Ethanol Productivity (Qp: g/L/h)

Table 4 presents the results of ethanol productivity by the yeast strains used. The productivities recorded at the level of the different samples were significantly different (P=0.000) whatever the strain considered. The analysis of these results shows that the alcohol productivity depends both on the type of microorganisms and on the enrichment. The best productivity obtained is (3.92±0.28 g/L/h) with the S3 strain followed by that of S0 (3.15±0.40 g/L/h). Alvarenga and al. obtained a productivity of (1.29 ± 0.01g/L/h) [6].

Table 4. *Ethanol productivity (Qp: g/L/h).*

Fermentation condition	Control	S0	S1	S2	S3
Ferment only	0.23 ± 0.02^{d}	2.33 ± 0.11^{a}	0.83 ± 0.06^{c}	0.31 ± 0.03^{d}	1.04 ± 0.09^{b}
Ferment+nutrient	0.23 ± 0.02^{d}	3.15 ± 0.40^{b}	2.33 ± 0.20^{c}	$2.80 \pm 0.33^{b,c}$	3.92 ± 0.28^{a}

In the same line, the values not sharing any letter are significantly different (P < 5%) according to ANOVA and Tukey's multiple comparison tests.

3.4.6. Ethanol Production Yield ($Y_{p/s}$: g/kg)

The quantities of ethanol produced from one kg of substrate used are given in Table 5. The production yield evaluated gave values of (21.11±0.24) to (165.47±1.02 g/kg) for non-enriched musts and values from (21.11±0.24) to (225.59±2.02 g/kg) for enriched musts. The results revealed a significant difference according to the strain used and according to the enrichment. The best ethanol production yield (225.59±2.02 g/kg) was recorded in the S0 yeast.

Table 5. *Ethanol production yield ($Y_{p/s}$: g/kg).*

Fermentation condition	Control	S0	S1	S2	S3
Ferment only	21.11 ± 0.24^{a}	165.47 ± 1.02^{e}	58.24 ± 0.60^{c}	21.68 ± 0.22^{b}	73.12 ± 0.07^{d}
Ferment+nutrient	21.11 ± 0.24^{a}	225.59 ± 2.02^{c}	85.30 ± 0.09^{b}	132.64 ± 1.01^{d}	185.87 ± 1.02^{c}

In the same line, the values not sharing any letter are significantly different (P < 5%) according to ANOVA and Tukey's multiple comparison tests.

3.4.7. Efficiency of Production Yield (E_y: %)

The efficiency of the ethanol production yield evaluated made it possible to have the results of Table 6. The analysis revealed that the efficiency rates obtained are significantly different regardless of the fermentation condition. The rates obtained vary from (3.91±0.04%) to (41.78±0.04%). The S0 strain was the most effective. This strain had revealed similar efficacy (42.94 ± 2.04%) during our investigations on cashew apple residues [12].

Table 6. *Efficiency of production yield (E_y: %).*

Fermentation condition	Control	S0	S1	S2	S3
Ferment only	3.91 ± 0.04^{e}	30.64 ± 0.03^{a}	10.79 ± 0.01^{c}	4.02 ± 0.04^{d}	13.54 ± 0.01^{b}
Ferment+nutrient	3.91 ± 0.04^{e}	41.78 ± 0.04^{a}	15.80 ± 0.02^{d}	24.56 ± 0.02^{c}	34.42 ± 0.03^{b}

In the same line, the values not sharing any letter are significantly different (P < 5%) according to ANOVA and Tukey's multiple comparison tests.

4. Conclusion

In the present study, the production of first generation bioethanol by batch fermentation using yeast strains was investigated on the juice extracted from pineapple peelings. The results revealed that the fermentation carried out in the presence of selected strains was more efficient than spontaneous one. Therefore, the use of the nutrient contributes to an improvement in the alcoholic fermentation reaction of pineapple peels must by strains of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*. The best ethanolic bioconversion performance was recorded in the enriched musts and in the presence of *Saccharomyces carlsbergensis* followed by the *Angel brand thermal-tolerant alcohol* (S3) strain of *Saccharomyces cerevisiae*. In this study, pineapple peeling was proved as one of the novel and potential raw material for biofuel production.

5. Recommendation

The energy recovery of biomass by biotechnological processes constitute a solution of choice for the use of agricultural products with low commercial value, waste from the agro-food industries, crop residues, etc. The realization of this study is a challenge environmentally and socio-economically important to promote agriculture and the biofuels sector.

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