



(RESEARCH ARTICLE)



STUDY OF THE PERFORMANCE OF THE FOUR STRAINS OF SACCHAROMYCES CEREVISIAE AND THE INFLUENCE OF UREA DURING THE PRODUCTION OF BIOETHANOL FROM THE FRUITS OF *CHRYSOPHYLLUM ALBIDUM*

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ABSTRACT

The present study aims to enhance agricultural resources and reduce post-harvest losses through the bioconversion of the fruits of the *Chrysophyllum albidum* in bioethanol. In this context, the fruits of the *Chrysophyllum albidum* were collected in Tori in southern Benin and underwent a series of treatments such as sorting, washing, separating the seeds from the apples. Then the grinding and mechanical pressing made it possible to obtain the juice from the fruits. Fermentation with levures *Angel brand super alcohol*, *Angel super alcohol* and *Angel brand Thermal-tolerant alcohol* and *Saccharomyces carlsbergensis* followed by a fraction distillation made it possible to obtain bioethanol. During the study, the fermentation performance of the different yeasts in the production of bioethanol from the wort of *Chrysophyllum albidum* were tested. The monitoring of fermentation kinetic parameters such as pH, acidity, density and Brix, showed a great variability of these parameters during the fermentation process. The distillation of the musts at the end of fermentation made it possible to obtain ethanol extraction rates (% v/v at 20°C) between 5.3 and 60.7; extraction efficiencies between 0.15 and 0.37; ethanol yields between 1.87 g/L/h and 4.64 g/L/h after fractional distillation. In conclusion, this study has made it possible to set up a process for the valorization of the fruits of *Chrysophyllum albidum* through its bioconversion ethanol.

Keywords: *Chrysophyllum albidum*; Bioconversion; Fermentation; yeasts; Fractional distillation; Bioethanol

1 INTRODUCTION

In recent decades, the world has been increasingly faced with an increase in energy demand. Indeed, our energy needs are mainly based on the exploitation of fossil fuels. This system is seeing its limits with the problems of increasing global energy needs, resource depletion, environmental impacts and global warming. Scientists, politicians and citizens are thinking about the implementation of an energy transition to get away from the current model and to build the foundations of alternatives that would make it possible to meet the energy needs of our societies in a sustainable, safe and environmentally friendly way [1]. The energy transition is based on the control of energy consumption and the development of renewable energies. Among the renewable energy sources, biomass has been used for thousands of years but is nevertheless a resource of the future. Its use is envisaged in particular to synthesize fuels that can replace products derived from fossil resources and thus reduce the production of greenhouse gases, of which the transport sector is the main emitter. Thus, among the biomass available in Benin, *Chrysophyllum albidum* is a domestic tree and often encountered in villages, especially in the South. It is used for many purposes and is marketed by the population. Thus, the fruits of *Chrysophyllum albidum*, harvested during the month of November and April in houses and in certain fields, are very early dumped in local markets to reach secondary markets thanks to the know-how of several rural women and collectors of these markets. This trade generates a large amount of money for the actors involved that helps them solve certain problems that arise for them. Unfortunately, despite the richness of this fruit, its production does not yet constitute a sector in Benin, due to a lack of organization of the sector. This state of affairs does not ensure the availability of the raw material. Moreover, during the picking period, a very large quantity of the fruit is destined for the trash. Barely 10% to 20% of the quantity of fruit is directly consumed [2]. Thus, it is necessary to valorize these unexploited fruits (80% to 90%) rich in sugar and lignocellulose. It is with this in mind that the present study aims to valorize the fruits of *chrysophyllum albidum* through its bioconversion into fuel ethanol.

2 MATERIAL AND METHODS

2.1 Collection of plant material and extraction of apple juice

Yellow morphotype *Chrysophyllum albidum* fruits were collected from fields and houses in South Benin. After collection, the apples and seeds are separated, followed by crushing and mechanical pressing for the extraction of juices used as a sweet substrate for fermentation. Photo 1 and Figure 1 show respectively the image and the technological diagram of the extraction of fruit juice from *Chrysophyllum albidum*



Photo 1: Fruits du *Chrysophyllum albidum*

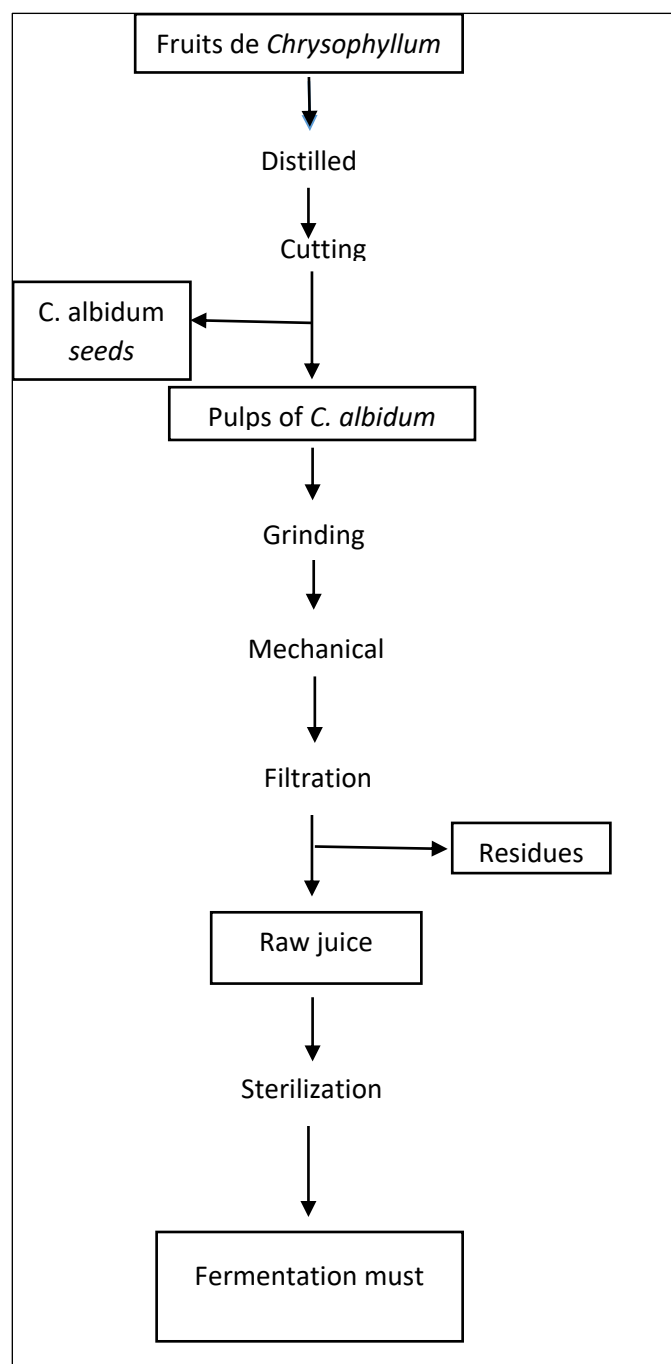


Figure 1: Wort extraction technology diagram

2.2 Preparation of the inoculum

The ferments used are made up of four yeast strains, three of which are freeze-dried belonging to the species *Saccharomyces cerevisiae* (Angel brand super alcohol, Angel super alcohol and Angel brand Thermal-tolerant alcohol) and *Saccharomyces carlsbergensis* marketed by the Chinese company "Ryan Wu / 邬善远 / Angel Yeast Co., Ltd., <http://en.angelyeast.com>". The inoculum was prepared by introducing 1.0 g of each of the dry (lyophilized) yeast strains into 9 mL of buffered peptone water.

2.3 Preparation of fermentation musts

In order to promote acclimatization of the yeasts to the culture medium, pre-fermentation was carried out by adding the inoculum to 1/10 of the total volume of sterile juice to be fermented and left under agitation for 24 hours in a closed chamber and in a controlled environment. Then the mixture was added to 9/10 of the remaining volume of sterile sweet substrate. After homogenization by rotary agitation, the performance of yeast strains in the presence and absence of growth factors was evaluated. Thus, urea (NH_2CONH_2), chosen as a growth factor, was added at a concentration of 4g/L. A control was carried out without the addition of growth factor.

2.4 Alcoholic fermentation

The different musts obtained are kept at 25°C under intermittent agitation. Fermentation is batch or batch. Each fermenter is kept hermetically sealed. The alcoholic fermentation of the musts took place over eight days. Periodic sampling followed by analysis made it possible to monitor the evolution of physico-chemical parameters such as pH, titratable acidity (expressed for the must as % acetic acid), density, Brix and total sugar content.

2.5 Distillation

When the evolution of the brix degree (end of fermentation) stopped, the ethanol contained in the musts was extracted by fractional distillation at a temperature of 79°C at the top of the column [3] (Kouwanou *et al*; 2022).

2.6 Analytical methods

The pH of the musts was measured using the HANNA pH-meter. The acidity of the musts was determined according to the AOAC (1984) method [4]. The total soluble dry matter rate (Brix) was determined using a PAL 3-ATAGO digital refractometer. The alcohol content (% v/v) of the musts at the end of fermentation is determined according to the pycnometric method recommended by AOAC (1984) [4]. Other parameters such as Limit Attenuation (AL), Ethanol productivity, average fermentation time and ethanol production yield were determined. The average fermentation times represent the time between the start of fermentation and the cessation of sugar consumption [5], the ethanol production yield (Rp) which is the ratio of the volumes of ethanol obtained per volume of fermented wort. These kinetic parameters are determined according to the following formulas: $AL = [(Initial\ Brix - Final\ Brix) / Initial\ Brix] \times 100$

2.7 Statistical Analyses

The analyses and extractions are done in triplicate. The data are synthesized using Microsoft Excel Worksheet 2013 and Origin Pro 8.5 software, which also allowed the calculation of different means and the determination of standard deviations. The analysis of variance (ANOVA) for the assessment of the significance of the difference between certain means is done with the SPSS 16.0 software. The method used to discriminate the means is that of the smallest significant difference at the $P < 5\%$ probability threshold.

3 RESULTS AND DISCUSSION

3.1 Evolution of the pH of the different musts during fermentation

Figure 2 shows the evolution of the pH of enriched and unenriched musts (Figs. 2a and 2b) of juice from *Chrysophyllum albidum* fruits during fermentation. Analysis of this figure shows a decrease in pH from 4.1 to values 2 and 2.1 respectively for strains S0 and S1 with unenriched musts during the first 48 hours of fermentation, followed by an increase in pH during the last 72 hours of fermentation. On the other hand, in strains S2 and S3, the decrease phase lasted 72 hours and the increase phase lasted 48 hours. The results obtained show a gradual increase in pH until the fermentation cessation phase. Akin [6], has shown the same evolution of pH during his work on alcoholic fermentation. He attributes this evolution of pH to two phenomena (assimilation of nitrogen and effect of ethanol on dissociation) which are mainly responsible for the evolution of pH during the fermentation of musts. On the other hand, the acidic character of the sweet must in fermentation is relative to the lactic fermentation phase. Indeed, the production of sweet must requires a significant phase of spontaneous lactic fermentation during which lactic acid bacteria, in particular the genera *Lactobacillus*, *Leuconostoc* and *Pediococcus*, produce organic acids [7; 8]. Thus, the alcoholic fermentation initiated by traditional ferments is essentially carried out by yeasts [9]. During this fermentation, the activity of lactic acid bacteria is generally negligible. This means that the organic acids produced during the souring phase undergo very little modification during the alcoholic fermentation of the *Chrysophyllum albidum* must. The acidic character of the fermenting *Chrysophyllum albidum* must would therefore be linked to the presence of organic acids, the main ones being oxalic acid, lactic acid, tartaric acid, malic acid and citric acid [10]. Also, Vaitheki and Deepa (2016) [11] had noticed the decrease in pH (6 to 5.3) during the alcoholic fermentation process carried out for 72 hours on the hydrolysates of pineapple peels obtained by thermal pretreatment in Tamilnadu, India. Gbohaida *et al*; 2016 [12] obtained a pH evolution similar to that obtained in our study during the alcoholic fermentation of pineapple peels from Benin.

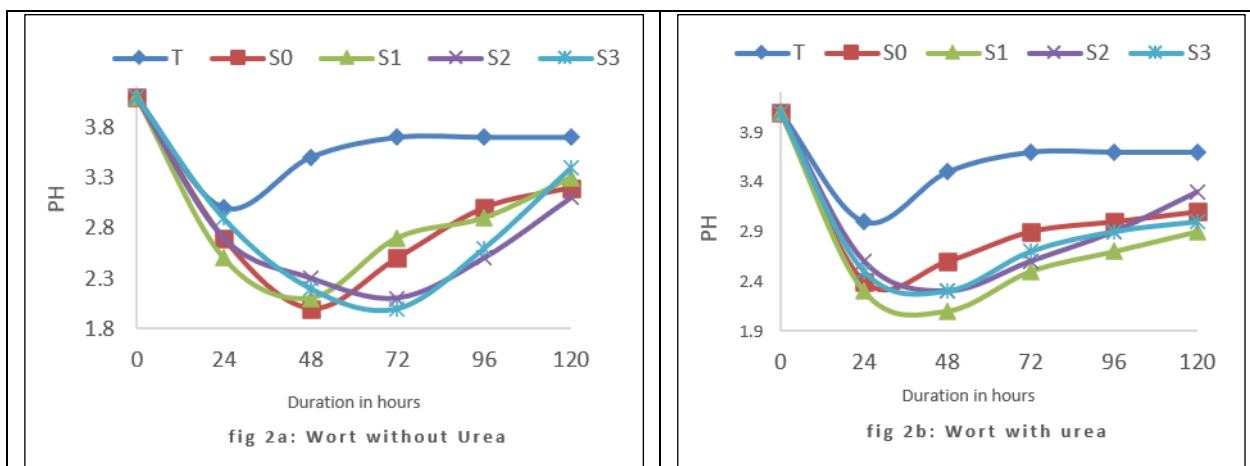


Figure 2: Evolution of the pH of enriched and unenriched musts during fermentation

3.2 Evolution of titratable density

Figure 3 presents the results on the evolution of the acidity of the fermenting musts. From the analysis of this figure, we observe an increase in acidity during the first 2 or 3 days of fermentation. We then note a progressive decrease in the acidity of the musts at the level of the different samples. When fermentation stopped, the acidity of the reaction medium decreased significantly. This could be due to the release of CO_2 from the fermentation medium. Aidoo's investigations [13] in Kumassi in Ghana on pineapple juices showed that during fermentation, the musts that received ferments were more acidic than the control until the end of the process when there was a decrease in the acidity of the fermentation media. The same observations are made by Gbohaïda *et al*; (2016) [14], during the fermentation of pineapple peelings.

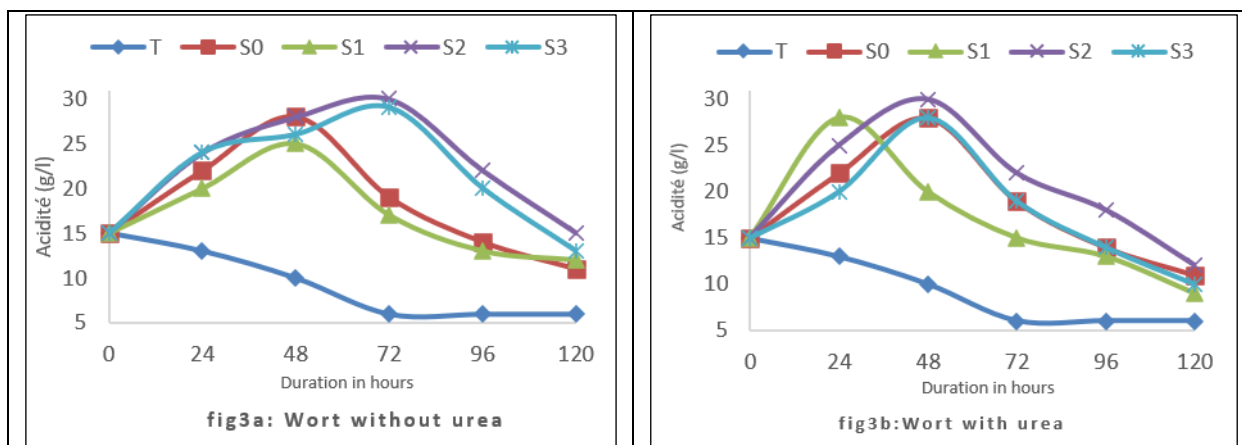


Figure 1: Evolution of the acidity of musts

3.3 Evolution of density

Figure 4 presents the results of the evolution of the density of fermenting musts. The analysis of this figure shows a decrease in the density of the different musts containing ferment during the first 48 hours of fermentation, followed by an increase during the last 72 hours of fermentation. All these results show that during fermentation, the evolution of the density of the musts follows a kinetic pattern of three stages: an acceleration phase, a slowing phase and a stabilization phase. This kinetic pattern can be associated with the growth of fermentative microorganisms which, on a compound medium rich in carbohydrates, achieve growth with a three-stage kinetics [15]. These fermentative microorganisms break down the substrate with the production of alcohol or acid metabolites which lead to a decrease in the density of the must [16]. The decrease phase can be explained by the transformation of sucrose into alcohol and the loss of mass in the form of CO_2 . In South Africa, Deenanath and *al* [17] also obtained a density of 1.05w/w for cashew apple juices. Akinwalé [18] had also obtained the same density (1.046w/w) in Nigeria.

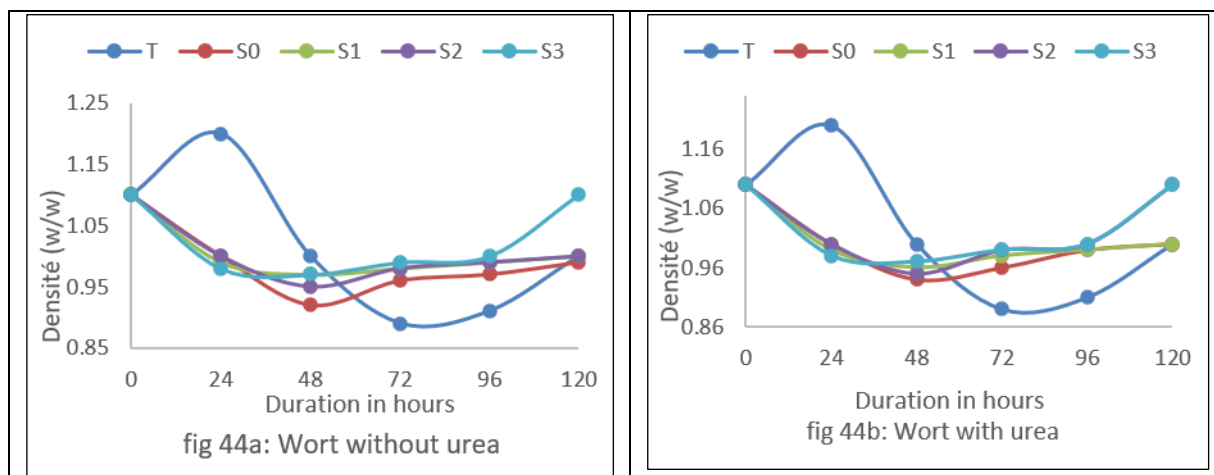


Figure 2: Evolution of the density of musts

3.4 Evolution of Brix

Figure 5 presents the results of the evolution of the Brix degree of the different fermenting musts. From the analysis of this figure, we note a decrease in Brix during the fermentation of the musts for the four strains used, as well as for the control. This decrease is more noticeable for the S0 strain than for the other three strains (S1, S2 and S3). The addition of ferment therefore has a significant influence on fermentation, particularly with the S0 strain; it can therefore be noted that in 4 days of fermentation, most of the sugars contained in the juice of the *Chrysophyllum albidum* are converted back into ethanol. According to several authors, 72 hours of fermentation or sometimes even less are sufficient to obtain the maximum amount of ethanol (Hajar *et al*; 2012) [19]. The present study showed that fermentation carried out in the presence of selected strains is more efficient than spontaneous fermentation, in the sense that the decrease in Brix was more pronounced in the musts fermented with the *Saccharomyces cerevisiae* than at the level of the must that has not received ferment (control).

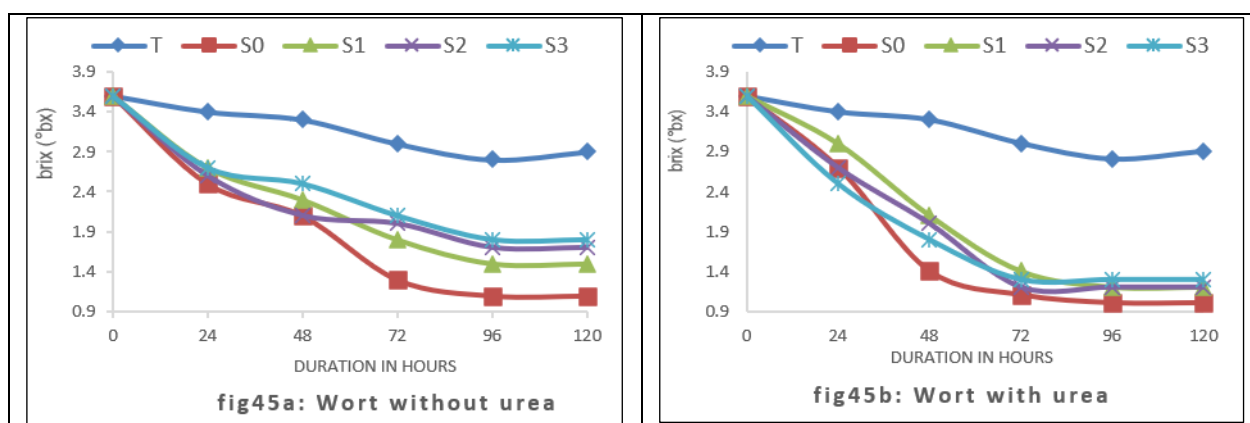


Figure 3: Evolution of Brix

3.5 Average fermentation time

Figure 6 shows the average fermentation time for each substrate according to the yeast strain used and the presence or absence of the growth factor. Each duration reflects the time between the start and the end of the fermentation process. The analysis of the results obtained shows that the four microbial strains used have variable fermentation times (between 72 h and 96 h) depending on the presence or absence of the growth factor. In other words, the enriched musts have a fermentation time of 72 h while the unenriched musts have a fermentation time of 96 h. It should also be noted that the control samples showed the longest fermentation times. It appears that, under the conditions of the study, the fermentation performance of the different microbial strains used is closely linked to the type of substrate and the presence or absence of growth factor in the fermentation musts. In the opinion of several authors, 72 hours of fermentation or sometimes even less is sufficient to obtain the maximum amount of ethanol (Hossain and *al*; 2010, De prados, 2010) [20 ; 21].

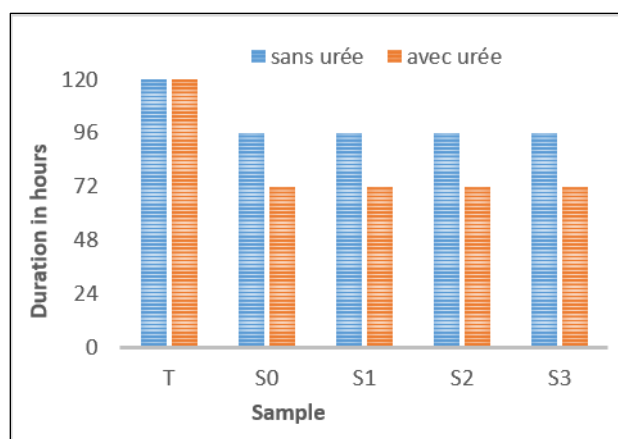


Figure 4: Fermentation time in hours

3.6 Limited attenuation

Figure 7 presents the results on the sugar consumption rate by the strains used. From the analysis of this figure, it can be seen that the sugar consumption rate is between 19.00% and 69.44% for the non-urea enriched samples. For this sample category, the highest value was recorded with the S0 strain, while the lowest value was recorded for the control sample. For the urea-enriched musts, the sugar consumption rates by yeasts ranged from 19.44% to 72.22%. It should also be noted that for this sample category, the highest value was recorded for the S0 strain and the lowest value for the control sample. In general, it can be noted that the yeast samples showed a higher consumption rate than the control sample. In addition, the urea-enriched samples showed better sugar consumption rates than the non-enriched samples. According to the work of Gbohaïda *et al.*, (2016) [12] the attenuation limit is between 69.5 and 75% for the fermentation of cashew apple juice and also between 16.38% and 72.41% for pineapple peels. We then note that our results are in agreement with the results reported by Gbohaïda *et al.*, (2016) [12].

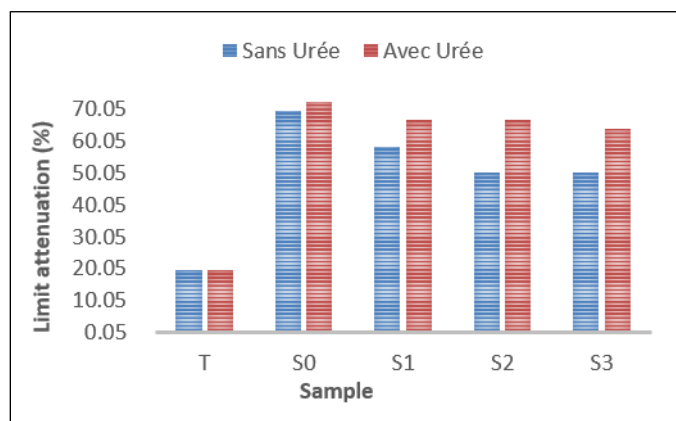


Figure 5: Sugar consumption rate

3.7 Alcoholic strength of musts

Figure 8 shows the results on the alcoholic strength of the musts of each sample at the end of fermentation. From the analysis of this figure, it can be seen that the alcoholic strengths of the musts are between 23.18 and 214.16 g/L for the unenriched musts and between 23.18 and 334.16 g/L for the urea-enriched musts. It should be noted that the highest values of the ethanol concentration were recorded in the samples fermented with the S0 strain, while the lowest value was recorded in the control sample. It can also be seen that the urea-enriched musts showed the best ethanol strengths at the end of fermentation, which justifies the importance of urea in the fermentation processes from the sugary substrates. Some of our results are in agreement with those obtained by Gbohaïda and *al.*, [12] on pineapple peelings. However, our values were higher than those reported by Pacheco and *al.* [22] which obtained an ethanol content of 19.82-37.83g/L from cashew apple juice in Brazil. The same is true for Karuppaiya and *al.* [23] in India, which had obtained an alcoholic strength of 15.64 g/L under optimal conditions (pH 6.5 and at 32°C with a fermentation time of 37 hours).

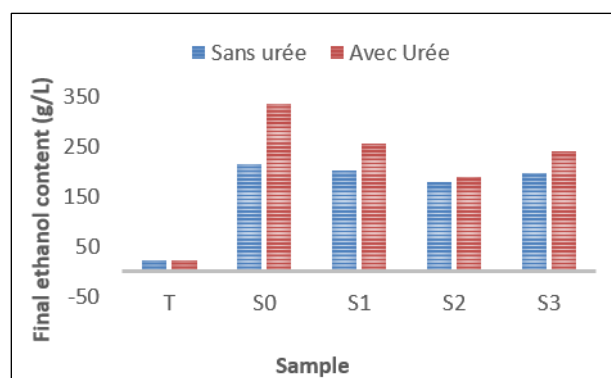


Figure 6: Alcoholic strength of musts of *Chrysophyllum albidum* at the end of fermentation

3.8 Ethanol Productivity

Figure 9 presents the results of ethanol productivity by the different strains used. From the analysis of this figure, it can be seen that the ethanol productivities for the non-urea-enriched musts are between 0.19 g/L/h and 2.23 g/L/h. On the other hand, for the urea-enriched musts, the productivities are between 0.19 g/L/h and 4.64 g/L/h. It can be seen that the lowest values are recorded for the control samples, while the highest values are recorded for the musts fermented with the S0 strain. In addition, it can be seen that it is the urea-enriched musts that have the best ethanol productivity. Our results are in agreement with those obtained with pineapple peelings and also with cashew apple musts by Gbohaïda et al.,[12]. These results are also similar to those of Pacheco et al., (2010) [22] who obtained ethanol yields ranging from 3.30 to 6.31 g/L/h with cashew apple juice in Brazil.

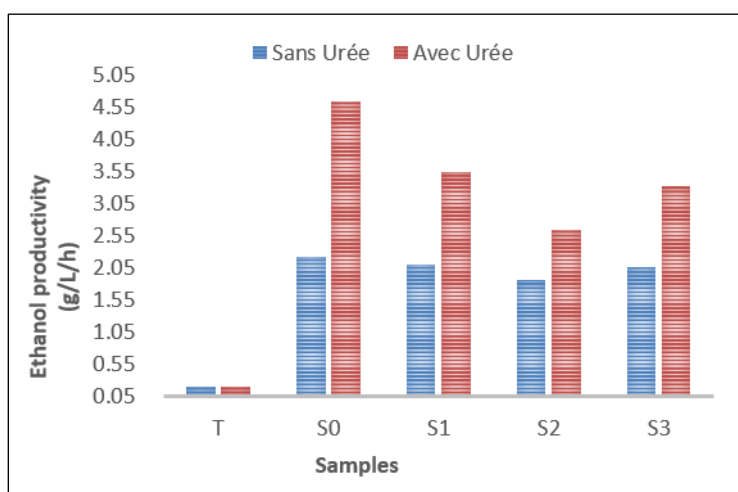


Figure 7: Ethanol productivity

3.9 Ethanol Production Yield

Figure 10 shows the results on the ethanol extraction yield at the level of the different fruit musts of the *Chrysophyllum albidum*. From the analysis of this figure, it can be seen that the non-urea-enriched samples had extraction yields of between 0.02 and 0.25 with the lowest yield value obtained at the level of the control sample. However, the highest value was obtained at the level of the S0 strain. As far as the urea-enriched musts are concerned, the extraction yields are between 0.02 and 0.37 with the highest value recorded at the level of the must fermented with the S0 strain. In general, it can be seen that the urea-enriched musts had higher extraction yields than the non-urea-enriched musts. Our results are in agreement with the results obtained by Gbohaïda and al[24] during the production of bioethanol from cashew apple pressing residues Also, our results on urea-enriched musts are similar to those obtained by Rocha and al., [25], in Brazil, on pressing residues of apples pretreated with dilute sulphuric acid.

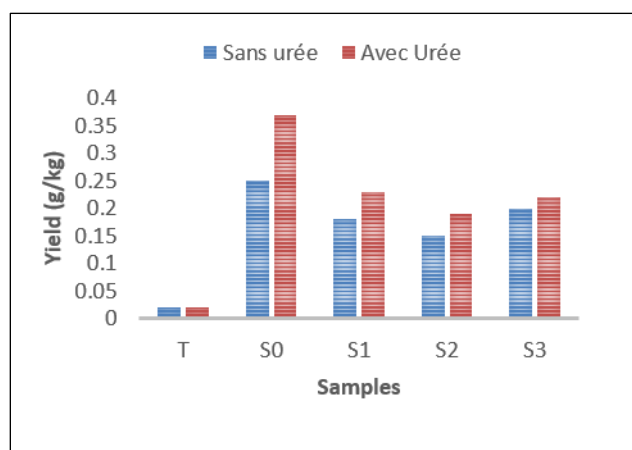


Figure 10: Ethanol yield

3.10 Alcohol content

Figure 11 shows the results of the alcohol content obtained in the different samples. From the analysis of this figure, it can be seen that the alcoholic degrees of distillates from musts not enriched with urea are between 5.3%V/V and 48.14%V/V, with the highest value recorded in the sample fermented with strain S0 and the lowest value recorded in the control sample. In terms of musts enriched with urea, it can be seen that the alcohol content is between 5.3%V/V and 60.70%V/V, with the highest value recorded in the sample fermented with strain S0 and the lowest value in the control sample. In general, it can be seen that samples enriched with urea had higher alcohol levels than those shown by unenriched musts. We can also say that the S0 strain is more efficient than the other strains. Our results are in agreement with those obtained for pineapple peelings and cashew apples by Gbohaïda et al., (2016) [12] in Benin. It should be noted that during spontaneous fermentation, the types of microorganisms involved are not controlled. They can be yeasts or lactic acid bacteria. Thus, the final product of fermentation (lactic acid or alcohol) depends on the type of microorganism [26]. These observations could justify the low alcohol content in the distillates resulting from the spontaneous fermentation of musts used in this study.

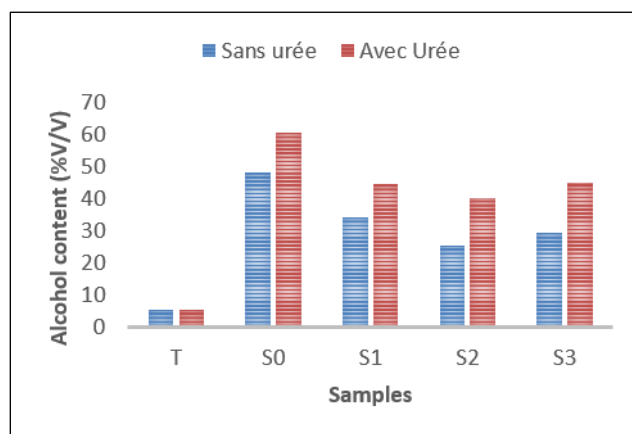


Figure 11: Alcohol content of distillates

4 CONCLUSION

This work then made it possible to evaluate the fuel potential of the fruits of *Chrysophyllum albidum*. The methodology followed consisted in studying the performance of the fermentation of the urea-enriched and non-urea-enriched musts of the fruits of *Chrysophyllum albidum* using different strains of *Saccharomyces cerevisiae*, as ferments. The strain of *Saccharomyces carlsbergensis* proves to be more efficient than the other strains used. Also, the different enriched musts have the best alcoholic degrees, which shows the importance of using urea during alcoholic fermentation.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. No funding was received.

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