Science Journal of Chemistry
2018; 6(6): 99-107
http://www.sciencepublishinggroup.com/j/sjc
doi: 10.11648/j.sjc.20180606.11
ISSN: 2330-0981 (Print); ISSN: 2330-099X (Online)

Evaluation of the Bioethanol Potential of *Nauclea Latifolia* (Sm.) Fruit Juice

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To cite this article:
Issiakou Mossi, Cosme Sagbo Kouwanou, Guévara Nonviho, Mahouglo Barnabé Houessou, Flora Adjahatode, Papin Sourou Montcho, Dominique Codjo Koko Sohounhloué. Evaluation of the Bioethanol Potential of *Nauclea Latifolia* (Sm.) Fruit Juice. *Science Journal of Chemistry*. Vol. 6, No. 6, 2018, pp. 99-107. doi: 10.11648/j.sjc.20180606.11

Received: November 6, 2018; **Accepted**: November 26, 2018; **Published**: December 20, 2018

Abstract: Commonly called African peach tree, the *Nauclea latifolia* (Sm.) of *Rubiaceae* family gives fruits twice a year. It is also a well known plant in sub-Saharan Africa in the traditional pharmacopoeia. The fruits of the African peach tree are one of the countless fruits of this continent which, in lethal period, are left in the nature where they rot, thus causing a big shortage to the farmers of our country and those of the sub-region West Africa. This is why the objective of this study is to promote the fruits of *Nauclea latifolia* (Sm.) through the bioconversion of their juice by fermentation into ethanol as biofuel. For this, different initial concentrations (1, 2, 3, 4 and 5g/l) of *Saccharomyces carlsbergensis* and three strains of *Saccharomyces cerevisiae* reference were used on the juice formulated to the proportion of 1.5L of distilled water per kilogram (1kg) of fruit. The monitoring of the parameters (pH, density and brix degree) of 4g/l urea-enriched juices, not only enriched revealed the performance of *Saccharomyces carlsbergensis* and *Saccharomyces cerevisiae* strains in the alcoholic fermentation of the *Nauclea latifolia* (Sm.) fruit juice. From this work, it appears that the best efficiencies of bioethanol productivity of 122.4±0.4, 119.4±0.3 and 119.2±0.2ml/kg of fruit were obtained respectively from the enriched mashes with *Thermal-tolerant alcohol* (2 and 3g/l) and non-enriched must to *Angel super alcohol* (2g/l). This study shows that *Saccharomyces cerevisiae*'s *Thermal-tolerant alcohol* and *Angel super alcohol* are more effective in the fermentation of *Nauclea latifolia* (Sm.) fruit juice into bioethanol.

Keywords: *Nauclea Latifolia*, Saccharomyces, Bioconversion, Must, Bioethanol

1. Introduction

Environmental pollution, ozone depletion and climate change become more and more worrying in the world [1, 2]. Since the 1750 industrial era, the average concentration of the greenhouse gases has increased dramatically in the atmosphere and carbon dioxide has reached 400ppm in 2015 [3]. Due to increased anthropogenic emissions, chlorofluorocarbons (CFC), hydrochlorofluorocarbons (HCFC) and hydrofluorocarbons (HFC) are increasing rapidly [4]. These greenhouse gases generally emitted by egines-smokes and by human activities, destroy the ozone layer [5]. Massive use of petroleum products and pesticides is polluting the environment. Moreover, the melting of glaciers at both poles, the modification of the polar axis of the earth and the general increase of the temperature of the globe cause the drying up of lakes and aquifers in Eurasia, in the region of the Caspian Sea then in India [3, 4]. The global disruption of temperature and rainfall is noticed everywhere on the planet. Natural disasters such as cyclones, floods, snow rains, rising sea levels and oceans are regularly observed [4, 6]. The incresing populations drew more from the water supplies and the oil well [7]. In

order to remedy the disastrous and the heavy consequences related to mineral fuel, that facing our planet is facing, several conferences, conventions and agreements on the environment and climate between Third World countries are taking place. Renewable energies are chosen as alternatives to these consequences [8-10]. In the biofuel sector, the biomasses that are little used in food or referred to the nature are increasingly targeted. This is the case of the fruits of *Nauclea latifolia* (Sm.) which, in the lethal period in the Republic of Benin, are left in the forest. *Nauclea latifolia* (Sm.), commonly known as African Peach Tree, of the *Rubiaceae* family, gives fruits twice a year [11]. It is also a well known plant in sub-Saharan Africa in the traditional pharmacopoeia [12, 13]. The conversion to bioethanol of juice could contribute to its promotion. The objective of this work is to ferment the juice of *Nauclea latifolia* fruit into bioethanol. Specifically, it was to:

i Follow the kinetic parameters of the alcoholic fermentation of the juice
ii Measure the performance of yeast strains used in fermentation
iii Optimize the bioethanol production of *Nauclea latifolia* (Sm.) fruits.

2. Materials and Methods

2.1. Materials

The fruits of *Nauclea latifolia* (Sm.) were used as plant material in the framework of the realization of this study. These fruits were collected in the town of Segbana in the Republic of Benin and were directly transported fresh canned to the laboratory where they are kept at -10°C in the freezer.

One strain of *Saccharomyces carlsbergensis* and three strains of *Saccharomyces cerevisiae* used in the alcoholic fermentation of *Nauclea latifolia* fruit juice were industrial and commercial yeasts of the Chinese company "Angel yeast Co., Ltd". These strains of *Saccharomyces cerevisiae* are:

i Angel brand Thermal-tolerant alcohol (Thermal-tolerant alcohol)
ii Angel brand super alcohol
iii Angel super alcohol.

2.2. Raw Material Preparation

A kilogram (1kg) of *Nauclea latifolia* (Sm.) fruit was chopped, crushed into particles and pressed using a mechanical press equipped with a filter in the presence of 1.5L of distilled water. The resulting juice was sterilized at 121°C for 15 minutes.

2.3. Preparation of Yeast Suspensions

The revivification of dry and active *S. cerevisiae* and *S. carlsbergensis* was done in buffered peptone water. One gram (1g) of strain is inoculated into 9ml of this water and homogenized for 30 minutes. Different masses of 1, 2, 3, 4 and 5g of each of these yeast strains used were revived and activated as ferments in the context of carrying out this work.

2.4. Fermentation

The sterilized *Nauclea latifolia* juice, cooled to room temperature (25°C) and distributed in fermentors, was seeded with yeast strains of *S. cerevisiae* and *S. carlsbergensis* at different concentrations (1–5g/l). We have juice enriched with urea (4g/l) and non-enriched musts. The must does not contain ferment and urea. During this fermentation the fermenters were kept closed for seven (7) days at room temperature (25°C).

2.5. Monitoring of Fermentation Parameters

Parameters such as the brix degree, the density and the pH of the musts were followed from the beginning to the end of the alcoholic fermentation. The brix level of a must was determined using a Palm Abbe 201 MISCO handheld refractometer. The pH was determined using an OHAUS ST10 digital pHmeter. The relative density at 25°C of musts was determined according to a standard method of the Association of Official Analytical Chemists (AOAC) [14].

2.6. Distillation of the Musts

The extraction of the ethanol was carried out by distillation of the musts using a vigorous QUICKFIT/FC3/13 column distiller of 85cm in length and 4.45cm in diameter. During the distillation the temperature was maintained at 79°C at the head of the vigreux column until the alcohol from the must of the heating flask was exhausted.

2.7. Attenuation Limit

It gives an estimate of the amount of sugars consumed or likely to be converted into alcohol during fermentation [15].

$$\mathrm{Al} = \frac{\text{Brix degree (initial)} - \text{Brix degree (final)}}{\text{Brix degree (initial)}} \times 100$$

2.8. Sugar Consumption Speed Rate

It expresses the amount of consumed sugar by a unit of fermentation time by the yeasts used.

$$V_{\mathrm{cons}} = \frac{\text{Brix degree (initial)} - \text{Brix degree (final)}}{\text{Fermentation time}}$$

The consumption speed rate of sugars is expressed in °Bx/h

2.9. Average Production of Ethanol

The average production of a must is the bioethanol yield of the latter. It gives the amount of bioethanol produced from 1kg of *Nauclea latifolia* (Sm.) fruits used [16].

$$\mathrm{R} = \frac{\text{Volume of ethanol obtained}}{\text{Fruit mass formulated as juice}}$$

Where R is expressed in ml/kg

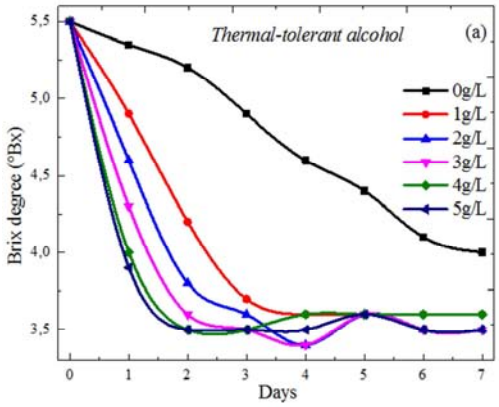
2.10. Statistical Analyzes

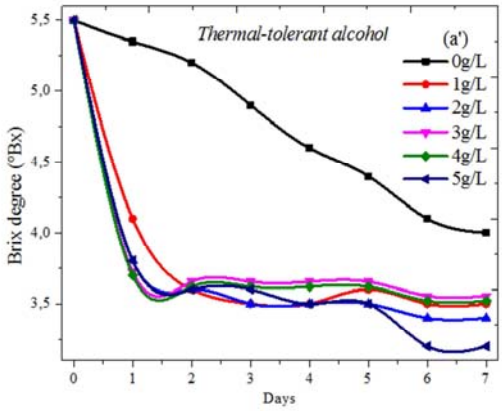
The tests were repeated three times and the data was processed using the Microsoft Excel 2010 software. The statistical analysis of the data and the comparison of the independent averages were made at the 5% threshold by SPSS 16.0.

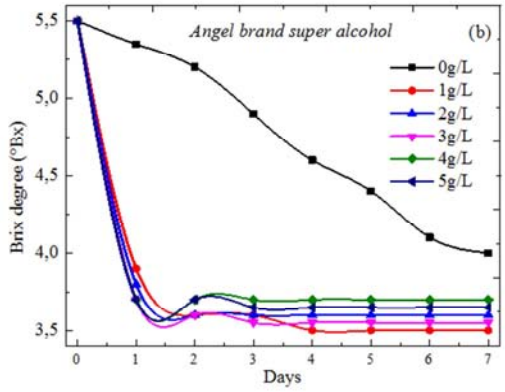
3. Results and Discussion

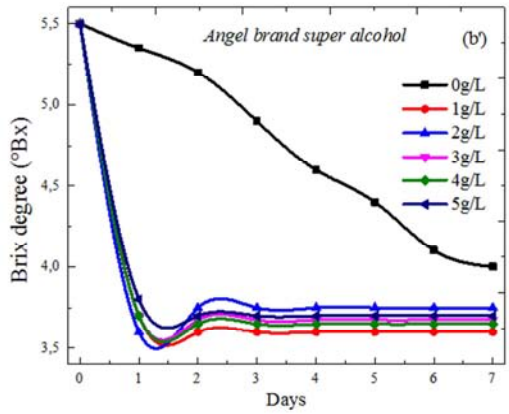
3.1. Brix Degree of Musts

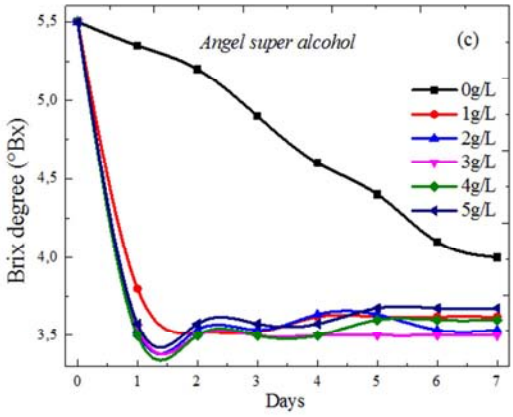
Figure 1 shows the variation of the brix degree of all the musts of the alcoholic fermentation. We observed a sharp drop in the rate of soluble solids from must to ferment compared to the sample must. In the case of *Thermal-tolerant alcohol* musts (figure.1a and figure.1a') the addition of urea (4g/l) had influence on the fall of their brix. The musts of concentrations 1 and 2g/l and enriched with urea fell in 48 hours whereas the non-enriched took 72 hours to reach the limit-fall of their brix degree. The variation of the Brix degree of the urea enriched and non-enriched musts, in the presence of *Angel brand super alcohol*, *Angel super alcohol* or *Saccharomyces carlsbergensis*, was uniform. In general, the brix level of must containing a strain of *Saccharomyces cerevisiae* drops from 5.5 to 3.5°Bx and those containing *Saccharomyces carlsbergensis* from 5.5 to 3.6°Bx. The maximum consumption of sugars by *S. cerevisiae* and *S. carlsbergensis* was respectively 48 and 72 hours of fermentation, with the exception of non-enriched musts with *Thermal-tolerant alcohol* (1 and 2g/l) and *S. carlsbergensis* (1g/l) which made 72 and 96 hours.

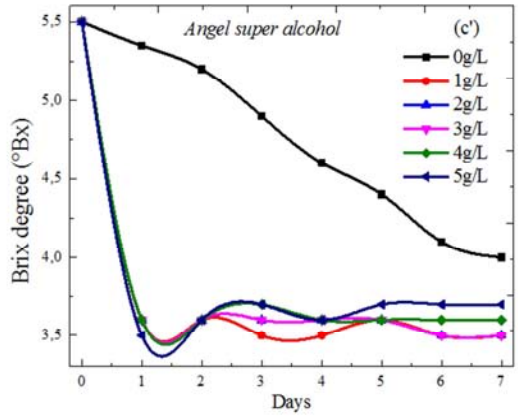


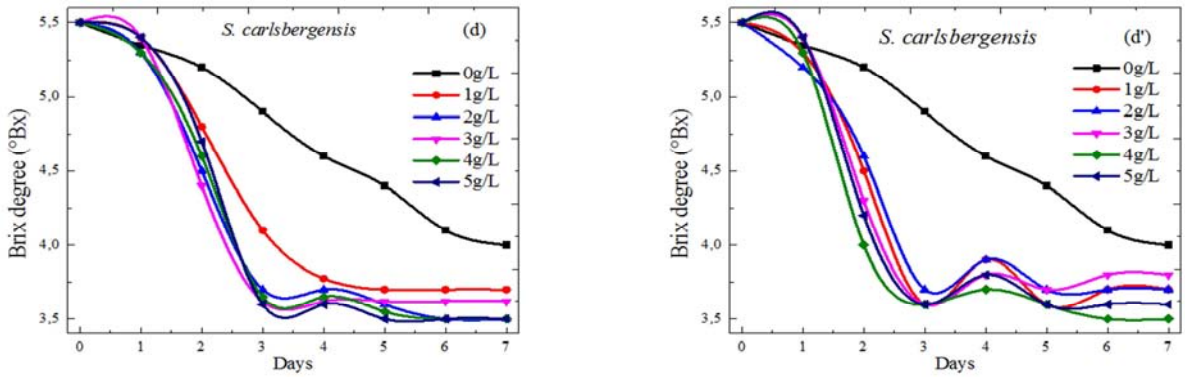












Musts without urea (figure 1a, 1b, 1c, 1d) Musts with urea (figure 1a', 1b', 1c', 1d')

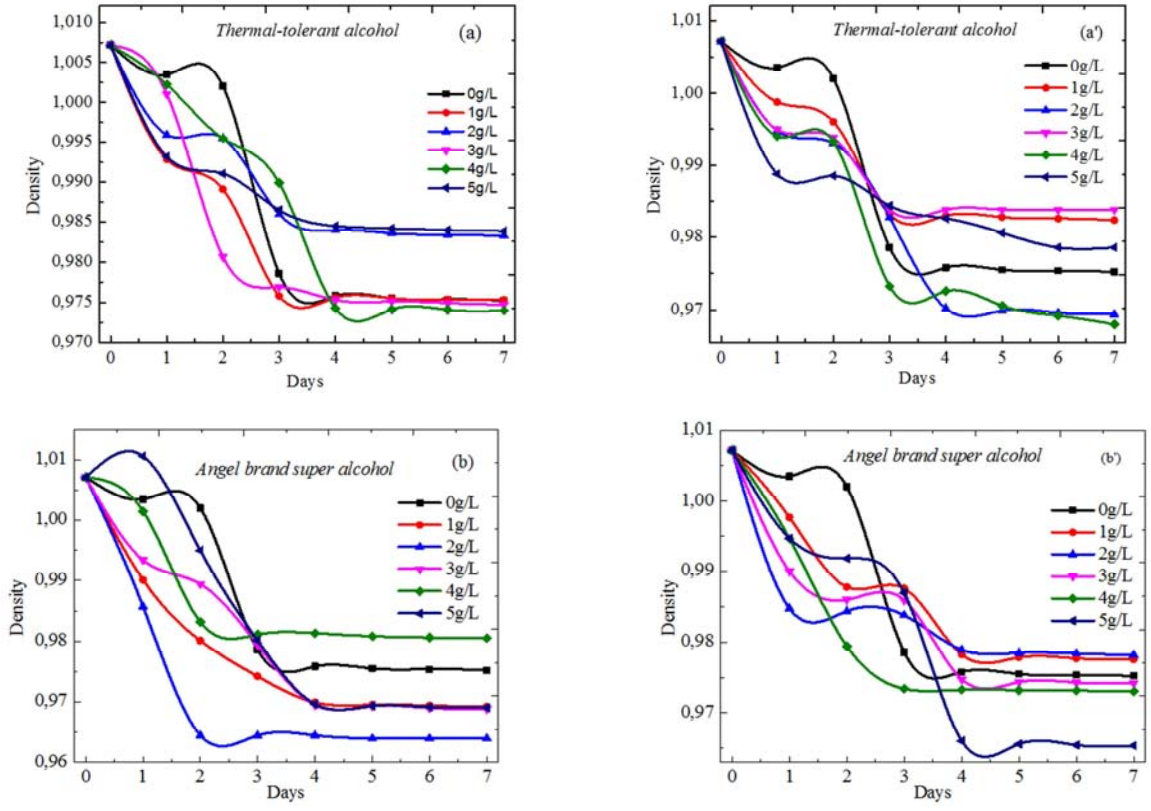
Figure 1. *Variation of Brix level of non-enriched musts and must enriched with 4g/l urea of Nauclea latifolia (Sm.) fruits in the presence of different concentrations of Thermal-tolerant alcohol, Angel brand super alcohol, Angel super alcohol and Saccharomyces carlsbergensis.*

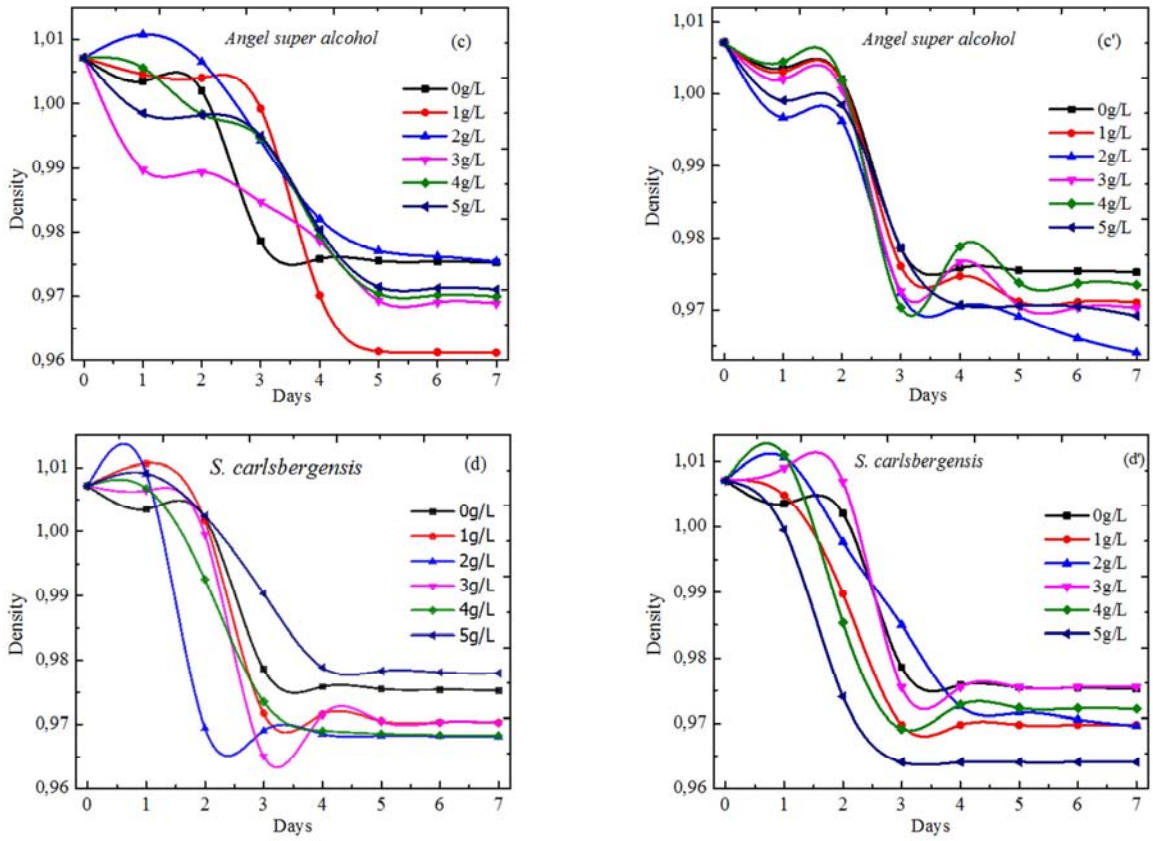
The variation of the brix level of musts containing *S. cerevisiae* presented two phases. The first phase of decay, which lasted 24 hours, showed a rapid decrease in brix. It showed the consumption of sugars by microbial strains. The second phase called the stabilization phase was observed after 24 hours of fermentation until it stopped. During this phase, the brix level of musts had not decreased. This constant variation of brix has virtually shown the end of sugar consumption. As for the musts containing *S. carlsbergensis*, their brix degree consisted in three phases of variation. During the acclimation phase, between 0 and 24 hours of fermentation, the layers adapt to the medium. The decay phase is between 24 and 72 hours and the stabilization phase starts on the third day until the end of fermentation.

As part of the evaluation of the fermentative potency of *Saccharomyces cerevisiae* and *S. carlsbergensis* in the production of bioethanol from cashew apple juice, Gbohaïda et al. (2016) note that the brix level of non-enriched and urea enriched musts (4g/l) decreases respectively from 13°Bx to 3.3 and 3.8°Bx [17].

3.2. Density

Figure 2 shows the evolution of the density of enriched and non-enriched urea musts of *Nauclea latifolia* (Sm.) fruits. It showed the gradual loss of the weight of fermenting areas. This triphasic evolution was observed during the fermentation. In general, the acclimation phase lasted 48 hours followed by the decay phase between 48 and 72 hours and the stabilization phase from the 72nd hour of fermentation. The density of the musts was followed from the beginning to the end of the fermentation. Initially of 1.007, it dropped to reach 0.961 observed non-enriched must with the *Angel super alcohol* 1g/l ferment (figure 2c).



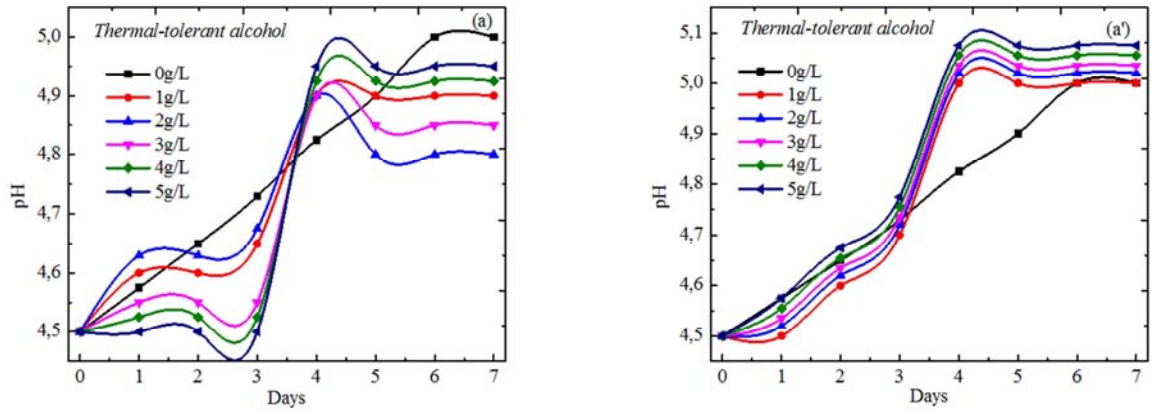


Musts without urea (figure 2a, 2b, 2c, 2d) Musts with urea (figure 2a', 2b', 2c', 2d')

Figure 2. *Evolution of the density of non-enriched musts and musts enriched with Nauclea latifolia (Sm.) fruit urea 4g/l in the presence of different concentrations of Thermal-tolerant alcohol, Angel brand super alcohol, Angel super alcohol and Saccharomyces carlsbergensis.*

3.3. pH of Musts

The pH monitoring of musts has been presented in figure 3 above. In the presence of *Thermal-tolerant alcohol*, *Angel brand super alcohol*, *Angel super alcohol* and *Saccharomyces carlsbergensis*, the musts showed an increase in pH during fermentation. The pH increased from 4.5 to 5.2 in 120 hours of fermentation. It stabilized for the *Thermal-tolerant alcohol* and fell for all the rest of the must containing ferment. In the case of the sample must, pH growth is linear.



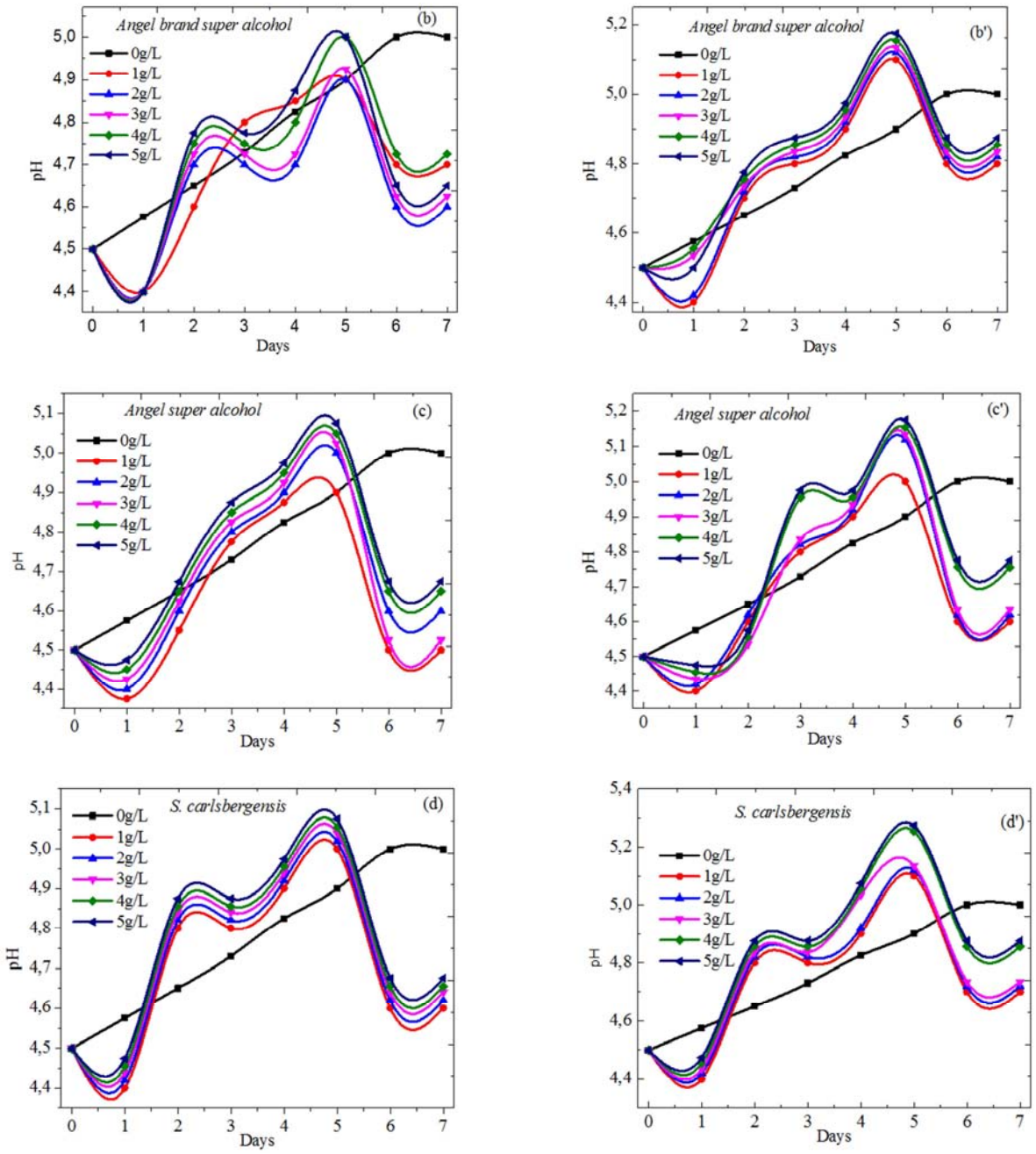


Figure 3. *PH monitoring of non-enriched musts and musts enriched with Nauclea latifolia (Sm.) urea 4g/l in the presence of different concentrations of Thermal-tolerant alcohol, Angel brand super alcohol, Angel super alcohol and Saccharomyces carlsbergensis.*

At the end of the fermentation, the aging and death observed on the yeast cells are due to the decrease of the intracellular pH, to the proton impulse or to intermediate reactions [18, 19]. The pace of pH observed from the fifth day could highlight this loss of yeast viability.

3.4. Fermentation Time

The average duration of fermentation is given in figure 4. The sample wort has an average duration of 144 hours compared with 72 hours for *S. carlsbergensis* except *S. carlsbergensis* (1g/l) which lasted 96 hours. When the concentration of *S. cerevisiae* strains used is low (1 g/l), the fermentation time is long. The high concentration (>2g/l) of *S. cerevisiae* accelerates the alcoholic fermentation. According to the work of Shen et al. (2012), Novidzro et al. (2013) then Gbohaïda et al. (2015) on the juices of lignocellulosic biomass in the presence of a strain of *S. cerevisiae*, a good fermentation can last between 24 and 144 hours [20-22]. According to Gubicza et al. (2016) and Appiah-Nkansah et al. (2018) the end of an alcoholic fermentation is marked by the stop of the consumption of sugars by the yeasts and by the stop of the fall of the weight of the fermentary medium [23, 24].

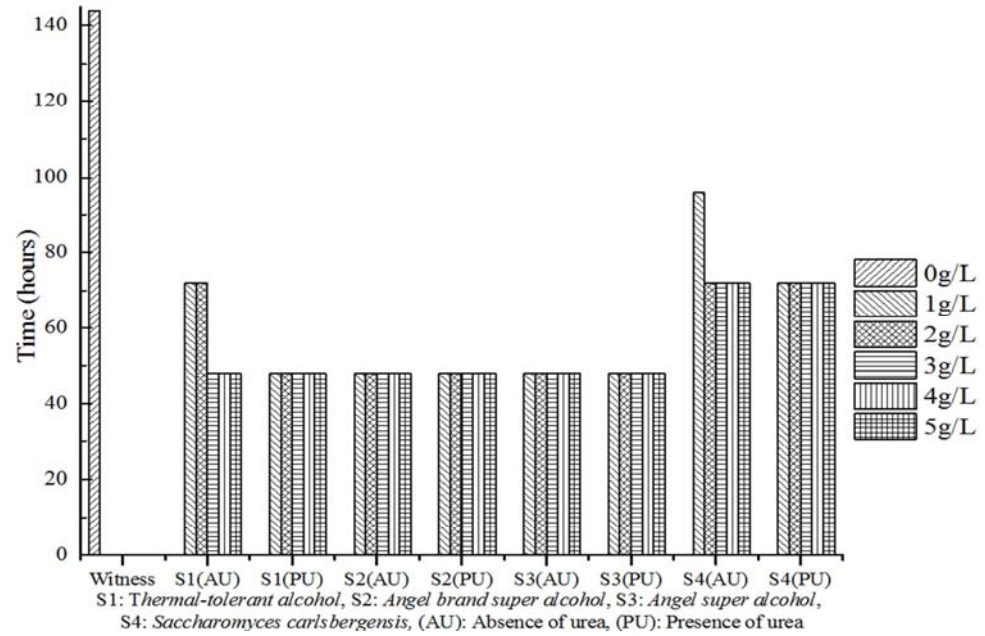


Figure 4. *Average duration of alcoholic fermentation of musts of Nauclea latifolia (Sm.) fruits.*

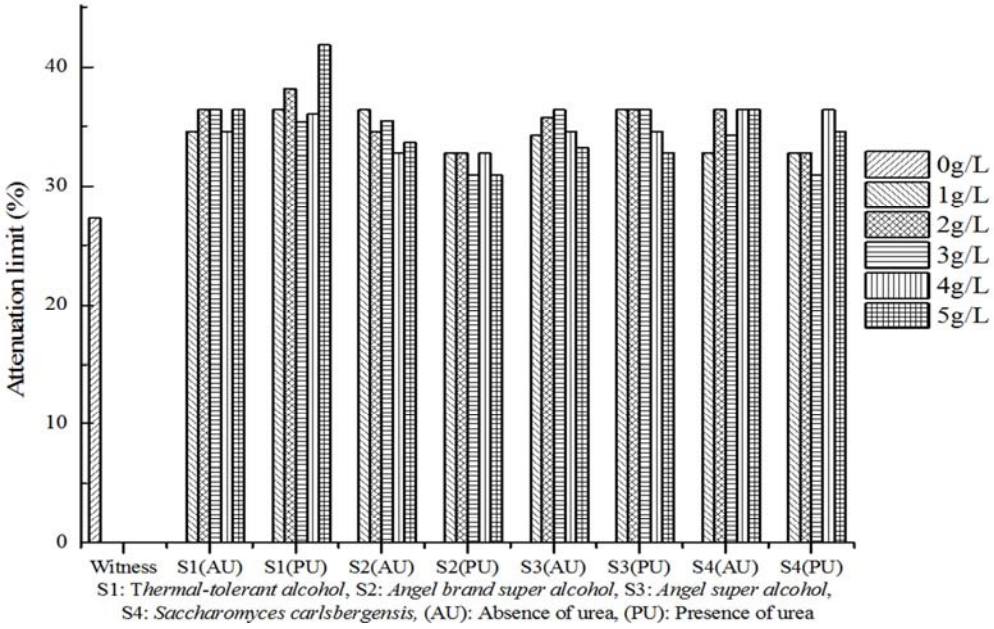


Figure 5. *Sugar consumption rate by strains of Saccharomyces cerevisiae and Saccharomyces carlsbergensis.*

3.5. Attenuation Limit

The average percentage of sugar consumption by the strains of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* was presented in Figure 5. The rod strips showed that this attenuation limit did not depend on the concentration of yeasts used. In general, it has been noticed in all musts a rate of consumption of sugars between 30.9 and 41.8%.

According to the work of Gbohaïda et al. on strains of *Saccharomyces cerevisiae* (*Angel brand super alcohol*, *Angel super alcohol* and *Angel brand Thermal-tolerant alcohol*) and *Saccharomyces carlsbergensis* in the fermentation of cashew apple juice the limiting attenuation is between 69.5 and 75% [17].

3.6. Sugar Consumption Rate

The average sugar consumption rate was given in Figure 6. The three strains of S. cerevisiae (*Angel brand Thermal-tolerant alcohol*, *Angel brand super alcohol* and *Angel super alcohol*) have a higher consumption rate than the *Saccharomyces carlsbergensis*. The highest value (0.08°Bx/h) was derived from the 4g/l urea-enriched must with *Angel super alcohol* (2g/l) and the lowest value (0.02°Bx/h) was from *S. carlsbergensis* (1g/l) without adding urea.

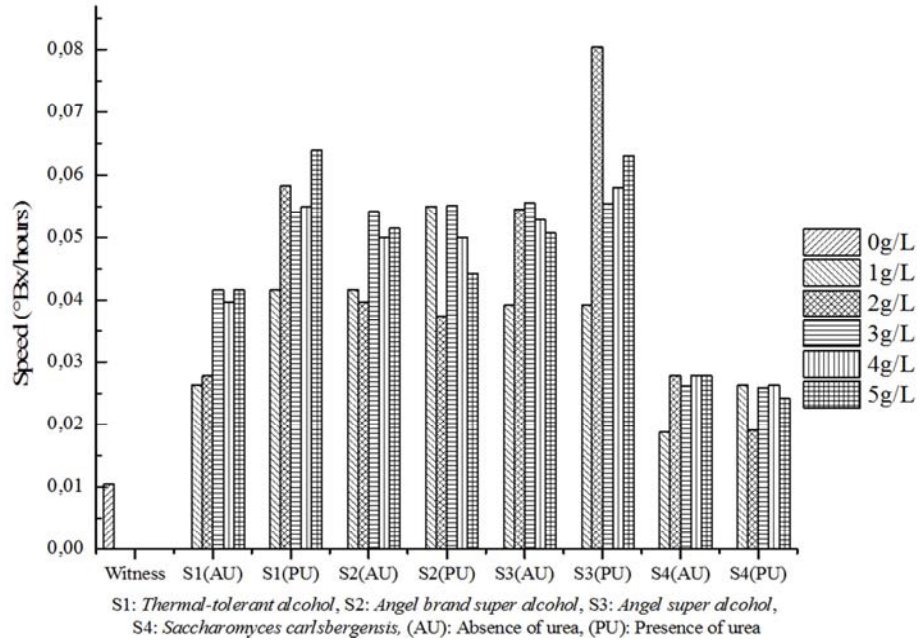


Figure 6. *Speed of sugar consumption by strains of Saccharomyces cerevisiae and Saccharomyces carlsbergensis.*

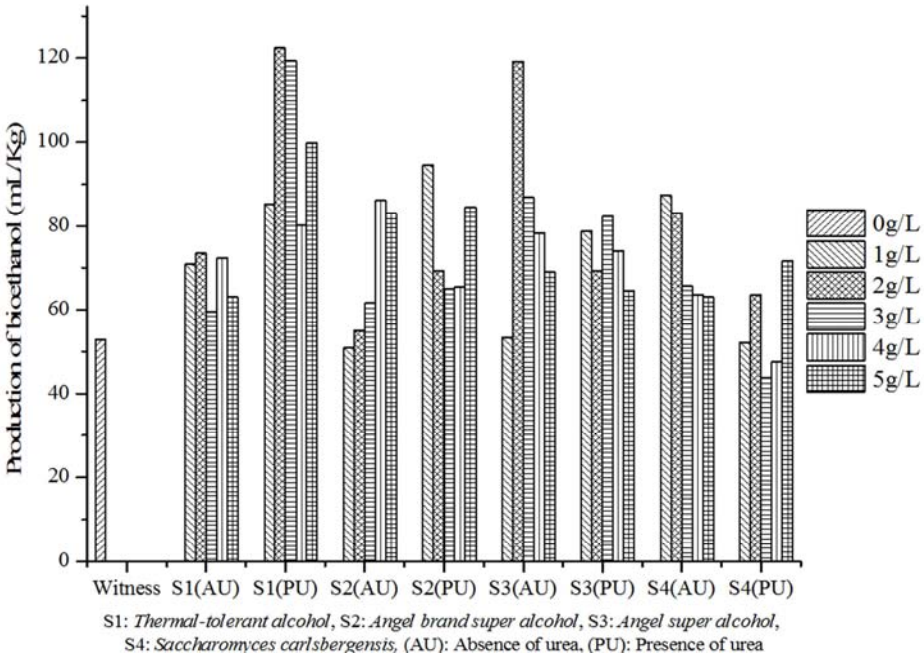


Figure 7. *Bioethanol production.*

3.7. Production Yield

The production yield of bioethanol by the strains of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* of *Nauclea latifolia* (Sm.) fruits in the presence is presented in figure 7. In the presence of urea, the strain *Thermal-tolerant alcohol* (of concentrations 2, 3 and 5g/l) showed its performance through production yields of 99.9±0.2, 119.4±0.4 and 122.4±0.4ml/kg recorded. In the absence of urea, it was the *Angel super alcohol* strain (2g/l) that provided 119.2±0.3ml/kg. The strain *S. carlsbergensis* (1 and 2g/l) yielded 87.4±0.3 and 83.2±0.3ml/kg in the absence of urea. Like lignocellulosic biomasses (cashew apples, *Balanites aegyptiaca*, *Curcubita pepo*, *Dialium guineense*, *Opilia amentacea* and *Sorghum saccharatum*) where strains of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* showed their ethanolic bioconversion performances [15, 25, 26], the fruits of *Nauclea latifolia* (Sm.) could contribute to the production of bioethanol in the presence of these yeasts.

4. Conclusion

Strains of *Saccharomyces cerevisiae* and *Saccharomyces*

carlsbergensis showed through this work their performance of bioethanol production of *Nauclea latifolia* (Sm.) fruits. These results show that the Angel brand must-have heat-tolerant alcohol (at concentrations 2 and 3g/l in the presence of urea) and *Angel super alcohol* (2g/l in the absence of urea) respectively provided 122.4±0.4, 119.4±0.4 and 119.2±0.3ml/kg. These strains may have a high affinity for *Nauclea latifolia* (Sm.) fruits. They could contribute on a large scale to the production of bioethanol. Thus, these fruits that were neglected in the wild in lethal times could contribute to the production of bioethanol fuel.

References

[1] Kyoto. Kyoto protocol to the United Nations framework convention on climate change United. FCCC/INFORMAL/83 GE.05-61647 (F) 070605 090605, 1998.

[2] New York. United Nations Framework Convention on Climate Change. New York Convention, May 9, 1992.

[3] Surendra A and Erik RI, (2016). Climate-driven polar motion: 2003–2015. *Science Advances*, 2(4): 1501693. DOI: 10.1126/sciadv.1501693.

[4] World Meteorological Organization (WMO). WMO Bulletin on Green house Gas, 2016.

[5] Montreal. Montreal Protocol on Substances that Deplete the Ozone Layer, Montreal Protocol, 16 September 1987.

[6] Paris. Conférence sur les changements climatiques. Conférence des Parties (COP) ; Bourget, France, 2015.

[7] Surendra A and Erik RI. Climate-driven polar motion: 2003–2015. *Science Advance;* 2(4), p 1501693, 2016. DOI: 10.1126/sciadv.1501693.

[8] Mali. Stockholm Convention on Persistent Organic Pollutants (POP), Regional Training Workshop for French-speaking African Countries on Polychlorides of Biphenyls (PCB) and Wastes Containing Persistent Organic Pollutants (POP). Report of March 2010. North-South Hotel, Bamako, Mali, March 8-11, 2010.

[9] Changwon. Ramsar COP10 Resolution X.25, Les zones humides et les «biocarburants». Changwon, République de Corée, 28 octobre au 4 novembre, 2008.

[10] Food and Agriculture Organization of the United Nations (FAO). Bioenergy, food security and sustainability: towards an international framework. High Level Conference on Global Food Security: The Challenges of Climate Change and Bioenergy. Rome, 3-5 jun 2008.

[11] Haudecoeur R, Peuchmaur M, Pérès B, Rome M, Taïwe MS, Boumendjel A, Boucherle A. Traditional uses, phytochemistry and pharmacological properties of African Nauclea species. *Journal of Ethnopharmacology;* 17(4), pp8728-8741, 2017.

[12] Badiaga M. An ethnobotanical, phytochemical and biological study of *Nauclea latifolia* Smith, an African medicinal plant harvested in Mali. Doctoral thesis, Université Blaise Pascal – Clermont Ferrand II. Français, 2011.

[13] Akoègninou A, van der Burg WJ et van der Maesen LJG. *Flore analytique du Bénin*. Université d'Abomey-Calavi, Cotonou & Wageningen, Backhuys Publishers, 2006.

[14] Association of Official Analytical Chemists. *Methods of Analysis*. Washington DC 18th Ed, 2003.

[15] Novidzro KM. Production of bioethanol by alcoholic fermentation of fruit juices of: *Balanites aegyptiaca*, *Curcubitapepo*, *Dialiumguineense* and *Opilliaamentacea*, PhD. thesis: chemistry of natural substances. University of Lomé (Togo); p.219, 2013.

[16] Mossi I, Adjou ES, Agbangnan DCP, Nonviho G, Adda MMC, Yehouenou BB and Sohounhloué DCK. Production of bioethanol from stems of *Sorghum saccharatum* monitoring kinetic parameters of fermentation. *J Chem Eng Mater Sci*; 9(2), pp9-16, 2018.

[17] Gbohaïda V, Mossi I, Adjou ES, Agbangnan Dossa CP, Wotto DV, Avlessi F, Sohounhloué DCK. Évaluation du pouvoir fermentaire de *Saccharomyces cerevisiae* et de *S. carlsbergensis* dans la production de bioéthanol à partir du jus de la pomme cajou. *Journal of Applied Biosciences;* 101, pp9643 – 9652, 2016.

[18] Kasemets K, Kahru A, Laht TM and Palme T. Study of the toxic effect of short and medium chain monocarboxylic acids on the growth of *Saccharomyces cerevisiae* using the CO2 auxo acceletostat fermentation system. *International Journal of Food Microbial;* 111, pp206-215, 2006.

[19] Ferreira BS, Cecilia RC, Van Keulen F and Cabral MS. Recombinant *Saccharomyces cerevisiae* strain triggers acetate production to fuel biosynthetic pathways. *Journal of biotechnology;* 109, pp159-167, 2004.

[20] Shen F, Hu J, Zhong Y, Michael LYL, Jack NS, Liu R. Ethanol production from steam pretreated sweet sorghum bagasse with high substrate consistency enzymatic hydrolysis. *Biomass Bioenergy;* 41, pp157-164, 2012.

[21] Novidzro KM, Agbodan KA, Koumaglo KH. Study of the performance of four strains of *Saccharomyces cerevisiae* during the production of ethanol from enriched sucrose musts. *J Soc Ouest-Afr Chim*; 35(12), pp1-7, 2013.

[22] Gbohaïda V, Mossi I, Adjou ES, Agbangnan Dossa CP, Yêhouènou BB, Sohounhloué DCK. Morphological and Physicochemical Characterizations of Cashew Apples from Benin for their use as Raw Material in Bioethanol Production. *International Journal of Pharmaceutic;* 35(2), pp7-11, 2015.

[23] Gubicza K, Nieves UI, William JS, Barta Z, Shanmugam KT, Ingram LO. Techno-economic analysis of ethanol production from sugarcane bagasse using a Liquefaction plus Simultaneous Saccharification and co-fermentation process. *Journal of Bioresource Technolog;* 208, pp42-48, 2016.

[24] Appiah-Nkansah NB, Zhang K, Rooney W, Wang D. Ethanol production from mixtures of sweet sorghum juice and sorghum starch using very high gravity fermentation with urea supplementation. *Industrial Crops and Product;* 111, pp247-253, 2018.

[25] Mossi I, Adjou ES, Gbaguidi BA, Agbangnan Dossa CP and Sohounhloué DCK. Valorization of extracts from sorghum stems (*Sorghum saccharatum* L.) by alcoholic bioconversion. *International Journal of Chemical Studies*; 5(6), pp1231-1234, 2017.

[26] Umashankar N, Mohan Chavan, Benherlal PS and Maruthesh AM. Standardization of fermentation process for the production of cashew wine. *International Journal of Science and Nature;* 5 (2), pp226-230, 2014.