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Valorization of fruit residues of *Nauclea latifolia* (Sm.) by enzymatic catalysis and alcoholic bioconversion

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Abstract
The present study aims to evaluate conditions for bioethanol production from fruit residues of *Nauclea latifolia* (Sm.). To do this, the juice obtained from the fruit residues of *Nauclea latifolia*, initially pretreated at 200 °C and hydrolyzed in the presence of enzyme (Pectinase), was fermented with three different commercial strains of *Saccharomyces cerevisiae* (Angel Brand Thermal-tolerant alcohol active dry yeast, Angel Brand Super alcohol active dry yeast, Angel Super alcohol active dry yeast). The monitoring of the kinetic parameters of fermentation indicated that the production yields depend on the yeast strains and the presence of compounds used as a growth factor such as Urea. The best yield of 34.48 $ml.kg^{-1}$ is obtained with Angel Brand Super alcohol active dry yeast strains, with addition of growth factor to the fermentation medium. The fruit residues of *Nauclea latifolia* (Sm.) hydrolyzed by the enzymatic process, therefore constitute an interesting substrate which can be used as alternative sources for bioethanol production.

Keywords: *Nauclea latifolia*, enzymatic catalysis, alcoholic bioconversion, bioethanol, Benin

Introduction
The most obvious manifestations of climate change are global warming, rising sea levels, drying up of lakes and lagoons, and increased greenhouse effects. Other foreseeable risks are the emergence of new species and the total disappearance of many species of flora and fauna due to their inability to adapt to different changes. Faced with these threats, most of the Third World countries have set up research programs for alternative sources of energy in general and biofuels in particular [1]. Especially since the fuel industry consumes more petroleum products and provides more greenhouse gases, it is essential to find substitutes for fossil fuels.
Bioethanol is one of the valuable substitutes for gasoline, which is the main fuel currently used in transportation. Indeed, it is accepted that the use of pure bioethanol instead of gasoline allows a reduction in carbon dioxide (CO_2) emissions of around 90% [2]. Beyond environmental issues, biofuels help to reduce the energy dependency of countries. An increase of the production of biofuel could make possible the reduction of petroleum and its derivatives products. In addition, the interest in producing of bioethanol also stems from the fact that it is a strategic energy substance whose use covers a wide range of industrial activities [3]. These include the manufacture of spirits, solvents, detergents, disinfectants, organic acids and chemical intermediates such as cosmetics, perfumes, cosmetics and pharmaceuticals [4]. Thus, the use of new resources and the improvement of yields are all challenges to be met in order to ensure the emergence of viable and sustainable processes.
Nauclea latifolia (Sm.) is a plant from the African pharmacopoeia whose root and barks are commonly used in the treatment of severe digestive disorders, gastrointestinal pathologies, abdominal pain (colic), certain parasitic and hepatobiliary disorders and digestive disorders [5, 6]. The roots, bark, trunk and leaves are used by people for the treatment of stomach aches and indigestion in children [7]. In Benin, the fruits of *Nauclea latifolia* (Sm.) are abandoned by the population and rot in large quantities every year. Thus, the objective of this study is to enhance this available plant resource through the production of bioethanol by enzymatic catalysis and alcoholic bioconversion.

Material and Methods

Collection of plant material

The fruit residues of *Nauclea latifolia* (Sm.) are collected by picking and by direct collection from trees, in the forest areas of Ségbana in northern Benin.

Pretreatment by steam explosion

The fruit residues collected are cut, crushed and pretreated by steam explosion according to the method described by [8]. In fact, the ground vegetal material are soaked with distilled water (0.75 $l.kg^{-1}$) and brought to a temperature of 250 °C in an electric saturated steam oven (Memmert brand 854 Schwabach) for 30 minutes.

Enzymatic hydrolysis and juice extraction

The enzymatic hydrolysis of the pretreated ground material is carried out using Pectinase from *Aspergillus niger*. Three different concentrations of enzymes (1; 2 and 3$g.kg^{-1}$) are used. Incubation is carried out at a temperature of 50 ± 1 °C for seven (7) days. Periodic sampling followed by physicochemical analyses are allowed to follow the evolution of degree Brix (°Bx) during the incubation period. After that, the plant material are pressed using a mechanical press equipped with a filter, in the presence of 0.5L of distilled water used as humectant. The collected juice is then sterilized at 121 °C for 15 minutes.

Alcoholic fermentation and distillation

The three lyophilized strains of *Saccharomyces cerevisiae*, used for alcoholic fermentation, are purchased from the company "Angel Yeast CO., LTD". These are *Angel Brand Thermal-tolerant alcohol active dry yeast*, *Angel Brand Super alcohol active dry yeast*, *Angel Super alcohol active dry yeast*. For the preparation of the fermentation musts, a preculture of the dry yeasts was carried out using peptone water (0.5g of dry $yeast.ml^{-1}$) for 30 minutes at 25 °C in order to revive the yeast cells. These revived yeasts are then used as a ferment in the preparation of the musts by using extracted juices obtained from *Nauclea latifolia*. For each strain of yeast, three different concentrations (3; 4 and 5$g.l^{-1}$) are used. The effect of growth factors on the fermentative activities of the different yeast strains used, was also evaluated by incorporating Urea (4$g.l^{-1}$) in the musts before incubation. Negative controls (musts without Urea and without ferments) were also carried out. The alcoholic fermentation is carried out in batch mode for seven (7) days at a temperature of 29 °C, and the some kinetic parameters (pH and Brix) are followed throughout the fermentation process. The pH is determined by direct measurement using the OHAUS ST10 brand portable pH meter, according to the method described by the NF ISO 1842 standards. The Brix degree is determined using a MISCO Palm brand portable refractometer Abbe 201. At the end of the fermentation process, the extraction of bioethanol was carried out by distillation using a column of vigreux QUICKFIT / FC3, and production yields (Rp) were determined.

Statistical Analyses

The data generated from these studies were analyzed using Statistical Analysis Software (SAS) and SYSTAT 5.05. The statistical analyses carried out were mean and standard deviation and analysis of variance (ANOVA).

Results and Discussion

Figure 1 presented the results of the evolution of Brix (°Bx) during the enzymatic hydrolysis process of fruit residues of *Nauclea latifolia*. Analysis of the results indicated that the increase in Brix (°Bx) during enzymatic hydrolysis depends on the enzyme concentration and the incubation time. Thus with an enzyme concentration of 3$g.kg^{-1}$, the best value of Brix (4.1°Bx) is obtained after an incubation time of 36 hours. These results also underlined the effectiveness of the steam pretreatment method used. Indeed, according to Ogier *et al*. [8], steam pretreatment results in partial hydrolysis of hemicellulose, melting of lignins and intensified the destruction of the plant wall, thus increasing the accessible surfaces for enzymes [9]. This is accompanied by a decrease in the degree of polymerization, but also by an increase in the crystallinity index of cellulose [10]. Thus, complex substrates such as lignocellulosic biomass, can therefore be hydrolyzed by the enzymatic complex (Pectinase), and their efficiency results in the synergistic action of three types of enzymes, in particular cellulases (endo 1.4 β-glucanases and exo 1.4 ß-glucanases) which hydrolyze celluloses to cellobiose and ß-glucosidases which hydrolyze cellobiose to glucose [8]. With a view to modeling the kinetics of enzymatic hydrolysis, models taking into account the structure of the substrate, have been developed in the literature in order to describe the influence of the morphology of the substrate on the hydrolysis reaction [11]. These are empirical models, models based on the Michaelis-Menten equation, mechanistic models and models taking into account the structure of the substrate; because the rate of hydrolysis depends closely on the intrinsic parameters of the enzymes but also on the reactivity of the substrate [8]. However, after a certain incubation time, there is a gradual slowing down of the enzymatic hydrolysis process, as indicated in the present study, by the stabilization of the Brix (°Bx) value of the samples during incubation (Figure 1). Then, according to Chauve [12], whatever the enzymes and the substrate used, a gradual decrease in the reaction rate is observed after a long period during the bioconversion process. Several factors could be responsible for this decrease and could be associated with the inhibition of enzymes by reaction products or with the non-productive adsorption of enzymes.

Figures 2 and 3 presented the results of the physicochemical parameters of the musts during the fermentation process. Results indicated that in the musts inoculated with selected strains of *Saccharomyces cerevisiae*, the pH increased very quickly during the first 48 hours of fermentation time when compared to the control samples (Figure 2). This rapid increase in pH therefore indicates a systematic onset of fermentation activity by the different strains of yeast with a much reduced latency phase. This fermentation model could be explained by the fact that the yeast strains used are from industrial origin and are further revived by peptone water in preculture before there used. Similar results are obtained when monitoring the evolution of the Brix (°Bx) in fermentation musts (Figure 3). In fact, the results obtained indicated a rapid decrease in the Brix (°Bx) during the first hours of fermentation in musts fermented with the different selected strains of *Saccharomyces cerevisiae*, when compared to the control.

This reduction in the Brix (°Bx) could result in a bioconversion of the fermentable sugars present in the fermentation musts.

However, after a relative period of 3 or 4 days, there is a stabilization of the values of kinetics parameters, which therefore indicated the cessation of the fermentation process. Novidzro *et al.* [4] have reported that the stopping of the reaction of an alcoholic fermentation is due to the lack of fermentable sugars in the medium, or either in response to the various stresses undergone by the microorganisms involved in the fermentation. Indeed, during the fermentation process, the yeasts transform the sugars into carbon dioxide and alcohol. This metabolism is characterized by a set of reactions that occur in the absence of oxygen as the final electron acceptor. Glucose, which is a carbohydrates with 6 carbon atoms, enters into the cell where it undergoes phosphorilations before being split into two (2) molecules. The latters enter into a series of reactions resulting in pyruvate which, in the absence of oxygen, is transformed into acetaldehyde and then into bioethanol excreted by the cell [13]. Table 1 presented the bioethanol production yields of the musts. Results indicated that the yield values obtained are between 8.95 and 34.48 $ml.kg^{-1}$. The best production yield is obtained in musts inoculated with the *Angel Super alcohol active dry yeast strain*, in the presence of Urea as growth factor. Indeed, taking into account the characteristics of this strain (given by the Manufacturer), it is a high quality yeast strain, specially selected for his high density alcoholic fermentation activity. It also is a yeast strain with a high reproductive capacity, a high fermentation rate and a high tolerance to a high level concentration of ethanol. However, the poor performance of the other yeast strains used in the present study could be due to the non-adaptation of these strains for the raw material (*Nauclea latifolia*) used, or to other parameters as reported by Bellissimi and Ingledew [14]. They reported that among the industrial active dry yeasts sold specifically for the production of alcohol, some have a significantly low viability and others have a high levels of contaminants (presence of anaerobic bacteria), resulting in a subsequent loss of ethanol yield during fermentation [14].

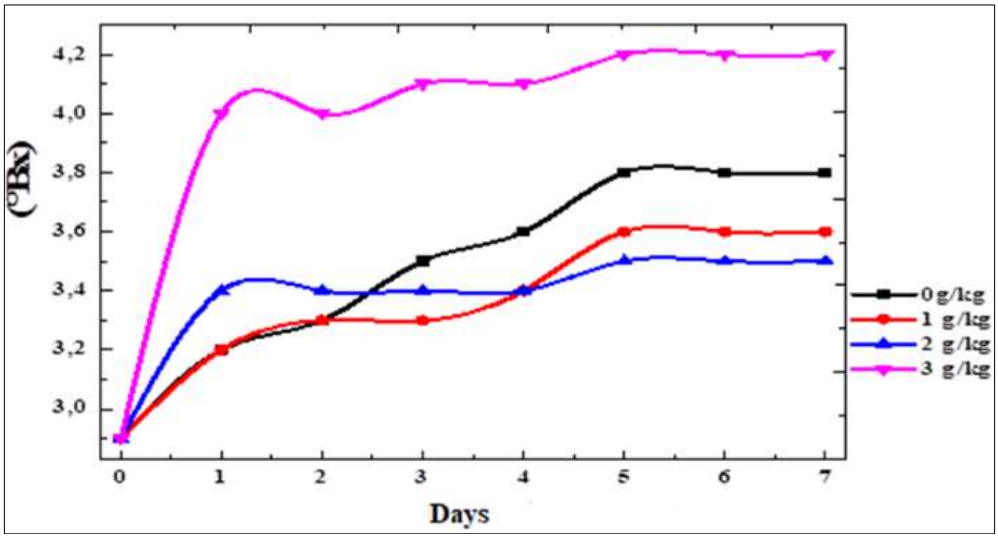
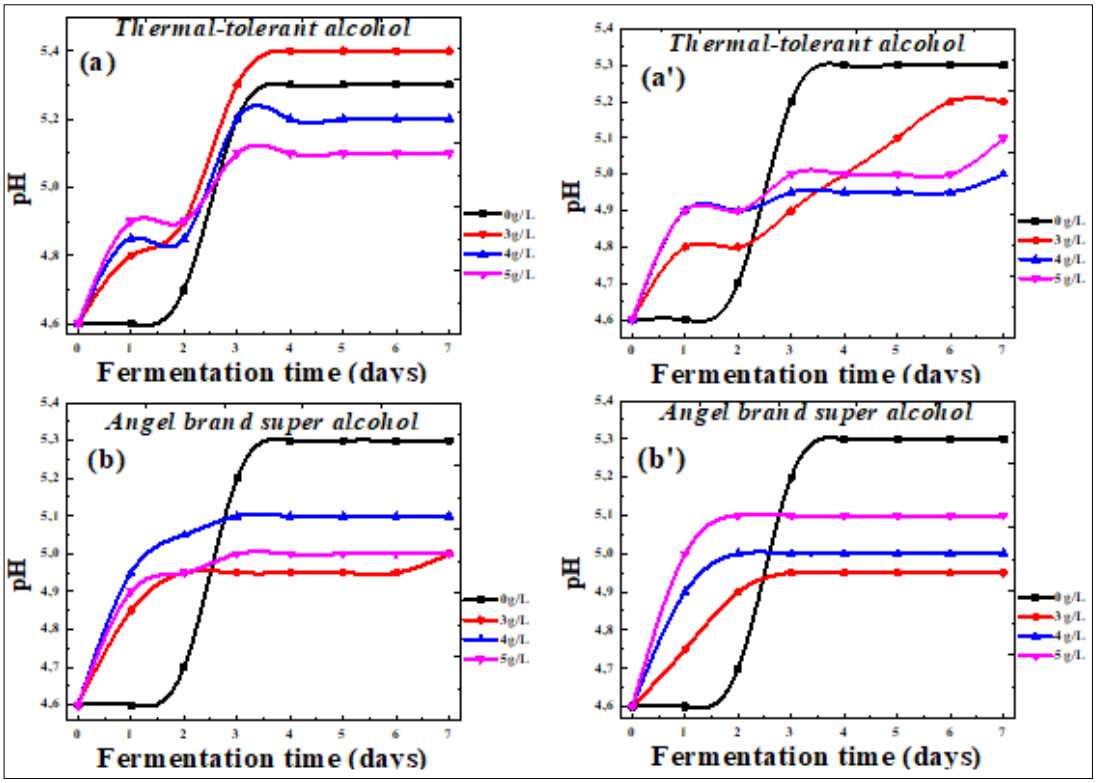
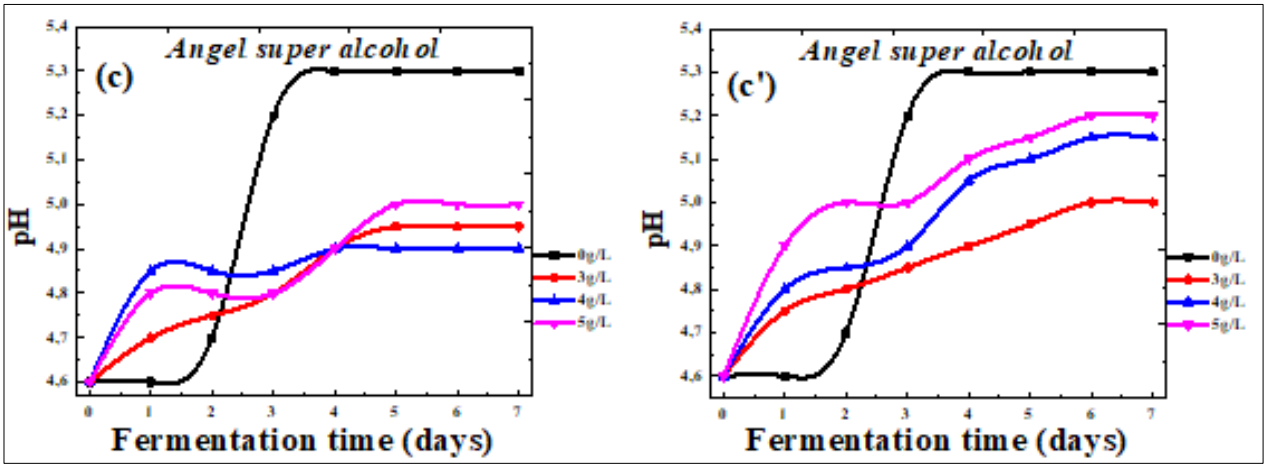


Fig 1: Evolution of the Brix (°Bx) during the enzymatic hydrolysis of fruit residues of *Nauclea latifolia* (Sm.)





a, b, c are musts without urea;
a', b', c' are musts with urea

Fig 2: Evolution of the pH of the musts during the fermentation processes of the fruit residues of *Nauclea latifolia* (Sm.)

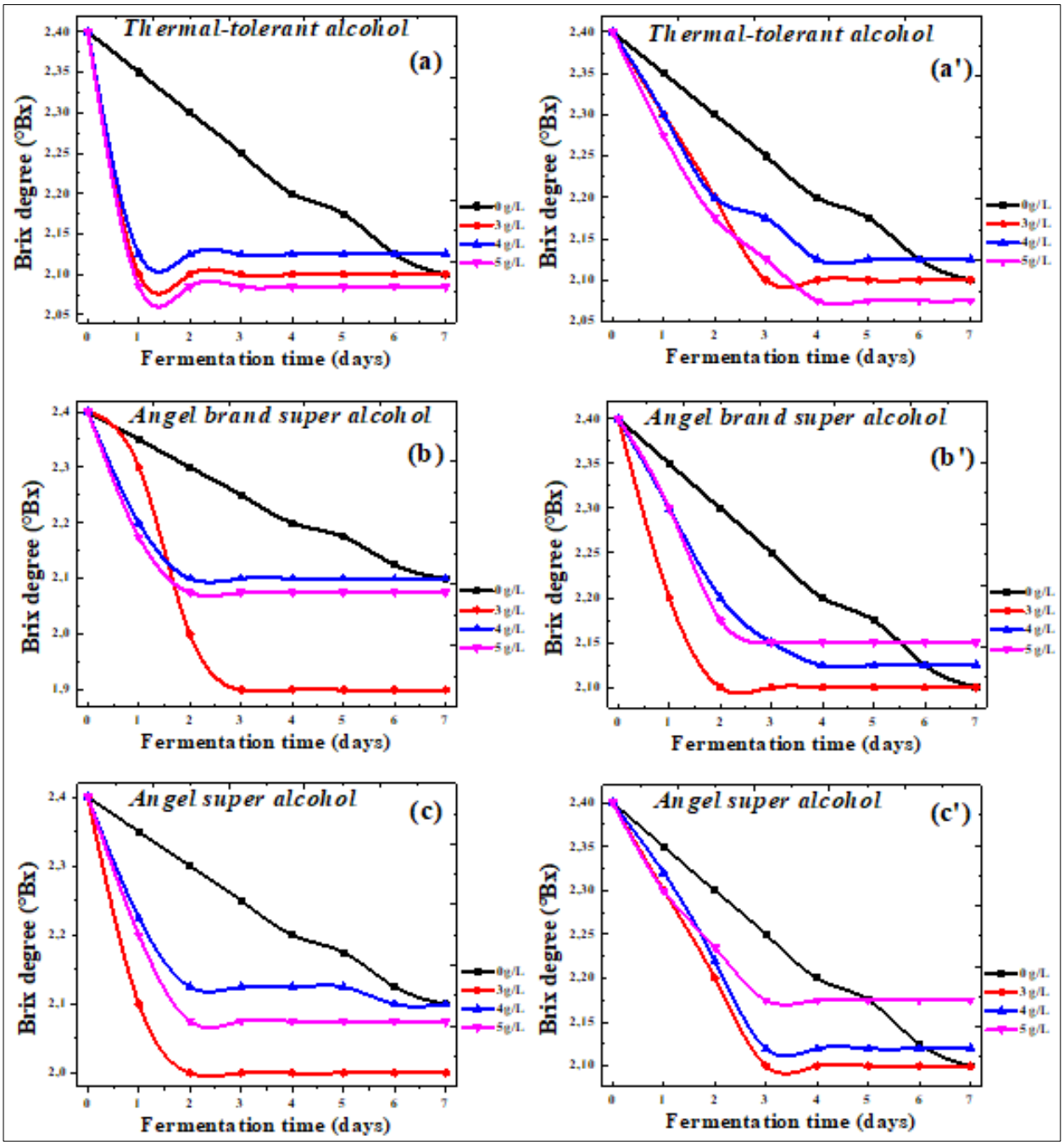


Fig 3: Evolution of the Brix (°Bx) of the musts during the fermentation processes of the fruit residues of *Nauclea latifolia* (Sm.)

Table 1: Yields of bioethanol production by different yeast strains during the fermentation processes of the fruit residues of *Nauclea latifolia* (Sm.)

		Initial concentration of yeast strains			
yeasts	**Urea**	**Control**	**3 g.l^{-1}**	**4 g.l^{-1}**	**5 g.l^{-1}**
Angel Brand Thermal-tolerant alcohol active dry yeast	Absence	8,95±0,07^{a}	12,66±0,08^{a}	14,82±0,07^{a}	21,52±0,11^{a}
	Presence	8,95±0,07^{a}	17,42±0,09^{b}	24,05±0,12^{b}	20,91±0,10^{a}
Angel Brand Super alcohol active dry yeast	Absence	8,95±0,07^{a}	22,09±0,10^{c}	23,18±0,10^{b}	20,70±0,09^{a}
	Presence	8,95±0,07^{a}	28,35±0,11^{d}	26,85±0,12^{c}	31,98±0,12^{b}
Angel Super alcohol active dry yeast	Absence	8,95±0,07^{a}	25,16±0,11^{e}	31,93±0,13^{d}	21,76±0,10^{a}
	Presence	8,95±0,07^{a}	26,19±0,11^{e}	34,48±0,13^{d}	34,27±0,09^{c}

Values with the same letter in the same column are not significantly different (p <5%) according to ANOVA and Tukey's multiple comparison tests

Conclusion

The present study underlined the interesting and valuable potential of the fruit residues from *Nauclea latifolia* through the production of bioethanol by enzymatic catalysis and alcoholic bioconversion. This study therefore offers a novel perspectives in the promotion and valorization of plant species whose fruits are of little interest to the population in Africa.

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