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Kosi Mawuéna Novidzro
(a) Laboratory of Plant Extracts and Natural Aromas (LEVAN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01BP 1515 Lomé 01, Togo
(b) Laboratory of Process Engineering and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01BP 1515 Lomé 01, Togo

Mamatchi Melila
Laboratory of Process Engineering and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01BP 1515 Lomé 01, Togo

Balbine Amoussou Fagla
School of Nutrition, Food Sciences and Technologies, Faculty of Agricultural Sciences, University of Abomey-Calavi, 01BP 526 Cotonou, Bénin

Kokouvi Dotse
(a) Laboratory of Plant Extracts and Natural Aromas (LEVAN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01BP 1515 Lomé 01, Togo
(b) Laboratory of Process Engineering and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01BP 1515 Lomé 01, Togo

Kossi Honoré Koumaglo
(a) Laboratory of Plant Extracts and Natural Aromas (LEVAN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01BP 1515 Lomé 01, Togo
(b) Laboratory of Process Engineering and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01BP 1515 Lomé 01, Togo

Corresponding Author:
Kosi Mawuéna Novidzro
(a) Laboratory of Plant Extracts and Natural Aromas (LEVAN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01BP 1515 Lomé 01, Togo
(b) Laboratory of Process Engineering and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01BP 1515 Lomé 01, Togo

Potentialities of bio-based ethanol production with tuberous roots of *Icacina senegalensis* in comparison with tubers of *Manihot esculenta* and *Ipomea batatas*

Kosi Mawuéna Novidzro, Mamatchi Melila, Balbine Amoussou Fagla, Kokouvi Dotse and Kossi Honoré Koumaglo

Abstract
Global warming is one of the manifestations of greenhouse gas emissions from fossil fuels consumption. To reduce the trend in transport sector, policymakers around the world are favorably supporting researchers in successful programs to replace conventional fuels with biofuels. Togolese flora has a potential for agro resources rich in convertible sugars into bioethanol. This study aims to promote the use of starch of *Icacina senegalensis*, for green energy production. The hydrolysis of the starches was carried out with 5.5% of H_2SO_4 and the hydrolysates were converted biologically into bioethanol using of *Saccharomyces cerevisiae* yeasts as ferment. The results show that sucrose is more alcoholic than sweet hydrolysates obtained from starches. However, taking into account the non-edibility and availability of raw materials, the tubers of *Icacina senegalensis* stand out as the best substrate for 1th generation bioethanol production. This results can be improve by enzymatic hydrolysis and/or enriching hydrolysates with nutritional supplements.

Keywords: Global warming; inedible plants; acid hydrolysis; green energy production

Introduction
Recurrent petroleum crises, air pollution with its drawbacks and the adverse effects of global warming due to the exorbitant consumption of fossil fuels (Kang *et al.*, 2019) [1] are endangering the global economy. It is mainly poor countries that continue to pay heavy tributes to the deterioration of the economic context caused by the excessive consumption of fossil energy resources. On the other hand, rich countries such as the USA and China, which are big consumers, are reaping the benefits to reach a high level of development without making appropriate commitments to limit their consumption of petroleum products.
The negative consequences of environmentally irresponsible management of terrestrial planetary energy resources are now tangible and can block the development of all mankind if no action is immediately taken. Paradoxically, petroleum resources are becoming increasingly scarce from year to year as the demand rises faster and faster (Ghosh and Nag, 2008; Allouache *et al.*, 2013) [2-3]. Global warming is one of the most spectacular manifestations of greenhouse gas (GHG) emissions from fossil fuel consumption. In order to reduce the trend in the transport sector, new sources of energy are currently being studied to replace fossil fuels. This is why recently, with soaring fuel prices, the government is supporting huge programs to substitute conventional fuels with the original one known as agrofuels or biofuels (Chavanne and Frangi, 2008) [4], such as bioethanol, biodiesel, bioturbosine, green hydrogen, among others. This alternative is gaining credibility on the part of policymakers and the international community. Petroleum origin (Becerra-Ruiz *et al*., 2019) [5].
This alternative has received credibility from policymakers and the international community as it is seen like one of the viable options for reducing the impact of the use and consumption of liquid fuels of petroleum origin (Becerra-Ruiz *et al*., 2019) [5]. Indeed, biofuels have great potential for environmental sustainability as part of the millennium goals for sustainable development. This is justified by the fact that they are produced from biomass, considered as a source of renewable energy thanks to the photosynthetic reaction mechanisms of chlorophyllous plants, algae and cyanobacteria. To this end, biofuels have played and will

continue to play an important role in reducing fuel consumption and GHG (Karvonen and Klemola, 2019) [6].

Among the biofuel production technologies currently practicable, at least three sectors can be distinguished, namely: the first generation (1G), the second generation (2G) and the third generation (3G). The 3G sector is the one that is currently being tested and which consists of using carbon dioxide captured by the biomass of algae and cyanobacteria with a very powerful capacity to fix solar energy and associated with photosynthesis technologies (Karvonen and Klemola, 2019) [6]. While the 2G sector is based on the use of lignocellulosic plant material, the most abundant renewable resource on earth (Finore *et al*., 2016, Zucaro *et al*., 2016, Kang *et al*., 2019) [1, 7-8] and and much cheaper compared to 1G (Karvonen and Klemola, 2019) [6], which could provide more sustainable energy production without harming food security and the environment (Di Donato *et al*., 2019) [9].

In fact, the 2G sector exploits non-food crops (Godin *et al*., 2019, Karvonen and Klemola, 2019) [6, 10], food crop residues and waste from wood-based or food-based industries, such as : wood chips, skins or fruit pressing pastes, respectively (Di Donato *et al*., 2019) [9]. However, the 2G processing methods are very complex (Karvonen and Klemola, 2019) [6], its costs are expensive and the production yields are still very low compared to the 1G, despite the many efforts made to develop them. For now, the industrial process of 2G biofuels producing is still in its infancy (Di Donato *et al*., 2019) [9]. As such, the future and sustainability of the 2G biofuel production is highly dependent on the development of current technologies (Di Donato *et al.*, 2019) [9]. However, the 1G buiofuels is obtained from food materials (Di Donato *et al*., 2019; Karvonen and Klemola, 2019) [6, 9], sweetened or starchy. It seems that it is currently the easiest methods to achieve and with good yields of biofuel production. However, its major disadvantage is that it is derived from edible raw materials. Thus, for ethical reasons, the use of food crops for energy purposes is imperatively prohibited because of the repercussions it can have directly on food security and biodiversity (Di Donato *et al*., 2019) [9].

Indeed, one of the explanations given for the increase in food prices recorded on the markets since 2006 (+ 24% in 2007 and + 53% in 2008, according to the FAO index for 55 products) was attributed to the rivalry stimulated by incentive policies leading to a competition between food needs and the production of biofuels for some products such as maize, sugar cane, wheat and oil palm. According to World Bank estimates, the contribution of agrofuels to price increases is between 15% and 75% in relation to the different types of raw materials used. Agrofuels can therefore be at the origin of tension between food and non-food valorisations of agricultural raw materials. That's why the expansion of bioenergy sector in developing countries must pose risks to the four dimensions of food security, namely: availability, access, stability and use (FAO, 2018) [11]. In the West African sub-region countries, access to food is the most sensitive aspect to which everything must be taken care.

Among the most sought liquid biofuels in the transport sector, bioethanol is currently the most consumed in the world (Allouache *et al*., 2013) [3] because as compared to fossil fuels, it reduces GHG (Verna *et al*, 2003, Kotaka *et al*, 2008) [12-13], responsible for the degradation of the ozone layer (Fossi *et al*., 2009) [14] at a rate of 30 to 85% and contributes to the reduction of the particles emitted in the atmosphere, up to a rate of 50% (Agrillo *et al*., 2013; Riccio *et al*., 2017) [15-16]. The United States of America and Brazil are the leading producers and users of bioethanol as fuel (Di Donato *et al*., 2019) [9].

Togo has a enormous potential in agroresources, rich in fermentable sugars that can be used for bioethanol production (sugar cane, cashew apple, sorghum, millet, maize, cassava) and oleaginous vegetable resources such as cotton, jatropha, peanuts and oil palm for use in biodiesel production. However, since the production of energy from most of these conventional agroresources for the 1G process does not receive favorable support from political authorities and the international community, it is imperative to exploit credible agroresources to boost the production of 1G biofuel production.

In this study, our approach consists in promoting the use of non-edible plants in the bioenergy sector by producing 1G bioethanol in order to make energy production profitable and minimise competition between energy resources and food products. To find answer for these two issues, our choice has focused on the tuberous roots of *Icacina senegalensis* Juss, commonly called false yam, for the production of bioethanol. The specific objectives were to determine the optimal conditions for the hydrolysis of starch extracted from tubers of this plant using sulfuric acid as a catalyst, to study the reaction of ethanol fermentation of hydrolysates and to evaluate the conversion yield of starch into bioethanol. However, in order to better appreciate this biofuel production, comparisons were made with sucrose and tubers of *Manihot esculenta* Crantz and *Ipomea batatas* Lam. The originality of this study lies in the fact that *Icacina senegalensis* is a raw material on which little research has been done, particularly on the use of its starch for bioethanol production. Thus, this agroresource, with regard to its toxicity for humans and its high starch content, has potential non-food valorisation potential among which bioethanol production can constitute an interesting economic and technological opportunity.

Material and Method

Framework

The work was carried out during the June 2010 to December 2010 at the Laboratory of Plant Extracts and Natural Aromas (LEVAN), located in the Department of Chemistry at the Faculty of Sciences of the University of Lomé (UL) Togo.

Plant material

The plant material that has been used is tuberised roots of *Icacina senegalensis*, harvested in May 2010 in Sotouboua; while the tubers of *Manihot esculenta* and *Ipomea batatas* used for comparisons were obtained during the same period at the Assiyéyé market in Adidogomé, the north-western part of Lomé city (Figure 1).

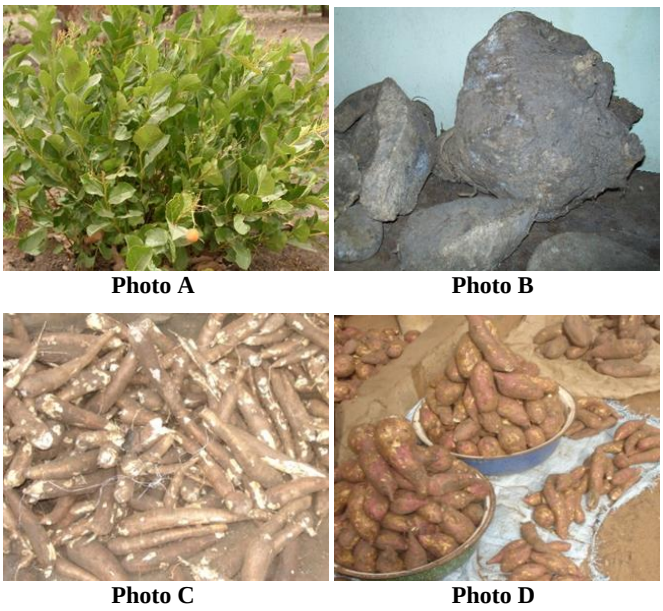

Photo A **Photo B**

Photo C **Photo D**

Fig 1: Plant of *Icacina senegalensis* (Photo A) and Tubers of *Icacina senegalensis* (Photo B), *Manihot esculenta* (Photo C) and *Ipomea batatas* (Photo D)

The process of producing bioethanol from tubers

In this study Separate Hydrolysis and Fermentation (SHF) was applied. It was carried out in three stages: the first consists in making an aqueous extraction of the starch from the tubers; in the second step, the extracted starch has been converted into fermentable sugars by hot acid hydrolysis and the last step concerns the ethanol fermentation of starch hydrolysates with yeasts of the genus *Saccharomyces cerevisiae*. After fermentation, the recovery of bioethanol produced was done by fractional distillation using a Vigreux column. The four different successive operations that had intervened in this bioethanol production from the tubers are illustrated in Figure 2.

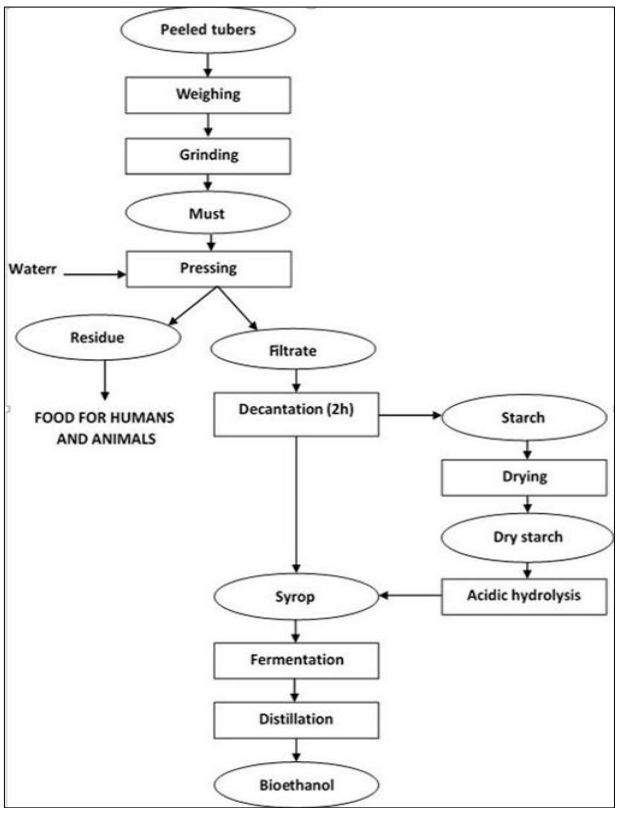

Fig 2: Diagram of bioethanol production from tubers

Starch extraction

The tubers (Icacina senegalensis, Manihot esculenta and Ipomea batatas) have been peeled, washed, dried and weighed. Then, they were first crushed in a mill, and were pressed after adding a volume of water of 1 L for about 5 kg of tubers to facilitate and increase the starch extraction efficiency. The starch milk obtained after filtration was allowed to stand for about two hours. The fresh starch deposited at the bottom of the container was recovered and then dried in the sun for a week, and then dried in an oven.

Calculation of the yields of starch extraction

The yields of starch extraction from tubers were calculated using Formula 1.

$$R = \frac{M_{DS}}{M_{(F/D)T}} x100\ \%$$

With: M_{DS} = mass of dry starch and $M_{(F/D)}$ = fresh or dry tuber mass.

Hydrolysis process of starches

Three hydrolysis alternatives exist: enzymatic hydrolysis, concentrated acid hydrolysis and· dilute acid hydrolysis (Sidiras and Koukios, 2004) [17].

Dilute acid hydrolysis was selected for the production of fermentable sugars obtained from starches via softer conditions than those in the case of concentrated acid. This process uses dilute acid concentration (up to 3 – 4%) in temperatures 100 – 240 °C (Waldron., 2010) [18]. The pH of the starch solutions was determined using a pH-meter (WTW/pH 330i), previously calibrated before any use.

Brix degree of the must's determination

The musts were analysed according to the standard method 934.01 of "Association of Official Analytical Chemists"(Sidney, 1984) [19] for the determination of the rate of soluble solids, expressed in Brix degree.

Fermentation process

Batch fermentation has been applied for the biological transformation of starch hydrolysates into ethanol. The bioreactor is an Erlenmeyer flask of 1 L of capacity, closed with a rubber stopper and in which the must has been fermented.

The conduct of the must's fermentation

Fermentation must be prepared from 250 mL of hydrolysate of starch or sucrose solution. The pH of the must was adjusted to a value close to 4 with a solution of NaOH (5%). Bioethanol was produced biologically. The musts were inoculated with a preculture conducted for 24 hours at laboratory temperature (28 °C - 32 °C) with one-tenth volume of must, and using the active dry yeast called Saf-levure as ferment and the ethanol fermentation was monitored in the dark for 168 hours with continuous stirring at 125 rpm using a magnetic stirrer.

Bioethanol production yields

The theoretical yield, called Gay-Lussac's yield (Bellarini, 2006) [20], of the bioethanol production from a starch or sucrose was calculated from the equation of acid hydrolysis of starch yielding glucose formation (Equation 1); then the ethanolic fermentation reaction of glucose (Equation 2) or sucrose (Equation 3) into ethanol.

$$(C_6H_{10}O_6)n + nH_2O \xrightarrow{(H_2SO_4,\triangle)} nC_6H_{12}O_6 \quad \text{(Equation1)}$$

$$C_6H_{12}O_6 \xrightarrow{\text{Yeasts}} 2CH_3\text{-}CH_2\text{-}OH + 2CO_2 \quad \text{(Equation 2)}$$

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{Yeasts}} 4CH_3\text{-}CH_2\text{-}OH + 4CO_2 \quad \text{(Equation 3)}$$

Results and Discussion

Water and volatile matter content, the rates of dry matter and starch content versus dry matter in tubers

In Figure 3 are shown the water and volatile matter (WVM) content, the rate of dry matter (DM) and starch content (SC) versus DM in tubers within three tubers used as raw materials in this study for bioethanol production.

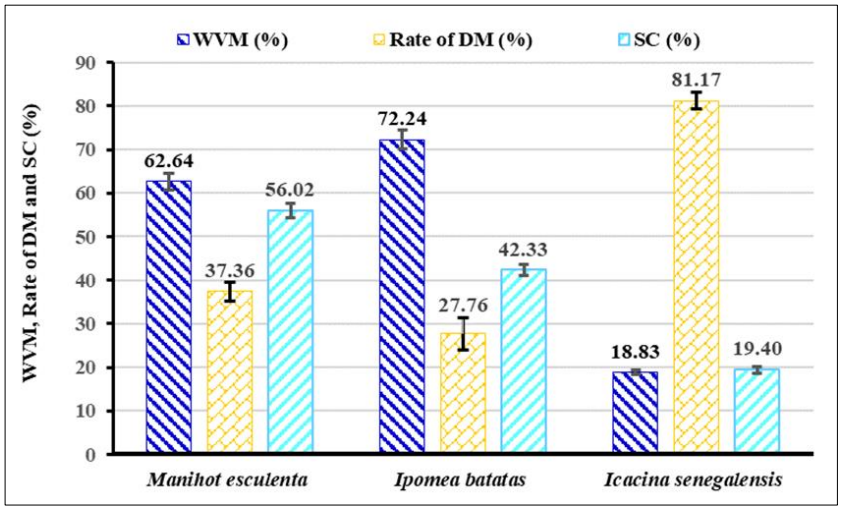

Fig 3: WVM content, the rate of DM and SC versus DM in tubers of *Icacina senegalensis* compared to *Manihot esculenta* and *Ipomea batatas*

Legend: WVM= water and volatile matter; DM = dry matter and SC = starch content.

In this work, it is found that the tubers of *Icacina senegalensis* are the richest in DM with a rate of 81.17%, while that of *Manihot esculenta* and especially that of *Ipomea batatas* have the lowest DM contents, with the values of 37, 36% and 27.76%, respectively. As the DM content is related to the WVM content, the tubers of *Ipomea batatas* have the highest WVM content, ie 72.24%; followed by those of *Manihot esculenta* and *Icacina senegalensis*, ie 32.64% and 18.84%, respectively. In addition, among the three starchy materials tested in this study, *Manihot esculenta* has the highest SC, ie 56.02%, thus the highest potential for ethanol production; followed in descendant order by *Ipomea batatas* (42.33%) and *Icacina senegalensis* (19.40%). However, some researchers have mentioned that there are varieties of *Manihot esculenta*, *Ipomea batatas* and *Icacina senegalensis* with SC of up to 86.59% (Lebot, 2009) [21], 83.8% (Ndiaye, 2009) [22] and 48.63% (Dei *et al*., 2011) [23] of MS, respectively. The use of these starch products is a major advantage for bioethanol production, compared to lignocellulosic materials that require very expensive pretreatment steps (Karvonen and Klemola, 2019) [6]. Nevertheless, the production of bioethanol from starch products is slightly more expensive than that with sweet raw materials which do not require hydrolysis step.

Influence of pH of the reaction medium on the starch's hydrolysis

The study depending on the pH of the reaction mixture of the hydrolysis of starchy solutions of *Manihot esculenta*, *Ipomea batatas and Icacina senegalensis*, with a concentration of 133.3 g/L, led to results in Table 1.

With a pH = 7 for the reaction medium, the reaction of starch hydrolysis is blocked (Table 1), because the heating of the starch solutions gave only agglutinated starch, whatever the source of the starches used in this study.

Table 1: Effect of pH on the physical appearance of starch hydrolysates

Types of starch	pH	Heating time (h)	Initial concentration (°Bx)	Obtained products	
				Hydrolysate appearance	Final Concentration (°Bx)
Manihot esculenta	7.00 ± 0.01	2	0.50 ± 0.01	Agglutinated starch	0.00 ± 0.01
	1.00 ± 0.01	2	0.60 ± 0.01	Syrup	11.60 ± 0.83
Ipomea batatas	7.00 ± 0.01	2	1.00 ± 0.01	Agglutinated starch	0.00 ± 0.01
	1.00 ± 0.01	2	1.02 ± 0.01	Syrup	11.90 ± 0.02
Icacina senegalensis	7.00 ± 0.01	2	0.02 ± 0.01	Agglutinated starch	0.00 ± 0.01
	01.0 ± 0.10	2	0.03 ± 0.01	Syrup	10.19 ± 0.26

However, with a very high acidic pH, ie pH ≈ 1.0, the hydrolysis of starches by heating gave syrups which are liquid solutions, with respective concentrations (measured in °Bx) equal to 11.60 ± 0.83, 12.20 ± 0.02 and 10.19 ± 0.26, for *Manihot esculenta*, *Ipomea batatas* and *Icacina senegalensis*.The difference between the concentrations of these syrups may be explained by the fact that the level of impurities varies according to the sources of the starches. Indeed, since the tubers of *Icacina senegalensis* are more ligneous compared to those of *Manihot esculenta* and *Ipomea batatas*, its hydrolysate has a lower soluble DM content. If the syrup of *Ipomea batatas* is slightly more concentrated in

soluble DM than that of *Manihot esculenta*, this can be justified by the fact that the tubers of *Ipomea batatas* are richer in free sugars, because some authors had indicated that *Ipomea batatas* tubers contain free sugars such as maltose, glucose, sucrose and fructose (Bradbury and Holloway, 1988) [24].

Description of the mechanism of the reaction of acid hydrolysis of starches

In starch grains, the two types of polymer mixed with varying proportions depending on the source of the starch are amylose and amylopectin. Starch granules also differ in size and form depending on their botanical source (Jane *et al*., 1994) [25].

Amylose, which represents 20-30% of the starch, is a polymer of D-Glucose units, linked together by α- (1→4) bonds, whith the degree of polymerisation between 1,000.00 and 5,000.00. Amylose is essentially composed of linear chains, while Amylopectin, the major starch compound, has a branched structure with high molar masses and degrees of polymerisation between 100,000.00 and 1,000,000.00 (Bellarini, 2006) [20]. As these two polymers are not fermentable for yeasts, it is compulsory first to convert them into fermentable sugars by acidic hydrolysis method or enzymatic hydrolysis method, before ethanol fermentation.

Enzymatic hydrolysis of starch is a method that is carried out under mild conditions such as low temperature (about 100 °C), normal pressure and a more or less neutral ambient pH (about 6-8) (Kolusheva and Marinova, 2006) [26]. At the same time the enzymatic hydrolysis is characterized by a high reaction yield, a stability of the enzymes through the denaturalising action of solvents, detergents, proteolytic enzymes, and a decrease in viscosity of the reaction medium at high temperatures, etc.(Manelius, 2005) [27].

According to the acid method, the mechanism of hydrolysis of amylose for example as proposed by Losev *et al*. (2003) [28], can be described as follows (Figure 4).

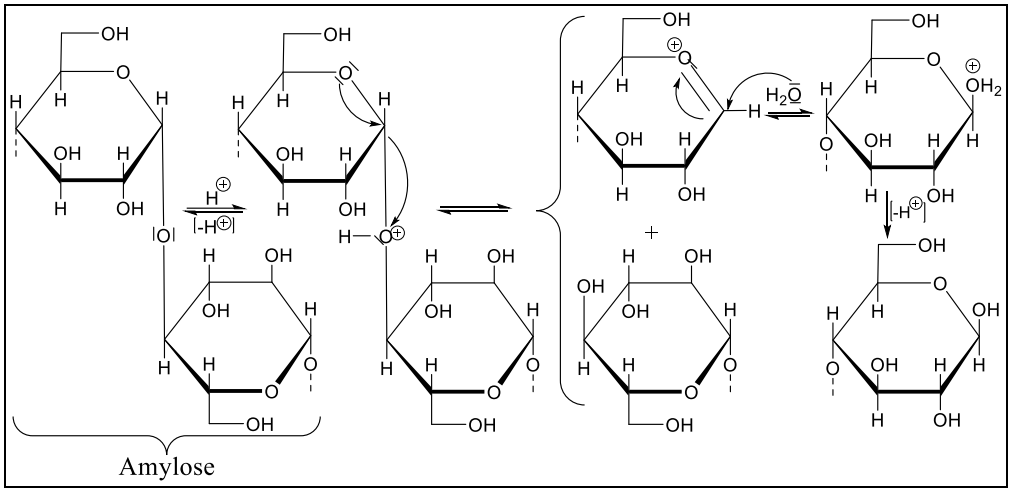

Fig 4: Drawing-directional mechanism for the hydrolysis of amylose

Variation of Brix degree of acidified starch solutions depending on heating duration

During the experimentations, the heating time is another important parameter that is necessary to improve the kinetics of the acid hydrolysis of starches. The results presented in Figure 5 show the Brix degree variation of the starch solutions as a function of heat time during acid hydrolysis of the starches.

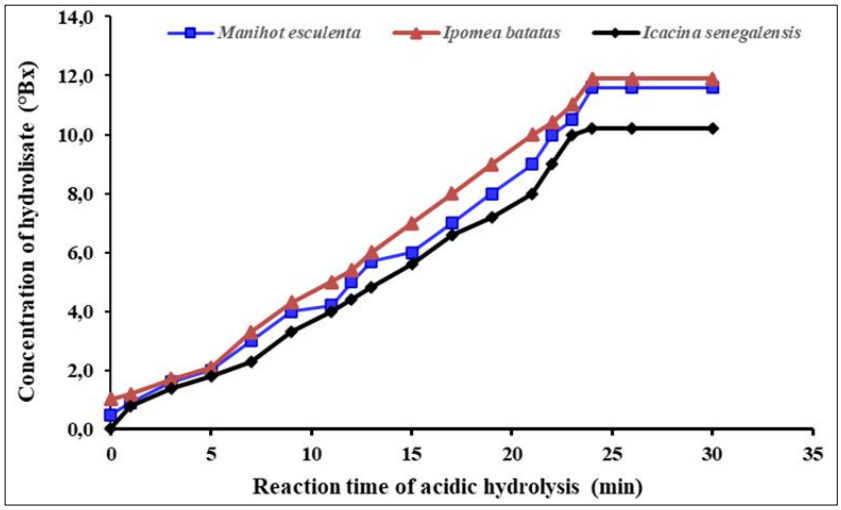

Fig 5: Variation of Brix degree as a function of the time of starch hydrolysis

Comparison of the curves in Figure 5 indicates that the acidic hydrolysis curve of *Ipomea batatas* starch is slightly above of that of *Manihot esculenta*; while that of *Icacina senegalensis* is below that of *Manihot esculenta*. However, in overall, it is found that Brix degree of each starch hydrolysate evolves gradually during the first 25 minutes before stabilising.

The profile of the curves during the first 25 minutes indicates that the conversion of starch polymers to monomeric sugars

by acidic catalysis is not instantaneous. Previous work has shown that this chemical transformation passes successively through three successive phases such as gelatinisation, resulting in the dissolution of starch grains to form a viscous suspension; liquefaction, resulting in partial hydrolysis of the starch, with a simultaneous loss of viscosity; and finally, saccharification, giving rise to the production of glucose and maltose according to the type of hydrolysis adopted (Ruiz *et al*., 2011) [29].

Evolution of the final Brix degree of the syrups according to the initial concentration of starch solutions compared to sucrose

Brix degree was measured after 30 minutes of acidic hydrolysis (pH ≈ 01) by heating the starch solutions. The results in Figure 6 show the Brix degree variations of the hydrolysates as a function of the initial concentration in each starch and in sucrose.

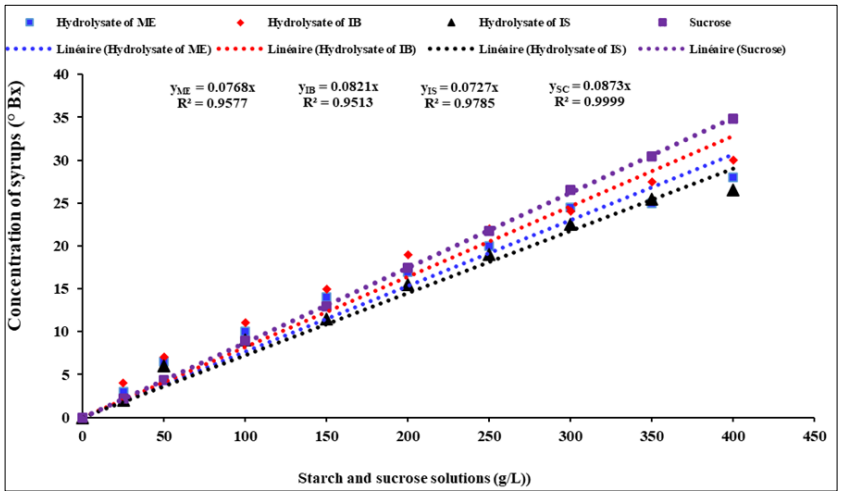

Fig 6: Variation of Brix degree as a function of initial starch concentration

Legend: ME = *Manihot esculenta*; IP = *Ipomea batatas* and IC = *Icacina senegalensis*

Generally, the concentration of each syrup obtained in this study is a linear function of the initial starch concentration of the corresponding solution. But, the slopes of these linear curves are not the same. Indeed, the hydrolysis curve of *Ipomea batatas* starch has the highest slope compared to the other two starches; while the starch of *Icacina senegalensis* is distinguished by the weakest slope. However, the curve representing the sucrose solution, with the same solute concentration as the starch solutions, appears above the others. This shows that the sucrose solution contains more soluble DM than those of the starch syrops.

Comparison of solutions of starches and their acidic hydrolysates with sucrose

Hot hydrolysis of starch solutions at the concentration of 133.3 g/L and with H_2SO_4 (5.5%) for 60 minutes gave syrups. The obtained results, compared to that of a sucrose solution with similar solute concentration, are illustrated on the histograms (Figure 7).

After the acidic hydrolysis of starch solutions with initial concentration equal to 133.3 g/L, the obtained syrups have a content of soluble DM (measured in Brix degree) slightly more or less low than that of sucrose solution of identical concentration. Maybe, the starch of *Icacina senegalensis* would contain more insoluble impurities than the other starches, since its syrup has a lower DM content of 10.19 °Bx. This can be explained by the fact that the tubers of Icacina senegalensis from which the starch has been extracted are more ligneous than the other tubers. On the other hand, if the starch syrup of Ipomea batatas is slightly more concentrated in soluble DM (11.90 °Bx) compared to that of Manihot esculenta (11.60 °Bx), this is due to the fact that Ipomea batatas tuber contain free sugars such as maltose, glucose, sucrose and fructose (Bradbury and Holloway, 1988) [24].

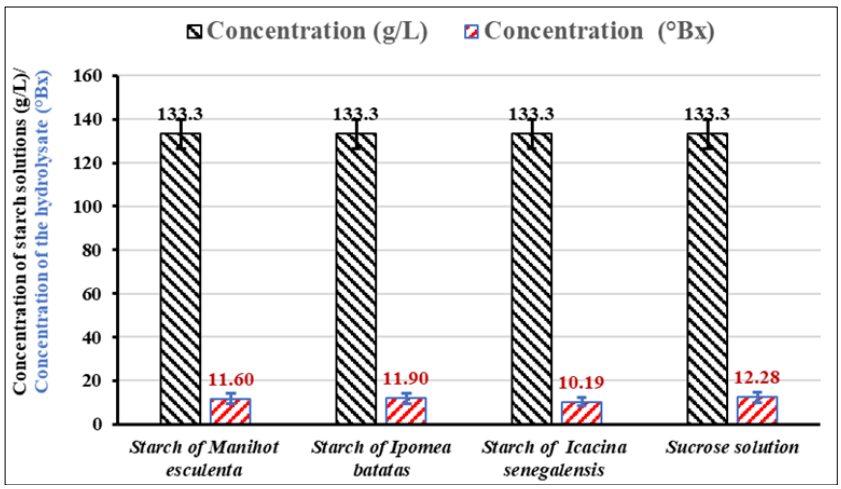

Fig 7: Comparison of Brix degree of hydrolysates with that of a solution sucrose of similar mass concentration

Brix degree variation of the musts according to fermentation time

For the same starch concentration equal to 133.3 g/L, after the hot acidic hydrolysis of the starches and the inoculation of the syrups with the same yeast level equal to 2%, the Brix degree variations during the ethanolic fermentation reaction of the starch hydrolysates of *Manihot esculenta*, *Ipomea batatas* and *Icacina senegalensis* in comparison with a sucrose solution are shown in Figure 8.

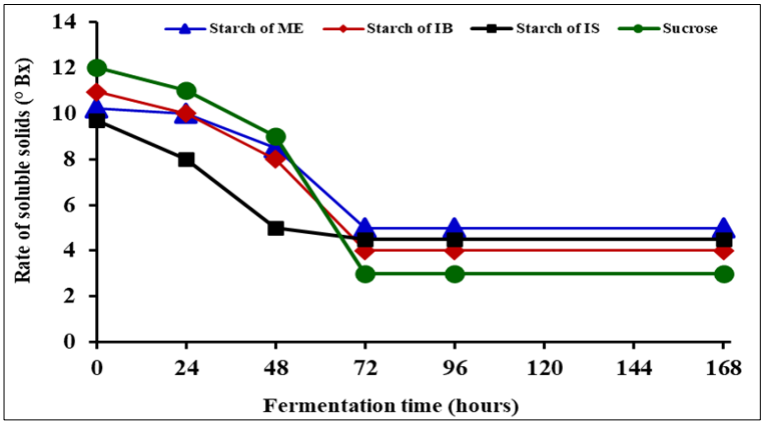

Fig 8: Evolution of Brix degree of the musts according to the fermentation duration

The rate of the decrease in Brix degree is not the same thing for starch syrops and sucrose solution. According to Ban *et al*. (1988) [30], the reduction of Brix degree during a fermentation gives useful information on the kinetics of the reaction, in particular on the four different phases of the reaction, namely: the phase of latency, the phase of the growth of the yeasts, the phase stationary and the decline phase.

Generally, all the curves have four distinct phases: the lag phase, the exponential growth phase of the yeasts, and the stationary phase followed by the decline phase (Novidzro, 2017) [31]. The first so-called latency phase (during the first 2 or 5 hours), characterised by a slow decrease in Brix at the beginning of fermentation, is not visible in Figure 8. However, the consumption of fermentable sugars begins at this stage. This consumption of sugars is primarily intended for the growth and multiplication of yeast cells (Novidzro, 2017) [31].

The 2nd phase, called the exponential growth phase of the yeast biomass, can be observed on the curves by a rapid reduction of Brix degree over a time interval between the 2nd or 5th hour until the 48th hour, depending on the fermentation capacity of each strain. At this stage, a rapid consumption of sugar molecules is observed, due to the yeast population which is very dense, younger and very active. It is during this phase that ethanol production was intensified in relation to the high consumption of sugars in syrups (Novidzro, 2017) [31].

The third phase, known as the stationary phase, which starts from the 48th hour and ends around the 72nd hour, differs from the other previous phases by a gradual slowdown in the reduction of the Brix degree. This is due to the fact that the cell growth becomes very slow, even blocked because of the aging of the yeast population. At this stage, the fermentative activity of the active cells is weaker, because the fermentable sugars become depleted, in addition to the inhibitory effects due to the increase in the concentration of ethanol, CO_2 and other by-products of the reaction (organic acids, glycerol, etc.) (Novidzro, 2017) [31].

Finally, the 4th phase, called decline phase, starts from the 72nd hour. It ends the process and is characterised by the stabilisation of the Brix degree at the values of 5.0 ° Bx, 4.0 ° Bx, 4.5 ° Bx and 3.0 ° Bx, respectively for starch syrups of *Manihot esculenta*, *Ipomea batatas* and *Icacina senegalensis*, then the sucrose. This phase marks a definitive end to the ethanolic fermentation reaction (Novidzro, 2017) [31].

Influence of heating time on the Brix decrease rate after fermentation

For the same initial starch concentration of *Icacina senegalensis*, ie 150.0 ± 0.1 g/L, but with different heating times, the Brix degree reduction rates after the ethanol fermentation of the syrups obtained by inoculating with the same level of yeast, ie 2%, are shown in Figure 9.

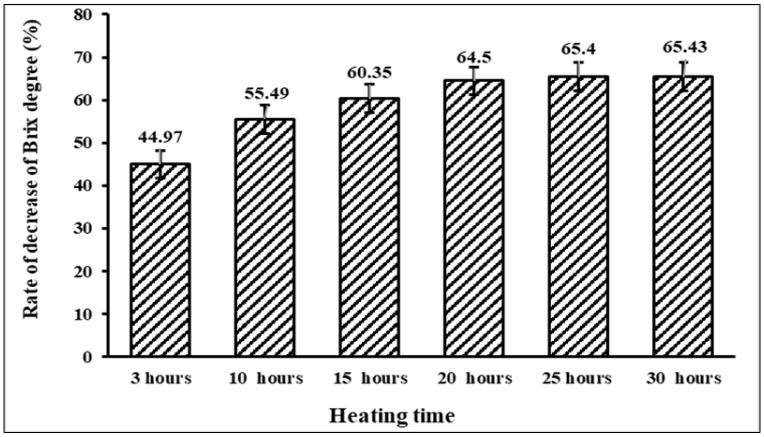

Fig 9: Variation of the decrease rate of Brix degree as a function of the heating duration of *Icacina senegalensis* starch syrups after ethanolic fermentation

Comparison of the Brix degree decrease rates of the syrups as a function of the heating time reveals that the maintenance of the starch hydrolysates beyond 25 minutes of heating without modifying the soluble DM content of the syrup, helps to improve the yield of ethanol fermentation. Indeed, when the heating time of the syrup goes from 3 hours to 30 hours, the Brix reduction rate of the syrup increases from 44.97 °Bx to 65.43 °Bx (Figure 9). As the decrease in Brix degree is a sign that indicates the consumption of fermentable sugars, the higher the rate of decline is, and so will be the yield of the bioethanol production in this work.

Yields of ethanol production

In Figures 9, 10 and 11, are shown the yields of ethanol production compared to starch masses with sucrose and with the masses of peeled tubers, in percentage and in kg of ethanol/ton of tubers peeled, respectively.

Knowing that 162 g of starch can provide by hydrolysis 180 g of glucose (Equation 1) and 100.00 g of glucose gives 51.11 g of ethanol (Equation 2) (Bellarini, 2006) [20], in this study the yields of starch-based bioethanol production with respect to the theoretical yield of Gay-Lussac are shown in Figures 10 to 13.

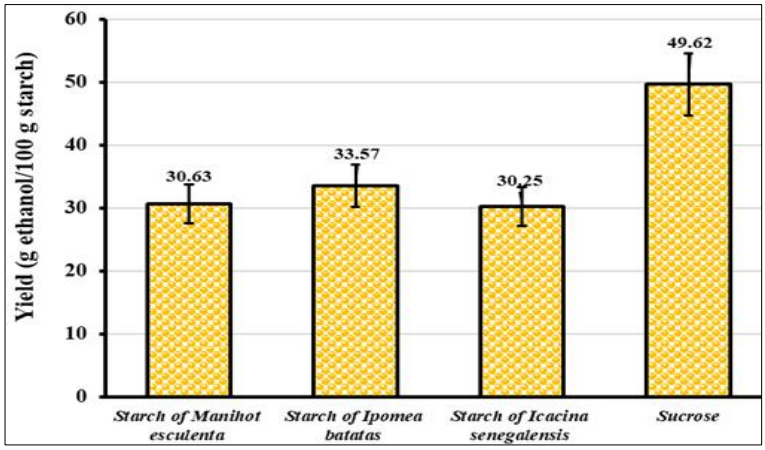

Fig 10: Yields of bioethanol production depending on dry starch masses in comparison with sucrose

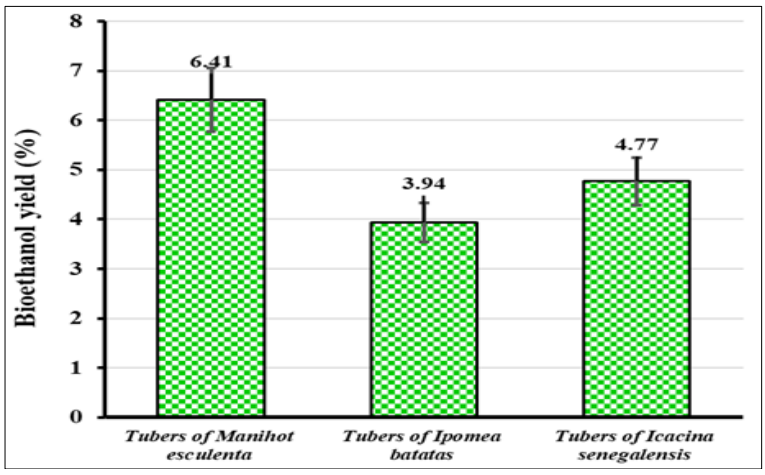

Fig 11: Yields of bioethanol production compared to fresh tubers masses

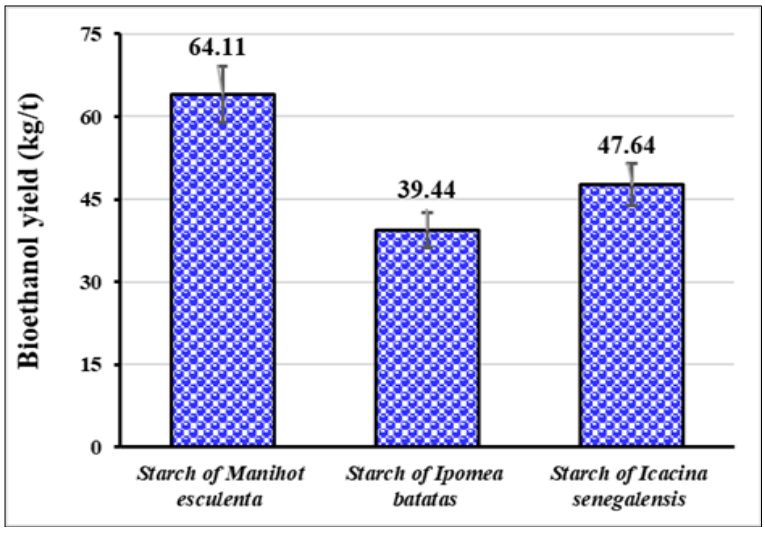

Fig 12: Yields of bioethanol production in relation to tuber masses (in kg/t of fresh tubers)

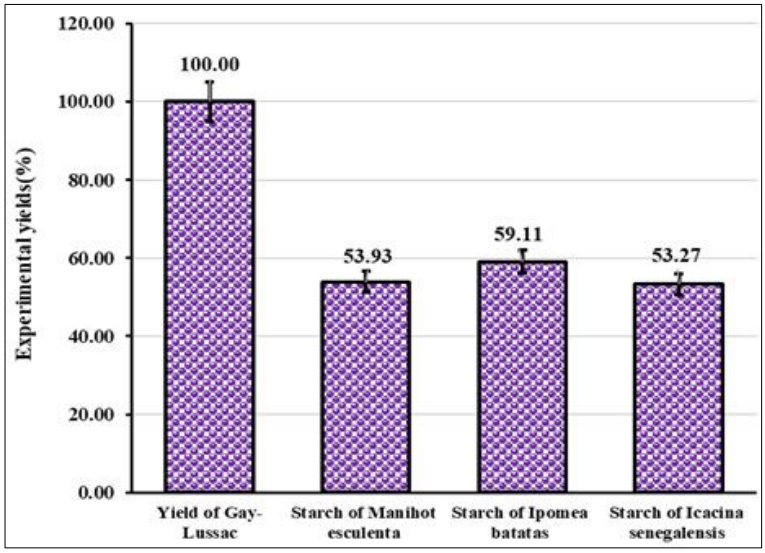

Fig 13: Experimental yields of bioethganol production based on starches

In this study, the yields of bioethanol production relative to starch masses are: 30.63 ± 0.52 g; 33.57 ± 0.46 g and 30.25 ± 0.89 g/100 g of starches, respectively for *Manihot esculenta*, *Ipomea batatas* and *Icacina senegalensis*; while that of sucrose (Equation 3) is 49.62 ± 0.82 g of ethanol/100 g of sucrose powder. This shows that sucrose is more alcoholic than starches of the tubers studied.

Compared to the theoretical yield of Gay-Lussac which is 56.79 g of ethanol/100 g of starch equivalent to 100%, in this study the experimental yields of bioethanol production based on the different types of starches are: 53.93±1.28%; 59.11±1.40% and 53.27±1.25%, respectively for *Manihot esculenta*, *Ipomea batatas* and *Icacina senegalensis*. These yields are much lower than the yield of Louis Pasteur, which must be 94.7% of Gay-Lussac's yield (Bellarini, 2006) [20]. This can be explained by the fact that either the hydrolysis reaction of the starches are not total or the yeasts involved in the ethanolic fermentation reaction can not assimilate all the sugars contained in hydrolysates of starches, because of the inhibitory compounds formed during acidic hydrolysis (Novidzro, 2017) [31].

In this study, the yield of bioethanol production with sucrose is 92.23 ± 1.52%. This yield obtained is therefore slightly less than Pasteur's yield. Indeed, due to the unavoidable production of various by-products and yeasts, Louis Pasteur had provided explanations that the loss of alcohol yield that corresponds to approximately 5.7%, would be caused by about 3% of sugars that are transformed into glycerol; 0.5% into succinic acids; 0.5-0.8% into fusel oils which are a mixture of amyl and propyl alcohols and their isomers; and 0.8-1% are consumed to ensure the development of fermentative microorganisms (Bellarini, 2006) [20].

Taking into account only the use of starches as raw materials for bioethanol production, it appears in this work that the conversion of *Ipomea batatas* starch into alcohol is slightly more profitable compared to that of *Manihot starch esculenta*; while those of *Manihot esculenta* starches and *Icacina senegalensis* are practically similar. However, when using the tubers as raw materials for bioethanol production, the yields of bioethanol production are 6.41 ± 0.11%; 3.94 ± 0.05% and 4.77 ± 0.14%, relative to the masses of fresh tubers, respectively for *Manihot esculenta*, *Ipomea batatas* and *Icacina senegalensis*. This equates to production yields of 64.11 kg; 39.44 and 47.64 kg of ethanol/ton of fresh tubers, respectively for *Manihot esculenta*, *Ipomea batatas* and *Icacina senegalensis*. Finally, it is rather the tubers of *Manihot esculanta* that give more bioethanol; followed in descendant order by the tubers of *Icacina senegalensis* and *Ipomea batatas*. Compared to dry tuber masses, these values correspond to 17.15 ± 0.29%; 14.19 ± 0.13% and 5.88 ± 0.17%; ie 171.57 kg; 141.93 and 58.76 kg of ethanol/ton of dry tubers respectively for *Manihot esculenta*, *Ipomea batatas* and *Icacina senegalensis*.

However, it is also necessary to compare the yields of bioethanol based on these starchy plants compared to the mass of tubers harvested per hectare, but also taking into account the duration of culture of each plant to know in which case profitability is really better.

Conclusion

At the end of our investigations, it appears in this study that among the three starchy materials tested, the tubers of *Icacina senegalensis* that are less rich in starch. In fact, the results of aqueous extraction show that the alcoholic potentials based on the starches of *Ipomea batatas* and *Manihot esculenta* exceed 2.18 times and 2.88 times that of *Icacina senegalensis starch*. Without addition of acid or with addition of acid but without heating, the hydrolysis reaction of the starches is completely blocked. However, heating a starch solution with a concentration of 133.3 g/L with H_2SO_4 (5.5%) for thirty minutes leads to a syrup whose soluble DM content is close to 12 °Brix, whatever the type of the used starch. The final Brix degree of the hydrolysates increases linearly as a function of the initial concentration of the starch solutions during acidic hydrolysis. All the obtained syrups after acid hydrolysis of the starch solutions have a content measured in Brix degree slightly more or less low compared to the sucrose solution of identical concentration. Starch hydrolysates are less fermentable than the sucrose solution, having the same initial concentration. In the long term and beyond 25 minutes of heating, the yield of the ethanolic fermentation of starch hydrolysates is improving more and more. Bioethanol production yields from starches of the three tubers studied here are 53%, 54% and 59% similar to the theoretical yield of Gay-Lussac, respectively for *Icacina senegalensis*, *Manihot esculenta* and *Ipomea batatas*; while that of sucrose is about 92.23%. These results show that sucrose is more alcoholic than starches of the tubers studied here. However, taking into account the criteria of non-edibility and the availability of the raw material, the tubers of *Icacina senegalensis* is

distinguished from the other tubers studied here as the best substrate for the 1G ethanol production.
The results presented in this study can be improved either by applying the enzymatic starch hydrolysis method and/or by enriching the starch hydrolyzate with yeast food supplements.

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Conflict of interest
The authors declare that they have no conflict of interest in relation to this article.

Authors contributions
KMN and KD designed the project, conducted the sampling and performed the experiments; KMN, MM and BAF analyzed the data and wrote the manuscript; KHK supervised all activities related to this article; all the authors gave their agreement to the final version of the manuscript and its publication.

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