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EXTRACTION PERFORMANCE OF JUICE AND BIOETHANOL PRODUCTION FROM SWEET SORGHUM (SORGHUM BICOLOR, (L.) MOENCH)

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ABSTRACT

The present work was initiated to determine extraction performance of juice and bioethanol production from sweet sorghum (Sorghum bicolor, (L.) Moench). Influence of impregnation of material on juice extraction was established. Study shows that juice obtained by pressing the stems of this plant contains 98.85 g/L of soluble sugars and 27.45 g/L of reducing sugars. The method of sugars extraction by squeezing is influenced by the temperature and the time of impregnation. Indeed, this work shows that, to improve the extraction of residual sugars, a bagasse / water ratio of 1/1, a temperature of 45°C and 30 min of impregnation, are optimal conditions for an efficient and optimal sugars extraction required for the production of ethanol. These conditions made it possible to obtain an overall extraction yield of the order of 68.203 and 91.189% respectively for total soluble sugars and reducing sugars. The production of ethanol by fermentation of the sweet sorghum juice in batch mode gave a yield of 91.2 % of the theoretical yield of Gay Lussac.

INTRODUCTION

Energy is the bedrock of development, a socio-economic, strategic and geopolitical issue of the day. Moreover, population growth and technological development are driving a continuous rise in global energy demand with a predominance of fossil fuels, the use of which leads not only to its depletion but also and above all to global warming. The transportation sector is responsible for one-third of greenhouse gas emissions with an upward trend, Alazard-Toux *et al.* (2011). Faced with this problem, the development of renewable and sustainable alternative fuels for transportation, produced from plant biomass, was quickly identified as a short-term means of action with interesting potential. For this purpose, fresh stem residues of sweet sorghum (*Sorghum bicolor* (L.) Moench) have a technological advantage because of the presence of a high concentration of free sugars, soluble and fermentable in the form of juice, (Ritter et al., 2008 ; Wang et al., 2013 ; Ishizaki and Hasumi, 2014) combined with a very high yield of green biomass estimated to be between 20 and 120 tonnes / ha depending on growing conditions (Saballos, 2008). The sugars contained in this juice are sucrose, glucose and fructose, in variable proportions depending on the variety (Schaffer and Gourley, 1982; Murray et al., 2009). Classified in fifth rank of cereals in the world (Mwithiga et al. 2006) and second in Africa (Taylor 2016), sorghum is one of the essential subsistence cereals for rural people in the Far North region of Cameroon, occupying an estimated area of 487 597 hectares in 2015 (MINADER, 2015). This significant quantity of biomass generated by the cultivation of this graminaceous cereal is not always very well valued. Moreover, the efficient and optimal extraction of the sugars contained in the stems of this plant is a major challenge for the potential contribution of this biomass to meet the energy needs of the future. This study aims to determine the composition of sugars, the optimization of the process of extracting the juice of fresh stems of a variety of the bicolor race of Sorghum bicolor grown in the Far North of Cameroon and to produce the second generation of bioethanol from the extracted juice.

MATERIAL AND METHODS

Vegebable material

The vegetable material used for this study consists essentially of the fresh stems of a sweet sorghum cultivar. The genus and species *Sorghum bicolor*, and *bicolor* race, this raw material was collected locally in a field of culture located on the outskirts of "PITOARE" district in the city of Maroua, chief town of the Far North region of Cameroon. The sowing was modeled on the rainy calendar of the region, and the sorghum was sown on June 27, 2017 and the harvest took place on November 11, 2017. It is during this harvest period that the collection of the stems was carried out.

Collection and pretreatment

The stems were previously harvested and collected and then received a series of pretreatment operations prior to the sap extraction step. Indeed, to improve the extraction efficiency of sorghum juice and its sugar content, it is important to eliminate leaves and panicles before the pressing stage (Rains *et al*., 1993). Pretreatment of the biomass begins *in situ*, once cut and the panicles recovered by the farmers, the stems were collected and cleared leaves, then transported to the laboratory to undergo a second pretreatment. Once in the laboratory, they were further peeled with a stainless steel knife to separate the fibrous material (bark) and the marrow. The marrow obtained was then minced and cut into small pieces of the order of one to three centimeters (Figure 1), before being introduced into a squeezer, it was ground and reduced to a few millimeters.

Figure 1. *Illustration of the operations (collection and pretreatment of the stems).*

Juice extraction and sampling

A 200 g sample of the chopped biomass was taken before the start of the extraction operation for the physicochemical characterizations. The rest of the biomass was weighed using a high precision scale (PRECISA 12000 D SCS), before the extraction of the sap. The extraction operation was carried out simultaneously with grinding and mechanical pressing using a CORONA squeezer. The center of the head of this squeezer is equipped with a screw which forces the marrow to pass between two disks, one of which is fixed and the other rotating, which grind the marrow more or less finely according to the adjustment. The residual juice contained in the ground material was separated from the bagasse by forcing its passage by simple mechanical pressing through two layers of filter cloth. Then the volume of the pressed juice was determined using a graduated cylinder and 80 mL was taken from a sampling bottle and kept cool in a freezer for possible analyzes of soluble sugars and dry matter content. The bagasse was collected in a plastic bin and weighed then 200 g were taken for further analysis.

Optimization of sugar extraction

To study the process of optimization of the extraction yield of sugars, the bagasse of the first pressing was impregnated with water at a ratio of 1/1 (bagasse / water ratio, expressed in kg of bagasse / liter of impregnation water) and squeezed again. Two parameters were taken into consideration during the experiment: the incubation temperatures (35, 40 and 45 ° C) and the impregnation time (0, 30 and 60 min). Which gives a 1x3x3 experience plan. The choice of study parameters was made based on results of previous work including that of Jia *et al.* (2013); Noura Saïed, (2016) and the aim is to deepen the study by focusing on the effect of the variables time, temperature, for a ratio 1/1, taken independently and on the simultaneous effect of the variables time-temperature

on the other hand. The volume of the juice recovered from the second pressing was determined each time using a graduated cylinder, 80 ml were taken and kept in the freezer for further analysis.

Determination of dry matter content

Samples taken from the biomass were placed in the oven set at 105 ° C for 24 hours and then cooled in the desiccator and weighed. The average value of the different measurements was then calculated. The dry matter content was calculated according to the formula below:

$$DM_C = \frac{D_m}{W_m} \times 100 \quad (1)$$

Where: DM_C = dry matter content; D_m = average dry mass; W_m = average of wet masses

The moisture content (M_c) contained in the samples before and after the pressing was calculated according to the following equation:

$$M_C = 100 - DM_C \quad (2)$$

This moisture content in the biomass samples before and after pressing was used to calculate the juice extraction rate of the biomass during pressing according to the following formulas:

$$E_1 = B_1 \times M_C \quad (3)$$

$$E_2 = B_2 \times M_C \quad (4)$$

$$J_{ER} = \frac{E_1 - E_2}{E_1} \times 100 \quad (5)$$

Where: E_1 = mass of juice contained in the biomass before pressing (g); E_2 = mass of juice contained in the biomass after pressing (g); B_1 = mass of biomass before pressing (g); B_2 = mass of biomass after pressing (bagasse) (g); M_C = moisture content in the sample (%); J_{ER} = Juice extraction rate of biomass (%).

Soluble sugars determination

To extract the sugars, the samples of the marrow and the bagasse of the first and second squeezing were dried by drying at 45 ° C for five days and crushed with a CORONA Moulinex then with a sieve, the grind marrow was sieved to remove particle greater than 1 mm. Extraction of the soluble sugars in the samples was performed by the zinc acetate method (2g / 100 mL) and potassium ferrocyanide (10.6 g/100 mL). The soluble sugars extracted was estimated using a spectrophotometer at 530 nm according to the DNS method (3,5 Dinitro Salicylic Acid) described by **Fischer et Stein (1961).**

The soluble sugars content from each test portion was determined by referring to the regression equation calibration curve: OD = a Q + b.

Where, OD = Optical Density and Q = amount of soluble sugars; a, b: Constants to be determined
The sugars content expressed in g / 100 g of DM is given by the relation:

$$Q = 100 \times Q_1 V_T / m\, V\, (100 - H°_r) \quad (6)$$

Where: Q_1: the amount of sugar in the test portion; V_T: the total volume of the extract; m: the mass of the test sample in (g); V: the sample volume analyzed; H°r: the residual water content

Determination of total sugars content

The total sugars in the samples were extracted by the 1.5 N sulfuric acid method, using 70 % ethanol, zinc acetate (2g / 100 mL) and potassium ferrocyanide (10.6g / 100 mL). The total sugars content was determined using a spectrophotometer at 450 nm by the phenol and sulfuric acid method (d = 1.83) described by **Dubois *et al*, (1956).**

Determination of reducing sugars content

The extracted reducing sugars content was determined using a spectrophotometer at 540 nm according to the DNS method (3,5 DinitroSalicylic Acid) described by **(Miller, 1956).**

Bioethanol Production

The second-generation bioethanol was produced by fermenting the extracted juice with the instant brewer's yeast (*Saccharomyces cerevisae*). The operation was carried out according to the fermentation method in batch mode at room temperature, in microreactors of 1.5 liters of useful volume. To prepare the leaven, the juice of sweet sorghum was diluted with distilled water at a rate of 20 g / L. The inoculum was prepared from the leaven which allowed the adaptation of the strain to the culture medium, then inoculated with a volume of yeast in a yeast concentration of the order of 2.8 g / L.

Fermentation kinetics

The kinetics of fermentation were studied through two parameters: the evolution of ethanol production and the consumption of fermentable sugars as a function of time. For this purpose, a series of sampling of the reaction medium was carried out (every 5 h) for physicochemical analyzes.

The evolution of the sugar content was determined by spectrophotometric determination according to the DNS method of **Fischer and Stein (1961)** and **Miller, (1956)**, respectively for soluble sugars and reducing sugars, described above.

The production of ethanol was followed according to the dichromate ion titration dosage method by the sodium thiosulfate solution.

Statistical analyses

Each experiment was repeated 3 times for physicochemical analyzes of the stems and juices component. Fermentation tests at different pHs were performed in duplicate. The results obtained were expressed in a form: $M \pm \sigma$, with σ the standard deviation and M the average. The determination of interdependence relationship between the study variables was performed by the one and two factor ANOVA method using SPSS version 20 software. Two analysis plans were made for the determination of the interdependencies between the study variables: the one-way ANOVA, to determine the effect of each of the categorical variables independently of the second one, on the dependent variable; and the two-way ANOVA to determine the simultaneous effect of the two independent factors or variables on the dependent variables. Statistical differences with a probability value less than 0.05 ($P < 0.05$) are considered significant. When the probability is greater than 0.05 ($P > 0.05$) the statistical differences are not significant.

RESULTS AND DISCUSSION

Physico-chemical characteristics of sweet sorghum stems

This study shows that the bicolor cultivar of sorghum studied has a water content at maturity, evaluated at 65.340% for the whole stem. This value is similar to that obtained in 2017 by Crépeau (2017) which was 65%. Moreover, the marrow obtained after the pretreatment operation has a water content evaluated at 77.091% or 10.751% more compared to that of stem with bark. With regard to the sugar content of this biomass, the analysis carried out on the samples of the stripped stalks reveals a sugars content evaluated on average at 494.829 g / kg DM for total sugars, 176.552 g/kg DM for total soluble sugars and 41.929 g/kg DM for reducing sugars. This analysis performed on the marrow shows a total sugar content estimated at 376.307 g/kg DM, which is lower than that of the stem with bark. However, the marrow has a much higher content of soluble sugars and reducing sugars compared to that of the stem with bark. It is evaluated at 228.779 g/kg DM and 55.470 g/kg DM respectively for soluble sugars and reducing sugars. This result is much higher than that obtained in Quebec by Noura Saïed,

(2016), where the soluble sugar content of the hybrid sorghum variety CSSH 45 was 129.39 g/kg DM. However, the soluble sugar content of the breed we studied is similar to that observed by Almodares and Hadi (2009) in Quebec with the hybrid CSSH 45, where they obtained average values of soluble sugar concentrations varying between 145 and 298 g/kg DM. These differences in the soluble sugars content of the stems observed in sweet sorghums can be explained by the difference in breeds, varieties and cultivars studied on the one hand and the difference in environments and growing conditions on the other. The physicochemical characteristics of the race studied are summarized in table 1.

Table 1. Physico-chemical characteristics of the studied biomass.

	Water content (%)	**Total sugars (g/kg DM)**	**Soluble sugars (g/kg DM)**	**Reducing sugars**	
				Content (g/Kg DM)	Rate relative to soluble sugars (%)
Stems with Bark	65.34	497.828 ± 2.141	176.552 ± 1.132	41.929 ± 2.157	23.74
Marrow	77.091	376.304 ± 2.558	228.779 ± 1.243	55.470 ± 2.437	24.24

Juice extraction and press efficiency

The screw press used for the pressing operation yielded an extraction rate of the order of 34.66% for the total soluble sugars and 49.89% for the reducing sugars as shown in table 2. These results are similar to those of Crépeau et *al*. (2013), who were able to extract, depending on the place of cultivation, an average of 30% and 57% of soluble sugars from the biomass of sweet sorghum, also using a screw press. Monroe et *al.* (1984) were able to extract 47.2% and 41.7% of the free sugars from sorghum stems after single squeezing, respectively using vertical and horizontal roller squeezers.

Table 2. Content of free sugars in the biomass and extraction rate of the first squeezing.

	Marrow	**Bagasse 1**	
	Content (g/kg DM)	Content (g/kg DM)	Extraction rate (%)
Soluble sugars	228.779 ± 1.243	149.462 ± 1.231	34.660
Reducing sugars	55.47 ± 1.603	27.791 ± 0.243	49.890

In addition, the effectiveness of the squeezing in the extraction rate of water is estimated at 76.573%. This result confirms that of Vincent et *al*. (2006) as well as those obtained by Crépeau et *al.*, (2013) who obtained an average water extraction rate ranging from 65.5% to 74.9%. This squeezer has on average a juice extraction yield of 0.598 L/kg, and a mass extraction rate of 74.080% (Table 3). This extraction rate is comparable to that obtained by Cosgrove *et al*. (2012) using a three-pair roller squeezer, where the extraction rate was between 70 to 80% and slightly higher than that of Mask and Morris (1991), who also used a three-pair roller squeezer and obtained an extraction rate of the order of 50 to 60%.

Table 3. Yield of the first biomass squeezing operation.

Extraction operation effectiveness

Juice extraction (L/kg)	Water extraction rate (%)	Mass extraction rate (%)
0.598	76.573	74.080

Influence of impregnation duration

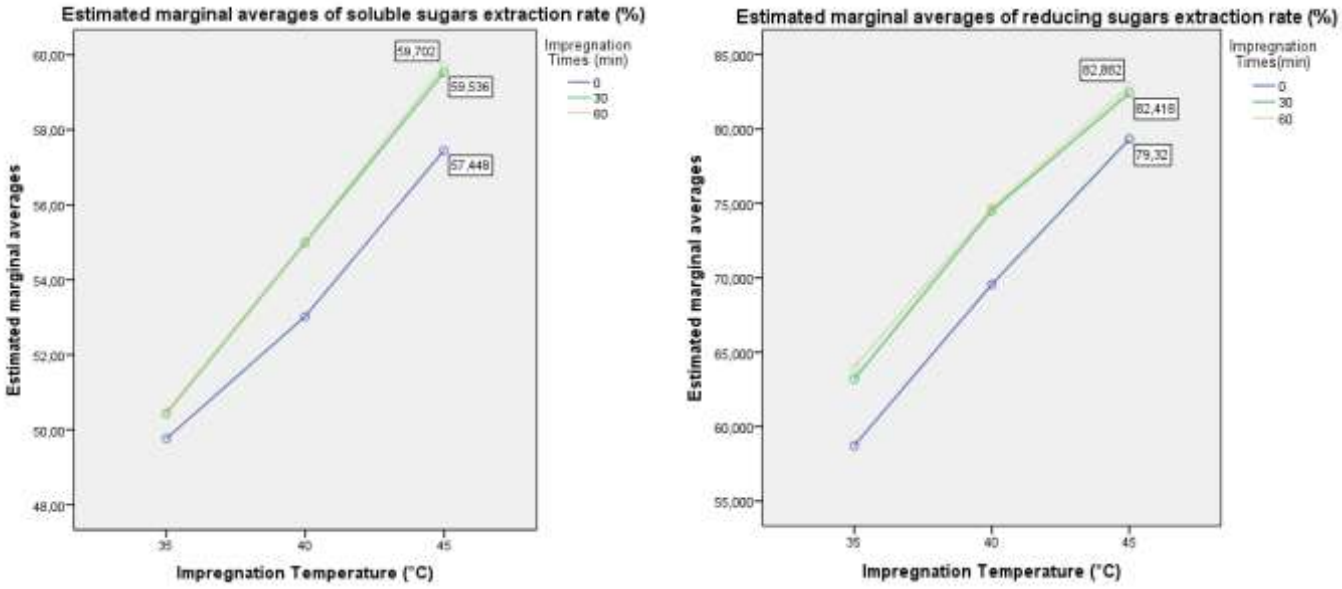
The results of data ANOVA indicate that: the impregnation time effect on the extraction rate, though with a growing trend among the three periods of observation, the difference of extraction rate of these three time is however not significant (F = 0.138; df = 8; P = 0.874) and (F = 0.204; df = 8; P = 0.821) respectively for soluble sugars and reducing sugars. It can be concluded that the variable impregnation time for the intake, regardless of the temperature does not significantly influence the extraction rate of soluble sugars and reducing sugars in the bagasse (P> 0.05), which confirms the results of Noura Saïed, (2016).

Influence of incubation temperature

It emerges from this study that the extraction rate of residual sugars increases with the increase of impregnation temperature of the first squeezing bagasse. It is evaluated on average at 50.225; 54.344 and 58.895% respectively for temperatures of 35, 40 and 45 ° C, however, this rate is much higher for reducing sugars. It is evaluated at 61.969; 72.915 and 81, 540% respectively for impregnation temperatures of 35, 40 and 45°C. ANOVA indicates that extraction rate means differ significantly between the three temperature ranges considered for this study (35, 40 and 45°C). (F = 55.526, df = 8; P = 0.000) and (F = 42.662, df = 8; P = 0.000) respectively for soluble sugars and reducing sugars. It can be concluded that the impregnating water temperature variable taken independently of time significantly influences the rate of extraction of soluble sugars and reducing sugars in the bagasse ($p < 0.05$).

Simultaneous effect of Time-Temperature variables

The interaction between the categorical variable and the co-varables shows statistically significant differences of averages. With: (F = 837.718, dd1 = 3, P = 0.000) and (F = 8.199, dd1 = 3, P = 0.022) respectively for soluble sugars and reducing sugars. It can therefore be concluded that the combination of effect of temperature-impregnation time variables significantly influences the extraction rate of soluble sugars and reducing sugars in bagasse. In addition, the results (Figure 2) indicate that for a solids / liquid ratio of 1/1, the temperature of 45 ° C and a duration of 30 minutes of bagasse impregnation, are the most optimal conditions for the maximum and efficient extraction for both soluble and reducing sugars. These conditions made it possible to obtain extraction rates evaluated on average at 59.536 and 82.418%, respectively for the soluble sugars and the residual reducing sugars contained in the bagasse of the first pressing. This represents an additional extraction rate of the order of 33.542 and 41.299% respectively, compared to the carbohydrate content of the initial biomass. This makes it possible to obtain an overall extraction yield of the order of 68.203 and 91.189% respectively for total soluble sugars and reducing sugars. These results were verified through the analysis of the evolution of the concentration of sugars in the juice of the second squeezing which reveals concentrations evaluated on average of 39.426 g/L and 14.823 g/L, respectively for soluble sugars and reducing sugars. These concentrations are sufficient for the satisfaction of the conditions of production of bioethanol during the fermentation process which are much higher than that obtained by Jia *et al*. (2013), where the maximum concentration of about 22.5 g/L was obtained under the most favorable conditions of the experiment.



a) Extraction rate of soluble sugars *b) Extraction rate of reducing sugars*

***Figure 2.** Illustration of the simultaneous effect of Time-incubation Temperature of bagasse on the extraction rate of residual sugars.*

In addition, there is a much higher concentration of reducing sugars in the juice of the second squeezing (37.59%) compared to the juice of the first squeezing (27.77 g/L). This increase in the rate of reducing sugars in the juice of the second pressing could be explained by the fact that the sucrose was hydrolysed into its two monomers, glucose and fructose under the action of invertase, an enzyme responsible for hydrolysis and present in most plant species, Noura Saïd, (2016).

Juice caractériscs

The results of the first squeezed juice analysis indicate concentrations averaged 98.85 g / L total soluble sugars and 27.45 g/L reducing sugars (Table 4). The analysis of these results shows that the juice consists of 27.77% in reducing sugars compared to the total soluble sugars. The soluble sugar content of the cultivar studied in this work is slightly lower than that of Daniel et *al*. (2017) who obtained a juice concentration of 100.3 g/L in soluble sugars from three cultivars (Dale, M81 E, and Theis) of sweet sorghum, grown in the United States. However, this content is higher than that obtained by Crépeau et *al*. (2013) who were able to extract a juice with a concentration of 60.9 g / L in soluble sugars from the biomass of sweet sorghum. However, a US study by Lingle et *al.*, (2012) found that the soluble sugar content of sweet sorghum juice was in the order of 53.5 g/L at 90 days after sowing and increased to 112 g/L at 140 days after sowing.

Table 4. Sugar Composition of juice.

Juice characteristics			
	Total soluble sugars	Reducing sugars	Sucrose
Content (g/L)	98.85	27.45	71.40
Rate (%)	100	27.77	72.22

Fermentation kinetics

The evolution of ethanol production follows a kinetic pattern of three times (Figure 3): a phase of low production; a phase of acceleration of production; and a deceleration and stabilization phase. This kinetic pattern of ethanol production can be associated with that of the growth of fermentative yeasts which, in a medium rich in carbohydrate where the physicochemical conditions of their growth are met, achieve growth with a three-stage kinetics, (Sutra, 1998). The final alcohol content during the stabilization phase is estimated at 9.32 g/L, with a production yield of 46.56%, or 91.2% of the theoretical yield of Gay Lussac. These results are consistent with the predictions of Louis Pasteur, reported by Ballerini *et al*. (2006). Moreover, the analysis of these results shows that, the rate of consumption of fermentable sugars in the reaction medium during the fermentation process, follows kinetics also in relation to the evolution of the rate of production of ethanol and microbial growth with inverse concavity.

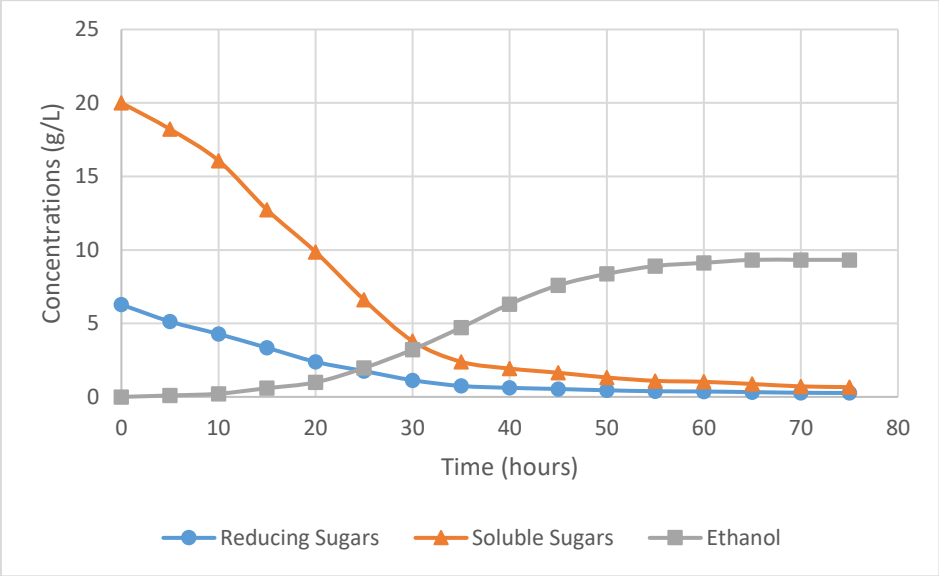


Figure 3. ***Illustration of fermentation kinetics.***

CONCLUSIONS

This study presents a valorization technique of the residues of culture, fresh stalks of sorghum in fact, for the production of a second generation biofuel, as alternative and durable source of energy for the transport. Physicochemical analyzes of the biomass showed that the bicolor stalks, studied, contain 65.34% of moisture, a concentration of sugars evaluated on average at 494.829, 176.552 and 4.229 g/kg DM, respectively for total sugars, total soluble sugars and reducing sugars. This study also shows that this juice contains mainly sucrose (72.22%) and 27.77% reducing sugars.

The study of the improvement of the sugar extraction process shows that a bagasse / water ratio of 1/1, an incubation temperature of 45 ° C and an impregnation time of 30 minutes are the optimal conditions for maximum and efficient extraction of the residual sugars from the bagasse of first squeezing. These conditions made it possible to extract an average of 59.536 and 82.418%, respectively, of soluble sugars and residual reducing sugars from the bagasse, with the second pressing juice concentration evaluated on average at 39.426 g/L and 14.823 g/L, respectively for soluble sugars and reducing sugars. These conditions made it possible to obtain an overall extraction yield of the order of 68.203 and 91.189% respectively for total soluble sugars and reducing sugars.

These results show that sweet sorghum is a multi-purpose cereal grass that can be used not only as a human food source, but also and most importantly as an alternative energy source. The valorization of sorghum stalks for the

production of second-generation bioethanol is a renewable and viable source of energy, which can make enormous contributions to the global energy mix.

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