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Research Article

Pilot Process for Obtaining Fructose from Industrial Cassava (*Manihot esculenta* C)

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Abstract: This study aimed to evaluate yields of obtaining fructose syrups from industrial cassava (*Manihot esculenta* C) at pilot scale since the varieties cultivated in the Colombian Caribbean have shown their benefits in the generation of different products at laboratory scale. However, to take advantage of the deficit in the domestic supply through industrial production, it is necessary to increase the size gradually, generating the least impact on its productivity. The yields of starches were obtained through mass balance. The kinetic evaluation of the processes of liquefaction and saccharification was made by quantifying the reducing sugars by the DNS technique. The production of fructose syrups was evaluated by the method of GOD/POD, using a completely randomized design with three levels of starch concentration (25, 30, 35% w/v, respectively) and five levels of industrial cassava varieties (Orense, Caiselli, Gines, Verónica, Tai). Wet extraction of the starch reported yields between 7.5 and 15.8% w/w. The highest yields in the enzymatic hydrolysis were obtained with the varieties Corpoica-Tai and Corpoica-Caiselli with Dextrose Equivalents (DE) of 94.97 and 93.41% respectively. In the isomerization stage, the best result was for the 30% w/v Corpoica-Tai variety with 104.56 kg of fructose/ton of fresh cassava, values 10% lower than those reported a laboratory level. The process is technically feasible requiring improvements, especially in the wet extraction stage of starch.

Keywords: Enzymatic processes, extraction, scaling, starch, yields

INTRODUCTION

Since the beginning of the 1980s, strategies have been developed to promote the modernization and strengthening of the cassava agribusiness in the Colombian Caribbean region (Ospina and Ceballos, 2002). One of the most successful was the development of different cassava varieties for industrial use, resulting in clones with yields of up to 30 ton/ha and dry matter percentages of 35% w/w (CIAT *et al*., 2004) allowing producers and processors to respond to market demands. These results demonstrate the potential of this tuber as a raw material for industrial use in activities of higher added value for food and non-food purposes such as obtaining native and modified starch, maltodextrins, glucose and fructose syrups, paper pastes, flocculating agents and lubricants in others (Glittemberg, 2012).

Motivated by these uses, laboratory-scale research has been carried out to allow the generation of products derived not only from cassava but also from other raw materials such as bananas (*Musa acuminata*), yams (*Dioscorea alata*), sweet potatoes (*Ipomoeas batatas*), malanga (*Colocasia esculenta*) and tiquisque (*Xanthosoma sagittifolium*). Evidence of this is the work done by Salcedo *et al*. (2009), which reports starch yields between 17.5 and 18.5% w/w using two varieties of industrial cassava (*Manihot esculenta* C), such as Corpoica-Orense and Corpoica-Tai. In the enzymatic hydrolysis stage they obtained DE of 95.32 to 94.12 from 30% w/w and starch solutions and in the isomerization step reveals that they can be obtained from 635.8 to 674.7 kgF/ton S. For the following year, (Salcedo *et al*., 2010) analyze the use of two varieties of diamond yam (*Dioscorea alata*) and hawthorn yam (*Dioscorea rotundata*), finding that yields of starch can be obtained between 13.7 and 16.2% w/w. The reported DE ranged from 96.84 to 98.28 from 30% w/w starch solutions and from 701.6 to 735.9 kgF/ton S from the isomerization step.

Similarly, Tovar (2010) reports yields of 22.84% of starch obtained using hawthorn yam (*Dioscorea rotundata* Poir). Bettin and Quintero (2010) informed close to 95% conversions for commercial maltodextrins to glucose syrups. In the saccharification stage, Morales *et al*. (2008) presented DE of 85.6% using cassava starch in a concentration of 40% w/v. Johnson *et al*. (2009) reported DE values of 95.16 and 98.52% for cassava and sweet potato respectively at 48 h reaction times. In work done by Hernández (2004), the

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production of fructose syrups from banana starch was evaluated obtaining a concentration of 63.3 mg/mL, which represented an average conversion of 41.3%. Finally, Salazar and Penaranda (2012) determined the production of DE from the tiquisque starch (*Xanthosoma sagittifolium*) with results between 98.7 and 99.4% from solutions at 40% w/w. These data show that cassava varieties cultivated in the Colombian Caribbean have benefits in the laboratory generation of different products. However, to guarantee its correct production at the industrial level, it is necessary to increase the scale gradually, maintaining greater control and generating the least impact on its productivity.

This correct transition is influenced by an appropriate design of the intermediate level, i.e., the pilot level if perfect scaling criteria are applied (Xia *et al*., 2016). The results of this state will allow verifying the technical viability reported at the laboratory level, in addition to determining the possible risks of its operation (Anaya-Durand and Pedroza-Flores, 2008). At this level, the study by Perez *et al*. (2004) which evaluated the yield of the starch extraction process from banana fruits (*Musa paradisiaca*) on a pilot scale, showed a reduction of 14% when compared to the laboratory results.

As of 2009, the region generates added value to industrial cassava production by obtaining native starches in an industrial plant with a capacity of 50 tons/day, as an example of the benefits described for this crop. Despite this progress, it is necessary to generate higher added value, through the generation of products such as maltodextrins and glucose and fructose syrups. Recent studies demonstrate the competitive advantages of these, as potential products for export to external markets (Ahcar-Olmos *et al*., 2011) and take advantage of the existing trade deficit in the fructose market, reaching a level of close to 2710 tons per year in the last decade (Yousef and Marco, 2015).

This research evaluated the yields of fructose syrups obtained from starches of five cassava varieties of industrial use using a full pilot process divided into two stages, a wet extraction of starch and a hydrolysis and isomerization reagent.

MATERIALS AND METHODS

Location: This research was developed in the Unitary Operations Plant of the University of Sucre, located at Granja El Perico, located at kilometer 7 of the Sampues-Sincelejo highway.

Materials and equipment: Five varieties of industrial cassava cultivated in the Colombian Caribbean and introduced by CIAT *et al*. (2004) were used: Corpoica-Orense, Corpoica-Caiselli, Corpoica-Gines, Corpoica-Verónica and Corpoica-Tai, supplied by the Association of Cassava Producers from the Savannahs of Sucre and Córdoba (APROYSA). The enzyme γ-amylase Liquozyme® Supra 2.2X was used in the liquefaction stage, the enzyme amyloglucosidase Dextrozyme® GA 1.5X was used in the saccharification process and the isomerization process the immobilized glucose-isomerase Sweetzyme® IT Extra was used.

The equipment used in the wet extraction stage of cassava starch was constructed based on the methodology proposed by Freddy and Dufour (1998) without using any scaling criteria since at the laboratory level these processes are performed manually. Among these is a Washer-Peeler with a capacity of 100 kg/h and water inlet at the rate of 100 L/h. In this, the cassava was fed into the chute to be introduced to the machine where the dirt and impurities were removed from the equipment with the aid of pressurized water and the spinning effect of the vertical axis cylinder. The used Mincer received the roots in the input hopper of materials where the size was reduced to a thickness of 0.02 m, as a result of the rotation of the cutting disc.

Then, the material was then sent to a Grater. The material to be treated in this operation was received and fed into the chute to transfer it to the cutting drum where the starch granules from the roots were released from the pressure generated by the rotation of the perforated cutting cylinder on the wall, producing coarse wet bran. The Extractor allowed the obtaining of solutions of starch. In this equipment, the bran was fed by the receiving chute, after mixing with water in a proportion of 2.1 kg of water for each kg of grated cassava. The starch is separated from the bran thanks to the spinning effect of the screw which drags it along the whole length of the equipment pressing it on the mesh net of 80, producing the solutions. Finally, a Sedimentation Tank was used to concentrate the solutions from 6.5 to 30% w/w. This equipment handled a volume of 0.27 m^3 in cylindrical form with a height/diameter ratio of 1 and a speed of rotation of the agitator type Rushton turbine of 45 rpm.

The design and construction of the equipment in the reactive stage of the pilot process was based on the criterion of geometric similarity scaling (Najafpour, 2007). A Stirred Tank Bioreactor was used for the liquefaction and saccharification operations, it was cylindrical with a working volume of 60 L, in a height/diameter ratio of 1 for a scale factor of 1:12 and a rotational speed of 120 rpm with a Rushton turbine-type agitator. The glucose syrups resulting from these processes were sent to an Adequacy System composed of a filter to separate materials that could cause clogging of the biocatalyst with a size of 5 to 10 μ, a column of activated carbon model KDF-10 American Filtre® brand and ion exchange column. For the glucose isomerization step, a Fixed Bed Bioreactor was used in a tube and shell type exchanger configuration, with a shell diameter of 0.33 m, a tube diameter of 0.023 m, a length of 2 m and with the capacity to process up to 40 L/h. All equipment used was made of stainless steel.

Methods of analysis: The determination of starch yields, as well as all by-products and residues from the wet extraction stage were performed through mass balances (Doran, 2013). Initially, the cassava was received and introduced into the Washer-Peeler. For each variety, three replicates were made, with 10 kg of cassava being fed to each replicate and a residence time of 10 min.

The yield of the cassava pulp was established with the weights that were taken before and after washing and peeling. A Metter Toledo® balance was used, expressing in kg of cassava pulp/kg of fresh cassava (kg CP/kg FC) as Eq. (1) shows that y_{CP} represents the cassava pulp yield, w_{FC} is the mass of fresh cassava in kg and w_H is the mass of the husk in kg:

$$y_{CP} = \frac{w_{FC} - w_H}{w_{FC}} \tag{1}$$

The yield of starch obtained in the Extractor and Settler expressed in kg of starch/kg of fresh cassava (kg S/kg FC). Was found with final weights of starch and initial cassava as shown in Eq. (2) Where y_S stands for the yield of starch, w_{FC} is the mass of fresh cassava in kg and w_{TR} is the mass of total residues in kg:

$$y_S = \frac{w_{FC} - w_{TR}}{w_{FC}} \tag{2}$$

With the obtained starches of each variety, solutions were prepared in concentrations of 25, 30 and 35%, w/v to analyze the kinetics of conversion of starches to glucose syrups. The liquefaction was carried out in the Agitated Tank Bioreactor using the Liquozyme® Supra 2.2X ά-amylase at a dose of 0.852 kg ETonS/S as recommended by Hernández *et al*. (2016a). The temperature was maintained at 90°C with steam coming from a boiler, stirring speed at 120 rpm and pH at 5.4 with Carlo Erba Reagents® sodium hydroxide solutions of 0.1N and RA Chemicals® acetic acid 0.1N. This first hydrolysis was performed in two stages; A primary at 60°C for 30 min and a secondary at 90°C for 120 min, evaluating the variation in DE level at 30 min intervals, quantifying the reducing sugars by the DNS method (Miller, 1959) with a spectrophotometer (Genesys 10s UV-VIS). At the end of this process, saccharification was evaluated using Dextrozyme® GA 1.5X glucoamylase at a dose of 1.052 kg ETonS/S as recommended by Hernández *et al*. (2016a), controlling the temperature at 60°C, stirring speed at 120 rpm and the pH at 4.5. After addition of the glucoamylase, samples were taken every 30 min for 2.5 h for the determination of the DE. To each of the glucose solutions the unhydrolyzed material was removed with the aid of a filter and the glucose concentrations were determined by the GOD/POD method described by Werner *et al*. (1970).

The evaluation of fructose syrup yields from starch was performed in the Fixed Bed Bioreactor, for which the equipment was loaded with the enzyme Sweetzyme IT Extra as recommended by Hernández *et al*. (2016b) controlling the temperature at 60°C and pH at 7.4. The solutions were circulated with a MasterFlex® peristaltic pump inside the equipment previously tempered at 60°C. Glucose concentrations at inlet and outlet were determined using the GOD/POD method described by Werner *et al*. (1970).

The yields of the fructose syrups were expressed in kg of fructose/ton of starch (kg F/ton S). These were calculated through Eq. (3) where $y_{F/S}$ represents the yield, $[G_i]$ and $[G_f]$ are the initial and final concentrations of glucose in kg/L respectively and [S] is the concentration of starch in kg/L:

$$y_{F/S} = \frac{[G_i] - [G_f]}{[S]} \tag{3}$$

Similarly, yields were expressed in kg of fructose per ton of fresh cassava (kg F/ton FC), using Eq. (4) where $y_{F/FC}$ represents the yield, y_S represents the yield of starch expressed in Eq. (2):

$$y_{F/FC} = \frac{[G_i] - [G_f]}{[S]} * y_S \tag{4}$$

Experimental design: To determine if there were differences between the treatments, a completely randomized experimental design was used with three levels of substrate concentration (25, 30 and 35% w/v, respectively) and five levels of industrial cassava varieties (Orense, Caiselli, Gines, Verónica and Tai) with three replicates for a total of 45 experimental units and 15 treatments. Results were analyzed by variance analysis and a comparison test with Tukey confidence level of 95% using the statistical package R.

RESULTS AND DISCUSSION

Starch yields: The results obtained, standard deviation, are shown in Table 1 during each step of wet starch extraction.

The highest yields of starch obtained at pilot scale were for the Corpoica-Tai, Corpoica-Verónica and Corpoica-Orense varieties, with values of 15.8, 15.1 and 13.6% respectively. These are below those reported by Salcedo *et al*. (2009), where a starch yield of 18% was obtained for the Corpoica-Tai variety and 17.5% for the Corpoica-Orense variety. They are also lower than those reported by Tovar (2010) who reported a yield of yam starch of 22.84%. These studies were carried out on a laboratory scale and as the scale increases, the values decrease, which is because, by

Table 1: Mass balance in obtaining wet starch with standard deviation

	Industrial varieties corpoica				
Material	Tai	Verónica	Ginés	Caiselli	Orense
Fresh cassava (kg)	10.00±0.10	10.00±0.59	10.00±0.13	10.00±0.47	10.00±0.41
Moisture fresh cassava (% w/w)	62.00±0.93	61.60±0.86	57.80±1.04	62.00±3.22	63.40±2.92
Husk and discards (kg)	2.55±0.05	3.08±0.06	3.42±0.08	4.46±0.23	3.25±0.19
Pulp (kg)	7.20±0.18	6.70±0.16	6.42±0.18	5.36±0.19	6.58±0.34
Reductions (kg)	0.25±0.01	0.22±0.01	0.16±0.00	0.18±0.01	0.17±0.01
Cassava pulp solution (kg)	21.60±0.76	20.10±0.68	19.30±0.73	16.10±0.43	19.70±1.32
Fibrous material (kg)	4.22±0.17	3.93±0.15	3.77±0.20	3.15±0.07	3.86±0.14
Starch grout (kg)	17.40±0.27	16.20±1.05	15.50±0.89	12.90±0.22	15.80±0.49
Residual water (kg)	14.40±0.69	13.40±0.79	12.90±0.93	10.70±0.14	13.20±0.38
Wet starch (kg)	2.98±0.09	2.77±0.11	2.65±0.11	2.21±0.08	2.72±0.10
Dry starch (kg)	1.58±0.10	1.51±0.11	0.75±0.05	1.17±0.08	1.36±0.03
Total cassava residues (kg)	8.42±0.08	8.49±0.08	9.25±0.09	8.83±0.09	8.64±0.12
Starch yield (% w/w)	15.80±0.79	15.10±0.74	7.51±0.36	11.70±0.67	13.60±0.22
Starch moisture (% w/w)	11.90±0.54	13.30±0.59	12.70±0.55	14.60±0.61	13.80±0.15

Prepared by researchers

Table 2: Yields of cassava pulp and starch and standard deviation

	Industrial varieties corpoica				
Parameter	Tai	Verónica	Gines	Caiselli	Orense
y_{CP} (kg PC/kg FC)	0.745±0.01	0.692±0.01	0.652±0.01	0.554±0.01	0.675±0.02
y_s (kg S/kg FC)	0.158±0.01	0.151±0.00	0.075±0.01	0.117±0.01	0.136±0.01

PC: Pulp cassava; FC: Fresh cassava; S: Starch; Prepared by researchers

increasing both the quantity of raw material to be processed and the size of the equipment, there is a greater amount of losses. Compared with the results of Perez *et al*. (2004) the reduction in yields at pilot scale was 14%, while in this study the decrease was only 4% on average.

These results also show that such processes generate grants percentages of fibrous material and shell represented by about 45%, affecting the final yield. For this reason, it is necessary to give them the appropriate treatment so that they do not become waste that contaminates the environment. Lozada *et al*. (2008) proposed that three strategies should be employed to have cleaner production in the starch extraction process, known as source reduction, harvesting and treatment. However, the husk and fibrous material are residues that cannot be reduced because they are a constituent part of the raw material. So the best alternatives are to use them in animal feed (Lu *et al*., 2012) and human nutrition (De Souza *et al*., 2014), in the recovery of residual starch to increase the yields of the process (Nair *et al*., 2011) and in other biotechnological uses (Texeira *et al*., 2012).

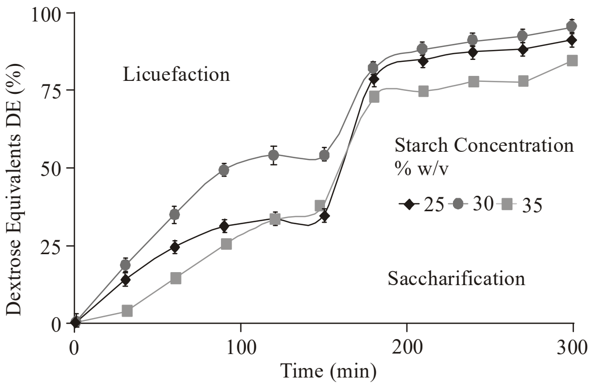
Analyzing Table 2, this shows that the highest y_{CP} were given for the same varieties, i.e., Corpoica-Tai with 0.75 kg CP/kg FC, Corpoica-Verónica with 0.692 kg CP/kg FC and Corpoica-Orense with 0.675 kg CP/kg FC. These values are below that reported by Tovar (2010), which obtained a yam yield of 0.854 kg YP/kg FY and by Salcedo *et al*. (2009) who achieved a yield for the Corpoica-Tai variety of 0.958 kg CP/kg FC and 0.93 kg CP/kg FC for the Orense variety. The difference between these values lies in the scaling. In the wash-peel stage, these two authors manually performed, minimizing the loss of pulp, whereas in this study the pulp was obtained using a pilot-scale washer-peeler with pulp losses due to the design and size of the equipment and the asymmetrical shape of the tubers.

Enzymatic hydrolysis of starches and glucose syrups: Figure 1 to 3 show the behavior in the production of DE throughout the processes of liquefaction and saccharification for all varieties and concentrations of starch studied. Differences were observed as a function of starch concentration, mainly reaching DE values between 84.20 and 94.97.

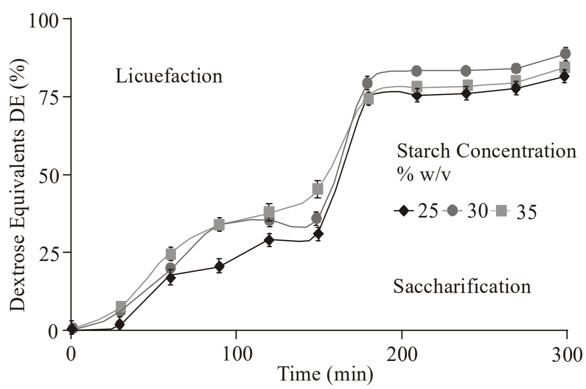
In the case of the Corpoica-Tai variety, the highest production was registered with the concentration of 30% w/v with 94.97 DE as shown in Fig. 1a. As shown in Fig. 1b, the best DE production in the Corpoica-Verónica variety was given for the initial concentration of 30% w/v with a value of 89.24 DE, followed by 85.13 DE for the concentration of 35% w/v.

At the end of the hydrolysis, the starch of the Corpoica-Ginés variety obtained a maximum yield of 92.54 DE for the initial concentration of 30% w/v, followed by 90.89 DE for 25% w/v and 88.68 DE for the solution of 35% w/v, as seen in Fig. 2a. Figure 2b shows that for the Corpoica-Caiselli variety, the highest production at the end of the saccharification process was presented with the concentration of 30% w/v with a value of 93.4 DE.

For the Corpoica-Orense variety, the greatest amount of DE produced was for the initial concentration of 30% w/v with a value of 91.58 ED, whereas for that of 35% w/v it was 90.23 DE as shown in Fig. 3.



(a)



(b)

Fig. 1: Production of DE from (a) Corpoica-Tai, (b) Corpoica-Verónica cassava starch varieties
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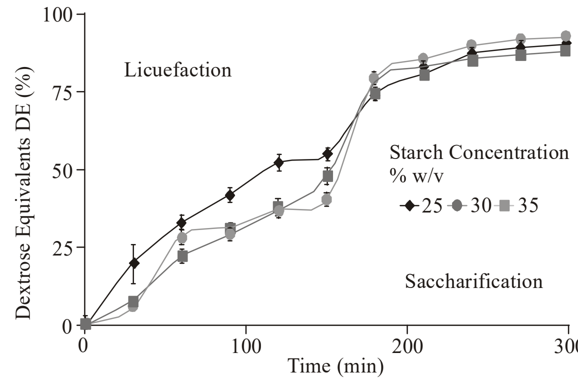
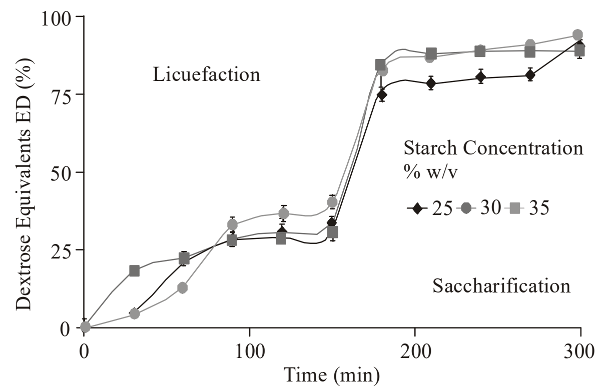


(a)



(b)

Fig. 2: Production of DE from (a) Corpoica-Gines, (b) Corpoica-Caiselli cassava starch varieties
Prepared by researchers

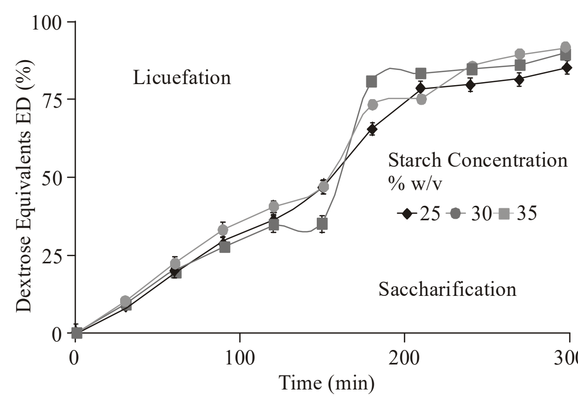


Fig. 3: Production of DE from Corpoica-Orense cassava starch variety
Prepared by researchers

Analyzing Fig. 1 to 3, it can be observed that the production of sugar reducers shows a similar behavior in all the varieties. The EDs have a relationship directly proportional to time. In the saccharification stage after 30 min, ED production tends to remain constant.

The highest production of DE was in the variety Corpoica-Tai with a value of 94.97 DE, followed by the Corpoica-Caiselli varieties with 93.41 DE and Corpoica-Ginés with 92.54 DE for the initial starch concentration of 30% w/v. These results are lower than those obtained by Salcedo *et al*. (2009, 2010) because of the difficulty of reaching and maintaining the temperature in this scale with the bioreactor used.

When comparing with the data reported by Morales *et al*. (2008), these obtained a lower amount of DE. This is due to the increase in the substrate concentration of the feed, a significant decrease in conversion occurs. In addition when the saturated catalysts are found there would be an insensitive reaction to increases in feed concentration with which employment of smaller starch concentrations is recommended (Khalilpour and Roostaazad, 2008). Similarly, Aguilar (2008) reached a lower value due to the low initial substrate concentration, compared to the concentrations used in this research and the type of enzyme used, taking into account that the enzyme Dextrozyme GA 1.5X has more performance than Dextrozyme GA.

Evaluation of fructose yields: Table 3 shows the average yields of fructose obtained in each of the cassava varieties at each concentration of starch. The

Table 3: Yields of fructose syrups, standard deviation and Tukey test results

Variety of cassava	Concentration % p/v	$y_{F/S}$ kg F/ton S	Tukey	$y_{F/FC}$ kg F/ton FC	Tukey
Tai	25	377.27±10.50	f	59.68±1.65	cde
	30	652.65±16.40	a	104.50±2.71	a
	35	616.28±18.10	a	99.21±3.31	a
Verónica	25	449.96±4.95	cd	67.94±0.74	bc
	30	485.12±11.10	bc	73.25±1.68	b
	35	419.73±9.65	def	63.38±1.46	cd
Gines	25	443.18±22.10	cde	33.24±1.66	h
	30	518.99±6.75	b	38.92±0.51	gh
	35	485.56±12.10	bc	36.42±0.91	h
Caiselli	25	398.93±17.10	ef	46.67±2.01	fg
	30	506.36±20.20	b	59.24±2.37	cde
	35	299.70±9.29	g	35.06±1.09	h
Orense	25	397.24±4.92	ef	54.02±3.52	def
	30	409.59±6.58	def	55.70±4.22	def
	35	385.38±10.10	f	52.41±4.01	ef

F: Fructose; FC: Fresh cassava; S: Starch; Values of the same column with different letters indicate a statistically significant difference ($p<0.05$); Prepared by researchers

variances revealed highly significant differences between treatments, with $p<0.05$ ($2.26*10^{-4}$) for yields of kg F/ton S and $p<0.05$ ($2.26*10^{-8}$) for yields of kg F/ton FC.

Finally, Tukey's mean comparison test determined that the best treatments correspond to Corpoica-Tai at 30% w/v and Corpoica-Tai at 35% w/v, since they present significant differences with the rest, represented by equal letters (a). Although there is no significant difference between them, the first one is selected as the best, because the performance is the variable response evaluated and it was sought to maximize it.

The 30% w/v selected CORPOICA-TAI treatment yields 652.65 kg F/ton S and 104.5 kg F/ton FC followed by the 35% w/v Corpoica-Tai treatment with values of 616.28 kg F/ton S and 99.21 kg F/ton FC. When comparing the yields obtained at laboratory scale by Salcedo *et al*. (2009, 2010), they obtained higher yields in a proportion of 10% affected mainly by the yields obtained in the wet extraction of the starch.

CONCLUSION

The best yields for obtaining wet starch at the pilot scale were for the Corpoica Tai, Corpoica Verónica and Corpoica Orense varieties, highlighting the high amount of fibrous material and peel.

The production of dextrose equivalents throughout the enzymatic hydrolysis process was affected by the starch concentration and the cassava variety, reaching values close to 90 ED. The 30% w/v Corpoica-Tai treatment presented the best results with values of 652.65 kg F/ton S and 104.56 kg F/ton FC. These results prove the technical viability at the pilot scale to generate products of higher added value from cassava.

RECOMMENDATIONS

It is necessary to improve the wet extraction process, especially in the grating and extraction processes. The application of scaling criteria related to transport phenomena and simulation will allow the design of bioreactors with greater control to develop hydrolysis and isomerization processes with higher productivity.

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CONFLICT OF INTEREST

Authors should disclose all financial/relevant interest that may have influenced the development of the manuscript.

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