Study of Carbohydrate Hydrolysis From Arracacha Roots (*Arracacia Xanthorriza* Bancroft) to Produce Fermentable Sugars

Estudio de Hidrólisis de Carbohidratos en Raíces de Arracacha (*Arracacia Xanthorriza* Bancroft) para Producir Azúcares Fermentables

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ABSTRACT

In Colombia, approximately 855 840 tons of arracacha are produced each year. The unsalable post-harvest arracacha root (*Arracacia xanthorriza* Bancroft) is not commercialized, mainly due to mechanical damage or small and misshapen roots. In this work, dry samples were characterized and subjected to two treatments: one using thermal hydrolysis, applying saturated steam at pressures of 0,1034 MPa, 0,2068 MPa, and 0,4137 MPa; and another one using hydrolysis with sulfuric acid in concentrations between 0,25-2,00 M. Then, the cake resulting from the hydrolysis and filtration process was enzymatically hydrolyzed (Liquozyme SC DS, Novozymes) at 1,5, 5 and 10 KNU/g (pH 6, 80 °C, 2 h). Fermentation inhibitors (acetic acid and furfural) were evaluated in the best pretreatment. The results showed that the treatment with sulfuric acid at 1,00 M (2 h) has high yields in reducing sugars added to enzymatic hydrolysis. The maximum level of fermentable carbohydrates per gram of dry sample (1,04 g/g) was also reached. Regarding the fermentation inhibitors of the reducing sugar, a higher concentration of acetic acid was found with a lower furfural content. Therefore, arracacha discards are a promising raw material to increase the supply of bioethanol.

Keywords: chemical hydrolysis, enzymatic hydrolysis, fermentation inhibitors, reducing sugars

RESUMEN

En Colombia se producen aproximadamente 855 840 toneladas anuales de arracacha. La raíz de arracacha postcosecha no vendible (*Arracacia xanthorriza* Bancroft) no se comercializa, principalmente debido a daños mecánicos o raíces pequeñas y deformadas. En este trabajo, las muestras secas fueron caracterizadas y sometidas a dos tratamientos: uno con hidrólisis térmica aplicando vapor saturado a presiones de 0,1034 MPa, 0,2068 MPa y 0,4137 MPa; y el otro de hidrólisis con ácido sulfúrico en concentraciones entre 0,25-2,00 M. Luego, la torta resultante del proceso de hidrólisis y filtración se hidrolizó enzimáticamente (Liquozyme SC DS, Novozymes) a 1,5, 5 y 10 KNU/g (pH 6, 80 °C, 2 h). Se evaluaron los inhibidores de fermentación (ácido acético y furfural) en el mejor pretratamiento. Los resultados mostraron que el tratamiento con ácido sulfúrico a 1,00 M (2 h) tiene altos rendimientos en azúcares reductores adicionados a hidrólisis enzimática. También se alcanzó el nivel máximo de carbohidratos fermentables por gramo de muestra seca (1,04 g/g). En cuanto a los agentes inhibidores de la fermentación del azúcar reductor, se encontró una mayor concentración de ácido acético con contenido furfural menor. Por tanto, los residuos de arracacha son una materia prima prometedora para incrementar la oferta de bioetanol.

Palabras clave: hidrólisis química, hidrólisis enzimática, inhibidores de fermentación, azúcares reductores

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Introduction

Arracacha roots (AR) or creole celery (*Arracacia xanthorrhiza* Bancroft.), are a tuber with high nutritional value that offers large amounts of minerals and carbohydrates compared to cassava (Londoño-Restrepo, Rincón-Londoño, Contreras-Padilla, Millan-Malo, and Rodriguez-Garcia, 2018; Otache, Ubwa, and Godwin, 2017). Furthermore, Colombia is

currently the world's largest producer of arracacha with an annual production of 855 840 t/year in 2018 (MinAgricultura and Agronet, 2018).

Moreover, *an* arracacha crops are characterized by having productive cycles throughout the year, and their quality is definitely affected by pre-harvest, harvest, and post-harvest processes. Poor implementations stemming from cultural habits influence arracacha in terms of sprout decomposition, production heterogeneity, and the frequency of crop diseases (Rana and Kumar, 2017), thus generating a higher volume of non-quantified waste in post-harvest, as well as creating low production yields, poor product quality, and, in turn, environmental emissions problems such as nitrous oxide, carbon monoxide, among others; these compounds contribute to the greenhouse effect (Galford et al., 2020; D. Huang et al., 2019).

Likewise, the use of these agricultural residues is quite extensive and highly studied, including the extraction of compounds of pharmaceutical interest (Didaskalou, Buyuktiryaki, Kecili, Fonte, and Szekely, 2017), fertilizers (Lupton, 2017), and fermentable sugars for the production of biofuels (C. Huang, Jeuck, and Yong, 2017; Nair, Lennartsson, and Taherzadeh, 2017).

Obtaining biofuel from vegetable waste is an alternative to solve issues with oil dependency that come from the 1970s energy crisis, where the fossil fuel supply decreased considerably, and the ensuing riots created the necessity to contribute to environmental management (Venn, 2016).

Even though there was a collapse in crude oil prices, which fell to negative values due to the current COVID-19 pandemic (Aloui, Goutte, Guesmi, and Hchaichi, 2020), there is still an interest in modifying oil-powered engines. This has led to research on the topic of ethanol as a fuel source for the automotive, energetic and agroindustry industry since the mid-nineteenth century until now (Awad et al., 2018).

Bioethanol increases gasoline octane, improving its combustion and efficiency in the conversion to mechanical energy. At the same time, it reduces fuel consumption by means of a higher release of free radicals (H, OH, and O) and reducing emissions of hydrocarbons (HC), carbon monoxide (CO), and nitrogen oxides (NOx) (Aditiya, Mahlia, Chong, Nur, and Sebayang, 2016; Awad et al., 2018). This complies with Colombian law nr. 693 of 2001 (Colombian Congress, 2001), which states that the automotive industry has to contain oxygenated components such as fuel alcohols, with the purpose of controlling air pollution.

Concerning the above, the ethanol production process is developed in four steps: pretreatment, fermentation, separation, and post-treatment of the liquid fraction (Zabed, Sahu, Suely, Boyce, and Faruq, 2017). As for pretreatments, benefits depend on the kind of material. They could be physical (increasing the surface area and pore volume) or chemical (alkali and acid). Their function is to decrease the degree of cellulose polymerization and its crystallinity, thus allowing for a major production of reducing sugars (Sarkar, Ghosh, Bannerjee, and Aikat, 2012). However, there are some significant disadvantages in the use of physical methods, such as high energy consumption, which is environmentally unfriendly and non-viable for a commercial process.

Chemical pretreatments have the presence of inhibitors in each of the mentioned stages (Zabed et al., 2017), which causes the delay of yeast activity and an extension of fermentation time. Due to hydrolytic pretreatments applied to vegetable material (like furfural, hydroxymethylfurfural, and acetic acid, among other compounds) (Maiti et al., 2018) it has a major negative effect on enzymes (Wojtusik, Villar, Zurita, Ladero, and Garcia-Ochoa, 2017).

In this context, the purpose of this paper is to study the hydrolysis of the carbohydrates present in the post-harvest agricultural discards of arracacha (unsalable roots) to obtain fermentable sugars, assessing inhibiting compounds (acetic and furfural acid), despite AR having phenolic compounds (Leja et al., 2013), which are major enzyme and microbial inhibitors that can potentially affect the production of ethanol during fermentation.

Methodology

Sample conditioning

AR samples were purchased from a crop in Cajamarca, Tolima, Colombia. Only roots that were not marketable, malformed, or with mechanical or pest damage were used in the tests. Samples were dried to an average humidity of 13% in a convection oven (60 °C, 72 h), and then the particle size was reduced to 1 mm using a hammermill.

Analytical techniques

The contents of moisture (method 925.10), crude protein (method 955.04), ether extract (method 963.15), crude fiber (method 962.09), and ash (method 941.12) were determined for each residue by means of the official methodology (AOAC, 2000). Also, neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose, and lignin were all quantified according to the Van Soest methodologies (P. Van Soest and Wine, 1968; P. J. Van Soest, 1963).

Total and reducing carbohydrate quantification were performed by extraction with absolute ethanol in dilution 1:3 (w/v), filtered on quantitative paper and refrigerated until use. The quantification of both total carbohydrates (TC) and reducing sugars was carried out using the Antrona (Leyva et al., 2008) and DNS (Miller, 1959) spectrophotometric methods using a UV-Vis Helios Gamma spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 620 nm and 540 nm, respectively.

Physicochemical pretreatment

The AR was submitted to pressures of 0,1034 MPa (100,55 °C), 0,2068 MPa (121,28 °C), and 0,4137 MPa (144,83 °C) (saturated steam), and it diluted in distilled water to a ratio of 1:15 for one hour. The steam supply was generated in a 2-inch vertical, pirotubular

boiler with a capacity of 2 BHP (Equipos y Calderas Industriales E U, Bogotá) (Carranza Saavedra, Alvarado Núñez, Méndez Reyes, Valenzuela Real, and Solanilla Duque, 2015).

Chemical pretreatment

The samples were hydrolyzed with sulfuric acid (H_2SO_4) at concentrations of 0,25, 0,50, 1,00, and 2,00 M in 1:15 dilution at a temperature of 60 °C, refluxing at the atmospheric pressure of Ibagué, in the department of Tolima, Colombia (Carranza Saavedra et al., 2015).

Residence time

The resistance time for sugar splitting in every case was performed during 1 h. Then, in order to observe the effect of residence time on the samples, the best pretreatment (physical or chemical) was selected, and the residence time was increased to 2 h of hydrolysis. Next, the contents of total and reducing carbohydrates were analyzed.

Enzymatic pretreatment

From the best pretreatment, the samples were submitted to enzymatic hydrolysis to increase their reducing carbohydrates (RC) with Novozymes' Liquozyme SC DS α-amylase at 1,5, 5, and 10 kilo Novo units (KNU)/g (one KNU was defined as the amount of enzyme that hydrolyzed 5,26 g of starch (soluble starch) per hour under Novozymes's standard conditions for α-amylase determination) with diacid phosphate buffer pH 6 at temperature of 80 °C and 2 h with constant agitation (150 rpm).

Samples were taken every 30 minutes. The enzyme was inactivated at 95 °C for 5 min, and the samples were later centrifuged at 5 000 rpm for 10 min, determining RC immediately (Rathore, Paulsen, Vidal Jr., and Singh, 2009). The rate constant for the formation of first-order fermentable sugars was also evaluated, which is directly proportional to the concentration of the substrate and can be evaluated at different concentrations of enzyme. It is described using Equation (1):

$$\frac{drc}{dt} = k * rc \tag{1}$$

Where, rc is the concentration of RC, k is the first order kinetic constant, and t is time. Additionally, the solution of the linearized Equation (1), where k is the slope, is described using Equation (2):

$$Ln\,[rc] = kt + Ln\,[rc_0] \tag{2}$$

Where rc_0 is the concentration of initial RC.

Inhibiting Compounds in Pretreated Liquid Fractions

Determination of acetic acid content: Acetic acid was quantified through titratable acidity (Cheng et al., 2008). The results are expressed as equivalent milligrams of acetic acid per milliliter (mgEAA/mL).

Furfural quantitative determination: Furfural quantification was implemented according to the methodology described by the AOAC (2000). 25 mL of pretreatment liquid were taken to be distilled through a fractionating column. The fraction was collected at boiling temperature (161,20 °C). Later, 1 mL of the distilled result was taken and gauged with alcohol (99,80%) to complete 10 mL. Sample absorbance, alcohol white, and the standard (furfural) 277 nm were determined in a spectrophotometer. The results are expressed as equivalent furfural milligrams per milliliter (FEmg/mL).

Statistical Analysis

The results obtained in the study were expressed as the mean and the standard deviation of three determinations for each pretreatment. Analysis of Variance (ANOVA) and Multivariate Analysis of Variance (MANOVA) at a 95% confidence level were used to compare the mean values of each determination among the different pretreatments.

The STATGRAPHICS Centurion XV statistical package was used for the analysis of the obtained results. The treatments were executed through randomized complete block design (RCBD) and averaged differences using the least significant difference (LSD) for each one. The coefficient of determination (Equation 3) was used to assess the goodness of fit of the linearization of Equation (1).

$$R^2 = 1 - \frac{\sum(y_i - f_i)^2}{\sum(y_i - \overline{y})^2} \tag{3}$$

Where f_i, y_i, and $\overline{y}$ are the model data, experimental data, and the mean of the experimental data, respectively.

Results and Discussion

Bromatological and carbohydrates analysis

Table 1 shows the results of the bromatological analysis, as well as the composition of cell walls in AR. The protein content in the studied samples was higher than in other types of arracacha (Londoño-Restrepo et al., 2018), and did not exceed the range between 4,00% and 8,00%. When added to the TC content, it represents a significant amount of calories that partly support the conversion of energy in animals that are fed with these residues.

The ethereal extract found to have the greatest amount was the yellow and purple arracacha plant (Londoño-Restrepo et al., 2018; Palacios, Morales, and Arias, 2011), with documented fat values of 0,90% (dry basis).

The crude fiber content was almost ten times higher than in other studies (Londoño-Restrepo et al., 2018; Palacios et al., 2011), which shows a promising composition for the extraction of RC. The ash contents had similar values as the reported cases of arracacha flour, which means it is an important source of minerals in use for human and animal food.

Table 1 shows the values of the study of the cell wall (ADF, NDF, hemicellulose, cellulose, and lignin) which evinces a

small amount of lignocellulosic material in its structure, as well as the fact that, according to studies, it contains between 75% and 90% starch on dry basis (Londoño-Restrepo et al., 2018; Palacios et al., 2011). Therefore, it was not necessary to carry out tests with sodium hydroxide. It was also observed that it did not contain detectable lignin in its structure.

The carbohydrates in unprocessed AR (control) were characterized in order to evaluate the increase or decrease in present sugars during the pretreatments. The studies reported 27,39% and 1,63% of total and reducing carbohydrates, respectively, a considerable amount compared to cane cachaza, penelera cane chuff, rice shell, Tahití lime (citrus latifolia), and common lemon (citrus aurantifolia), which are reported as feedstock to obtain ethanol, especially by way of fermentation (Sánchez-Riaño, Barrero, and Murillo, 2010).

Table 1. Physicochemical analysis of AR

| Parameters	% w/w
Moisture	$13,50 \pm 1,4$ [a]
Ash	$4,30 \pm 0,8$ [bc]
Ethereal extract	$2,50 \pm 1,7$ [c]
Raw fiber	$13,20 \pm 0,7$ [a]
Crude protein	$8,00 \pm 0,7$ [b]
ADF	$5,80 \pm 0,5$ [b]
NDF	$2,30 \pm 1,6$ [c]
Hemicellulose	$8,60 \pm 2,1$ [b]
Cellulose	$3,50 \pm 1,0$ [c]
Lignin	$0,00$ [d]
Total available carbohydrates	$27,39 \pm 1,5$
Reducing carbohydrates	$1,63 \pm 0,1$

Results are the mean of three determinations ($n = 3$), $\pm$ represent standard deviation. Similar lower-case letters indicate that there is no statistical difference between treatments ($p < 0,05$).

Source: Authors

Pretreatments

Physicochemical pretreatment: Figure 1 shows the different pressures at which the AR were treated to obtain reductive carbohydrates at different temperatures. The MANOVA reported significant differences ($p < 0,05$) between the total and reducing carbohydrates by physicochemical treatment with a 95% level of significance.

Now, elevating the temperature of the starchy material proved its sensitiveness, which showed an increase in the quantification of TC with a significant pressure differences ($p < 0,05$) between 0,10 MPa ($0,52 \pm 0,03$ g/g), 0,20 MPa ($0,53 \pm 0,01$ g/g), and 0,41 MPa ($0,67 \pm 0,05$ g/g) with respect to the control sample. However, the pressure at 0,41 MPa allows an upper splitting of high molecular weight carbohydrates, attributed to hydrolyzed starch by the action of water molecules, which speed up the absorption at high temperatures, causing the swelling of the starch and breaking the granule; releasing the amylose and amylopectin molecules; and later, breaking their glycosidic bonds (Kong et al., 2016).
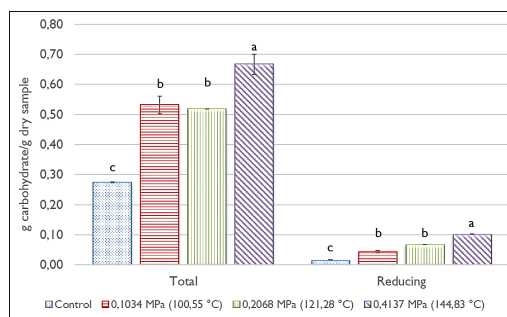
Figure 1. Total and reducing carbohydrate contents of arracacha obtained at different pressures with saturated steam. Vertical bars represent standard deviation. Similar lower-case letters indicate that there is no statistical difference between treatments ($p < 0,05$). **Source:** Authors

On the other hand, the variability of the results in terms of RC is shown in Figure 1, indicating that there are significant differences ($p < 0,05$) in the RC obtained at 0,41 psi in comparison with the control sample. Moreover, the simple sugars do not exceed $0,10 \pm 0,01$ g/g because of amylose and amylopectin molecules are released only in the rupture of starch granule, causing the breakup of 1,4 and 1,6 glycosidic bonds, respectively, but in small proportions (Shigematsu et al., 2017).

Chemical pretreatment: Figure 2 shows the sugar content (total and reducing) of arracacha when submitted to chemical hydrolysis. There seems to be a proportional or consequent behavior with the increase in acid concentration during the pretreatment. However, the 2,0 M acid concentration had an observed decline of total and reducing carbohydrates with a significant statistical effect ($p < 0,05$). TC reached their highest hydrolysis point at 1,0 M ($0,73 \pm 0,03$ g/g). A similar behavior was observed in RC at an acid concentration of 1,0 M with a $0,33 \pm 0,03$ g/g quantification. This phenomenon could be due to the degradation of amylose chains, and even amylopectin chains by H_2SO_4, which, as a consequence, releases total and reducing sugars (Li et al., 2020).

Furthermore, the increase of H_2SO_4 concentration for the hydrolysis of arracacha samples was harmful, because carbohydrates began to degrade, turning into unwanted compounds for fermentation (phenols and furfurals) (Guerrero, Ballesteros, and Ballesteros, 2017).

Increase in residence time: In pretreatments (physicochemical and chemical), it was evident that hydrolysis with H_2SO_4 at a 1,0 M concentration is the most effective method to obtain RC from arracacha discards.

Judging from the assessment of the residence time, higher yields were obtained at two hours with $0,52 \pm 0,09$ g/g (RC) compared to the one-hour treatment. This confirms a decrease in TC ($0,56 \pm 0,1$ g/g), possibly due to the rupture of the hydroxyl groups present in starchy materials (Peñaranda Contreras, Perilla Perilla, and Algecira Enciso, 2008).

Enzymatic pretreatment: The enzymatic hydrolysis for arracacha residues was implemented with α-amylase after choosing the best acid hydrolysis pretreatment (H_2SO_4 1,0
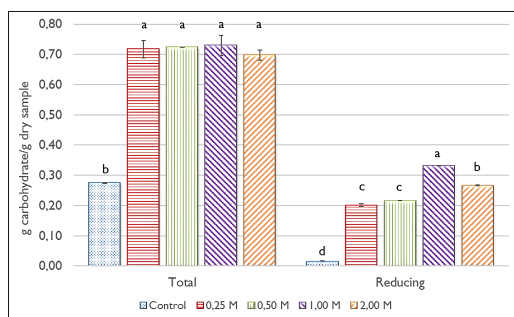
Figure 2. Total and reducing carbohydrate contents of arracacha obtained at different concentrations of H_2SO_4. Vertical bars represent standard deviation. Similar lower-case letters indicate that there is no statistical difference between treatments ($p < 0,05$).
**Source:** Authors

M, 2 h). Figure 3A shows the enzyme kinetics when it reacts with the substrate. An ANOVA revealed a statistic interaction ($p < 0,05$) with a 95,0% level of confidence between the fermentable sugar concentrations present in the hydrolyzed sample (g/g) and the enzyme concentration, showing a considerable increase in the monomeric sugars, due to the action that the enzyme exerts along any point of the carbohydrate chain (1-4 glucosidic bonds), hydrolyzing them in dextrin from amylose (Oliveira, Pinheiro, Fonseca, Cabrita, and Maia, 2019).

Therefore, the maximum conversion from substratum to dextrose is obtained within 2 hours, and in absence of a significant difference, according to Fisher's LSD method, between 1,5 and 5,0 KNU/g of concentration of the α-amylase enzyme, it is understood that 1,5 KNU/g is the concentration at which the enzyme works better, and the best results from enzymatic hydrolysis (0,56 ± 0,05 g/g) are obtained. Also, the highest concentration of the α-amylase enzyme might not have been attained because it could have been inhibited by the relation between enzyme and substrate, *decreasing the hydrolysis speed of starch to glucose* (Zhang et al., 2020).

Low values were obtained for corn starch hydrolysate with a reducing sugar content of 12-14% with the same enzyme and operation time, but without pretreatment (Z. Li, Wang, and Shi, 2019). Thus, it is possible that the enzyme conversion with pretreatment could find suitable applications in productions of sugars and chemicals made by the fermentation of sugars.

Alternatively, it is important to characterize the reaction kinetics for a reducing sugar gain. Contents of RC increase as part of the aging process, as shown in Figure 3A. First-order kinetics correctly described an increase in reducing sugars ($R^2 > 0,95$, 1,5 KNU/g in Equation (1)), as shown Figure 3B.

Chemical compounds react and convert to other species, and the rate constant indicates the speed of the change in concentration. The half-life time of each chemical species is an indicator of the time that a given compound increases its concentration by a factor of 0,02 (k) to 1,5 (NKU/g). Therefore, it is a standardized parameter that indicates the time at which a compound is degraded to simple sugars.
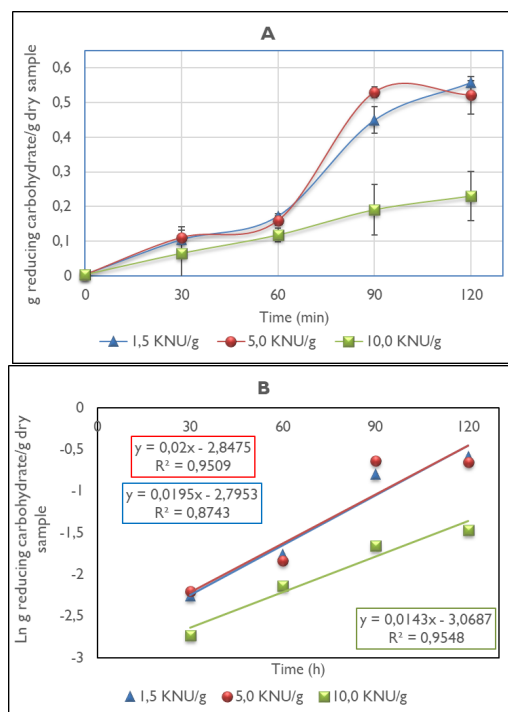
Figure 3. Hydrolysis of carbohydrates in AR with α-amylase enzyme. A: Enzymatic kinetics of reducing carbohydrate B: Kinetic analysis of the reducing sugar content. Vertical bars represent standard deviation. Lines in A are a guide for the eye and in B represent first-order fitting according to Equation 1.
Source: Authors

Evaluation of inhibiting agents

The assessment of the inhibiting compounds present in starchy material was made from AR hydrolysates. It was then concluded that this is the most efficient method to obtain reducing sugars using the chemical pretreatment with H_2SO_4 at 1,0 M and 2 h of residence time. In the hydrolyzed samples, an acetic acid formation was observed, due to the conditions of the chemical pretreatment applied to the roots (3,12 ± 0,11 mgEAA/mL). This result is associated with presence of acetyl groups by deacetylation of hemicelluloses during the pretreatment. These are not degraded despite the prolonged storage time, and they were possibly entirely hydrolyzed to form acetic acid by the action of the acid environment in which the pretreatment was carried out (Maiti et al., 2018), regardless of the residence time at which it was submitted.

There was also an effect (0,28 ± 0,002 mgEF/mL) of H_2SO_4 concentration, temperature, and residence time in the formation of the environment that fosters pentose dehydration, until their degradation to furfural in pre-treated starchy waste. This could be explained by the way in which many monomeric sugars (pentoses) were possibly hydrolyzed during the process of acid pretreatment in AR; these sugars would be degraded to this inhibiting compound (furfural) (Maiti et al., 2018). Lastly, AR have phenolic compounds within their structure, which act well as major enzymes and microbial inhibitors.

Conclusions

The physicochemical pretreatment with distilled water under high pressures turns out to be a soft method of carbohydrate hydrolysis compared to chemical pre-treatment in H_2SO_4 at 1,0 M with quantification of 51,8% of RC by dry sample of roots and a residence time of 2 h.

A further pretreatment with α-amylase increases the fermentable sugar yield with arracacha discards, reaching a conversion of 1,04 g of fermentable sugars per gram of dry vegetable material.

The fermentation of non-detoxified hydrolysates is characterized by a limited productivity and yield, due to the presence of a variety of compounds that act as potential inhibitors of the cellular metabolism, such as furfural and acetic acid found in AR after the hydrolysis with diluted H_2SO_4. Therefore, the use of mechanisms that remove most of these toxic compounds in the hydrolysates is necessary, thus improving the metabolism of the microorganisms responsible for starchy material fermentation.

Furthermore, the identification of the RC type that is generated in the hydrolysis is used to establish the appropriate biological mechanism for fermenting ethanol, considering the detoxification tests before the fermentation.

This study of carbohydrate hydrolysis in non-marketable food significantly shows the ease of taking advantage of agricultural waste and converting it into clean energy, thereby contributing to the continuous development of producing fermentable sugars as a workhorse in the production of bioethanol.

Acknowledgements

References

Aditiya, H., Mahlia, T. M. I., Chong, W., Nur, H., and Sebayang, A. (2016). Second generation bioethanol production: A critical review. *Renewable Sustainable Energy Reviews, 66*, 631-653. 10.1016/j.rser.2016.07.015

Aloui, D., Goutte, S., Guesmi, K., and Hchaichi, R. (2020). COVID 19's impact on crude oil and natural gas S&P GS Indexes. SSRN. 10.2139/ssrn.3587740

AOAC. (2000).Official Methods of Analysis. (17th. ed.). Gaithersburg, USA: Association of Official Analytical Chemists.

Awad, O. I., Mamat, R., Ali, O. M., Sidik, N., Yusaf, T., Kadirgama, K., and Kettner, M. (2018). Alcohol and ether as alternative fuels in spark ignition engine: A review. *Renewable Sustainable Energy Reviews, 82*, 2586-2605. 10.1016/j.rser.2017.09.074

Carranza Saavedra, D., Alvarado Núñez, J. A., Méndez Reyes, D. A., Valenzuela Real, C. P., and Solanilla Duque, J. F. (2015). Pretratamiento de residuos de plátano (Musa paradisiaca (L.) AAB) y arracacha (Arracacia xanthorrhiza Bancroft) para la obtención de azúcares fermentables. *Revista Venezolana de Ciencia y Tecnología de Alimentos, 6*(1), 019-035. http://oaji.net/articles/2017/4924-1495586231.pdf

Cheng, K.-K., Cai, B.-Y., Zhang, J.-A., Ling, H.-Z., Zhou, Y.-J., Ge, J.-P., and Xu, J.-M. (2008). Sugarcane bagasse hemicellulose hydrolysate for ethanol production by acid recovery process. *Biochemical Engineering Journal, 38*(1), 105-109. 10.1016/j.bej.2007.07.012

Congreso de Colombia (2001). LEY 693 de 2001. Por la cual se dictan normas sobre el uso de alcoholes carburantes, se crean estímulos para su producción, comercialización y consumo, y se dictan otras disposiciones. September 27, 2001. D.O. Nr. 44564. http://www.secretariasenado.gov.co/senado/basedoc/ley_0693_2001.html

Didaskalou, C., Buyuktiryaki, S., Kecili, R., Fonte, C. P., and Szekely, G. (2017). Valorisation of agricultural waste with an adsorption/nanofiltration hybrid process: from materials to sustainable process design. *Green Chemistry, 19*(13), 3116-3125. 10.1039/c7gc00912g

Galford, G. L., Peña, O., Sullivan, A. K., Nash, J., Gurwick, N., Pirolli, G., Richards, M., White, J., and Wollenberg, E. (2020). Agricultural development addresses food loss and waste while reducing greenhouse gas emissions. *Science of The Total Environment, 699*, 134318. 10.1016/j.scitotenv.2019.134318

Guerrero, A. B., Ballesteros, I., and Ballesteros, M. (2017). Optimal conditions of acid-catalysed steam explosion pretreatment of banana lignocellulosic biomass for fermentable sugar production. *Journal of Chemical Technology Biotechnology, 92*(9), 2351-2359. 10.1002/jctb.5239

Huang, C., Jeuck, B., and Yong, Q. (2017). Using pretreatment and enzymatic saccharification technologies to produce fermentable sugars from agricultural wastes. In Kalla, V. C. and Singh, L. (Eds.) Waste Biomass Management–A Holistic Approach (pp. 15-38) Berlin, Germany: Springer.

Huang, D., Cao, G., Geng, Y., Wang, L., Chen, X., and Liang, A. (2019). Impact of agricultural waste return on soil greenhouse gas emissions. *Applied Ecology Environmental Research, 17*(1), 1321-1335. 10.15666/aeer/1701_13211335

Kong, W., Zhang, T., Feng, D., Xue, Y., Wang, Y., Li, Z., Yang, W., and Xue, C. (2016). Effects of modified starches on the gel properties of Alaska Pollock surimi subjected to different temperature treatments. *Food Hydrocolloids, 56*, 20-28. 10.1016/j.foodhyd.2015.11.023

Leja, M., Kamińska, I., Kramer, M., Maksylewicz-Kaul, A., Kammerer, D., Carle, R., and Baranski, R. (2013). The content of phenolic compounds and radical scavenging activity varies with carrot origin and root color. *Plant Foods for Human Nutrition, 68*(2), 163-170. 10.1007/s11130-013-0351-3

Leyva, A., Quintana, A., Sánchez, M., Rodríguez, E. N., Cremata, J., and Sánchez, J. C. (2008). Rapid and sensitive anthrone–sulfuric acid assay in microplate format to quantify carbohydrate in biopharmaceutical products: Method development and validation. *Biologicals, 36*(2), 134-141. 10.1016/j.biologicals.2007.09.001

Li, H., Yan, S., Mao, H., Ji, J., Xu, M., Zhang, S., Wang, J., Yingli, L., and Sun, B. (2020). Insights into maize starch degradation by sulfuric acid from molecular structure changes. *Carbohydrate polymers, 229*, 115542. 10.1016/j.carbpol.2019.115542

Li, Z., Wang, D., and Shi, Y. C. (2019). High-Solids Bio-Conversion of Maize Starch to Sugars and Ethanol. *Starch-Stärke, 71*(1-2), 1800142. 10.1002/star.201800142

Londoño-Restrepo, S. M., Rincón-Londoño, N., Contreras-Padilla, M., Millan-Malo, B. M., and Rodriguez-Garcia, M. E. J. I. (2018). Morphological, structural, thermal, compositional, vibrational, and pasting characterization of white, yellow, and purple Arracacha lego-like starches and flours (Arracacia xanthorrhiza). *Journal of Biological Macromolecules, 113*, 1188-1197. 10.1016/j.ijbiomac.2018.03.021

Lupton, S. (2017). Markets for waste and waste–derived fertilizers. An empirical survey. *Journal of Rural Studies, 55*, 83-99. 10.1016/j.jrurstud.2017.07.017

Maiti, S., Gallastegui, G., Suresh, G., Sarma, S. J., Brar, S. K., Drogui, P., LeBihan, Y., Buelna, G., Verna M., and Soccol, C. R. (2018). Hydrolytic pre-treatment methods for enhanced biobutanol production from agro-industrial wastes. *Bioresource Technology, 249*, 673-683. 10.1016/j.biortech.2017.09.132

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*(3), 426-428. 10.1021/ac60147a030

MinAgricultura and Agronet. (2018). Reporte: Área, Producción y Rendimiento Nacional por Cultivo. https://www.agronet.gov.co/estadistica/Paginas/home.aspx?cod=1

Nair, R., Lennartsson, P. R., and Taherzadeh, M. J. (2017). Bioethanol production from agricultural and municipal wastes. In Pandey, A., Larroche, C., Angeles Saronman, M., and Du, G. (Eds.) Current developments in biotechnology and bioengineering (pp. 157-190). Amsterdam, Netherlands: Elsevier.

Oliveira, H. M., Pinheiro, A. Q., Fonseca, A. J., Cabrita, A. R., and Maia, M. R. (2019). Flexible and expeditious assay for quantitative monitoring of alpha-amylase and amyloglucosidase activities. *MethodsX, 6*, 246-258. 10.1016/j.mex.2019.01.007

Otache, M. A., Ubwa, S. T., and Godwin, A. K. (2017). Proximate analysis and mineral composition of peels of three sweet cassava cultivars. Asian Journal of Physical Chemical Sciences, 1-10. 10.9734/AJOPACS/2017/36502

Palacios, R., Morales, M., and Arias, G. C. (2011). Evaluación químico bromatológica de tres variedades de Arracacia xanthorrhiza "arracacha". *Ciencia e Investigación, 14*(2), 12-14. 10.15381/ci.v14i2.3161

Peñaranda Contreras, O. I., Perilla Perilla, J. E., and Algecira Enciso, N. A. (2008). Revisión de la modificación química del almidón con ácidos orgánicos. *Ingeniería e Investigación, 28*(3), 47-52. http://www.scielo.org.co/pdf/iei/v28n3/v28n3a06.pdf

Rana, M., and Kumar, S. (2017). Arracacha. In M. K. Rana (Ed.), Vegetable Crop Science (pp. 923-932). Boca Raton, FL: CRC Press.

Rathore, S., Paulsen, M. R., Vidal Jr, B., and Singh, V. (2009). Monitoring liquefaction unit operation in dry-grind ethanol process: Factors affecting hydrolysis and methods for analysis. *Transactions of the ASABE, 52*(5), 1639-1647. 10.13031/2013.29115

Sánchez-Riaño, A., Barrero, C. R., and Murillo, E. (2010). Perspectivas de uso de subproductos agroindustriales para la producción de bioetanol. *Scientia et Technica, 3*(46), 232-235. 10.22517/23447214.25

Sarkar, N., Ghosh, S. K., Bannerjee, S., and Aikat, K. J. R. e. (2012). Bioethanol production from agricultural wastes: an overview. *Renewable Energy, 37*(1), 19-27. 10.1016/j.renene.2011.06.045

Shigematsu, T., Furukawa, N., Takaoka, R., Hayashi, M., Sasao, S., Ueno, S., . . . Iguchi, A. (2017). Effect of high pressure on the saccharification of starch in the tuberous root of sweet potato (Ipomoea batatas). *Biophysical Chemistry, 231*, 105-110. 10.1016/j.bpc.2017.04.012

Van Soest, P. and Wine, R. (1968). Determination of lignin and cellulose in acid-detergent fiber with permanganate. *Journal of the association of official analytical chemists, 51*(4), 780-785. 10.1093/jaoac/51.4.780

Van Soest, P. J. (1963). Use of detergents in the analysis of fibrous feeds. 2. A rapid method for the determination of fiber and lignin. *Journal of the Association of Official Agricultural Chemists, 46*, 829-835. 10.1093/jaoac/46.5.829

Venn, F. (2016). The oil crisis. New York, NY: Routledge.Wojtusik, M., Villar, J. C., Zurita, M., Ladero, M., and Garcia-Ochoa, F. (2017). Study of the enzymatic activity inhibition on the saccharification of acid pretreated corn stover. *Biomass Bioenergy, 98*, 1-7. 10.1016/j.biombioe.2017.01.010

Zabed, H., Sahu, J., Suely, A., Boyce, A., and Faruq, G. (2017). Bioethanol production from renewable sources: Current perspectives and technological progress. *Renewable Sustainable Energy Reviews, 71*, 475-501. 10.1016/j.rser.2016.12.076

Zhang, B., Li, H., Wang, S., Junejo, S. A., Liu, X., and Huang, Q. (2020). In Vitro Starch Digestion: Mechanisms and Kinetic Models. In S. Wang (Ed.), Starch Structure, Functionality and Application in Foods (pp. 151-167). Singapore: Springer Singapore.