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OBTAINING FERMENTABLE REDUCING SUGARS BY CHEMICAL HYDROLYSIS OF MESQUITE POD FRACTIONS

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Abstract: Fermentable sugars can be obtained from a wide variety of lignocellulosic residues. To obtain it, a pre-treatment is necessary to remove lignin and hemicellulose. In this work, the effect of dilute hydrolysis and its relationship with temperature, time and agitation as the only factor in the release of reducing sugars from the whole meal of the mesquite pod was initially evaluated. It was observed that, as the reaction time increased (2 to 4 h), the concentration of reducing sugars decreased by 39.5 %, 59.75 %, and 57.8 % compared to a treatment for 4 h at 0.5, 1, and 2 N. of acid. On the other hand, when the temperature increased from 60 to 90 °C, no significant difference was observed compared to an acid concentration of 1N, obtaining an average of 66.8 g/L of reducing sugars. When studying agitation as the main factor on hydrolysis, it was observed that the concentration of reducing sugars was lower compared to the treatments at different temperatures and times. When evaluating the best hydrolysis conditions of the four mesquite pod fractions studied, it was observed that the highest amount of reducing sugars was obtained in the mesocarp with 38 g/L followed by the pericarp, integral and endocarp, obtaining 18.3, 15.07 and 2.6. g/L respectively at 2 h, 60 °C and 0.5 N acid without stirring.
Keywords: Mesquite pod, hydrolysis, reducing sugars

INTRODUCTION

Bioethanol as a metabolic product can be obtained from the fermentation of sugars found in combined plant products in the form of sucrose, starch, hemicellulose and cellulose through the action of microorganisms (Karimi et al., 2006). At present it has been found that fermentable sugars can be obtained from lignocellulosic residues such as; bagasse, cane waste, rice straw; crop residues such as alfalfa and grass or forest residues such as wood, and paper residues, among others (Betiku & Taiwo, 2015; Chen et al., 2015; Mishra et al., 2012). Lignocellulosic materials contain hemicellulose cellulose which are bound to lignin (Balat et al., 2008). Specifically, cellulose chains are composed of a homopolysaccharide composed of β-D-glucopyranose units linked by α(1-4) glycosidic bonds and are intertwined in such a way that neither water nor enzymes can penetrate, while Hemicellulose is a mixture of polymerized monosaccharides with sugars such as glucose, mannose, galactose, xylose, arabinose, and galacturonic acid that serve as a connection between cellulose and lignin fibers (Petersson et al., 2007). On the other hand, lignin has a highly branched structure and is often highly resistant to conversion by microorganisms and chemical agents (Balat et al., 2008; Talebnia et al., 2010).

Since lignocellulose does not contain sugars that are available for bioconversion to bioethanol; a pre-treatment is necessary to remove lignin and hemicellulose (Balat et al., 2008). The use of chemical pretreatments with acid are the most common (Zhou et al., 2021). The acid medium attacks polysaccharides, especially hemicellulose, which is easier to hydrolyze compared to cellulose (Cardona et al., 2010). Dilute acid hydrolysis allows the destruction of the structure of the lignocellulosic material, increasing the amount of released sugar monomers (Zhou et al., 2021). This treatment is carried out under acid conditions of between 0.5 and 1% sulfuric acid or hydrochloric acid with temperatures between 120 and 200 °C, pressures between 15 and 75 psi and reaction times from 30 min to 2 h (Kumar et al., 2009). The hydrolysis reaction consists of two reactions, the first converts the cellulosic material to sugars and the second converts the sugars to other chemical compounds, many of which can inhibit microbial growth (Zhou et al., 2021). Despite its effect, acid treatments are necessary to have a greater surface area (Dagnino et al., 2013), since it has the ability to increase porosity to improve its digestibility (Harmsen et al., 2010).

The genus *Prosopis*, (mesquite); it is a tree considered as the woody resource par excellence (Corona-Castuera et al., 1997). As a non-timber product, mesquite has a fruit called mesquite pod, used as food for various types of livestock, while the pods are consumed as flour or fermented drinks as human food (Rodríguez Sauceda et al., 2014). Mesquite pods can be mechanically fragmented to obtain the exocarp, which is the outer layer that covers the spongy part known as mesocarp, while the endocarp or hard inner layer is the one that protects the seed (Peña-Avelino et al., 2014). The endocarp is composed of cellulosic polysaccharides (40%) and lignin (17%) as main components in P. *glandulosa* (Pasiecznik et al., 2001). In the present work, the effect of temperature, time, agitation and acid concentration on the chemical hydrolysis of the mesquite pod in obtaining reducing sugars for fermentation was evaluated.

METHODOLOGY

OBTAINING THE SAMPLE

Mesquite pod samples were obtained from the Universidad Politécnica de Francisco I. Madero. These were previously treated and dehydrated, obtaining 4 fractions. Wholemeal flour, mesocarp flour, endocarp flour and pericarp flour, each fraction was stored in jars for use under low humidity conditions.

CHEMICAL HYDROLYSIS

From the whole mesquite pod flour, chemical hydrolysis was carried out in 125 ml flasks with 50 mL of H2SO4 at a concentration of 0, 0.005, 0.5, 1 and 2 N with a ratio of 10% w/v of flour, each experiment was performed in triplicate. To evaluate only the effect of temperature; the flasks were incubated for 2 h at 30, 60 and 90°C respectively without shaking. The effect of time on the hydrolysis process was carried out in flasks with 50 mL of H2SO4, these were incubated for 24h at 30°C; and for 1, 2, 4 h at a constant temperature of 60°C, without stirring. The effect of shaking was determined in flasks with a working volume of 50 mL of H2SO4 at a constant temperature of 30°C for 2h with orbital shaking at 0, 70, 140, and 180 rpm. The concentration of reducing sugars was determined using the dinitrosalicylic acid method described by Miller, (1959) using glucose as a standard at a concentration of 2 g/L.

EVALUATION OF THE EFFECT OF HYDROLYSIS ON THE MESQUITE POD FRACTIONS

To evaluate the effect of hydrolysis on the 4 fractions of the mesquite pod. Initially, 500 mL of a 10% w/v solids solution of each of the fractions (flour: integral, pericarp, mesocarp, and endocarp) were placed, the solutions were placed under constant magnetic stirring (180rpm) at room temperature for 1 h, were later placed in incubation for 2 h at 60°C to extract the soluble sugars from each of the fractions studied. The supernatant was filtered off and the filtrate was subjected to a drying process at 70°C for 24 hours until a constant weight solid was obtained. The solids obtained were ground and a sample of 10% w/v flour free of soluble sugars was placed in 125 ml flasks with 50 ml of H2SO4 at a concentration of 0.5 N. The experiments were placed in triplicate incubation at 60 and 90°C for two hours placing a treatment with only water as control. At the end of the treatment, the flasks were cooled and neutralized with NaOH. The neutralized samples were subjected to centrifugation for phase separation. The supernatant was vacuum filtered using

Whatman paper no. 1. The concentration of reducing sugars in the pretreatment and in the hydrolyzate was determined.

RESULTS

EFFECT OF HYDROLYSIS CONDITIONS ON THE WHOLE MEAL OF THE MESQUITE POD

The effect of hydrolysis time was evaluated with the four concentrations of acid, compared to water. It was observed that, as the reaction time increased from 2 to 4 h, the concentration of reducing sugars decreased by 39.5%, 59.75%, and 57.8% compared to treatment for 4 h at 0.5, 1, and 2 N of sulfuric acid. On the other hand, no differences were observed between the results obtained in the treatments with 1 N and 2 N of acid (Figure 1). The results showed that the treatment at 1 h did not present significant amounts of reducing sugars, obtaining 5.62 g/L in the treatment with water, while in the treatment at 2 h, 16.54 g/L were obtained. In a study; Kong-Win Chang et al., (2018) observed that at 97 °C, the glucose yield increased with reaction time up to a maximum of 76%, stabilizing at 60 min, while at 121 °C the maximum yield was of 78% after 20 min followed by a decrease at 60 min. This phenomenon was probably due to the simultaneous occurrence of the saccharification of amorphous cellulose combined with the degradation of glucose.

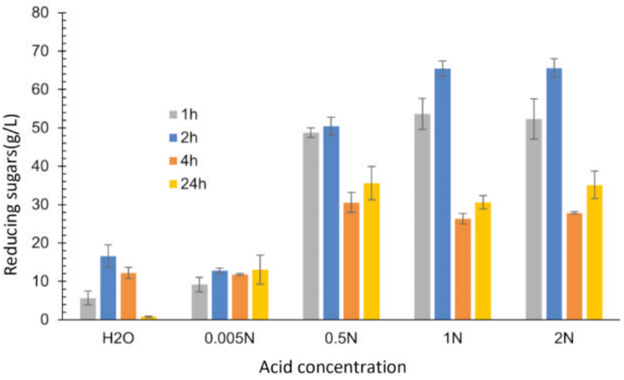

Figure 1. Effect of temperature on the hydrolysis of wholemeal flour from the mesquite pod at 60°C.

At 30°C for 24h, the amount of reducing sugars obtained was approximately 1 g/L, and as the temperature increased and the treatment time decreased, a significant increase was observed (Figure 2). On the other hand, when the temperature increased from 60 to 90°C, no difference was observed compared to an acid concentration of 1N, obtaining an average of 66.8 g/L of reducing sugars. At 90°C it was found that there were no differences between the hydrolysis treatments at 0.5 and 1N, while at 0.005 N the temperature directly influenced the increase in the amount of reducing sugars released. Additionally, at 30°C it was observed that the amount of sugars decreased slightly between the treatments with water and with an acid concentration of 0.005 N, obtaining 14.29 and 10.07 g/L respectively (Figure 2). This effect could be mainly due to the fact that the presence of acid at a very low concentration degraded the soluble sugars present under the tested conditions, due to the fact that the glycosidic bonds of the main chains are more stable than those of the branched chains, therefore hydrolysis acid caused excessive degradation of the released monosaccharides due to the treatment time (Liu et al., 2021).

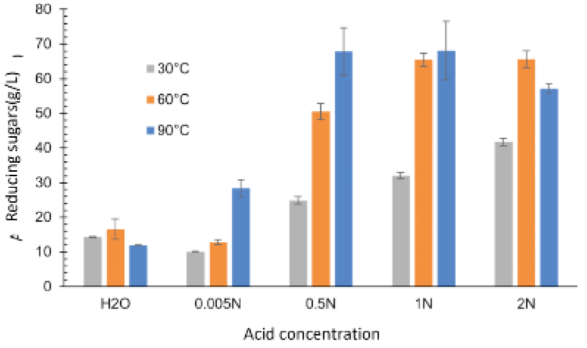

Figure 2. Effect of temperature on the hydrolysis of wholemeal flour from the mesquite pod after 2 h of treatment.

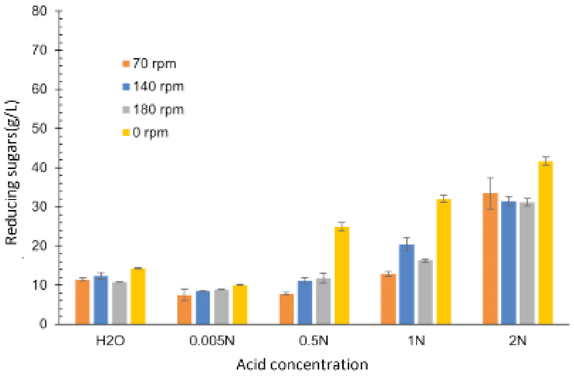

Figure 3. Effect of agitation on the hydrolysis of the whole meal of the mesquite pod after 2 h of treatment.

When evaluating the effect of stirring as the main factor on the hydrolysis of the mesquite pod wholemeal, it was observed that the concentration of reducing sugars was generally lower compared to the treatments at different temperatures and times (Figures 1 and 2). The decrease in the amount of reducing sugars was 28% for the treatment at 0.005 N of acid with the agitation speeds evaluated compared to the treatment with water. The maximum values were found at an average concentration of 32.05 g/L in the 2 N acid treatment. The low amount of reducing sugars obtained compared to the previously performed treatments could be mainly due to the fact that the movement of the fluid within the system followed the movement of the agitator limiting hydrolysis (Ramos M., 2014). The increase in the amount of reducing sugars in the tested treatments was gradual, with a linear trend between the treatments from 0.5 to 2 N at 140 and 180 rpm, the same as the treatment without stirring for a period of 24 hours of reaction; while for the treatment at 70 rpm the amount of sugars increased from 12.89 to 33.48 g/L without a linear trend (Figure 3).

It has been previously described by Morales-delaRosa et al., (2014) that the low reaction temperature, the use of a higher concentration of sulfuric acid increased the release of glucose linearly except in the highest concentration (2.5-12.5 mol /L) where the glucose concentration dropped slightly when long reaction times were used. This effect could be observed when changing the agitation from 70 to 140 rpm of orbital agitation.

EFFECT OF HYDROLYSIS CONDITIONS ON MESQUITE POD FRACTIONS

The four mesquite pod fractions underwent a pretreatment in which the soluble sugars of each of the fractions were extracted, observing that the integral fraction of the mesquite pod presented the highest concentration of reducing sugars (57.72 g/L), followed by the mesocarp (34.38 g/L), pericarp (32.17 g/L), while the endocarp showed the least amount of soluble sugars under the tested conditions (Figure 4).

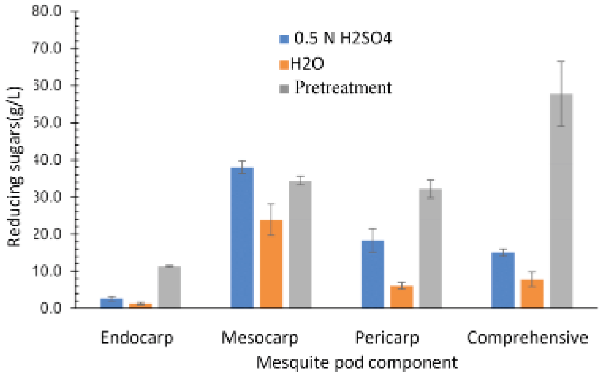

Figure 4. Effect of pretreatment and hydrolysis conditions on mesquite pod fractions at 60°C, 2h.

It was observed that the highest amount of reducing sugars in the treatment with 0.5 N of acid was obtained in the mesocarp flour with 38 g/L followed by the pericarp, integral and endocarp, obtaining 18.3, 15.07 and 2.6 g/L respectively. When comparing the experiments with the treatment with water, a significant amount of reducing sugars was observed in comparison to the treatment with acid. However, the acid treatment increased by 37.18, 66.72 and 48.12% the release of reducing sugars for the mesocarp, pericarp and integral fractions respectively with respect to the treatment with water. The results showed the need for a second pretreatment to extract the greatest amount of reducing sugars from the studied raw material.

CONCLUSIONS

From the hydrolysis conditions evaluated, it was observed that orbital agitation did not increase the release of reducing sugars, this could be due to the oscillatory movement of the fluid within the experiments carried out. However, hydrolysis at a temperature between 60 and 90°C for 2 h at a concentration of 0.5 and 1 N acid produced the highest amount of reducing sugars released. Additionally, the use of a pretreatment showed that the wholemeal flour followed by the mesocarp and pericarp were the fractions with the highest amount of soluble sugars and released the highest amount of sugars in an acid hydrolysis treatment, obtaining up to 38 g/L in the mesocarp fraction.

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