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EFFECT OF DRYING TEMPERATURE ON *AGAVE TEQUILANA* LEAVES: A PRETREATMENT FOR RELEASING REDUCING SUGARS FOR BIOFUEL PRODUCTION

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ABSTRACT

The leaves of *Agave tequilana* Weber variety Blue represent a viable, inexpensive, and renewable source of lignocellulosic biomass and fructans for the production of second generation biofuels. The objective was to study the effect of drying temperature on the release of reducing sugars for the agave leaves. It was found that with pretreatment-drying at 100°C for 30.5 ± 1.0 min had a maximum of the release of reducing sugars with a 66% increment compared to 60°C. An aqueous extract obtained from the powder of the leaves after drying did not show the presence of furfural and hydroxymethylfurfural compounds. Phenolic compounds were detected in order of 120.8 ± 1.0 mg L^{-1} below 1 g L^{-1} reported to cause inhibition of the alcoholic fermentation. In addition the drying of the leaves also can be used as preservation of agave leaf for biomass storing. The results show that pretreatment-drying allow increase the release of reducing sugars, avoids thermal degradation and does not produce significant concentrations of fermentation inhibitors.

PRACTICAL APPLICATIONS

The research in industrial waste materials has received special attention worldwide because it is potential for production of biofuels. This study propose a simple method of drying that acts as a thermal pretreatment of *Agave tequilana* Weber variety Blue leaves that increases the release of reducing sugars, avoids thermal degradation, not produce significant concentrations of fermentation inhibitors for ethanol production and can be used as a preservation method for storing biomass of the agave leaves. The results would be useful not only in the energy field but also in the alimentary and pharmaceutical industry. Fructans and phenolic compounds found in the agave are used as functional ingredients in the food industry and also bioactive compounds that by themselves promotes future research for the *Agave tequilana*.

INTRODUCTION

The research in biomass materials has been an interesting topic of study mainly because it's potential for the production of biofuels. Today, the fossil fuels provide about 80% of global energy demand and is estimated that will increase by 56% in 2040 (EIA, 2014). The increment on the energy demand will have a negative impact on environmental sustainability, this scenario has attracted interest for the use of non-fossil, renewable, and less polluting fuels. Special emphasis had been put on developing biofuels that comes

from industrial waste materials non appropriated for food purposes (Kim and Dale, 2004; Hahn-Hägerdal et al., 2006; Prasad et al., 2007; Linde et al., 2008; Metzger and Hüttermann, 2009; González García et al., 2010; Arrizon et al., 2012). At the present time one of the most important biofuels is ethanol, a renewable product obtained from energy crops and lignocellulosic material. In this sense the leaves of *Agave tequilana* Weber variety Blue has been studied as an useful biomass for producing biofuels (Davis et al., 2011; Montañez Soto et al., 2011; Escamilla-Treviño, 2012; Murugan and Rajendran, 2013; Huitrón et al., 2013; Villegas Silva et al., 2014; Li et al., 2014; Mielenz et al., 2015). The agave leaves are agricultural crop residues and a by-product of the tequila industry, representing ~38% of total weight of the plant, which containing fructans and lignocellulosic material that are suitable source for the obtention of sugars (Iñiguez Covarrubias et al., 2001; Mancilla-Margalli and López, 2002; Mancilla-Margalli and Lopez, 2006; Waleckx et al., 2008; Arrizon et al., 2010; Li et al., 2012). This material can be used to produce ethanol but first it requires physical, chemical, or biological pretreatments for the release of fermentable sugars. The goal of the pretreatment is to process the lignocellulosic material in order to break the lignin structure down and disrupt the crystalline structure of cellulose as a result acids or enzymes can easily access and hydrolyze the cellulose (Sun and Cheng, 2002; Mosier et al., 2005; Hendriks and Zeeman, 2009; Alvira et al., 2010;Agbor et al., 2011). The pretreatments however could have a negative side effect for the production of ethanol. Recent studies on the juice extracted from the agave leaves with pretreatments using temperatures above 100°C and/or employing dilute acid reports that the yeast *Saccharomyces cerevisiae* did not grow on this samples (Villegas Silva et al., 2014). It is worth to mention that for the agave leaves, there is still a lack of a systematic study of the compounds that have an inhibitory effect for alcoholic fermentation generated during pretreatment. Most of the research of *Agave tequilana* Weber variety Blue was conducted on the head of the plant, because is the part of the plant used for the tequila production. Traditionally Agave head are steam cooked in brick ovens for 36 hr to hydrolyzed the fructans in order to increase the amount of reducing sugar. Oftentimes the traditional steam cooked process generates compounds that will inhibit the alcoholic fermentation, such as hydroxymethylfurfural and furfural (Palmqvist et al., 1999; Palmqvist and Hahn-Hägerdal, 2000; Mancilla-Margalli and López, 2002; Lopez et al., 2003; Waleckx et al., 2008). The pretreatment of biomass is a key step for subsequent enzymatic hydrolysis and fermentation steps and therefore in the yield of ethanol. The present manuscript proposes a simple method of drying that acts as a thermal pretreatment that improves the extraction of sugars, prevents the degradation of carbohydrates, does not produce significant concentrations inhibitory compounds for hydrolysis and fermentation, does not require the addition of chemical compounds, and also preserve the material for storage. The drying of biological materials is one of the thermal treatment most commonly used to improve the product stability, as the drying significantly reduces the water activity (a_w) of the material, reduces the microbiological and enzymatic activity and minimizes the physical and chemical changes during storage (Geankoplis, 1998; Romero-Peña and Kieckbusch, 2003; Ertekin and Yaldiz, 2004; Doymaz, 2005; Vega et al., 2005; Montes et al., 2008; Mota et al., 2010; Arslan et al., 2010).

MATERIALS AND METHODS

Material

Agave tequilana Weber var. Azul plants of 8 years of ages which were harvested at the same time from a cultivation zone around Culiacan, Sinaloa, Mexico (24°52′50″ N, 107°21′20″ W). Plants were randomly selected and the leaves were cut from the head or piña. The samples were transported under ambient conditions (25°C ± 2°C; 80% relative humidity) from the field to the laboratory. The leaves were then washed first with water and liquid soap and then with chlorinated water (10 mg L^{-1}) to eliminate impurities. Afterwards the leaves were dried using absorbent paper towels for a 15 min at room temperature (23°C ± 2°C). Then the spines, on the end and along the margins of leaves, were manually removed with a knife in order to have a safer manipulations of the leaves. Finally the samples were kept under refrigeration (12°C ± 2°C; 95% relative humidity) for <1 day before they were dried and grinded to obtain a powder.

Preparation from the Raw Material

The agave leaf size were cut with a commercial slicers (Hobart, 1612E, USA), calibrated at 1 mm. The thicknesses of the slices then were verified employing precision Vernier (Uchida, M0-1, Japan).

Effects of the Temperature of Drying

The slices obtained from the leaves of agave were placed in trays constituted of a metallic insect screen on an aluminum frames. The slices were dried using a convective oven with the air velocity, set to 1.45 m s^{-1}, which was measured using a digital anemometer (Omega, HHF91, USA). The frames allow to expose both surface of the agave slices to the air flow. Four temperatures were used (60, 80, 100, and 120°C) in order to determine the chemical composition as a function of drying temperature. The drying process was monitored *in situ* by measuring the mass using a digital analytical balance (Sartorius, SAR TE124S, Germany, 1×10^{-4} g

precision) to determine the time necessary to reach a specific moisture in the material. The data was recorded and stored using a proprietary software (Sartorius, SartoCollect V-1.0, Germany). The drying process was stopped when the mass reaches a constant weight (equilibrium condition).

Obtaining the Powder of *Agave* Leaves

After the drying process was completed the obtained biomaterial was milled using a blade mill (Molinos Pulvex, Mexico, D.F., Mexico) and sieved (50 mesh) to obtain a particle size $\leq$0.3 mm. The powder produced was stored in polyethylene bags for later analysis.

Characterization *Agave* Leaves Powder

Chemical composition. The proximate analysis of the powder of agave leaves were obtained for each of the drying temperatures and were quantified according to the official methods of analysis of the AOAC International (AOAC, 2012); moisture (925.09), ash (923.03), lipids (923.05), protein (979.09), crude fiber (962.09). The carbohydrates were determined by taking the difference of the other compounds. The qualitative identification of starch was conducted using the Lugol's iodine test. Analyses were performed in triplicate.

Physico-chemical analysis. Water activity (a_w) was measured for the agave leaves powder with an Aqualab hygrometer (Aqualab CX-2, Decagon, Pullman, USA) previously calibrated with neutral distilled water ($a_w = 1.00$) and a saturated NaCl solution ($a_w = 0.75$ at 25°C). The hydrogenic potential was measured using a digital potentiometer (Hanna model HI 2211, Mexico) according to the official methods of analysis of AOAC International (AOAC, 2012). Analyses were performed in triplicate.

Aqueous extract (AE) of *agave* leaves powder. The aqueous extract of agave leaves powder was performed with a solution of powder and distilled water at a solid:liquid ratio of 1:10 and then stirrer (Eppendorf, thermomixer comfort, Hamburg, Germany) at 60°C, 37 rad s^{-1} for 30 min. The obtained suspension was then centrifuged (Eppendorf, 5810R, Hamburg, Germany), at RCF of 3,220 $\times$ *g* for 30 min at 4°C and the supernatant liquid was then filtered with a nylon membrane with a 45 $\times$ 10^{-8} m pore size (Millipore, SLHN033NK Millex, México) in preparation for the analysis.

Total and reducing sugars quantification. Total sugar content (TSC) was determined using the method of phenol-sulfuric at 490 nm (Dubois et al., 1956). The sugar content was obtained by comparing the absorbance of sample against a standard curve of fructose (Sigma–Aldrich, purity $\geq$ 99%). The total fructans content was determined using a commercial enzyme Fructozyme (Novozyme, Bagsvaerd, Denmark), employing an enzyme concentration of 2% in acetate buffer solution pH 5.0 with respect to the AE from agave leaves powder, then stirrer (Eppendorf, Thermomixer comfort, Hamburg, Germany) at 50°C, 37 rad s^{-1} for 24 h. Reducing sugars of all agave leaves powder samples were determined using 3,5-dinitrosalicylic acid (DNS) reagent: 0.5 cm^3 of AE was mixed with 0.5 cm^3 of DNS reagent (10 g DNS, 300 g $KNaC_4H_4O_64H_2O$, and 16 g NaOH per liter of distilled water), the mix was shaken an then heated for 10 min at 100°C. The reaction was stopped by immersion in ice for 10 min, and then the reducing sugars were obtained comparing the absorbance of sample at 540 nm against a standard curve of fructose. The absorbances were measured in a spectrophotometer (Thermo Spectronic, Genesys 20, USA). The samples were analyzed in triplicate.

High performance liquid chromatography. Sugars were identified and quantified in the AE of agave leaves powder by high performance liquid chromatography (HPLC). Using a 1220 infinity LC (Agilent Technologies, USA) and a column Aminex HPX-87°C (300 mm $\times$ 7.8 mm; Biorad, Hercules, CA) at 50°C and using a refractive index detector. Elution was performed with 5 $\times$ 10^{-3} mol L^{-1} H_2SO_4 (J.T. Baker, NJ, purity $\geq$95%) at a flow rate of 8 $\times$ 10^{-3} cm^3 s^{-1}, the sample volume injected was 20 mm^3. Glucose, fructose, sacarose and arabinose from Sigma–Aldrich (St. Louis, MO) were employed as standards. The samples were diluted in distilled water (100 g L^{-1}) and then filtered with a nylon membrane with a 45 $\times$ 10^{-8} m pore size (Millipore, SLHN033NK Millex, México) before they were injected. The samples were analyzed in triplicate.

Phenolic and furfural compounds considered as inhibitors in the fermentation alcoholic were identified and quantified in the AE of agave leaves powder by HPLC. Using equipment infinity LC 1220 (Agilent Technologies, USA) and a Zorbax Eclipse Plus-C18 column (250 mm $\times$ 4.6 mm, 0.005 mm column particle size; Agilent Technologies, USA) at 30°C, and a UV/VIS detector. The elution was performed with 17.5 $\times$ 10^{-2} mol L^{-1} acetic acid and 98.6 $\times$ 10^{-2} mol L^{-1} methanol (JT Baker, NJ, 99% purity) at a flow rate of 0.5 cm^3 min^{-1}, the volume of sample injected was 20 mm^3. The samples were diluted in distilled water (100 g L^{-1}) and then filtered through a nylon membrane with a 45 $\times$ 10^{-8} m pore size (Millipore, SLHN033NK Millex, México), the first milliliter through the filter was discarded and the remaining volume was taken into a vial (Agilent Technologies, USA). Furfural, hydroxymethylfurfural, 2-furoic acid, hydroquinone, 4-hydroxybenzaldehyde, pyrocatechol, phenol, 4-hydroxybenzoic acid, vinyl acid, syringic acid, 4-hydroxyacetophenone, vanillin, acetovanillin, acetosyringone, and coniferyl aldehyde were used as standard.

Samples were analyzed in triplicate. Phenolic and furfural compounds were expressed in mg L^{-1} of AE from agave leaves powder.

Composition of Lignocellulosic Materials

Klason lignin. The quantification of lignin was performed according to the modified method of Klason lignin. Which consists of using 72% H_2SO_4 (J.T. Baker, NJ, purity $\geq$ 95%) to hydrolyze and dissolve the polysaccharides contained in the sample and the insoluble fraction, which precipitates, is quantified as lignin. The analysis was performed in triplicate.

Holocellulose. The holocellulose content (cellulose and hemicellulose) was determinated according to the proposed method by Wise et al. (1946). Lignin sample is degraded and solubilized when is treated with $NaClO_2$ (Merck, Darmstadt, Germany) and $C_2H_4O_2$ (J.T. Baker, NJ, 99% purity) and the insoluble fraction represent the holocellulose content. Per each gram of agave leaves powder it was added 19.8 cm^3 of sodium chlorite, followed by 0.2 cm^3 of glacial acetic acid. The mixture was stirred and heated at 70°C on a hot plate and magnetic stirrer (Thermo Scientific, SP131010-33Q Cimarec Digital, USA) for 9 h. After reaction, the mixture was vacuum filtered on a glass fiber filter to separate the liquid from the solids, washed with distilled water at room temperature until the pH of the filtrate was nearly neutral. The solid was dried in an oven at 60°C to constant weight. Then, the sample was transferred to a desiccator for 15 min. The holocellulose content as the remaining residue was determined gravimetrically. The analysis was performed in triplicate.

Infrared Spectroscopy Analysis

For the analysis of the powder of agave the Fourier Transform Infrared Spectroscopy (FTIR) was employed, using an Alpha FTIR from Bruker in the range of 375–7500 cm^{-1} with a resolution of 2 cm^{-1} and a DTGS detector (deuterated triglycine sulfate), the data obtained was analyzed using proprietary software. Cellulose, fructose, and glucose from Sigma–Aldrich (St. Louis, MO) were used as standards.

Statistical Analysis

For the statistical analysis a completely random design was used. The analysis of variance was performed for every response variable using the drying temperature as the factor. The least significant difference (LSD) was used as a multiple range test with a confidence level of 95% ($P < 0.05$) to obtain differences between means.

TABLE 1. MOISTURE, DRYING TIME, PH, AND WATER ACTIVITY (a_W) FOR THE *AGAVE TEQUILANA* LEAVES POWDER

T_{dry} (°C)	Moisture (%)	Drying time (min)	pH	a_w
60	2.97 ± 0.2^a	66.5 ± 1.3^a	4.86 ± 0.13^a	0.38 ± 0.03^a
80	3.07 ± 0.3^a	49.0 ± 1.0^b	4.98 ± 0.07^a	0.33 ± 0.01^a
100	3.17 ± 0.2^a	30.5 ± 1.3^c	4.80 ± 0.13^a	0.34 ± 0.02^a
120	3.12 ± 0.2^a	19.5 ± 1.0^d	4.75 ± 0.12^a	0.36 ± 0.02^a

Mean $\pm$ standard deviation, different letters in columns indicate significant difference (LSD, $\alpha = 0.05$).

RESULTS AND DISCUSSION

Effects of the Temperature of Drying

The leaves were dried and processed to obtain a powder with the double purpose of having a common starting point from which all the studies were carried out, and to store and avoid microbiological contamination of the raw material. Furthermore our results suggest the drying temperature could be considered as a physical pretreatment. To support this hypothesis, the effect of the drying temperature on the chemical composition of the biomaterial was analyzed. During the drying processes the temperature of 60, 80, 100, and 120°C were applied until a 3% of moisture in leaf was achieved (Table 1). The 3% moisture was low enough obtain a material that can easily be converted into a powder using a milling process, a little higher moisture was jagging the blades of the mill. Furthermore at this moisture level the powder is microbiologically stable, according to recommended values of moisture for flour in the Codex Alimentarius ($\leq$15% moisture). Agave leaf presented an 84% $\pm$ 0.3% initial moisture and after the drying treatment ~81% moisture was eliminated. The drying time required to reach the required moisture decreased ($P < 0.05$) as the drying temperature increases. The drying time was reduced ($P < 0.05$) by up to 70.7, 54.1, and 26.3% for 120, 100, and 80°C with respect to process 60°C. This is consistent with was reported in the literature for food, at higher temperatures a shorter drying process is required (Geankoplis, 1998). For pH and a_w no significant difference was observed (Table 1) between different drying temperatures. The pH of dried agave leaf has values from 4.75 to 4.98, these values falls between the limits of 4.5 to 6.0 appropriate for the yeast to growth (Adams and Moss, 2000). The a_w in the agave leaves dried at different temperatures show values from 0.33 to 0.38, these values are below the limit of 0.62 for a_w reported in the literature that will reduce the microbiological and enzymatic activity preventing the contamination of the material, physical and chemicals changes are also minimized under this conditions (Rahman, 1999), therefore the material is stable for storage.

TABLE 2. CHEMICAL COMPOSITION APPROXIMATE OF *AGAVE TEQUILANA* LEAVES POWDER

Chemical components (% dry matter)						
T_{dry} (°C)	Moisture	Carbohydrates	Crude fiber	Ash	Protein	Fat
60	2.97 ± 0.2	54.1 ± 1.0	30.6 ± 1.1	7.18 ± 0.3	3.36 ± 0.1	1.70 ± 0.1
80	3.07 ± 0.3	51.9 ± 1.5	32.5 ± 1.3	7.25 ± 0.4	3.40 ± 0.2	1.73 ± 0.2
100	3.17 ± 0.2	52.0 ± 1.2	32.1 ± 1.6	7.45 ± 0.3	3.45 ± 0.1	1.79 ± 0.3
120	3.12 ± 0.2	51.1 ± 14	32.8 ± 1.2	7.51 ± 0.4	3.48 ± 0.2	1.86 ± 0.1

Mean ± standard deviation, a significant difference was not found when comparing different treatments (60, 80, 100, and 120°C) for each chemical component. (LSD, $\alpha = 0.05$).

Characterization of Agave Leaves Powder

Chemical composition. Carbohydrates, crude fiber, ash, protein, and lipids in the agave leaves powder showed no significant difference ($P < 0.05$) for the four drying temperatures 60, 80, 100, and 120°C, which is reported the in Table 2. Carbohydrates and crude fiber were the main constituents agave leaves powder about 52 and 32%, respectively, values which are consistent with those reported in the literature (Iñiguez Covarrubias et al., 2001; Montañez Soto et al., 2011). It is noteworthy to mention that agave leaf showed the absence of starch in the qualitative test of Lugol's iodine. The above result corroborates that fructans are the principal carbohydrates used as an energy reserve for this kind of plants. Sugars identified in agave leaves powder by HPLC were glucose, fructose, sucrose and arabinose.

Reducing sugars. In the powder of agave leaves the content of non-structural carbohydrates corresponds to direct reducing sugars (DRS) and fructans with a mass fraction of 51.6%. Structural carbohydrates are represented by the hollocellulose correspond to a mass fraction of 17.1%, this results are shown in Table 3. Comparing the content of total nonstructural carbohydrates among the four drying temperatures shows no significant differences ($P < 0.05$). Is observed that as the drying temperature increases to 80, 100, and 120°C there is an increase ($P < 0.05$) of the content of direct reducing sugars (DRS) 23.4, 65.5, and 55.5%, respectively, compared to the process at 60°C. But, when increasing the drying temperature to 120°C did not show an increase ($P < 0.05$) in DRS content with respect to temperature of 100°C. We associate the decrease of the DRS as a consequence of Maillard reaction from an interaction between amino compounds, usually amino acids or proteins, and the liberated reducing sugars since a browning of the color of the powder obtained was observed. The analysis of the fructans in agave leaves powder shows that the higher the drying temperature the lower content of fructans is. This behavior has been reported for hydrolysis of fructan employing temperatures above 80°C (Castellanos Pérez et al., 2012). Therefore, it is noted that for the drying temperature of 120°C the powder of agave leaves has a lower ($P < 0.05$) fructan content compared to treatments at 60 and 80°C. Moreover, the analysis of the structural carbohydrates composition shows no significant differences of the agave leaves powder for the four drying temperatures ($P < 0.05$). Holocellulose content in agave leaves powder was detected to be up to a mass fraction of 17.1%, this is desirable because the cellulose and hemicellulose are polymers of reducing sugars that can be easily hydrolyzed to obtaining reducing sugars. Comparing the results for the four drying temperature applied to agave leaf we conclude that the drying process at 100°C produces the higher benefits, because the release of reducing sugars was increased by 66% and the drying time was reduced by 54% to reach a 3% of the moisture ($P < 0.05$) in the agave leaf with respect to the process at 60°C.

TABLE 3. CONCENTRATION OF NON-STRUCTURAL CARBOHYDRATES (NSC), STRUCTURAL CARBOHYDRATES (SC) AND LIGNIN OF *AGAVE TEQUILANA* LEAVES POWDER

Content (% dry matter)					
	NSC			SC	
T_{dry} (°C)	Total	DRS	Fructans	Hollocelulose	Lignin
60	51.39 ± 0.98^a	9.27 ± 0.18^a	42.12 ± 0.60^a	16.96 ± 0.80^a	15.74 ± 0.40^a
80	51.05 ± 0.92^a	11.44 ± 0.20^b	39.61 ± 0.60^b	17.13 ± 0.60^a	15.97 ± 0.50^a
100	51.57 ± 0.64^a	15.34 ± 0.32^c	36.23 ± 0.90^c	16.57 ± 0.70^a	15.83 ± 0.40^a
120	50.97 ± 0.92^a	14.41 ± 0.27^d	36.56 ± 0.80^c	17.09 ± 0.60^a	15.78 ± 0.40^a

DRS: direct reducing sugars.
Mean ± standard deviation, different letters in columns indicates significant difference. (LSD, $\alpha = 0.05$).

Phenolic and furfural compounds. Phenolic and furfural compounds considered as inhibitors in the alcoholic fermentation were identified and quantified in the aqueous extract of agave leaves powder (Table 4) processed at 100°C. The drying pretreatment in agave leaf did not show the presence of furfural and hydroxymethylfurfural compounds, indicating that there is no degradation of the reducing sugars. Phenolic compounds detected in the aqueous extract of agave leaves powder generated during pretreatment of drying were 4-hydroxybenzoic acid, pyrocatechol, 2-furoic acid, vanillin and acetovanillin at a concentration of 86.8 ± 3.8 mg L^{-1}, 1.5 ± 0.1 mg L^{-1}, 3.5 ± 0.4 mg L^{-1}, 27.1 ± 0.4 mg L^{-1} and 1.9 ± 0.3 mg L^{-1}, respectively. These compounds are low compared to the concentrations of 1 g L^{-1} that have been reported in the literature to cause inhibition in the alcoholic fermentation (Palmqvist and Hahn-Hägerdal, 2000; Taherzadeh and Karimi, 2011; Jönsson et al., 2013; Zha et al., 2014; Mitchell et al., 2014), therefore it is not expected that these pretreatment will have a negative effect for the production of ethanol.

Fourier transform infrared spectroscopy. The FTIR spectra of the powder of agave leaves for the different drying temperatures is reported in Fig. 1, we observe that they have the same qualitative aspect, which indicates that the chemical and structural composition is similar for all temperatures. The peaks at 3385, 2924, 1619, 1425, 1054 cm^{-1} corresponds to the same family and coincides with the chemical bonds expected for the know compounds in the powder. The spectra shows a strong absorption in the 3500–3200 cm^{-1}, typical of the OH bonds, and absorptions bands that correspond to the carbonyl at 1900–1580 cm^{-1}, chemical bridges for monomers —*C*—*O*—*C*— at 1150–1050 cm^{-1}; cyclical alcohol at 1065–1015 cm^{-1} and the *C*—*O*—*C*, related to the presence of oxygen in asymmetric rings at 950–890 cm^{-1}. The result of the FTIR helps to corroborate that the drying process did not induce degradation or changes in the chemical composition of the agave leaves.

TABLE 4. QUANTIFICATION OF THE PHENOLIC AND FURFURAL COMPOUNDS OF AQUEOUS EXTRACT FROM *AGAVE TEQUILANA* LEAVES POWDER DRIED AT 100°C

Compounds	Concentration (mg L^{-1} of extract)
4-Hydroxybenzoic acid	86.8 ± 3.8
Pyrocatechol	1.5 ± 0.1
2-Furoic acid	3.5 ± 0.4
Vanillin	27.1 ± 0.4
Acetovanillin	1.9 ± 0.3
Hydroxymethylfurfural	ND
Furfural	ND
Hydroquinone	ND
4-Hydroxybenzaldehyde	ND
Phenol	ND
Vinyl acid	ND
Syringic acid	ND
4′-Hydroxyacetophenone	ND
Acetosyringone	ND
Coniferyl aldehyde	ND

Mean ± standard deviation.
ND = not detected.

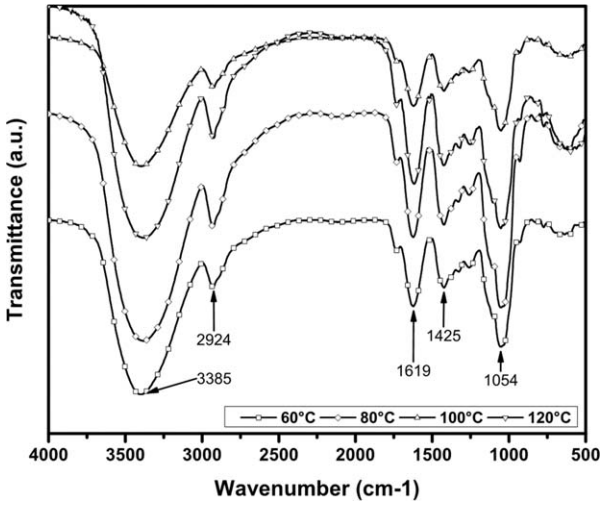

FIG. 1. SPECTRA FTIR: *AGAVE TEQUILANA* LEAVES DRYING TEMPERATURE 60°C, 80°C, 100°C, AND 120°C

CONCLUSIONS

The results obtained in this study show the advantages of using drying as a physical pretreatment for *Agave tequilana* Weber variety Blue leaves because it increases the release of reducing sugars, prevents thermal degradation and not produce significant concentrations of related compounds with an inhibitory effect in the fermentation process. It is found that for the drying temperature of 100°C there is an increase of 66% of the release of reducing sugars and a reduction of 54% of drying time required to reach a 3% of moisture in agave leaf with respect to 60°C. The drying pretreatment did not generate furfural and hydroxymethylfurfural compounds, indicating that there is no degradation of the reducing sugars in the aqueous extract. Phenolic compounds detected in the aqueous extract of agave leaves powder generated during the pretreatment of drying were 4-hydroxybenzoic acid, pyrocatechol, 2-furoic acid, vanillin and acetovanillona at a concentration of 86.8 ± 3.8 mg L^{-1}, 1.5 ± 0.1 mg L^{-1}, 3.5 ± 0.4 mg L^{-1}, 27.1 ± 0.4 mg L^{-1}, and 1.9 ± 0.3 mg L^{-1}, respectively. These compounds are low compared to the concentrations of 1 g L^{-1} that have been reported in the literatures to cause inhibition in alcoholic fermentation.

In addition the drying can be used as a preservation method of agave leaf for biomass storing and transporting, minimizing the deterioration produced by chemical reactions and microbiological contamination of the material. The

results show the benefits of the use of drying as a physical pretreatment of the agave leaves for biofuel production. Finally the agave leaves, due to its chemical composition, have a huge potential not only in the energy field but also in the alimentary, pharmaceutical and materials thermoplastics industry. Fructans and phenolic compounds found in the agave are used as functional ingredients in the food industry and also bioactive compounds that by themselves promotes future research for the *Agave tequilana*.

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