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# Kinetic Study of the Thermal Hydrolysis of *Agave salmiana* for Mezcal Production

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Supporting Information

**ABSTRACT:** The kinetics of the thermal hydrolysis of the fructans of *Agave salmiana* were determined during the cooking step of mezcal production in a pilot autoclave. Thermal hydrolysis was achieved at different temperatures and cooking times, ranging from 96 to 116 °C and from 20 to 80 h. A simple kinetic model of the depolymerization of fructans to monomers and other reducing sugars and of the degradation of reducing sugars to furans [principally 5-(hydroxymethyl)furfural, HMF] was developed. From this model, the rate constants of the reactions were calculated, as well as the pre-exponential factors and activation energies of the Arrhenius equation. The model was found to fit the experimental data well. The tradeoff between a maximum fructan hydrolysis and a critical furan concentration in allowing for the best ethanol yield during fermentation was investigated. The results indicated that the thermal hydrolysis of agave was optimal, from the point of view of ethanol yield in the ensuing fermentation, in the temperature range of 106−116 °C and the cooking range time of 6−14 h. The optimal conditions corresponded to a fructan hydrolysis of 80%, producing syrups with furan and reducing sugar concentrations of 1 ± 0.1 and 110 ± 10 g/L, respectively.

**KEYWORDS:** agave cooking, reducing sugars, 5-(hydroxymethyl)furfural, mezcal

## INTRODUCTION

Mezcal is an alcoholic beverage of Mexico that is obtained from the fermentation and distillation of the syrup of cooked agaves. Mezcal is manufactured according to a procedure that is similar to the production of tequila. The main difference between the production of mezcal and tequila is that the raw material used in tequila is *Agave tequilana* Weber, var. azul, commonly called blue agave.[1] Alternatively, the Mexican Government Norm NOM-006-SCFI-2005[2] suggests that a broad variety of agave species can be used in the production of mezcal; however, the most common are *Agave angustifolia, Agave esperrima, Agave weberi, Agave potatorum,* and *Agave salmiana*. The production of mezcal is regulated by the Designation of Origin, which is a federal law that establishes regions of mezcal production.[3] Under this law, the Mexican states of Oaxaca, Guerrero, San Luis Potosí, Zacatecas, Durango, and Tamaulipas and the town of San Felipe (Guanajuato state) are authorized to manufacture mezcal.[4,5]

The production of mezcal begins with the harvest of agave plants that are more than 8 years old. After removal of the leaves and roots, agave heads, which correspond to the stem and leaf base, are obtained. They are shaped like pinecones and are locally known as "piñas". The heads, which are rich in fructans, are cooked in traditional ovens or autoclaves to hydrolyze the fructans and release fermentable sugars, principally fructose. After the thermal treatment is complete, agave heads are thus milled to obtain a syrup that consists primarily of fructose, glucose, xylose, and maltose.[6] This syrup is fermented with native or selected yeast strains in open-top oak vats. The fermented syrup is distilled in pot stills to obtain mezcal (locally known as young mezcal) with an ethanol concentration of 36−55% (v/v).[7−9]

The thermal treatment of fructans is of interest because it is the first step in the production of mezcal and it requires significant amounts of energy. Fructans are the main reserve polysaccharides in agave plants[6] and are concentrated in the stem and base of the leaves. They are synthesized by crassulacean acid metabolism[6] and correspond to polymers or oligomers composed mainly of fructose units attached to a sucrose molecule.[9] In blue agave and other agave species, complex mixtures of highly branched fructans, where fructose moieties are connected by $\beta(2 \rightarrow 1)$ and $\beta(2 \rightarrow 6)$ bonds, have been reported.[9−12] During the cooking step, fructans are hydrolyzed to produce monomers or oligosaccharides with lower degrees of polymerization. Nattorp et al.[13] found that the depolymerization of polysaccharides by hydrolysis begins with protonation of the glycosidic oxygen, followed by breakage of the glycosidic bond, resulting in the formation of a cyclic carbocation. The carbocation is stabilized by a pair of electrons on a water molecule, and two new molecules are formed during the depolymerization process. Mancilla-Margalli and López[14] monitored the production of volatile compounds during the cooking of blue agave by gas chromatography−mass spectrometry (GC−MS) and observed a variety of different compounds including alcohols, aldehydes, amino acids, furans, organic acids, pyrans, sulfur compounds, terpenes, and furanones. Volatile products of the thermal treatment of agave such as phenylethyl alcohol, vanillin, α-damascenone, and

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linalool are a source of the organoleptic characteristics of tequila.[15] Furans are derived from the thermal degradation of hexoses and pentoses, and the most abundant furan formed during the cooking of agave heads is 5-(hydroxymethyl)furfural (HMF). In addition, furfural, 2-furanmethanol, and 2-furancarboxylic acid are also commonly produced during the thermal treatment of agave.[14]

The results of previous studies suggest that furfural and HMF have an inhibitory effect on yeast growth and ethanol production in fermentative processes.[16] Palmqvist et al.[17] found that the addition of furfural to cultures of *Saccharomyces cerevisiae* caused a decrease in cellular replication without inhibiting cellular activity (replicative inactivation). The absence of glycerol production during furfural reduction suggests that furfural acts as an alternative redox sink and oxidizes excess NADH formed during biosynthesis. Modig et al.[18] found that high concentrations of furfural inhibit essential enzymes such as pyruvate dehydrogenase and aldehyde dehydrogenase. Under anaerobic conditions, yeast metabolizes furfural to furfuryl alcohol; however, under aerobic conditions, furanoic acid is produced, and HMF is metabolized to form 5-(hydroxymethyl)furfuryl alcohol.[16] Sárvári et al.[19] suggested that the ability of yeast to convert furans into less toxic compounds is a crucial criterion for the selection of microorganisms used in ethanol production. Moreover, studies conducted by Palmqvist and Hahn-Hägerdal[16] revealed that a furfural concentration of 1.922 g/L reduced the specific growth rate of *Saccharomyces cerevisiae* to 36% and reduced alcohol production by 66%. Although a furfural concentration of 5.093 g/L completely inhibited growth, similar concentrations of furfuryl alcohol did not significantly affect fermentation. Alternatively, in a study conducted by Choteborska et al.,[20] a furfural concentration of 6.27 g/L and an HMF concentration of 0.2 g/L did not result in significant inhibition.

When agave heads are cooked in traditional kilns heated by firewood, the process takes 2−3 days. Alternatively, in ovens heated by steam at a temperature near 100 °C, the process is complete within 1−2 days; however, in an autoclave at temperatures up to 120 °C, the cooking process is complete within 8−12 h.[7,21] Although the thermal treatment of agave is a key step in the production of mezcal, the process has not been thoroughly investigated, and relationships between the operating conditions, the degree of hydrolysis, the generation of furans, and the effect of furans on fermentation have not yet been examined. Moreover, the Official Mexican Standards on alcoholic beverages require that the concentration of furfural must be less than 4 mg per 100 mL of anhydrous ethanol.[1,22] To promote international marketing of mezcal, these restrictions must be strictly enforced.

The objectives of this study were (i) to determine kinetic parameters of the thermal hydrolysis of fructans from *A. salmiana* in a laboratory autoclave by adapting a simple kinetic model, (ii) to evaluate the production of reducing sugars and furan compounds during thermal hydrolysis, and (iii) to determine the best range of cooking temperature and cooking time for a tradeoff between the maximum fructan hydrolysis and the critical furans concentration allowing for the best ethanol yield.

**Kinetic Model.** The cooking of agave heads is a heterogeneous reaction in a vegetal matrix with coupled heat and mass transport. Rigorous modeling requires a complete understanding and characterization of the depolymerization and degradation reactions, as well as the formulation of heat- and mass-transfer equations. The thermal properties of agave heads are unknown, but other similar processes have been studied by employing simplified models that describe the experimental data. In these simplified models, the vegetal matrix is considered to be a homogeneous system without transport restrictions, and hydrolysis and degradation reactions are considered lumped reactions. By applying these assumptions, the full reaction mechanism can be omitted from the rate equation. The simplified model used in this study was proposed by Saeman[23] and is based on the hydrolysis and saccharification of wood

$$\text{cellulose} \xrightarrow{k_h} \text{glucose} \xrightarrow{k_d} \text{degradation compounds} \quad (1)$$

Equation 1 depicts the hydrolysis of cellulose into free glucose and the partial transformation of glucose into degradation products. However, this model is applicable to other systems and was recently applied to the hydrolysis of lignocellulosic wastes.[23−26] For this study, the model was rewritten as

$$\text{fructans} \xrightarrow{k_h} \begin{bmatrix} \text{fructose} \\ \text{glucose} \\ \text{others} \end{bmatrix} \text{reducing sugars} \xrightarrow{k_d} \begin{bmatrix} \text{furfural} \\ \text{5-(hydroxymethyl)furfural} \\ \text{others} \end{bmatrix} \text{furans} \quad (2)$$

where the fructans (P) are hydrolyzed into monomers (reducing sugars, M), and the reducing sugars can undergo partial degradation to form furans (D), with $k_h$ and $k_d$ as the rate constants of hydrolysis and degradation, respectively. In the model, hydrolysis and degradation are assumed to be irreversible processes. The model assumes that fructans of different molecular weights can be described as a lumped variable. Here, this concentration is estimated as the maximum amount of monomers that can be achieved if full depolymerization occurs minus the amount of these monomers (M) present at any given time. M is given mainly by fructose and glucose, reducing monomeric sugars, although, in their monitoring, as described below, some short-chain polymers with reducing ends are also included. According to Waleckx et al.,[9] in the syrup collected after the thermal hydrolysis of fructans from blue agave, glucose and fructose account for 80−90% of the measured reducing sugars. Furthermore, the synthesis of glucose from other cellulosic sources can be considered negligible, as insoluble structural polymers hydrolysis occurs at temperatures above 150 °C.[13] The mathematical models of the reaction velocities can be written as

$$\frac{d[P]}{dt} = -k_h[P] \quad (3)$$

$$\frac{d[M]}{dt} = k_h[P] - k_d[M] \quad (4)$$

$$\frac{d[D]}{dt} = k_d[M] \quad (5)$$

with the initial conditions ($t = 0$)

$$[P] = P_0, [M] = M_0, [D] = 0 \quad (6)$$

$$P_0 = M_F \frac{180n - 18(n-1)}{180n} \quad (7)$$

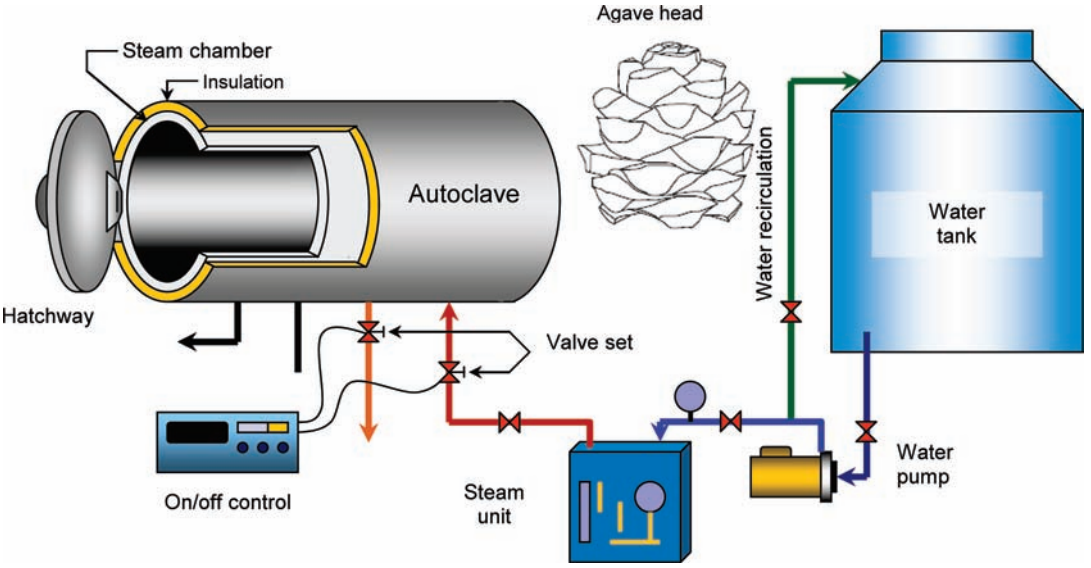


**Figure 1.** Experimental system used to study the kinetics of agave cooking. A depiction of an agave head is also shown.

where $M_0$ and $M_F$ are the initial and final concentrations, respectively, of reducing sugars; $P_0$ is the initial concentration of fructans; and $n$ is the degree of polymerization.

## MATERIALS AND METHODS

**Microorganisms.** In this study, agave syrups were fermented by *Kluyveromyces marxianus*, *Clavispora lusitaniae*, and *Kluyveromyces marxianus* var. *drosophilarum*. This consortium of microbes was developed at the Bioengineering Laboratory, Instituto Tecnologico de Celaya, Celaya, Guanajuato, México.[27] The strains were dispersed in a solution of 1:4 (v/v) glycerol/NaCl (85%) and were preserved in Corning tubes at −80 °C in an ultrafreezer (Revco, model ULT-1386).

**Inoculum.** The microorganism strains were propagated separately in Petri dishes containing potato dextrose agar (PDA, Becton Dickinson & Company, Sparks, MD) and were incubated for 48 h at 28 °C. They were then transferred to Erlenmeyer flasks containing 100 mL of potato dextrose broth (PDB, Becton Dickinson & Company, Sparks, MD) and were incubated in an orbital shaker (Forma Scientific Co. Marietta, OH) at 100 rpm for 24 h at 28 °C to obtain preinoculums. The inoculums used for agave syrup fermentation were formulated with 3.3 mL of these preinoculums.

**Cooking Kinetics.** Eight-year-old heads of *Agave salmiana* were collected from the northern region of Guanajuato, México, and were used as raw materials in the experiments. Each agave head was divided into cubical pieces that were 10 cm per side, and two pieces were used in each experiment. The cooking assays were performed in the semiautomatic system shown in Figure 1, which included an electrical steam generator (Sussman Electric Boiler) and a cylindrical horizontal autoclave made of stainless steel. The autoclave had an internal diameter of 0.40 m and a length of 0.80 m and was provided with a steam recirculation concentric jacket insulated with mineral wool. The apparatus included a hermetic hatchway for rapid opening. The temperature of the autoclave was maintained by feeding steam into the jacket, and a solenoid valve governed by a controller-type on−off switch was used to regulate the amount of steam (B&C Electronics TR 7615).

In the cooking studies, pieces of agave heads were placed inside the autoclave, and steam was injected toward the interior of the chamber until the desired temperature was attained. However, throughout the cooking process, steam was supplied to the jacket to maintain the established temperature. For sampling, the steam was evacuated from the autoclave, the hatchway was opened, and the samples were quickly removed. After the samples had been collected, the hatchway was closed, and the operation was continued at the fixed temperature. To obtain the agave syrup, each piece was mechanically milled, and the quantity of reducing sugars (denoted Red) was determined by the dinitrosalicylic acid (DNS) method.[28] The concentration of furan compounds (denoted Fur) was determined by a technique developed by Martínez et al.[29] The cooking experiments were performed at 96, 100, 106, 112, and 116 °C for 80, 62, 32, and 20 h; samples were taken at different periods of time, depending on the total run time. During the experiment at 116 °C, samples of syrups were analyzed by high-performance liquid chromatography (HPLC) (LC 1150, GBC, Scientific Equipment Ltd., Hampshire, IL) to separate and subsequently quantify fructose and glucose. HPLC analysis was conducted on a 250 × 4.6 mm (at 30 °C) SGE Exsil amino column with isocratic elution [AcN/$H_2O$ 75:25 (v/v); 1.2 mL/min]. The standard solutions were prepared with D-(−)-fructose and D-(+)-glucose (Merck, Darmstadt, Germany).

**Determination of Rate Constants.** The model described by eqs 3−7 was solved analytically, yielding the following expressions

$$[\mathrm{P}] = P_0(\mathrm{e}^{-k_h t}) \tag{8}$$

$$[\mathrm{M}] = \frac{P_0 k_h}{k_d - k_h}(\mathrm{e}^{-k_h t} - \mathrm{e}^{-k_d t}) + M_0 \mathrm{e}^{-k_d t} \tag{9}$$

$$[\mathrm{D}] = P_0\left(1 - \frac{k_d \mathrm{e}^{-k_h t} - k_h \mathrm{e}^{-k_d t}}{k_d - k_h}\right) + M_0(1 - \mathrm{e}^{-k_d t}) \tag{10}$$

For each of the experiments, the rate constants of the reaction ($k_h$ and $k_d$) were obtained by applying a nonlinear regression analysis using the Optimization Toolbox of MatLab v6.1 (The MathWorks Inc.). An analysis of variance was conducted to evaluate the fit of the model to the experimental data, according to the procedure described by Constantinides.[30] For each experiment, the pre-exponential factor and activation energy of the Arrhenius equations for hydrolysis and degradation were calculated from the rate constants of the reaction.

**Fermentation.** In the fermentation studies, eight syrups with different furan concentrations were prepared from distilled water and fresh syrup with an initial concentration of 190 g/L reducing sugars and 1.1 g/L furans. To increase the concentration of furans, the syrups were progressively cooked at 106 °C. These syrups maintained an initial

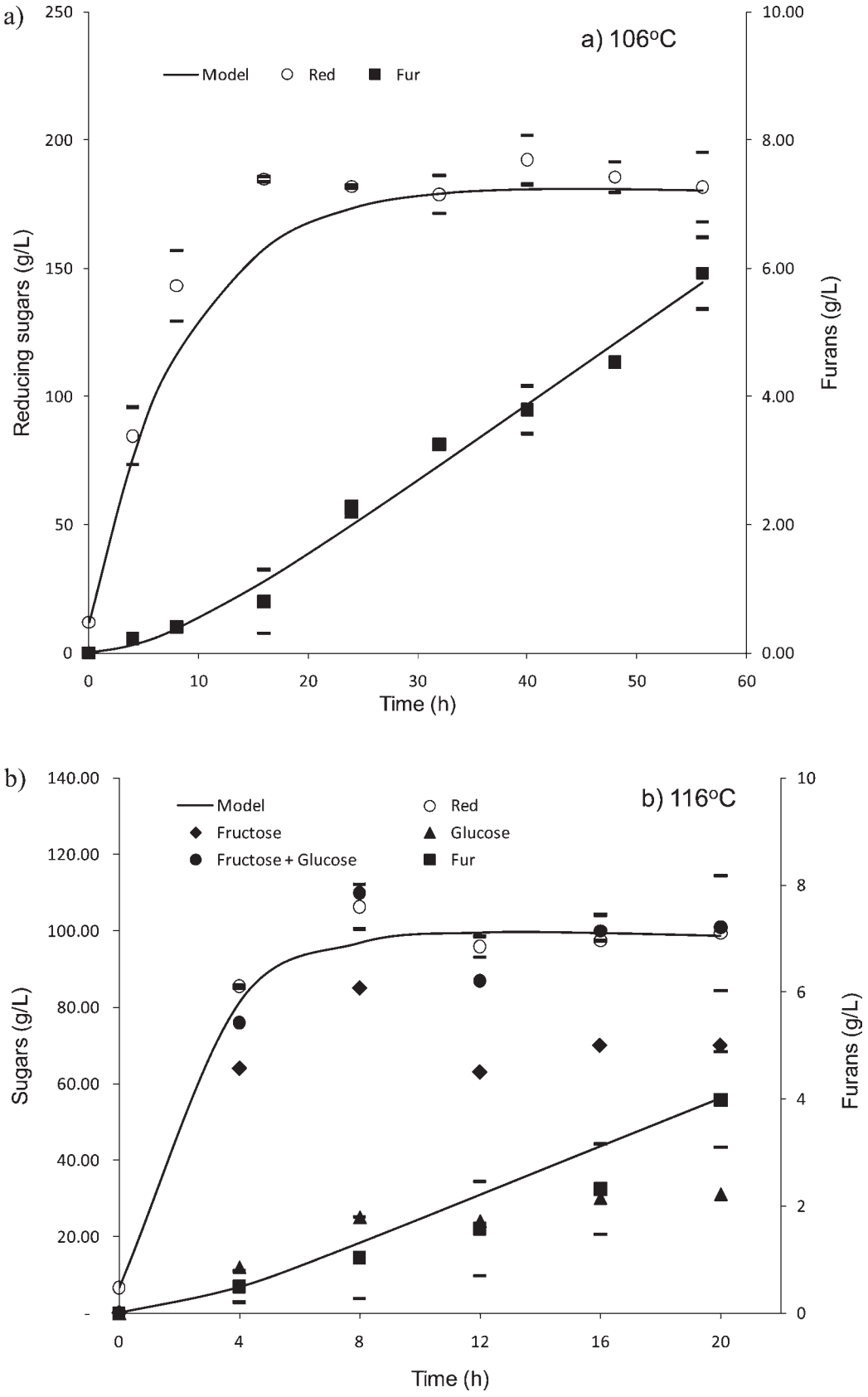


**Figure 2.** Kinetics of the thermal hydrolysis of *Agave salmiana* in an autoclave at (a) 106 and (b) 116 °C. Symbols indicate the experimental data, and lines represent the model solutions. Plot b includes data on fructose and glucose obtained by liquid chromatography.

Table 1. Rate Constants of Hydrolysis and Degradation of Fructans of *A. salmiana*[a]

| | | | $R^2$ | | $F$ | | |
|---|---|---|---|---|---|---|---|
| temperature (°C) | $k_h$ ($10^{-3}$ min$^{-1}$) | $k_d$ ($10^{-5}$ min$^{-1}$) | reducing sugars | furans | reducing sugars | furans | $F_{0.95,v1,v2}$ |
| 96 | 0.579 ± 0.00 | 0.381 ± 0.02 | 0.986 | 0.988 | 4.668 | 3.323 | 3.07 |
| 100 | 0.726 ± 0.00 | 1.000 ± 0.00 | 0.998 | 0.967 | 3.324 | 2.260 | 2.90 |
| 106 | 1.917 ± 0.36 | 1.096 ± 0.22 | 0.959 | 0.982 | 1.446 | 0.305 | 3.29 |
| 112 | 2.647 ± 0.00 | 1.949 ± 0.00 | 0.986 | 0.971 | 1.714 | 0.933 | 3.29 |
| 116 | 6.325 ± 3.10 | 3.796 ± 2.51 | 0.965 | 0.966 | 0.252 | 0.236 | 4.53 |

[a] Statistical parameters of correlation between experimental data and model: $R^2$, correlation coefficient; $F$, relationship between variance for lack of fit and experimental variance; $F_{0.95,v1,v2}$, parameter distribution $F$ with a confidence interval of 95% and degrees of freedom.

Table 2. Kinetic Parameters[a] of Hydrolysis and Degradation for Different Plant Materials

| temperature (hydrolysis type), and substrate | $k_h$ (min$^{-1}$) | $k_d$ (min$^{-1}$) | $E_h$ (kJ/mol) | $E_d$ (kJ/mol) |
|---|---|---|---|---|
| 96−116 °C (thermal), agave heads. | $(5.79 \times 10^{-4})-(6.32 \times 10^{-3})$ | $(3.63 \times 10^{-6})-(3.795 \times 10^{-5})$ | 139.08, $\ln(k_{h0}) = 37.77$ | 118.43, $\ln(k_{d0}) = 26.31$ |
| 100 °C (HCl), sorghum straw[33] | $(8.52 \times 10^{-3})-(5.64 \times 10^{-2})$ | $(4.30 \times 10^{-4})-(7.60 \times 10^{-4})$ | | |
| 100−128 °C ($HNO_3$), sugar cane[25] | $(6.00 \times 10^{-3})-(5.30 \times 10^{-2})$ | $(6.00 \times 10^{-4})-(3.90 \times 10^{-4})$ | 104, $\ln(k_{h0}) = 30.4$ | |
| 100−125 °C ($H_2SO_4$) sugar cane[26] | $(1.88 \times 10^{-2})-(8.22 \times 10^{-3})$ | $0.00-(8.90 \times 10^{-3})$ | 109, $\ln(k_{h0}) = 31.6$ | |
| 160−220 °C (thermal), mannan[13] | $(1.80 \times 10^{-4})-(8.00 \times 10^{-2})$ | $(2.50 \times 10^{-3})-(2.96 \times 10^{-1})$ | 113, $\ln(k_{h0}) = 24.8$ | 140, $\ln(k_{d0}) = 33.2$ |

[a] $k$, reaction rate constants; $E$ and $k_0$, activation energies and pre-exponential factors, respectively, of the Arrhenius equation; h and d, subscripts denoting hydrolysis and degradation, respectively.

concentration of approximately 120 g/L of reducing sugars (Red), and the concentration of furans (Fur) ranged from 0.655 to 4.762 g/L, with increments of approximately 0.5 g/L. A 150 mL sample of each syrup was fermented in an Erlenmeyer flask with addition of 10 mL of the inoculums previously mentioned. The cultures were incubated for 96 h in an orbital shaker (Forma Scientific Co., Marietta, OH) at 28 °C and 50 rpm. For each of the experiments with different concentrations of furans, we used nine identical cultures in flasks. Every 12 h, a flask was used as a sample, not to affect the reaction volume and to avoid possible contamination during sampling. The samples were filtered and centrifuged to eliminate biomass and solids from the syrups, and the concentrations of reducing sugars and furans in the supernatant were determined. The remaining part of each sample was distilled, and the concentration of ethanol in the distillate was determined by a spectrophotometric technique based on the oxidation of ethanol with potassium dichromate in acidic medium, as described by Caputi et al.[31] All fermentation samples were analyzed in duplicate.

**Response Surface Construction.** Using the solution of the proposed mathematical model, fitted with the experimental data, numerical simulations were performed. The cooking temperature was studied in the range from 95 to 125 °C, and the cooking time was varied from 4 to 20 h. The resulting response surface allows one to study the tradeoff between increasing the degree of hydrolysis of fructans versus the formation of furans that might inhibit the ensuing alcoholic fermentation.

Thus, the diagram facilitates the location of the optimal cooking conditions for a given maximum allowed concentration of furans.

## RESULTS AND DISCUSSION

**Cooking Kinetics.** Figure 2 shows an example of the experimental results for the cooking kinetics at 106 and 116 °C, as well as the predicted concentrations of reducing sugars and furans. The results indicate that the model fits the experimental results and that furans were generated steadily during cooking. When the highest concentration of reducing sugars was reached, the production of furans kept increasing.[32] This indicated that, once the hydrolysis of fructans was complete, the degradation reactions predominated during cooking. Comparable results were also observed at 96, 100, and 112 °C. The graphics for these runs can be found as Supporting Information for this article.

Figure 2b also shows the glucose and fructose concentrations obtained by HPLC for comparison with the results obtained by the DNS technique. In fitting the proposed simplified with the data from DNS, one assumes that a lumped variable, namely, reducing sugars, properly describes the depolymerization of fructans. From this figure, one can see that glucose and fructose, measured by HPLC, account for 88−94% of the reducing sugars as measured by DNS. Furthermore, the average apparent degree of polymerization was measured as 4, following the procedure suggested by Waleckx et al.[9] This value is lower than those reported for blue agave,[9,10] where fructose is present in a higher proportion than glucose.

Table 1 shows the estimated rate constants of fructan hydrolysis and monomer degradation. The results indicate that the rate constants of hydrolysis were 2 orders of magnitude greater than the rate constants of degradation. Moreover, both rate constants increased with an increase in temperature. In all cases, the coefficients of regressions ($R^2$) were greater than 0.9. The $F$ parameter, which is the ratio between the variance for lack fit of the model and the experimental variance, was close to or lower than $F_{0.95,v1,v2}$; thus, at a confidence level of 95%, the model adequately represented the experimental data. However, the model provided the poorest fit to the experimental data at 96 °C, which can be attributed to an increased resistance to heat transfer.

Table 2 reports the activation energies and natural logarithm of the pre-exponential factors of the Arrhenius equation, which were obtained from the rate constants of fructan hydrolysis and monomer degradation at different temperatures. The rate constants obtained in this study were lower than those for the hydrolysis of other vegetable matrixes in the presence of inorganic acids at high temperatures. However, the energies of activation and the natural logarithms of the pre-exponential

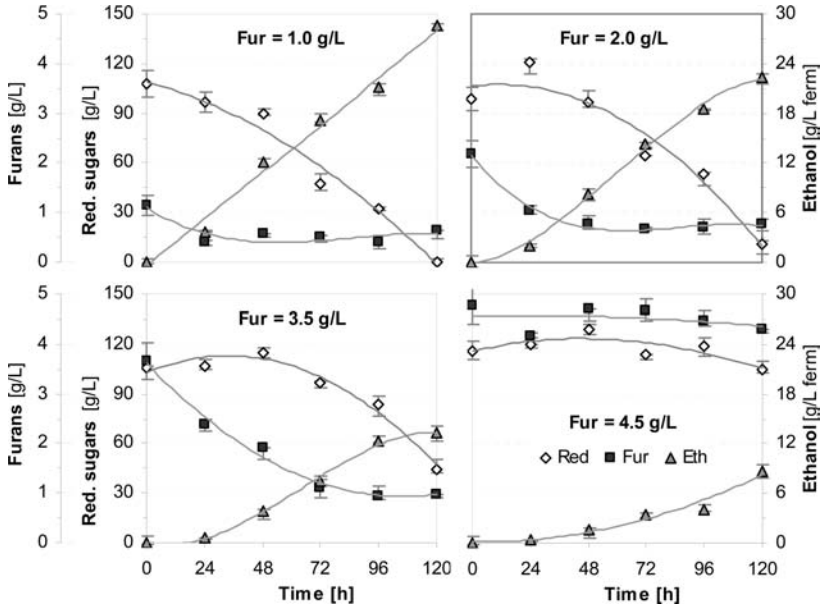


**Figure 3.** Fermentation kinetics of the syrup of cooked agave containing different concentrations of furans: (a) 1.0, (b) 2.0, (c) 3.5, and (d) 4.5 g/L. Symbols indicate the experimental data, and lines represent general trends.

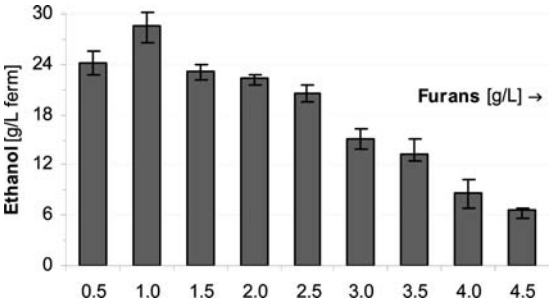


**Figure 4.** Effect of furan concentration on the production of ethanol in the fermentation of the syrup of cooked agave for 120 h.

factors of the Arrhenius equation were similar to those reported in the literature.

**Fermentation Studies.** Figure 3 shows the kinetics of the fermentations of different agave syrups and the corresponding ethanol productions at initial concentrations of 120 g/L for reducing sugars and 1.0, 2.0, 3.5, and 4.5 g/L for furans. At initial furan concentrations of 1.0, 2.0, and 3.5 g/L, a significant decrease in the concentration of furans was observed over time, in parallel with the consumption of reducing sugars and the production of ethanol. Yields of ethanol production decreased with increasing initial concentration of furans. This result is in agreement with those reported by Sárvári et al.,[19] who found that yeast are able to transform furfural into less toxic compounds that have a lower inhibitory effect on alcoholic fermentation. Nevertheless, at an initial furan concentration of 4.5 g/L, the microorganisms could not significantly metabolize the furfural, and the consumption of reducing sugars and the rate of ethanol production decreased dramatically. Apparently, high concentrations of furans can inhibit the activity of essential enzymes including alcohol dehydrogenase, aldehyde dehydrogenase, and pyruvate dehydrogenase.[18]

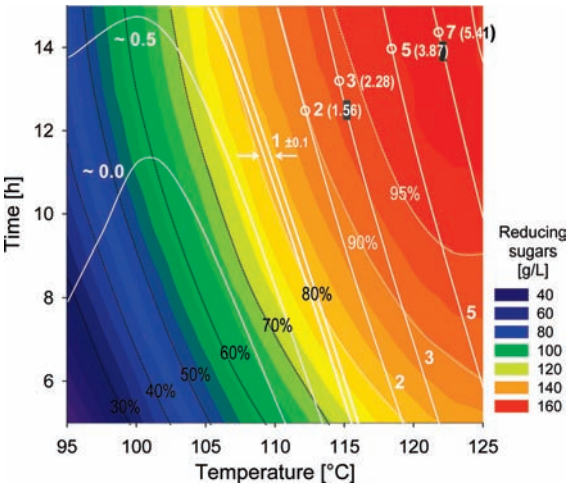


**Figure 5.** Response surface diagram of the thermal treatment of agave, including the generation of reducing sugars and furans and the percent hydrolysis.

Figure 4 shows a summary of the effect of the concentration of furans on ethanol production during fermentation. The results indicate that ethanol production reached a maximum when the concentration of furans was approximately 1 g/L and then gradually decreased as the concentration of furans increased. However, at a furan concentration of 0.5 g/L, only 80% of the maximum amount of ethanol was obtained. Sárvári et al.[19] reported that the metabolism of *Saccharomyces cerevisiae* is modified in the presence of furfural, dramatically reducing the production of ethanol and glycerol; however, this phenomenon was observed only at specific furfural concentrations. The lowest furan concentrations can cause a normal metabolic flux of yeasts, where the formation of biomass prevails over ethanol production. Alternatively, at higher concentrations of furans, the yeasts use

the furans as a substrate and convert them into furfuryl alcohol or other less inhibitory compounds, allowing larger amounts of ethanol to be produced (higher ethanol yields). Moreover, high concentrations of furfural modified cellular metabolism by inhibiting enzymes that participate in glycolysis and reducing the final ethanol yield.

**Response Surface Diagram.** Figure 5 shows that the complete hydrolysis of fructans also leads to the generation of furans, which have a negative effect on ethanol production as described above. The results indicate that the thermal hydrolysis of fructans of *A. salmiana* was optimal in the range of temperature from 106 to 116 °C and the range of cooking times from 6 to 14 h. The furan concentration that is most suitable for ethanol production (1 g/L, as showed in Figure 4) corresponds to approximately 80% hydrolysis and is indicated by a double contour line. In the production of mezcal, after milling the cooked agave, the bagasse was washed with water to remove retained sugars, and the resulting solution was mixed with concentrated syrup to obtain a fermentation broth with a concentration of reducing sugars of 90−120 g/L. The dilution process reduces the initial furan concentration, avoiding significant reduction in ethanol yields. The concentration of furans in diluted syrup with a reducing sugar concentration of 120 g/L is shown in parentheses in Figure 5.

## ■ ASSOCIATED CONTENT

**Supporting Information.** Kinetics of the thermal hydrolysis of *Agave salmiana* in an autoclave at 96, 100, and 112 °C. This material is available free of charge via the Internet at http://pubs.acs.org.

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