
Ingeniería de alimentos

Revista Mexicana de Ingeniería Química

Vol. 14, No. 3 (2015) 615-622


EFFECT OF TEMPERATURE AND PH ENVIRONMENT ON THE HYDROLYSIS OF MAGUEY FRUCTANS TO OBTAIN FRUCTOSE SYRUP

EFECTO DE LA TEMPERATURA Y EL PH DEL MEDIO EN LA HIDRÓLISIS DE FRUCTANOS DE MAGUEY PARA LA OBTENCIÓN DE JARABES FRUCTOSADOS

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Received November 23, 2014; Accepted November 2, 2015

Abstract

Fructan contents were analyzed in mezcal maguey juice (*Agave salmiana*), which were characterized and subjected to termal hydrolysis process. A set of experiments were conducted to compare the effect of the hydrolysis for 10 h at three temperatures (90, 100 and 110 °C) and three juice pH values (2.5, 4.6, 5.5). The hydrolysis process was monitored by measuring the release of sugars and the residual substrate by High Performance Liquid Chromatography (HPLC).The results obtained with a mathematical model that describes the kinetics of the degradation or hydrolysis of fructan and fructose liberation proposed. The temperature and pH of the sample exert significant effect on the kinetics. The main product of the hydrolysis of fructan is fructose and hydrolysis degree is between 27.28 and 92.10%.

Keywords: fructan, agave, fructose, termal hydrolysis, kinetics.

Resumen

Se analizaron fructanos contenidos en jugo de maguey mezcalero potosino (*Agave salmiana*), los cuales fueron caracterizados y sometidos a un proceso de hidrólisis térmica. Un conjunto de experimentos se llevaron a cabo para comparar el efecto de la hidrólisis durante 10 h a tres temperaturas (90, 100 y 110 °C) y a tres valores de pH del jugo (2.5, 4.6, 5.5). El proceso de hidrólisis fue monitoreado midiendo la liberación de azúcares y el sustrato residual por HPLC. Con los resultados obtenidos se propuso un modelo matemático que describe la cinética de la degradación o hidrólisis de fructano y la liberación de fructosa. La temperatura y el pH de la muestra ejercen efecto significativo en las cinéticas. El principal producto de la hidrólisis del fructano es la fructosa y el grado de hidrólisis varío entre 27.28 y 92.10%.

Palabras clave: fructano, agave, fructosa, hidrólisis térmica, cinética.

1 Introduction

The mezcal maguey (*Agave salmiana*) is a plant with multiple applications, from pre-Hispanic times has been used as food, building materials, fibers for clothing, but mainly as a source of fermentable sugars for the production of alcoholic beverages. The A. salmiana has a wide distribution in the Potosi-Zacatecas plateau and is probably the most Agave species of economic importance in the region, as it is mainly used as raw material in the production of mezcal and as fodder for cattle (Aguirre *et al*., 2001).

During photosynthesis process, plants use the energy of sunlight to carry out a series of chemical reactions that can transform carbon dioxide (CO_2) and water (H_2O) in the atmosphere, into simple sugars (Smith and Smith, 2002). These sugars such as fructose and glucose are used by the plant as a source of immediate energy for metabolic functions; from the polysaccharide sugars untapped different functions are synthesized, the most common polysaccharides in plants are cellulose, with a structural function constitutes the bulk of the plant cell wall; starch, used as backup power (Devlin, 1980) and fructans present

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in the maguey where they are used as osmoprotectants during the dry season and as a source of energy to hydrolyze and get fructose and glucose (Badui-Dergal, 1999; González-Díaz *et al*., 2006). Fructans begin to be synthesized in the leaves of agave by enzymes sucrose:sucrose 1-fructosyltransferase (1-SST) and fructan:fructan 1-fructosyltransferase (1-FFT) (Huazano, 2009), and a gradient of increasing concentration is observed as approaches the stem (commonly called "pineapple") where it is stored (Michel-Cuello *et al*., 2008).

Molecular structures of fructans vary depending on the species from which they come, even his structural elucidation has been proposed as a taxonomic marker (Bonnet *et al*., 1997). In species of the genus *Agave* have been reported more than one type fructan; Sanchez-Marroquin and Hope (1953) and Bhatia and Nandra (1979) reported the presence of a fructan inulin linked β (2-1) as the main reserve carbohydrate in Agave tequilana and *Agave americana* respectively. Aspinall and Das Gupta (1959), Satyanarayana (1976) and Dorland *et al*. (1977) reported that the *Agave vera cruz* has a mixture of fructans with widely branched structure, a molecule of glucose and not only internal link β (2-1), but also link β (2-6). Wang and Nobel (1998) indicate that in the *Agave deserti* observed the presence of both fructan with Degree of Polymerization (DP by its acronym) of 5 as fructans with a DP of 3 with one molecule of intermediate glucose known as neokestose. These values are similar to those reported by Romero-Lopez *et al*. (2015) about fructans in *Agave atrovirens* with a degree of polymerization between 5 to 10 DP. In 2003, Lopez *et al*., proposed the molecular structure of fructans present in *Agave tequilana* Weber Var. Blue, this molecule has three types of fructans: inulin, levan and Neoinulin, showing a DP of between 3 to 29 and β bonds (2-1) and β (2-6).

The content of fructans in plants of the *Agave* genus is considerable (*A. salmiana*: 69%, *A. tequilana*: 73% [dry basis]) (López *et al*., 2003; Mancilla-Margalli *et al*., 2006); for this reason, since pre-Hispanic times, maguey carbohydrates have been used as a substrate in the production of alcoholic beverages like mezcal, sotol, bacanora and tequila (Mancilla-Margalli *et al*., 2006; Waleckx *et al*., 2008; Ávila-Fernández *et al*., 2009; García-Soto *et al*., 2011). Currently the fructans is also being used as raw material for obtaining fructose syrups, product has gradually replaced the refined sugar and is being implemented as a low-calorie sweetener in foods and beverages (Forshee *et al*., 2007), in addition to use this sweetener in food transmits functional properties such as taste, color, solubility and inhibition of crystallization (Borges da Silva *et al*., 2006; Tomotan and Vitolo, 2007).

However, the relative sweetness of the fructans is negligible, therefore it is necessary to carry out a hydrolysis in order to break up these carbohydrates and release simple sugars, in its most fructose (Ortiz, 2006). It is important to note that fructans have the ability to withstand human gastrointestinal digestion (Alvarado *et al*., 2014).

During hydrolysis, from fructans emerge monomers or oligosaccharides with smaller DP; this release begins with the addition of a proton (H^+) (protonation) to the glycosidic oxygen, thereby breaking the glycosidic bond and allows the formation of a carbocation (C^+) cyclic; subsequently stabilized carbocation intervening an electron pair donated by a water molecule, which enables the formation of two molecules (monomers or oligosaccharides) (García-Soto *et al*., 2011; Forshee *et al*., 2007; Borges da Silva *et al*., 2006; Tomotan and Vitolo, 2007; Ortiz, 2006; Nattorp *et al*., 1999).

To hydrolyze fructans, many companies are using the traditional method of making tequila or mezcal, whole or cut pineapples are cooked in brick ovens for 36 to 48 hours or in autoclaves for 12 hours (González-Hernández *et al*., 2012), reaching both process temperatures above 100 °C. The efficiency of hydrolysis is very low, since the heat transfer is not uniform, so that some of the carbonized pine cones, while other incomplete hydrolysis; moreover, it has been found that under these conditions the cooking unfavorable phenomena as the Maillard reactions and the formation of compounds such as phenols, furfural and hydroxymethyl furfural are favored from the thermal degradation of pentoses and hexoses (Mancilla-Margalli *et al*., 2002; Waleckxy *et al*., 2008). In the same way, the enzymatic hydrolysis reduces the use of chemicals for the mezcal industry and its consequent environmental and health impacts (García-Rivero *et al*., 2015).

Increase the efficiency of heat transfer and control of the acidity of the medium in which is conducted the thermal hydrolysis is an alternative to improve the process and get better returns; therefore the aim of this study was to investigate the effect of temperature and pH on the hydrolysis kinetics of fructans in *Agave salmiana* juice to determine the best conditions for obtaining fructose syrups.

2 Materials and methods

2.1 Maguey juice extraction

The maguey cones or heads were collected in the Ejido of Zaragoza de Solís in the town of Villa de Guadalupe, San Luis Potosí, México. We worked with mature plants between seven and nine years old, and with a period of approximately one year neutered. Pineapples were cut into small pieces and treated with steam to remove as much as possible saponins and passed through a horizontal mill consists of three rollers. The extracted juice is heated 80 °C for 10 min to inactivate the saponins still present. Immediately passed through a stainless steel filter press with 2 inputs and 2 outputs, using as a filter medium of bleached pulp, two filtrations were performed with pore size of 22 and 4 microns. 1% of diatomaceous for removing fibers and solid particles are applied.

2.2 Carbohydrates analysis by HPLC

Determination of carbohydrates (glucose, fructose, sucrose and fructans) in the juice and during the hydrolysis process was carried out according to the HPLC method proposed by Zuleta and Sambucetti (2001), for that purpose Waters 600 chromatographic equipment is utilized (Milford MA. USA) composed degacificer, quaternary pump, compartment for thermal control of the column and refractive index detector (Waters 410). As stationary phase ion exchange column Aminex HPX- 87C (7.8 mm ID x 300 mm, Bio -Rad Hercules, Ca.) was used. The mobile phase is HPLC grade water flow handled with 0.6 ml/min. The volume of each injection was 50 μL (injector completely full). The temperature of the column compartment was maintained at 75 °C, and in the laboratory at 20 °C. The samples were filtered with nylon membrane filters (0.45 μm) coupled 5 mL polypropylene syringes, both from Waters (Milford, CT.) and analyzed immediately. Quick Start Empower 5.0 program was used for system control and data analysis. Reference sugars (glucose, sucrose, fructose, galactose, lactose and maltose) were from Sigma-Aldrich (CAR-11 carbohydrates kit). As standard fructan, inulin from chicory (Sigma-Aldrich, I2255, Germany) was used. Chromatographic separation time was 18 min. The carbohydrates present in the samples were identified by comparison with retention times of carbohydrate standards. All carbohydrates could be separated in a single run.

Quantification of carbohydrates was developed based on calibration curves for sugars the range was 0.1 to 3.2 % (r=0.9996) and 0.2 to 8.0 % fructan (r = 0.9999). The correlation coefficients (r) were calculated based on the refractive index obtained for samples and standards for each carbohydrate to estimate the consistency of the amplitude detector in terms of concentration. All determinations were carried out in duplicate, a mixture of standards were injected daily to identify variations in calibration.

2.3 Thermal hydrolysis

Thermal hydrolysis experiments in Agave juice are carried out in polypropylene tubes with stopper 125 mL and were placed in Pyrex beakers 3L. The vessels containing the vials were introduced into a vertical cylindrical retort (Mexican Engineers), the autoclave has a hermetic quick opening hatch. The temperature is maintained by feeding steam to the interior, and a solenoid valve controlled by a control switch on-off type is used to regulate the amount of steam (Honeywell, Morristown, NJ.).

Preliminary tests allowed to establish a full factorial design considering 3 different temperatures (90, 100 and 110 °C) and three pH values (2.5, 4.6 and 5.5). Two replicates were performed for each experimental condition and 27 runs were performed. The hydrolysis process was monitored to quantify sugars like fructose, glucose and residual fructans by HPLC method mentioned above. Samples were taken periodically and were filtered with Whatman # 42 filter paper with 2.5 μm pore size and then with a nylon membrane filter of 0.45 μm pore size to remove impurities and to obtain suitable for sugar analysis samples.

2.4 Mathematical model

The Agave fructan hydrolysis process is regarded as a heterogeneous reaction in a vegetable matrix. To formulate a mathematical model is necessary to know the reactions of depolymerization and release of other compounds as well as the formulation of the equations of heat and mass transfer. On the background investigations García-Soto *et al.* (2011) we can consider the maguey juice contained in vials as a homogeneous system without transport constraints, and whose reactions of hydrolysis and degradation are considered localized. The base equation for the formulation of this model was established by Seaman (1945) and is based on the hydrolysis and saccharification of wood Eq. (1). This equation shows the process of hydrolysis of cellulose to glucose releasing free transformation and subsequent partial degradation products. However, this model is applicable to other systems and was adapted by García-Soto *et al.* (2011) for the hydrolysis of fructans Eq. (2).

$$Celulose \xrightarrow{K_h} Glucose \xrightarrow{K_d} Degradation\ \ products \tag{1}$$

$$Fructan \xrightarrow{K_h} \begin{bmatrix} Fructose \\ Glucose \\ Others \end{bmatrix} Reducing\ \ sugars \xrightarrow{K_d} \begin{bmatrix} Furfural \\ hidroximetilfurfural \\ Others \end{bmatrix} Furans \tag{2}$$

Where fructans (P) are hydrolyzed and released reducing sugar monomers or (M), these may undergo a partial degradation and produce furans (D), are also observed in the hydrolysis rate constant (K_h) and degradation rate (K_d), where the model, hydrolysis and degradation are irreversible processes. From these equations the mathematical model established for the reaction rates are defined as:

$$\frac{d[P]}{dt} = -k_h[P] \tag{3}$$

To initial conditions ($t = 0$):

$$[P] = P_0 \tag{4}$$

The model described in Equations 3 and 4 was solved analytically, so that the following expression is obtained:

$$[P] = P_0 e^{-kht} \tag{5}$$

The kinetic constant value (k_h) is obtained by applying a nonlinear regression analysis by Scilab 5.2.1 integral scheduled method (Le Chesnay Cedex, France). The coefficient of determination (R^2) was used as the first criterion to determine the accuracy of the fit between the experimental data and the proposed model.

2.5 *Statistical analysis*

They conducted an analysis of variance (ANOVA) with a confidence level of 99 % (p <0.01) with Modde 7.0 statistical program (Umetric AB). A linear mathematical model was used to analyze interactions effect of temperature (T) and acidity (A) at the rate constant K.

$$y = \beta_0 + \beta_1 T + \beta_2 A + \beta_3 T \cdot A \tag{6}$$

3 Results and discussion

3.1 *Carbohydrate content in maguey juice*

The HPLC results showed that fructan and fructose have a concentration of 4.440 and 0.5685 mg/ml respectively. The separation column Aminex HPX-87C is capable of separating all sugars present in the juice of *Agave* with good resolution in a single run of 20 min. The resolution was good, allowing all components were identified and quantified. Sucrose, glucose and fructose were identified with the elution times of 8.1 min, 9.8 min and 12.7 min respectively. The main peak of the chromatogram coincides with the peak and the elution time (6.7 min), corresponding to chicory inulin used as a standard. A minor peak with an elution time of 7.5 minutes was detected shortly before the peak of sucrose that could be considered a low DP fructan and fructooligosaccharide. However, practical considerations with respect to this work, these two peaks were quantified as total fructan.

3.2 *Analysis of the kinetics of hydrolysis*

As shown in Figure 1, the degradation of substrates gradually occurred and after 2 h of hydrolysis, fructose was the main product. Figure 1B illustrates that when the pH is adjusted to 2.5 the rate of degradation of the substrate increases and reached within 2 h the highest possible degradation of the substrate. The degree of hydrolysis of substrate (%) is defined as the ratio of substrate consumed/amount of substrate used * 100. Thus, at the end of the thermal process, the values of 79.3% and 92.1% were calculated for the experiments 6 and 8, respectively. This disparity in the efficiency of the hydrolysis may be explained by the difference in the acidity of the substrate. However, the percent hydrolysis range reached in this study is similar to those described for the thermal hydrolysis of fructans contained in *Agave tequilana* (Waleckx *et al.*, 2008).

3.3 *Kinetics of hydrolysis*

The fructan degradation and release of fructose experimental kinetic in time of hydrolysis function is also seen in Figure 1. At 90 °C and pH 5.5, the fructan hydrolysis is carried mostly within the first hour of hydrolysis; during the kinetics, the release of fructose increases gradually and according to their behavior in the graph, the hydrolysis apparently continues after

10 h (Fig. 1A). A 100 °C and pH 2.5, the highest fructan hydrolysis level occurs during the first hour in the kinetics, this is due to acid hydrolysis was carried out by adjusting the pH of the juice; and after 2 h, both fructan concentration as the release of fructose is stabilized (Fig. 1B); acids function as catalysts in the hydrolysis of polymers that have the ability to break the bonds of the heterocyclic monomers in the polymer chains (Saeman, 1945; Laopaiboon *et al*., 2010) unfortunately, to adjust the pH to 2.5 is carried out by acid hydrolysis which has as results in the release of other compounds such as lignin and acetic acid (Lavaracky *et al*.,2002; Rodríguez-Chong *et al*., 2004; Cheng *et al*., 2008; Perez *et al*., 2013; De los Rios-Deras *et al*., 2015).

In samples adjusted to pH 2.5, the higher efficiency of hydrolysis was observed at 100 °C and not at 110 °C as expected, the same phenomenon was observed by Laopaiboon (2010) by hydrolyzing acid the bagasse cane sugar. In Agave juice sample a natural pH value

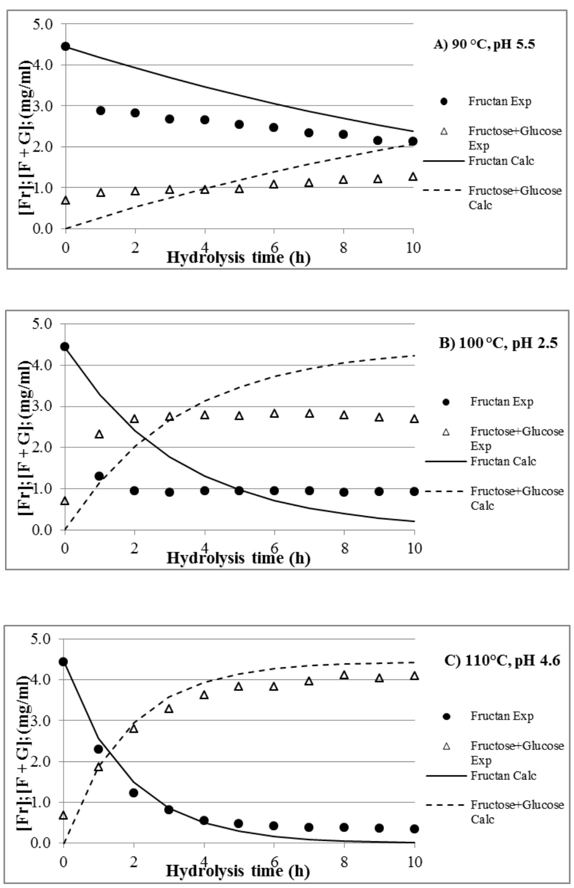


Fig. 1. Kinetics fructan degradation and fructose release in temperature and pH. function.

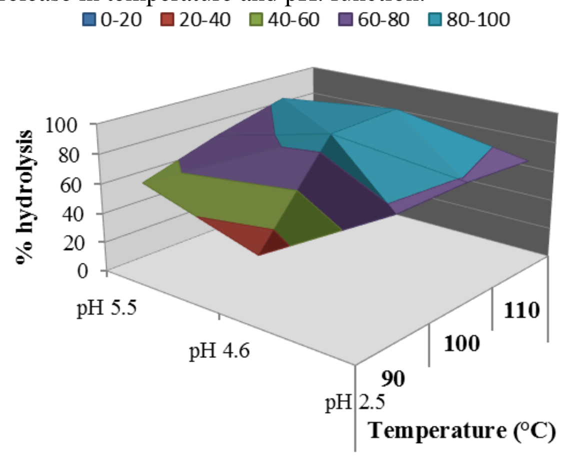


Fig. 2. Response surface plot of experimental efficiency (%) in agave fructan hydrolysis depending of solution temperature and pH.

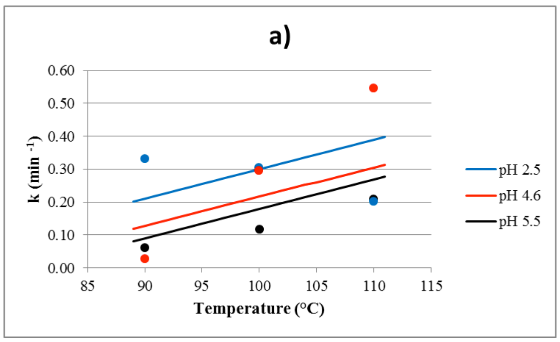
(4.6) and 100 °C, the rate of degradation of substrate is less than pH 2.5, but the degree of hydrolysis of the substrate is greater, this is achieved at approximately 6 hours of thermal hydrolysis. The substrate hydrolysis degree under the experimental conditions used will of 27.28 % (90 °C, pH 4.6) to 92.10 % (110 °C, pH 4.6).

The pH has an effect on the initial rate of substrate degradation since juice samples with pH 2.5 exhibit greater hydrolysis rate, this is more than the adjustment of acidity of the effect of temperature, because this rate is only observed during the first hours and then tends to stabilize. The juice samples under temperature 110 °C have more efficient hydrolysis, results that agree with Garcia-Soto *et al*., (2011) who found the higher thermal efficiency Agave fructan hydrolysis is in a temperature range from 106 to 116 °C. Although the initial rate of hydrolysis is greater in samples with pH of 2.5, do not reach the percen hydrolysis of the samples at pH 4.6 and 5.5 (Fig. 2). Similar behavior was observed by Waleckx *et al*. (2008) with the thermal hydrolysis of the fructans from *Agave tequilana*.

Simulated curves are also shown in Figure 1. When calculating the values of the rate constants (k_h) corresponding to the coefficients of determination (R^2) values are between 0.43 and 0.99 (Table 1), so that the fit between experimental and simulated values. An analysis of variance (ANOVA) with a 99% confidence level (p <0.01) revealed that affected both by the temperature (T), and for the value of the acidity (pH). The values of the rate constants as a function of temperature and pH can be observed in Figure 3.

Table 1. Rate constant values (k_h) and their respective value of R^2 determined by the linear regression method for each experimental condition.

Temperature (°C)	pH	k_h (min^{-1})	S_o (mg/ml)	R^2	P (%)
90	2.5	0.3309	3.2489	0.4377	192.2624
	4.6	0.0283	4.3626	0.8108	3.8017
	5.5	0.0623	3.5657	0.6846	6.6713
100	2.5	0.3047	3.2863	0.5079	141.4516
	4.6	0.2943	4.2989	0.9901	22.9203
	5.5	0.1175	3.5503	0.7896	11.9504
110	2.5	0.2017	2.9373	0.4447	55.2069
	4.6	0.5453	4.2956	0.9605	402.621
	5.5	0.2103	3.6915	0.8642	25.7296



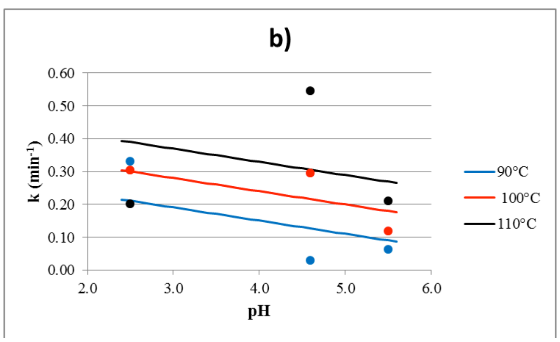


Fig. 3. Rate constant variation (k_h) in temperature (a) and pH (b) function.

The rate of hydrolysis of fructans is favored as the temperature increases (Saucedo-Luna *et al*., 2010) and the pH is adjusted to the natural acidity of the juice (Figure 3).

Conclusions

According to the results obtained in this work, the thermal hydrolysis may be of industrial interest for the large scale production of high fructose syrup under appropriate conditions of temperature and acidity. The *Agave salmiana* may be promising feedstock for industrial scale production of fructose syrup.

Nomenclature

kh	hydrolysis rate
kd	degradation rate
P	fructans
M	reducing sugars
D	furans
T	temperature
A	acidity
B*i* (i=0, 1, 2, 3)	regression coefficients model

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