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METHANOL PRODUCTION KINETICS DURING AGAVE COOKING FOR MEZCAL INDUSTRY

CINÉTICA DE PRODUCCIÓN DE METANOL DURANTE LA COCCIÓN DEL AGAVE EN LA INDUSTRIA DEL MEZCAL

A. Solís-García[1], P. Rivas-García[2], C. Escamilla-Alvarado[2], R. Rico-Martínez[3], M.G. Bravo-Sánchez[1], J.E. Botello-Álvarez[1]*

[1]*Departamento de Ingenieria Bioquimica, Instituto Tecnologico de Celaya, Av. Tecnologico y A. Garcia Cubas, zip 38010, Celaya, Guanajuato, Mexico.*

[2]*Departamento de Ingenieria Quimica, Facultad de Ciencias Quimicas, Universidad Autonoma de Nuevo Leon, Av. Universidad S/N, Cd. Universitaria, zip 64451, San Nicolas de los Garza, Nuevo Leon, Mexico.*

[3]*Departamento de Ingenieria Quimica, Instituto Tecnologico de Celaya, Av. Tecnologico y A. Garcia Cubas, zip 38010, Celaya, Guanajuato, Mexico.*

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Abstract

Cooking of agave is a basic operation in the production of mezcal in which the agave fructans are transformed into monosaccharides, and then these are converted to ethanol during fermentation. During these stages important minority compounds are generated such as the methanol, which is generated throughout the demethoxylation of the pectin in agave and that is toxic to humans, being strictly regulated in alcoholic beverages. This work evaluated the methanol production during the cooking of *Agave angustifolia*, from 92 to 122°C for 12 to 18 h. A first order kinetic model was developed, finding that the energies of activation of 90.64 and 115.96 kJ/mol for demethoxylation in stem and leaves, respectively. During cooking, the degree of demethoxylation of pectin reached 92%. It was estimated that most of the methanol produced was removed onto the water steam that was generated during cooking.

Keywords: agave, methanol, demethoxylation, activation energy.

Resumen

El cocimiento de agave es una operación básica en la producción de mezcal, los fructanos de reserva se transforman a azúcares simples que son convertidos a etanol en la fermentación. En estas etapas se generan compuestos minoritarios importantes como el metanol, que es un compuesto tóxico cuya presencia en bebidas alcohólicas se regula por rigurosas normatividades. En este trabajo se estudió la generación de metanol durante el cocimiento de *Agave angustifolia* para la producción de mezcal en el intervalo de 92 a 122ºC y tiempos de tratamiento de 12 a 18 h. Durante el cocimiento, el metanol es generado por la desmetoxilación de la pectina presente en el agave. El aporte de este trabajo es la construcción de un modelo cinético de primer orden y la determinación de las energías de activación de 90.64 y 115.96 kJ/mol para la reacción de desmetoxilación en las fracciones de tallo y hojas, respectivamente. Durante el cocimiento el grado de desmetoxilación de la pectina alcanza 92%. Se estimó que la mayoría de metanol liberado se elimina en el vapor de agua generado durante el cocimiento.

Palabras clave: agave, metanol, desmetoxilación, energía de activación.

1 Introduction

Mezcal is a distilled alcoholic beverage that along with tequila are the most commercially representative of the traditional drinks that are produced in Mexico from different species and varieties of agave. In the year 2015 about 2.5 million of liters of mezcal at 45% v/v of ethanol were produced (COMERCAM, 2015). The production of mezcal is integrated by stages such as cooking, grinding, extraction, fermentation, distillation and aging (Díaz-Montaño *et al*., 2008; Waleckx *et al*., 2008). The agave heads (which are the stem and the base of the leaves), are harvested after 6 to 8 years of culturing. The agave heads are composed of fructans as carbohydrates of reserve. These are polymeric molecules of fructose and glucose, whose degree of polymerization and structure has been studied in different species of

* *Corresponding author. E-mail*: enrique.botello@itcelaya.edu.mx

agave (Arrizon *et al*., 2010; López *et al*., 2003). The cooking stage is the thermal treatment with steam of the agave heads in autoclave or masonry ovens for hydrolyzing the fructans to produce concentrated syrups of fructose, which are the input to the fermentation stage. In addition to the fructans and structural carbohydrates, agave heads present other compounds of different nature such as fatty acids, terpenes, phenolic compounds and others, which suffer transformation processes during cooking (Mancilla-Margalli *et al*., 2002).

Cooking is a complex process affected by temperature and the own agave acidity (Garcia-Soto *et al*., 2011); in this stage there is a wide and diverse amount of compounds that have sensorial importance in the final product (Mancilla-Margalli *et al*., 2002; Benn and Peppard, 1996), as well as other compounds that may present harm to the consumer health, such as the 5-hydroxymethylfurfural and methanol (Capuano and Fogliano, 2011; Luttrell and Conley, 2011; Vale, 2007).

Methanol is a toxic compound for humans that is present in many alcoholic beverages such as beers, wine and spirits (Cabaroglu, 2005). It has been determined that the origin of methanol in these products is the demethoxylation of the pectin during the different stages of its processing by thermal or enzymatic effects (Wu *et al*., 2007; Hang and Woodams, 2010). The sanitary standards in all countries set the limits of the methanol content in alcoholic beverages (Wu *et al*., 2007). The Mexican Official Norm on mezcal specifications set as upper limit the concentration of 300 mg per 100 mL of anhydrous ethanol (SE, 1997).

The causes owing to methanol production have been investigated in agave industry (Soto-García *et al*., 2009; De León-Rodríguez *et al*., 2008; Cedeño, 1995). In mezcal production, Soto-García *et al*. (2009) found that the methanol content is affected by the presence or absence of lignocellulosic residues in the agave syrup. De León-Rodríguez *et al*. (2008) also stated that the production of methanol was inherent to the alcoholic fermentation due to the presence of lignocellulosic residues. Furthermore, they mentioned that during the fermentation, the methanol content is reduced due to its high volatility and CO_2 release. As for the tequila industry, Cedeño (1995) mentioned that methanol content is due to the enzymatic hydrolysis of pectin.

Tequila and mezcal industries have been technified with special interest in fulfilling the national and international norms in order to offer a high quality product, harmless to the consumer. In recent studies on methanol and other compounds content found in alcoholic beverages made from agave (SS, 2015; SE, 2012; SE, 1997), it has been reported that the methanol value is always lower than that of the limit specification (De Léon-Rodríguez *et al*., 2006; Lachenmeier *et al*., 2006); however, the further reduction of methanol content is still a challenge for the producers and researchers.

In this work, we have studied the production of methanol during the cooking of *Agave angustifolia* plants for mezcal production, through the assessing of the demethoxylation kinetics in the vegetal matrix at 92-122 ºC temperatures. The final objective was to improve the understanding of demethoxylation and methanol production phenomena that in future may serve to develop practical recommendations to reduce the content of this compound in alcoholic spirits made from agave such as mezcal.

2 Materials and methods

2.1 Vegetal substrate and cooking

Agave angustifolia eight year old heads were cultured in Comonfort municipality at Guanajuato state in Mexico. The heads of agave of 50 kg were separated from the stem and leaves to study each fraction separately. The agave was cut in cubic pieces of 0.5 kg, which were cooked in a horizontal 0.4 m diameter and 0.8 m long on stainless steel autoclave with temperature control and saturated steam injection system as described by García-Soto *et al*. (2011). Four cooking assays were performed on duplicate at 92, 102, 112 and 122 ºC, 12-18 h. Solid and liquid samples were withdrawn every 3 h, first evacuating the steam from autoclave and promptly removing the samples, after which the operation was continued (García-Soto *et al*., 2011). Humidity content was determined in fresh and processed trunks. The solid agave samples were removed from autoclave and were ground in a food processor (Champion Juicer 2000, Plastaket MFG. Co., Inc., California, USA). Then the material was press filtered manually, hence obtaining syrups free from suspended solids. Thus, each agave fraction originates three phases: a residual solid phase, a liquid solution: fructose syrup and a vapor phase is lost during cooking.

2.2 Analysis

Different extraction procedures were used for the methanol content determination in fresh and cooked solids, and syrups.

2.2.1 Determination of methoxylated groups

The fresh and cooked solid agave samples, were subjected to acid digestion to release methanol. The acid digestion was performed at 94 ºC on 5.0 g of agave sample, 200 mL water and 35 mL HCl (HCl ACS, Fermont, 37.30%). Distillation was carried out simultaneously until achieving 80 mL distillate. The methanol content in the distillate, reported in relation to the cooked agave dry weight, was determined by gas chromatography (below described).

2.2.2 Determination of methanol in syrup

Regarding the determination of methanol in the aqueous fructose solution, the syrup was first centrifuged at 12,500 rpm, 5 min, then it was filtrated in Whatman paper grade 1. Afterwards, 100 mL of the filtrated syrup were boiled until retrieving 30 mL distillate. The methanol content was determined by gas chromatography.

The methanol transferred to vapor phase during cooking was estimated by the difference between the methoxylated groups (expressed as methanol) in agave matrix before cooking, and the remaining in each solid residue and syrup samples.

2.2.3 Gas chromatography

The gas chromatography analyses were run in Clarus 500 equipment (Perkin Elmer) with Flame Ionization Detector (FID), manually injecting 1 μL sample on a splint ration 20:1. A 30 m x 0.25 mm capillary column AT-WAX (Alltech) was used. Carrying was nitrogen chromatography grade at 14 psig. The injection port and detector were maintained at 250 ºC. The oven program was 40 ºC 5 min, 10 ºC per minute ramp until 130 ºC. A calibration curve methanol-water from 0 to 1000 mg/L was performed using as internal standard hexanol (Sigma Aldrich).

2.3 Kinetic model

The demethoxylation model for agave was built using the kinetic data of the Methoxylated Groups (MG) expressed as methanol released in acid digestion during the different cooking runs. The reaction model proposed is shown in Equation 1. The methoxyl groups (-O-CH_3) are strongly bound to a vegetal matrix (R); during the thermal treatment demethoxylation and consequent methanol formation occurs, which remains in liquid or is transferred to vapor phase.

$$R-O-CH_3 \underset{\Delta}{\longrightarrow} R+CH_3-OH_{(solution)}+CH_3-OH_{(steam)} \quad (1)$$

Equation 2 is the proposed kinetic expression for demethoxylation. The content of methoxylated compounds bound to vegetal matrix are expressed in mg of methanol produced for each methoxylated group present in 1 kg vegetal matrix dry weight; t is the cooking time and K_{DM} is the rate constant.

$$\frac{dGM}{dt} = -K_{DM}GM \quad (2)$$

At initial conditions (Eq. 3), the solution of Equation 2 is Equation 4, where GMo is the methoxylated groups content in vegetal matrix before the cooking.

$$t = 0, \quad GM = GM_0 \quad (3)$$

$$GM = GM_0 e^{-K_{DM}t} \quad (4)$$

The experimental values of GM *vs.* t were used for determining the K_{DM} constant rates for each cooking temperature. The Arrhenius linearized equation was used for determining the activation energies and the pre-exponential factors for the demethoxylation of agave in stem and leaves.

3 Results and discussion

The generation and distribution of methanol from the stem, in solution and vapor phase −denoted as steam−, during cooking at 112 ºC of *Agave angustifolia* is shown in Figure 1. The amount of methoxylated groups, expressed in equivalent of methanol, appears to be released by acid digestion on the stem of agave. The methanol content from the fructose syrup solution that was obtained by pressing the cooked agave pieces. Besides the amount of methanol that was transferred to the vapor phase. At this temperature, approximately half the methanol remains in the vegetal structure as methoxylated groups, whereas the other half is transferred to the steam phase. A small amount of methanol remains in the fructosate solution, which is the main product of cooking.

Table 1. Distribution of methanol after cooking in stem / leaves

Cooking conditions	**Methanol in solids phase (%)**		**Methanol in solution (%)**		**Methanol in steam (%)**	
Temperature time	Stem	Leaf	Stem	Leaf	Stem	Leaf
92 °C 18 h	47.7	53.29	0.71	1.79	51.59	44.93
102 °C 18 h	46.71	19.7	1.37	2.69	51.92	77.7
112 °C 12 h	35.9	11.08	1.02	0.88	63.08	88.05
122 °C 12 h	17.87	7.21	1.01	0.7	81.13	92.09

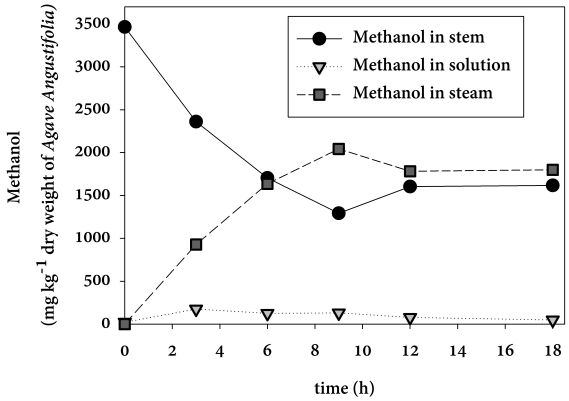

Fig. 1. Generation and distribution of methanol during *Agave angustifolia* cooking at 112 ºC.

In modern industry mezcal and tequila, agave pineapples are cooked in hermetic autoclaves with steam injection. At the end of cooking, the autoclaves are opened to allow the release of vapors and cooling agave pineapples. The syrup is filtered to remove any particles and pieces of agave. In some industries, the fructose syrup is partially evaporated before fermentation. These practices allow significant removal of the methanol.

Some artisanal producers introduced agave residual solids in the fermentation vat to achieve an anaerobic environment and depolymerization of fructans and leaching of free fructose by enzymatic pathways. This practice is negative because it favors the enzymatic demethoxylation of lignocellulosic materials and pectins, with the generation and leaching of methanol into syrup during fermentation. Indeed, Soto-García *et al*. (2009) found that when agave cooked solids were introduced to the must, the amount of methanol increased during fermentation.

In the analyzed samples of fresh agave it was found that average concentrations of methanol that can be generated by demethoxylation in acid digestion of agave are 3,548 and 3,098 mg/kg dry weight of stem and leaf, respectively. The distribution of methanol and methoxylated groups after cooking is shown in Table 1; it is observed that a considerable fraction of methoxylated groups was retained in the vegetal matrix at low temperatures. The generation of methanol and its transfer to the steam phase was favored as temperature increased. The data indicate that most of methanol generated was transferred to steam phase, and only a small fraction remained in the fructose solution.

The content of methanol in the fructose syrup is critical to ensure the accomplishment of the Mexican Official Norms (SS, 2015; SE, 2012; SE, 1997). These norms specify that the methanol to anhydrous ethanol ratio must be lower than 300 mg/100 mL. The elimination of methanol by distillation is in function to the ability to separate methanol and ethanol. Prado-Ramírez *et al*. (2005) when distilling alcoholic beverages derived from agave, reported that the methanol did not performed as a light compound in respect to ethanol, instead it remained during all the distillation until the exhaustion of ethanol. According to our work group experience, the elimination of methanol is hard to accomplish through conventional distillation.

Before proceeding the fermentation, the content of methanol must be carefully regarded as its concentration in fructose syrup often keeps a determined ratio to the concentration of ethanol in the end of fermentation. For instance, in our work group in 12ºBrix syrups uncontrolled fermentations yielding 3.5ºGL ethanol, the critical methanol concentration in fructose was 105 mg/L, whereas in controlled fermentations with 6.0ºGL ethanol production, methanol concentrations lower than 180 mg/L were allowed in the fermented must to ensure the fulfilling of the end-product norm.

In Figure 2, the experimental data of demethoxylation of *Agave angustifolia* stem and leaves cooked at 122 ºC are shown. It is observed that concentration of methoxylated groups diminished rapidly during the first four hours of cooking, and in

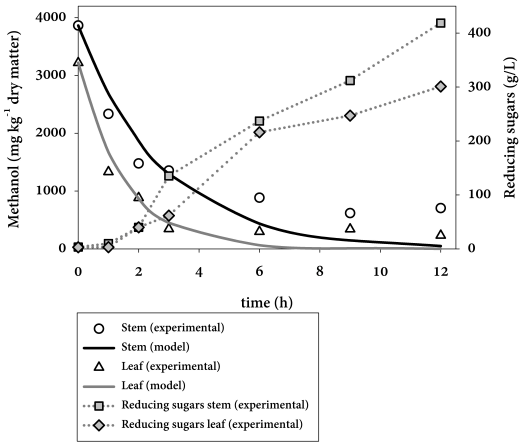

Fig. 2. Demethoxylation and reducing sugars production during cooking of *Agave angustifolia* at 122 ºC.

the end, important amounts of these groups remained attached to the vegetal structure. This may indicate that methoxylated groups are present in diverse forms inside the vegetal structure, some of which are recalcitrant to thermal treatment. The solid lines represent the predictions of the proposed model, where it is observed that the experimental points are represented adequately by the model during the first hours, however there is a lack of fit to the experimental points in the last hours of the experiment. In Figure 2 it is also represented the generation of reducing sugars during the cooking. The released sugars, mainly fructose, are produced from the thermal hydrolysis of fructans, which are the main reserve carbohydrates of agave (Garcia-Soto *et al*., 2011). It may be observed that depolimerization of fructans as main reaction during cooking occurs simultaneously with the demethoxylation and generation of methanol. This phenomenon may indicate that both reactions have similar activation energies.

Some authors have mentioned that methanol occurrence in alcoholic beverages is due to pectin demethoxylation (Arslan *et al*., 2015; Hang and Woodams, 2010; Wu *et al*., 2007). The thermal degradation of pectin by hydrolysis reactions, β-elimination and demethoxylation have been studied to understand the effect of thermal treatments on vegetal texture (Sila *et al*., 2016) However, in the literature the production of methanol from agave cooking has not yet been sufficiently studied. Neither has it yet been proposed a kinetic model that allows to evaluate the

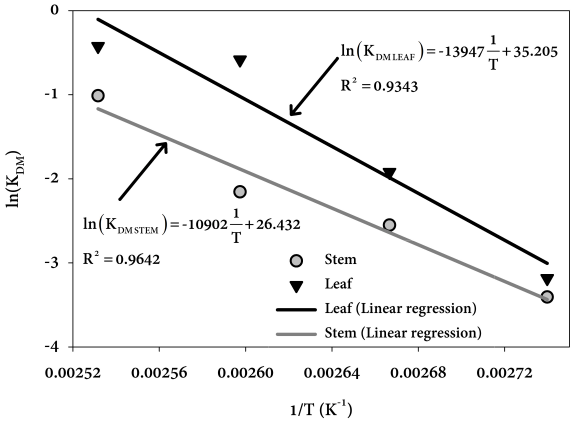

Fig. 3. Arrhenius equation adjustment of *Agave angustifolia* demethoxylation in leaf and stem.

parameters and to understand the phenomenology of the process.

In the Figure 3 the Arrhenius equations for the demethoxylation of *Agave angustifolia* in leaves and stem are represented, whereas Table 2 shows a summary of kinetic parameters during the thermal treatment of different plants obtained herein and in other works. It may be observed that the activation energies of the pectin demethoxylation (95-136 kJ/mol) from diverse sources are very close to the values determined in this work and others. It may be observed that the activation energies for other reaction related to the thermal treatment of plants are very close (82.9-139.06 kJ/mol). This suggests that the different degradation reactions during the thermal treatments are occurring concomitantly in the vegetal matrix. This in turns implies that it would be very difficult to achieve the depolimerization of agave fructans –the main objective of cooking- without the production of methanol at a certain temperature. On the other hand, the differences observed in the kinetic parameters of demethoxylation (Figure 3) in the steam and leave fractions, may be due mainly to the difference in the vegetal structures, which may offer different thermal transfer resistances inwards the cooking vegetal.

The methanol concentration in the alcoholic beverage may be controlled through a careful evaporation of the fructose syrup before the fermentation stage. As it has been reviewed, an important amount of methoxylated groups may be retained in the vegetal structure after cooking, which may be prejudicial when artisanal producers introduce the solid cooked agave residues in the fermentation tanks.

Table 2. Kinetic constants in pectin degradation reactions

Vegetal source/ *Degradation reaction*	**Temperature (ºC)**	**Reaction rate constant ($\bullet 10^{-3}$ min^{-1})**	**Activation energy (kJ/mol), pre-exponential factor**	**Reference**
Agave angustifolia stem/ *Demethoxylation*	92 102	0.553 - 0.690 1.30 - 2.44	90.64, ln(A_o)=26.43	This work
Agave angustifolia leaf *Demethoxylation*	112 122	1.93 - 9.29 6.06 - 10.9	115.96, ln(A_o)=35.5	
Green beans/ *Enzymatic demethoxylation*	35 - 65	--	112	26
Tomatoes/ *Enzymatic demethoxylation*	--	--	97	
Citrus pectins/ *Demethoxylationmethoxy*	75 - 110	1.20 - 9.0	98	27
Citrus pectins/*β–elimination*	--	--	136	
Citrus pectins/*Acid hydrolysis*	--	--	95	
Carrot/*β-elimination*	90 - 110		82.9-129.3	25
Agave salmiana/ *Thermal hydrolysis*	106 - 116	0.579 - 6.32	139.08, ln (A_o)=37.77	7

Conclusion

The first order kinetic models adjust adequately to the demethoxylation and methanol generation during the cooking of *Agave angustifolia* in autoclave for the production of mezcal. Arrhenius equation shows a good correlation between the constant rate and the temperature range 92 - 122 ºC. The activation energies where 90.64 and 115.96 kJ/mol for the demethoxylation of stem and leaves, respectively. During the cooking process, the most of methanol generated is dissipated in the vapor phase, whereas another proportion is dissolved in the aqueous solution of the fructose either retained in the agave head or condensed in the bottom of the autoclave. After cooking and pressing of the head to obtain the fructosate syrup, in the solid residues remains a considerable portion of the methoxyl groups. At 92 ºC after 18 h cooking, the demethoxylation was only 50%.

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