Vol. 18, No. 2 (2019) 543-554
Revista Mexicana de Ingeniería Química

DEXTRAN HYDROLYSIS AND ITS RHEOLOGY IN MASHES FROM BIOETHANOL PRODUCTION PROCESS

HIDRÓLISIS DE DEXTRANA Y SU REOLOGÍA EN MOSTOS PROVENIENTES DEL PROCESO DE PRODUCCIÓN DE BIOETANOL

E. Oropeza-De la Rosa, L.G. López-Ávila, G. Luna-Solano, G.R. Urrea-García, D. Cantú-Lozano*

Tecnológico Nacional de México, Instituto Tecnológico de Orizaba, División de Estudios de Posgrado e Investigación. Laboratorio de Procesos. Oriente 9, No.852 Colonia Emiliano Zapata Sur, C.P. 94320 Orizaba, Veracruz, México.

Received: December 7, 2018; Accepted: February 25, 2019

Abstract

Sugar cane molasses are, locally, the main raw material in bioethanol production process, which is affected by hydrocolloids presence, mainly dextran, raising fluids viscosity, generating clogging and embedding problems in equipment. The main goal of this study is to enzymatically degrade dextran contained in bioethanol production process mashes, and obtain important rheological parameters. This, with the aim, in a global study, of being able to reduce the sludge generation and its incrustation in equipment and distillation columns. Physicochemical, microbiological and rheological characterizations were carried out at mash and fermented mash samples. Dextran degradation was accomplished by enzymatic hydrolysis in presence of dextranase enzyme, evaluating the enzyme-substrate relation, substrate type, and temperature effects, applying a factorial design. Both mash and fermented mash showed a non-Newtonian behavior, with a flow behavior index greater than one ($n > 1$), fitting well to Herschel-Bulkley model. The highest enzyme activity was observed at 40 ºC, obtaining a lower viscous product (0.017 Pa s, 28.978% of reduction) and a lower dextran concentration product (173.220 ppm, 64.767% of reduction).

Keywords: dextran, dextranase, molasses, mash, bioethanol, rheology.

Resumen

Las melazas de caña de azúcar son, localmente, la materia prima principal en el proceso de producción de bioetanol, el cual es afectado por la presencia de hidrocoloides, principalmente dextrana, aumentando la viscosidad de fluidos, generando obstrucciones y problemas de incrustación en equipo. El objetivo principal de este estudio es degradar enzimáticamente la dextrana contenida en mostos provenientes del proceso de producción de bioetanol, y la obtención de parámetros reológicos de importancia. Lo anterior con el fin de, en un estudio global, poder reducir la generación de lodos y su incrustación en equipo y columnas de destilación. Una caracterización fisicoquímica, microbiológica y reológica fue llevada a cabo en muestras de mosto y mosto fermentado. La degradación de dextrana fue realizada mediante hidrólisis en presencia de enzima dextranasa, evaluando la relación enzima-substrato, tipo de substrato y efectos de la temperatura, aplicando un diseño factorial. Ambos mostos mostraron comportamiento no-Newtoniano, con un índice de comportamiento de flujo mayor a 1 ($n > 1$), ajustándose bien al modelo de Herschel-Bulkley. La mayor actividad enzimática fue observada a 40 ºC, obteniendo un producto con menor viscosidad (0.017 Pa s, 28.978% de reducción) y un producto con menor concentración de dextrana (173.220 ppm, 64.767% de reducción).

Palabras clave: dextrana, dextranasa, melaza, mosto, bioetanol, reología.

1 Introduction

Due to the fuel actual demand, high costs and the growing worldwide environmental awareness, the biofuel alternative have become a major stake holder in meeting global future energy needs (Naveen, *et al*., 2014).

Biofuels in Mexico, as bioethanol, have taken more importance in matter of bioenergy obtained by non-fossil sources. It has been established that by 2024 the share of non-fossil sources in electricity generation will be 35% (SENER, 2015). However, the increase in this biofuel request, forces to accelerate the construction of new production plants and to implement process improvements (Marriaga, 2009).

* *Corresponding author. E-mail*: dencantu@gmail.com
Tel. 272-72-57056
https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n2/Oropeza
issn-e: 2395-8472

Industries that involve sugar cane processes, such as bioethanol production, present different operational problems within the process due to hydrocolloids presence, mainly dextran, minimizing their production potential: fluids viscosity increase, clogging generation, equipment embedding problems, energy loss, among others (Abraham, *et al*., 2016; Bashari, *et al*., 2013; Chou, 2000; Khalikova, *et al*., 2005; Ninchan, *et al*., 2017; Rodríguez-Jiménez, 2005; Trost and Steel, 2002).

Dextran is a neutral and a branched naturally occurring polysaccharide composed of a main linear chain of α-1,6 glycosidic linkages with a few branches of α-glucopyranose at positions 0-2, 0-3 or 0-4 (Iqbal, *et al*., 2017). This biodegradable polymer is synthesized by lactic acid bacteria mainly belonging to the Leuconostoc genera (Kasaai, 2012; Zarour, *et al*., 2017). Furthermore, dextran is a unique exopolysaccharide which has high shear rate and water solubility (Iqbal, *et al*., 2017).

Dextran causes not only sugar lost, but also processing problems (Abraham, *et al*., 2016; Gibriel, *et al*., 2014; Bashari, *et al*., 2013b; Kaur and Kaler, 2008; Rodríguez-Jiménez, 2005). Hydrocolloids, as dextran, modify the rheology of the system, which includes two basic properties; flow behavior (viscosity) and mechanical properties (Sánchez-.Paz, *et al*., 2018). Rheologically, it has been demonstrated that dextran is a serious issue within bioethanol production, minimizing its production potential (Oropeza-De la Rosa, *et al*., 2017).

Locally, the main raw material in bioethanol production process is sugar cane molasses. In order to carry out bioethanol production from sugar cane molasses it is necessary to have several operations summarized in three stages: mashes preparation, continuous fermentation and distillation - rectification. Dextran represents a major problem within the process (mainly in the first distillation column inner wall) due to its quantity, nature and molecular weight.

Dextran degradation entails a number of glycosyl hydrolases with different specificities and modes of action. These enzymes are called endo- and exodextranases. Dextranase (1,6-α-D-glucan-6-glucanohydrolase) is an enzyme which catalyzes hydrolysis of α-(1-6)-d-glycoside linkages in random sites of dextran (Ninchan, et,al., 2017; Bashari, *et al*., 2013). Dextranases have important industrial applications since these enzymes can depolymerize various troublesome microbial dextran deposits (Bashari, *et al*., 2016; Khalikova, *et al*., 2005; Rodríguez-Jiménez, 2005) and catalyzes the degradation of dextran in to low molecular weight fractions (Ninchan, et,al., 2017; Zohra, *et al*., 2015; Zohra, *et al*., 2013).

The hydrolysis of dextran by enzyme is a promising and often used method to minimize the described processing problems (Abraham, *et al*., 2016). Dextranase enzyme usage is the most efficient method for dextran hydrolysis in the sugar sector (Purushe, *et al*., 2012; Zhao, *et al*., 2014). Its usage in the sugar industry was argued more than 30 years ago. In 1972 Inkerman and James presented preliminary studies results of industrial application (sugar mill) where an enzyme preparation, Glucanase D-1, produced by Phizer Chemicals company was used for dextran high concentrations hydrolysis in mixed juice. During 80's decade, companies producing industrial enzymes began to produce dextranase preparations for sugar process usage, however in many cases, no clear information is available about their handling (Bashari, *et al*., 2013; Rodríguez-Jiménez, 2005). There is confusion prevailing about which dextranase to use and how and where to add the dextranase (Bashari, *et al*., 2013; Eggleston, *et al*., 2011; Eggleston, *et al*., 2009). Unfortunately, not many published results about its industrial application (sugar process and distillation process) are found (Bashari, *et al*., 2013; Eggleston, *et al*., 2009; Eggleston and Monge, 2005; Fadel, *et al*., 2016; Rodríguez-Jiménez, 2005, Zhao, *et al*., 2014). Some researchers are working towards optimization of dextran application in sugar manufacture (Bashari, *et al*., 2013; Eggleston, *et al*., 2011; Eggleston, *et al*., 2009; Zhao, *et al*., 2014) but not so for bioethanol production process.

Treatments with dextranase increase the level of reducing sugars and produce a rapid decrease in substrate viscosity (Bashari, *et al*., 2013c; Gibriel, *et al*., 2014; Zohra, *et al*., 2015). Hence, determining rheological properties is imperative in order to know if dextran hydrolysis was carried out. Rheology is well stablished as the science of the deformation and flow of matter: It is the study of the manner in which materials respond to applied stress or strain (Steffe, 1996).

Aiming to improve bioethanol process efficiency, it becomes necessary to enzymatically degrade dextran and study its behavior through rheology, thereby, reduce sludge generation and embedding problems in equipment and distillation columns.

2 Materials and methods

Mash and fermented mash samples were collected from a local distillery factory in Orizaba, Veracruz, Mexico. Mash is the conditioned molasses prior fermentation process and Fermented mash is the generated mash after fermentation stage and before distillation process.

2.1 Physicochemical characterization

A physicochemical characterization was carried out for both mashes. Parameters were determined as follows: pH was determined by potentiometry, SST and SSV by technique 2540 Solids/Standard Methods, viscosity by rheometry, density by pycnometry, protein content by Kjeldahl method, reducing sugars content by Fehling method, CODT by technique 5220 D Chemical Oxygen Demand/ Standard Methods, color by Hunter parameters and dextran content by NMX-F-476-SCFI-2011 technique (SENER, 2011). Each determination was made with replica.

2.2 Microbiological characterization

Leuconostoc mesenteroides is known for dextran producing from sugar cane (Abraham, *et al*., 2016; Gibriel, *et al*., 2014; Khalikova, *et al*., 2005; Zarour, *et al*., 2017). Aiming to know the possible presence of this dextran synthesizing microorganism, a microbiological characterization of different samples was necessary: molasses, mash (at various points during fermentation process) and fermented mash. We attempted to identify the lactic acid bacteria *Leuconostoc mesenteroides*, in order to know if there is any additional dextran increase regarding the one that comes from the previous process. Besides this, these microorganisms are sucrose consumers, which is the raw material in bioethanol production.

In order to achieve that, molasses, mash at different fermentation points (fresh mash, fermentation tanks 4,5,7 and 10) and fermented mash samples were sterile obtained. Immediately, they were taken to be analyzed. Samples serial dilutions were performed using pre-sterilized 0.1% (w/v) peptone water (Madigan, *et al*., 2004). Prior sampling, different culture media were prepared, sterilized and solidified: Nutritive Agar for mesophilic bacteria growth (in which *L. mesenteroides* is found) (Duarte, *et al*., 1982), MRS Agar for exopolysaccharide-producing lactic bacteria growth (Cerutti, *et al*., 2000) and MSE media (Mayeux, Sandine and Elliker), a specific media for microorganisms growth and gum production (Cuervo-Mulet, *et al*., 2010). A count of formed colonies for each culture medium was made. The colonies were macroscopically characterized (color, shape, appearance), microscopically characterized (cell morphology, clustering, mobility) and specific microbial testing including Gram staining, capsule staining and dextran formation were carried out.

2.3 Rheological characterization

Steady state rheological properties were assessed using an Anton Paar Physica MCR301 rheometer, and Rheoplus/32 V2.81 software for data capture and analysis. For both mashes, it was used cylinder Peltier (C-PTD200-SN80123149) and stirrer geometry (ST22-4V-40-SN10120) due to their nature and suspended solids. Rotational tests were performed at 25 ºC, varying shear rate ($\dot{\gamma}$) from 0 to 1000 s^{-1}. As response variables were defined viscosity (η) and shear stress (σ). Every experiment was made with replica. Fitted constitutive rheological models for the shear rate- shear stress relation were obtained by Rheoplus/32 V2.81 software. The equations reliability was evaluated by linear regression analysis obtaining determination coefficients (R^2).

2.4 Dextran degradation trough enzymatic hydrolysis

Dextran is a chemically and physically complex polymer; its breakdown is carried out by a variety of endo- and exodextranases (Zohra, *et al*., 2015). Dextranases are produced by various microorganisms, but fungal dextranase has attracted much attention due to higher enzyme activity (Bashari, *et al*., 2013). In order to degrade dextran present in samples, an enzymatic hydrolysis reaction was performed, using a fungal endoenzyme dextranase (ENMEX Dextranfree) at 1:5 mL dilution with water (recommended dilution by the manufacturer).

The efficiency of dextranases in the factory depends on the pH, Brix, temperature, retention time, agitation, dextran concentration and dosage (Eggleston, *et al*., 2011; Ninchan, *et al*., 2017). Taken this into account, a completely randomized and replicated three-factor and two-level (23) factorial design was settled, where substrate (mash and fermented mash), substrate-enzyme relation (1:10 and 1:100 mL) and temperature (25 and 40 ºC) were the factors to be analyzed. Dextran content and viscosity,

as response variables, were evaluated. Experiments were carried out keeping reaction time (2 h) and stirring (167 rpm) constant. Subsequently, enzymatic kinetic of the best results (in terms of viscosity and dextran content reduction) were sustained for a total time of 5 hours.

Since measuring the enzyme activity of a dextran-hydrolyzing enzyme can sometimes be difficult due to the large variability of available substrates and because the reaction product is an undefined mixture of sugar polymers, a wide spectrum of methods are used (Khalikova, *et al*., 2005). For the present study, dextran content was determined by means of NMX-F-476-SCFI-2011 technique (SENER, 2011) and viscosity analysis. A rheological behavior analysis with its fitted constitutive rheological models was performed as well.

3 Results and discussion

3.1 Physicochemical characterization

Table 1 shows obtained values for both mashes. From the obtained values, the following was observed: both samples present similar acid pH, which it is suitable for the later enzymatic degradation (Bashari, *et al*., 2013; Eggleston and Monge, 2005; Ninchan, *et al*., 2017; Rodríguez-Jiménez, 2005). The pH value is a determining factor in the expression of enzymatic activity. In the case of fungi derived dextranases, the optimum pH value in general are slightly acid (Zhao, *et al*., 2014). Regarding to density, a slight decrease was observed (1.0329 g/cm^3 mash to 1.0297 g/cm^3 fermented mash) which, together with total suspended solids value (SST) (9.13 to 7.66 g / L) and reducing sugars value (46.7742 to 26.4846 g / L), reflects the solids (sugars) transformation to bioethanol during fermentation process. Both samples present a high amount of organic matter.

Allusive to dextran content, it decreases from 491.650 to 352.785 ppm; it is inferred that this is a precipitation consequence due to ethanol content (within fermented mash), since dextran is insoluble on it (Khalikova, *et al*., 2005). This represents a problem at the industry, causing high amounts of solids (dextran included) at the fermentation tanks bottom. As for color, different values were observed for each Hunter parameter: L * (lightness / darkness), a * (greenness / redness) and b * (yellowness / blueness). Fermented mash presented a slightly lighter (L*), reddish (a*) and bluish (b*) color. This color change might be caused by the sucrose transformation into ethanol and its quantity. Viscosity also decreases from 0.0264 Pa s (mash) to 0.0195 Pa s (fermented mash) indicating solids content decrease.

Table 1. Mash and Fermented mash physicochemical values.

Caracterization parameters	**Mash**	**Fermented mash**
pH [-]	5.18	5.17
Density [g/cm3]	1.0329	1.0297
Viscosity [Pa s]	0.0264	0.0195
SST [g/L]	9.13	7.66
SSV [g/L]	5.99	4.87
CODt [g/L]	114.72	114.32
Protein content [%]	1.0818	1.0454
Humidity [%]	76.6	91.39
Reducing sugars content [g/L]	46.7742	26.4846
Dextran content (ppm)	491.65	352.785
Hunter color parameters		
L	0.96	1.18
a	0.35	0.63
b	−0.1	−0.39

3.2 Microbiological characterization

Numerous colonies were formed on the different culture media. It was not found the lactic bacteria investigated in molasses, fresh and fermented mash analyzed samples. It was inferred that bacteria existence (*Leuconostoc mesenteroides*) in molasses is unlikely due to its sugars high content. High sugars concentration in molasses affects the osmosis process within the cell membrane (Rios, *et al*., 2005). Regarding to fresh mash, this has just been diluted, conditioned and inoculated for fermentation, so it is not feasible to find the lactic bacteria yet. Finally, fermented mash already have ethanol content, inhibiting the investigated bacteria.

Concerning sample from the fermentation thank 4, some colonies showed typical *Leuconostoc mesenteroides* characteristics and their microbial tests were positive. It was concluded that, lactic bacteria *Leuconostoc mesenteroides* does exist within the fermenting mash, although in minimal amount, increasing dextran quantity and contributing to the incrustation problem. Under favorable temperature and humidity conditions, the dextransucrase hydrolyses the sucrose, forming dextrans (Abraham, *et al*., 2016; Bashari, *et al*., 2013).

3.3 Rheological characterization

3.3.1 Mash

Mash at 25° C showed a non-Newtonian behavior. Its flow behavior index greater than one ($n > 1$) confirmed dilatancy (shear thickening behavior) and its flow curve was well fitted ($R^2 = 0.9950$) to Herschel-Bulkley constitutive equation (Barnes, 2000), Eq. (1).

$$\sigma = \sigma_0 + K\dot{\gamma}^n \tag{1}$$

where σ is shear stress (Pa), σ_0 denotes yield stress (Pa), K is the consistency index (Pa s^n), γ is shear rate (s^{-1}), and n is the flow behavior index for Herschel-Bulkley model (dimensionless) (Cantú-Lozano, *et al*., 2000). σ_0 is regarded to be an indication of the specific value of stress exerted on the mash when it begins to flow (Liu, *et al*., 2016); thus, such materials exhibit a solid-like behavior for shear stresses below σ_0 and then flow for shear stresses above σ_0 (López-Durán, *et al*., 2013). Herschel-Bulkley model describe the flow behavior of non-Newtonian fluids with yield stresses in a steady shear, fluids with high molecular weight and with many internal degrees of freedom (Barnes, 2000).Table 2 shows the obtained values using Rheoplus/32 V2.81 software.

$$\sigma = 0.115 + 3.136 \times 10^{-4}\dot{\gamma}^{1.719} \tag{2}$$

Viscosity value was 0.0263 Pa s at 25 ºC; Herschel-Bulkley fluids viscosities strongly depend on the shear rate (Barnes, 2000). The shear thickening behavior shown is due to its suspended solids content, and it is confirmed by the yield stress and the consistency index presented. Eq. (1) predicts a shear thickening behavior once the fluid starts flowing (López-Durán, *et al*., 2013) and is useful to predict flow in any other situation, where the same general range of shear rates applies (Barnes, 2000). Viscosity at this stage is very important, because it is needed to be ideal for the yeast fermentation. In order to carry out the fermentation function, the microorganism (*Saccharomyces cerevisiae*) needs to have suitable conditions: pH, temperature, viscosity, nutrients, water among others (Cantú-Lozano, 1987).

3.3.2 Fermented mash

Fermented mash at 25 ºC behaved as dilatant fluid as well, showing a flow behavior greater than one ($n > 1$). The Herschel-Bulkley model (Barnes, 2000), Eq. (3), described the shear stress-shear rate relation ($R^2 = 0.9860$). Table 3 shows experimental parameters.

$$\sigma = 0.072 + 1.326 \times 10^{-4}\dot{\gamma}^{1.815} \tag{3}$$

Fermented mash viscosity value was 0.0194 Pa s at 25 ºC. As observed, mash apparent viscosity at 25 ºC (0.0263 Pa s) decreased with fermentation process, ending with fermented mash apparent viscosity of 0.0194 Pa s. Mash σ_0 value (0.115 Pa) and K value (3.136×10^{-4} Pa s^n) decrease to fermented mash σ_0 value (0.072 Pa) and K value (1.326×10^{-4} Pa s^n). The consistency index K is considered as an indicator of the viscous native of the system (Soto-Caballero, *et al*., 2016).

Table 2. Herschel-Bulkley experimental model parameters for mash at 25 °C.

Model	σ_0 **[Pa]**	K_{HB} **[Pa** s^n**]**	**n [-]**	R^2
Herschel-Bulkley	0.115	3.136×10^{-4}	1.719	0.9950

Table 3. Herschel-Bulkley experimental model parameters for fermented mash at 25 °C.

Model	σ_0 [Pa]	K_{HB} [Pa s^n]	n [-]	R^2
Herschel-Bulkley	0.072	1.326×10^{-4}	1.815	0.9860

The flow behavior index (n) increase from 1.719 to 1.815 meaning a dilatancy increase of mash. All of the above confirms that solids concentration decreases due to the alcohol production from sucrose. The higher the solid content, the stronger the colloidal forces and network strength (Liu, *et al*., 2016).

3.4 Dextran degradation trough enzymatic hydrolysis

Table 4 shows the obtained results, in terms of dextran reduction, of enzymatic hydrolysis runs for mash (M) and fermented mash (FM) samples, according to the experimental design proposed. It is important to note that molasses were not included because high sugars concentration (60º Brix) reduces the enzymatic activity between 30 and 40% and the reaction times increase in comparison to the juice treatment. Dextranases activity is stable up to 25-30º Brix. In addition, an increase in Brix makes it necessary to increase the enzyme dose added and decrease the hydrolysis yield (Bashari, *et al*., 2013; Eggleston, *et al*., 2011; Rodríguez-Jiménez, 2005). Juice application are more efficient and economical than adding them (dextranases) to molasses (Gibriel, *et al*., 2014; Eggleston and Monge, 2005).

Initial dextran values, obtained through physicochemical characterization, were considered: mash presented 491,650 ppm of dextran content, while fermented mash presented 352,785 ppm. Table 4 shows dextran content after enzymatic hydrolysis treatment.

It is clear that temperature affects dextran hydrolysis, reaching higher reduction values at 40 °C, agreeing with those reported in literature (Bashari, *et al*., 2013; Gibriel, *et al*., 2014; Ninchan, *et al*., 2017; Eggleston and Monge, 2005; Rodríguez-Jiménez, 2005; Zhao, *et al*., 2014). From Table 4, it is observed that the greatest reductions in dextran content (64%) are achieved at 40 °C with fermented mash as substrate.

Then, it was evaluated the combined effect of substrate type, enzyme-substrate relation and temperature on dextran content. Table 5 shows the statistical analysis (ANOVA), obtained by NCSS 2000 software.

It is concluded that factor A (substrate) and C (temperature), as well as the interaction between both factors, have an effect upon dextran content. It should be noted that, due to the high values of statistic F_0, only the factor A (F_0 = 423.38) is considered to have a significant effect on the response variable.

Table 4. Dextran content values after enzymatic hydrolysis treatment.

Run	Substrate	[E:S]	T [°C]	Dextran [ppm]$^{\text{STD DEV}}$	Reduction [%]
1	M	[1:10]	25	$325.510^{6.265}$	33.792
2	FM	[1:10]	25	$198.861^{11.649}$	43.631
3	M	[1:100]	25	$309.850^{7.077}$	36.977
4	FM	[1:100]	25	$204.795^{7.403}$	41.95
5	M	[1:10]	40	$248.037^{11.012}$	49.55
6	FM	[1:10]	40	$174.626^{6.191}$	64.482
7	M	[1:100]	40	$230.731^{6.942}$	53.07
8	FM	[1:100]	40	$173.220^{11.525}$	64.767

Table 5. ANOVA of dextran content results.

	Degrees of freedom	Sum of squares	Mean square	F_0	Prob. level	Power α= 0.05
A: Substrate	1	32 836.970	32 836.970	423.38	0.000*	1
B: Relation	1	203.312	203.312	2.62	0.144	0.297642
AB	1	352.341	352.341	4.54	0.0656	0.466205
C: Temperature	1	11 267.560	11 267.560	145.28	2.000×10^{-6}*	1
AC	1	2 539.228	2 539.228	32.74	4.430×10^{-4}*	0.998611
BC	1	19.618	19.618	0.25	0.628	0.073028
ABC	1	7.473	7.473	0.1	0.764	0.058712
S	8	620.477	77.56			
Total (adjusted)	15	47 846.980				
Total	16					

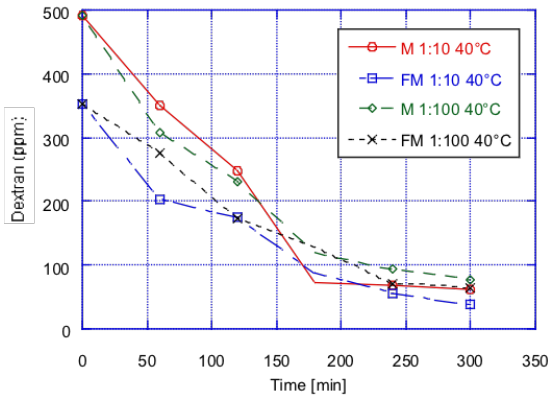

Fig. 1. Dextran degradation with respect to enzymatic hydrolysis time, at high temperature level.

Dextran degradation tendencies with respect to enzymatic hydrolysis time, at a high temperature level, are shown in Fig. 1. From the above figure, it is possible to observe that, in all cases, dextran content decreased substantially in the first 180 min, reaching average values of 110 ppm. As well, stabilization was achieved after that time, reaching average minimum values of 70 ppm. It was noted that the substrate that showed the greatest degradation was the fermented mash (FM), and there is not a strong significance regarding E:S relation factor, as it was known through the statistical analysis.

From the data analysis, we concluded that fermented mash (FM) was the substrate in which the highest removal was obtained, which is considered of interest in the study, since fermented mash will be treated before entering the distillation column. Regarding temperature, higher value (40 °C) showed greater removal. It is important to note that, at industry, fermented mash has similar temperatures once the fermentation has concluded. As can be elucidate in ANOVA results table, the enzyme-substrate relation factor is not that significant in this study. Hence, for resource optimization purposes, the higher value (1:100) was taken into account, where a smaller amount of enzyme is taken in a larger substrate volume.

It is concluded that fermented mash (FM) is the most suitable substrate in order to carry out the treatment at greater scales due to its highest removal achieved (in addition to its optimum pH, Brix and favorable temperature at industrial operating conditions). From the best results obtained, a new experimental design will be developed, in order to optimize enzyme at dextran degradation process.

3.4.1 Dextran enzymatic-hydrolysis rheology

Dextranases reduce the molecular mass and therefore the viscosity of juices (Abraham, *et al.*, 2016; Khalikova, *et al.*, 2005; Ninchan, *et al.*, 2017). Table 6 depicts obtained results after enzymatic treatment, in terms of viscosity, for mash and fermented mash samples, according to the proposed experimental design. There were taken as initial values those obtained in physicochemical characterization, where the mash presented 0.026 Pa s, while fermented mash presented 0.019 Pa s of viscosity.

The dextran effect on viscosity has been studied and it was found to increase with an increase in molecular weight, and decrease with increase in branching (Kaur and Kaler, 2008). The lowest viscosity value was obtained with fermented mash as substrate, [1:10] relation and 40 ºC of temperature, in agreement with the treatments that obtained the greatest dextran reduction.

Table 6. Viscosity values for enzymatic hydrolysis.

Run	Substrate	[E:S]	T [°C]	Dextran [ppm]$^{\text{STD DEV}}$	Reduction [%]
1	M	[1:10]	25	$0.022^{7.071E-5}$	15.72
2	FM	[1:10]	25	$0.019^{3.535E-4}$	0.256
3	M	[1:100]	25	$0.024^{1.414E-4}$	10.985
4	FM	[1:100]	25	$0.020^{0.000}$	−2.564
5	M	[1:10]	40	$0.019^{7.071E-5}$	28.978
6	FM	[1:10]	40	$0.017^{0.000}$	12.308
7	M	[1:100]	40	$0.020^{0.000}$	25.758
8	FM	[1:100]	40	$0.018^{7.071E-5}$	10

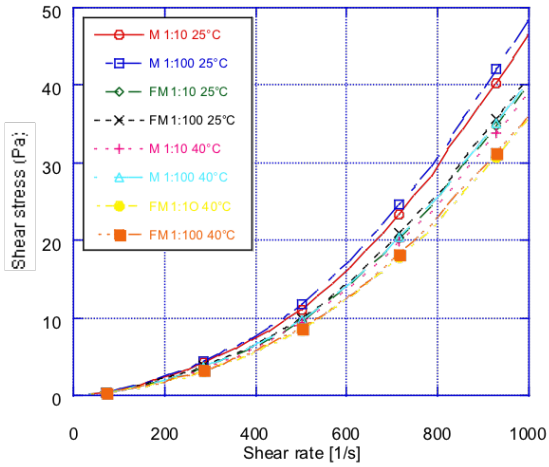

Fig. 2. Rheogram of samples treated by enzymatic hydrolysis.

Since fermented mash has less solids content due to ethanol production, the interaction between the particles decreases. At higher temperature lower viscosity; this is linked to the incidence of a freer molecule to molecule interaction at elevated temperatures. The viscosity of a liquid is a function of the intermolecular forces that restrict molecular motion. These forces depend on the intermolecular spacing which determine the free volume and are affected by changes in temperature. At higher temperatures, the thermal energy of the molecule increases with increase of intermolecular distances, causing reduction of intermolecular forces and consequently the viscosity decreases (Hasan and Nurhan, 2004; Soto-Caballero, *et al.*, 2016).

The largest viscosity reduction was obtained with mash, [1:10] relation and 40 °C. In addition, rheological behavior of each experiment is presented graphically through rotational flow curves (rheograms) (Fig. 2).

It is clear settled that all the samples presented a similar rheological behavior, they did not present a direct proportionality between shear stress and shear rate, meaning a non-Newtonian behavior. They were found to present a dilatant (shear thickening) behavior ($n > 1$) and their flow curves were well fitted to Herschel-Bulkley model (Eq. (1)). Table 7 shows the rheological experimental models obtained, together with their parameters and determination coefficients, obtained with a confidence level of 95%.

The shear thickening behavior presented by the samples is due to its suspended solids content and it is confirmed by yield stress and consistency index presented. With enzyme hydrolysis, yield stress (σ_0) of both mashes decreased (similar results regarding yield stress were obtain by Liu *et al.* (2016)), meaning a solid content decrease (dextran). Furthermore, the consistency index decreased on both mashes regarding initial values (before dextran hydrolysis) meaning a dextran content decrease (Soto-Caballero, *et al.*, 2016). Thus, both viscosity and rheological parameters demonstrated that dextran decreases through enzymatic hydrolysis.

Table 7. Rheological experimental models obtained from treated samples.

Sample	Experimental model	R^2
M [1:10] 25 ºC	$\sigma = 0.086 + 1.120 \times 10^{-4}\dot{\gamma}^{1.862}$	0.992
M [1:100] 25 ºC	$\sigma = 0.093 + 1.228 \times 10^{-4}\dot{\gamma}^{1.855}$	0.993
FM [1:10] 25 ºC	$\sigma = 0.059 + 1.140 \times 10^{-4}\dot{\gamma}^{1.836}$	0.991
FM [1:100] 25 ºC	$\sigma = 0.064 + 1.326 \times 10^{-4}\dot{\gamma}^{1.817}$	0.990
M [1:10] 40 ºC	$\sigma = 0.063 + 9.707 \times 10^{-5}\dot{\gamma}^{1.856}$	0.992
M [1:100] 40 ºC	$\sigma = 0.068 + 1.053 \times 10^{-4}\dot{\gamma}^{1.850}$	0.992
FM [1:10] 40 ºC	$\sigma = 0.043 + 1.016 \times 10^{-4}\dot{\gamma}^{1.835}$	0.990
FM [1:100] 40 ºC	$\sigma = 0.029 + 1.155 \times 10^{-4}\dot{\gamma}^{1.818}$	0.990

Conclusions

Different physicochemical parameters were obtained in order to know mash and fermented mash composition and to understand its behavior. Dextran initial values were also obtained for comparisons. Through microbiological characterization, it was concluded that *Leuconostoc mesenteroides* bacteria does exists, although in a minimal amount, in fermenting mash; thus, it increases dextran amount and contributes to the sludge generation and incrustation problem in equipment (first distillation column inner wall). Important rheological parameters were obtained through rheological characterization.

Regarding dextran degradation through enzymatic hydrolysis, it was concluded that fermented mash (FM) is the most suitable substrate to carry out dextran treatment at higher scales due to its highest removal achieved. As it was observed in ANOVA results, the enzyme-substrate relation factor is not that significant in the study; thus, the higher value (1:100) was taken into account for future optimization purposes.

Treated samples (M and FM) did not present a direct proportionality between shear stress and shear rate, meaning a non-Newtonian behavior. Samples showed a dilatant (shear thickening) behavior ($n > 1$) and their flow curves were well fitted to Herschel-Bulkley model. Through both physicochemical and rheological analysis, it was concluded that dextran degradation is carried out more efficiently on fermented mash (in addition to its optimum pH, Brix and favorable temperature at industrial operating conditions) and it will be an important output in terms of efficiency improving at industrial scale. This study is part of a wide research in terms of improving bioethanol production process.

Acknowledgements

The authors thank the local distillery factory, FABRICACIÓN DE ALIMENTOS TENERIFE, S.A de C.V. for providing the necessary material for our study. The authors thank Tecnológico Nacional de México-TecNM for the financial support [project key: 5822.16-P]. In the same way, the authors thank CONACyT for the scholarships granted with numbers 383226 and 619085.

Nomenclature

A	substrate
B	substrate-enzyme relation, mL
C	temperature, ºC
FM	fermented mash
K	consistency index, Pa s^n
M	mash
n	flow behavior index, (dimensionless)
v	volume, mL
w	weight, g
Greek symbols	
$\dot{\gamma}$	shear rate, s^{-1}
τ	shear stress, Pa
τ_0	yield stress, Pa
η	Viscosity, Pa s

References

Abraham, K., Hagen, S., Schlumbach, K., Rohde, A., Floter, E. (2016). Dextranase application in sucrose solutions - towards a better understanding. *International Sugar Journal 118*, 582-588.

Bashari, M., Jin, Z., Wang, J., Zhan, X. (2016). A novel technique to improve the biodegradation efficiency of dextranase enzyme using the synergistic effects of ultrasound combined with microwave shock. *Innovative Food Science and Emerging Technologies 35*, 125-132.

Bashari, M., Tounkara, F., Abdelhai, M.H., Lagnika, C., Xu, X., Jin, Z. (2013). Impact of dextranase on sugar manufacturing and its kinetic on the molecular weights of remaining dextran. *Sugar Technology 15*, 84-93.

Bashari, M., Lagnika, C., Ocen, D., Chen, H., Wang, J., Xu, X., Jin, Z. (2013b). Separation and characterization of dextran extracted from deteriorated sugarcane. *International Journal of Biological Macromolecules 59*, 246-254.

Bashari, M., Eibaid, A., Wang, J., Tian, Y., Xu, X., Jin, Z. (2013c). Influence of low ultrasound intensity on the degradation of dextran catalyzed by dextranase. *Ultrasonics Sonochemistry 20*, 155-161.

Cantú-Lozano, D., Rao, M.A., Gasparetto, C.A. (2000). Rheological properties of noncoehesive apple dispersion with helical and vane impellers: Effect of concentration and particle size. *Journal of Food Process Engineering 23*, 373-385.

Cantú-Lozano, D. (1987). Caracterización de propiedades reológicas en caldos de fermentación. *La Agroindustria en México 2*, 749-761.

Cerutti de Guglielmone, G.I., Diez, O.A., Cárdenas, G.J., Oliver, G. (2000). Sucrose utilization and dextran productions by *Leuconostoc mesenteroides* isolated from sugar industry. *The Sugar Journal 62*, 36-41.

Chou, C.C. (2000). *Handbook of Sugar Refining: A Manual for the Design and Operation of Sugar Refining Facilities*. Wiley, New York.

Cuervo-Mulet, R.A., Ledesma, J.A., Durán-Venegas, J.A., Argote-Vega, F.E. (2010). Aislamiento y control microbiano de *Leuconostoc mesenteroides*, en un ingenio para optimizar el rendimiento de azúcar y etanol. *Facultad de Ciencias Agropecuarias 8*, 31-40.

Duarte, E., Guillermo, A., Polanco, N., López, M. (1982). *Análisis Microbiológico de Productos Azucareros*. Editorial científico-Técnica, Cuba.

Eggleston, G., Dilks, A., Blowers, M., Winters, K. (2011). Successful application of dextranse in sugar beet factories. In: *Proceedings of the American Society of Sugarbeet Technologists Meeting*, March 2-6, 2011, Albuquerque, New Mexico. p. 1-16

Eggleston, G., Monge, A., Montes, B., Stewart, D. (2009). Application of dextranases in sugarcane factory: Overcoming practical problems. *Sugar Tech 11*, 135-141.

Eggleston, G. and Monge, A. (2005). Optimization of sugarcane factory application of commercial dextranases. *Process Biochemistry 40*, 1881-1894.

Fadel, M., Zohri, A.N.A., El-Dean, A.M.K., Auob, A.H., Deiab, B. (2016). Enhancing etanol yield from sugar cane molasses fermentation by addition of depolymerising enzymes. *Indian Journal of Applied Research 6*, 291-294.

Gibriel, A.Y., Amin, A.A., Yassien, N. M., El banna, H.A., Khaled, F.M. (2014). Application of immobilized P. aculeatum dextranase in sugarcane processing. *International Journal of Current Microbiology and Applied Sciences 3*, 1147-1160.

Hasan, T. and Nurhan, A. (2004). Mathematical model for prediction of apparent viscosity of molasses. *Journal of Food Engineering 62*, 281-289.

Iqbal, S., Marchetti, R., Aman, A., Silipo, A., Ul Qader, S.A., Molinaro, A. (2017). Enzymatic and acidic degradation of high molecular weight dextran into low molecular weight and its characterizations using novel Diffusion-ordered NMR spectroscopy. *International Journal of Biological Macromolecules 103*, 744-750. http://dx.doi.org/10.1016/j.ijbiomac.2017.05.073

Kaur, S. and Kaler R.S.S. (2008). Dextran and its effect on the flow behavior of molasses and crystallization rate. *Journal of Food Engineering 86*, 55-60.

Kasaai, M. R. (2012). Dilute solution properties and degree of chain branching for dextran. *Carbohydrate Polymers 88*, 273-381.

Khalikova, E., Susi, P., Korpela. T. (2005). Microbial dextran-hydrolyzing enzymes: Fundamentals and applications. *Microbiology Molecular Biology Reviews 69*, 306-325.

Liu, J.B., Yu, D., Zhang, J., Yang, M., Wang, Y., Wei, Y., Tong. J. (2016). Rheological properties of sewage sludge during enhanced anaerobic digestion with microwave-H_2O_2 pretreatment. *Water Research 98*, 98-108.

López-Durán, J.J., Pérez-González, J., Marín-Santibañez, B.M., Rodríguez-González, F. (2013). A new method to determine the yield stress of a fluid from velocity profiles in a capillary. *Revista Mexicana de Ingeniería Química 12*, 121-128.

Madigan, M.T., Martinko, J. M., Parker, J. (2004). *Brock Biología de la Microorganismos*, PEARSON, Prentice Hall, Décima edición.

Marriaga, N. (2009). Indicadores de desempeño de la destilación de bioetanol. *DYNA 76*, 85-92.

Naveen, K.M., Ravichandra, P., Rama, R.B., Vijai, K.G. (2014). Current bioenergy researches: Strengths and future challenges. In: *Bioenergy Research: Advances and Applications*, (V. Gupta, M. Tuohy, C. Kubicek, J. Saddler y F. Xu, eds.), Pp. 1-21. Elsevier.

Ninchan, B., Vanichsriratana, W., Sriroth, K. (2017). Investigation of the optimized dextran-degrading enzyme conditions on the decomposition of different molecular weights of pure dextran using response surface methodology. *Archives of Industrial Biotechnology 1*, 8-19.

Oropeza-De la Rosa, E., López-Ávila, L.G., Luna-Solano, G., Cantú-Lozano, D. (2017). Bioethanol production process rheology. *Industrial and Crops Products 106*, 59-64.

Purushe, S., Prakash, D., Nawani, N.N., Dhakephalkar, P., Kapadnis, B. (2012). Biocatalytic potential of an alkalophilic and thermophilic dextranase as a remedial measure for dextran removal during sugar manufacture. *Bioresource Technology 115*, 2-7.

Ríos, C.A., Fajardo, M., Pérez, J.C. (2005). *Evaluación de una Cepa de Levadura para Fermentar Diferentes Concentraciones de Miel Apis mellifera*. Estación experimental apícola, Cuba.

Rodríguez-Jiménez, E. (2005). La dextranasa a lo largo de la industria azucarera. *Biotecnología Aplicada 22*, 11-19.

Sánchez-Paz, L.A., Dublán-García, O., Díaz-Ramírez, M., Arteaga-Arcos, J.C., Domínguez-López, A., Pérez-Alonso, C. (2018). Effect of added gelatin on rheologycal and textural properties of a pound cake reduced in margarine. *Revista Mexicana de Ingeniería Química 17*, 777-789.

SENER. (2015). *Energías Limpias*. Dirección general de energías limpias. Disponible en: https://www.gob.mx/cms/uploads/attachment/file/36175/Bolet_n_Energ_as_Limpias_Dos.pdf. Accesado: 25 agosto 2018.

SENER. (2011). NMX-F-476-SCFI-2011-Método para la determinación de dextrana en azúcares y materiales azucarados, México. Disponible en: https://www.gob.mx/cms/uploads/attachment/file/114866/NMX-f-476-SCFI-2011.pdf. Accesado: 18 marzo 2017.

Soto-Caballero, M.C., Valdez-Fragoso, A., Salinas-López, A.N., Welti-Chanes, J., Verardo, V., Mújica-Paz, H. (2016). Rheological parameters of xanthan gum/pectin solutions as a function of temperature and composition. *Revista Mexicana de Ingeniería Química 15*, 859-868.

Steffe, J.F. (1996). *Rheological Methods in Food Process Engineering*. Second Edition. Ph. D., P.E. Freeman Press. USA.

Trost, L. W. and Steel, F. M. (2002). Review article: Control of microbiological losses prior to cane delivery and during sugar processing. *International Sugar Journal 104*, 118-123.

Zarour, K., Llamas, M.G., Prieto, A., Rúas-Madiedod, P., Dueñas, M.T., Fernández de Palencia, P., Aznar, R., Kihalb, M., López, P. (2017). Rheology and bioactivity of high molecular weight dextrans synthesised by lactic acid bacteria. *Carbohydrate Polymers 174*, 646-657.

Zhao, Z.G., He, X., Yu, S.J., Atsuya, H. (2014). Optimizing conditions for dextranase application of sugarcane mixed juice using response surface methodology. *International Sugar Journal*, 50-55.

Zohra, R.R., Aman, A., Ansari, A., Haider, M.S., Ul-Qader, S. A. (2015). Purification, characterization and end product analysis of dextrandegrading endodextranase from *Bacillus licheniformis* KIBGE-IB25. *International Journal of Biological Macromolecules 78*, 243-248.

Zohra, R.R., Aman, A., Zohra, R.R., Ansari, A., Ghani, M., Ul-Qader, S. A. (2013). Dextranase: Hyper production of dextran degrading enzyme from newly isolated strain of *Bacillus licheniformis*. *Carbohydrate Polymers 92*, 2149-2153.