

Optimization of the dextrane enzymatic hydrolysis process in sugar cane juice (*Saccharum officinarum*) using surface response methodology.

Optimización del proceso de hidrólisis enzimática de dextrano en jugo de caña de azúcar (*Saccharum officinarum*) utilizando la metodología de superficie de respuesta.

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ABSTRACT

Dextran is a homopolysaccharide of glucose, formed in sugarcane juice during post-harvest and is responsible for the low yields of evaporation and crystallization in the sugar industry. The enzymatic saccharification of dextran has been noted for its satisfactory yields; However, this enzymatic process requires an adequate selection of the enzyme and its concentration, as well as the control of the environmental conditions of the biocatalysis. The objective of the present investigation was to optimize the process of enzymatic hydrolysis of dextran from sugarcane juice using the response surface methodology. A central composite rotational design with three independent factors of five levels was used: enzyme concentration, reaction time and temperature, and as a response variable: reducing dextran concentration. The commercial enzyme Dextranfree Xe was used which was kinetically characterized. The experiment consisted of two stages, first a simulated cane juice medium was used to determine the optimal conditions and these were then applied to the real sugar cane juice. The optimal environmental conditions for dextran hydrolysis in the simulated cane juice turned out to be: enzyme concentration 224 ppm, reaction time 32 minutes and reaction temperature 36 °C, to achieve 70 % dextran reduction. Finally, the optimized conditions were validated by applying them to the sugarcane juice, obtaining a reduction of 69.2 % in dextran concentration; said process being effective for its application in the industry.

KEY WORDS: Hydrolysis, Dextranfree Xe, optimal conditions, dextran reduction.

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RESUMEN

El dextrano es un homopolisacárido de glucosa, formado en el jugo de caña de azúcar durante la postcosecha y es responsable de los bajos rendimientos de la evaporación y cristalización en la industria azucarera. La sacarificación enzimática del dextrano se ha destacado por sus rendimientos satisfactorios; sin embargo, dicho proceso enzimático requiere de una adecuada selección de la enzima y su concentración, así como el control de las condiciones ambientales de la biocatálisis. El objetivo de la presente investigación fue optimizar el proceso de hidrólisis enzimática de dextrano del jugo de caña de azúcar mediante la metodología de superficie de respuesta. Se utilizó un diseño central compuesto rotacional con tres factores independientes de cinco niveles: concentración de enzima, tiempo y temperatura de reacción y como variable respuesta: la reducción de la concentración de dextrano. Se utilizó la enzima comercial Dextranfree Xe la cual fue caracterizada cinéticamente. El experimento consistió en dos etapas, primero se utilizó un medio simulado de jugo de caña para determinar las condiciones óptimas y éstas aplicarlas después al jugo real de caña de azúcar. Las condiciones ambientales óptimas de hidrólisis del dextrano en el simulado de jugo de caña resultaron ser: concentración de enzima 224 ppm, tiempo de reacción 32 minutos y temperatura de reacción 36 °C, para alcanzar un 70 % de reducción de dextrano. Finalmente, las condiciones optimizadas fueron validadas aplicándolas al jugo de caña de azúcar, obteniéndose una reducción del 69.2 % de concentración de dextrano; siendo dicho proceso efectivo para su aplicación en la industria.

PALABRAS CLAVE: Hidrólisis, Dextranfree Xe, condiciones óptimas, reducción de dextrano.

Introduction

Sugarcane (*Saccharum officinarum*) is one of the globally relevant raw materials for sugar (sucrose) production (Lu *et al.*, 2017). However, during the post-harvest period and the processing of sugarcane stalks, this raw material undergoes the reduction of its sucrose content due to various factors such as climatic conditions, waiting times between harvest and processing, sugarcane variety, microorganisms present, among others (Misra *et al.*, 2020a), resulting in irreparable losses according to each stage of its origin influenced by chemical degradation (16 %), enzymatic degradation (22 %), and microbiological degradation (62 %). In this sense, *Leuconostoc mesenteroides* is the microbiological species that develops most rapidly in sugarcane, breaking the sucrose molecule through the enzyme dextranase, thus producing dextran, one of the most damaging problems, also causing mechanical damage to equipment, pipes, pumps, among others (Petit, 2021).

Dextran is an extracellular homopolysaccharide composed of glucose units linked predominantly by α -(1,6) glycosidic bonds and also containing α -(1,2), α -(1,3), and α -(1,4) bonds (Khalikova *et al.*, 2005). The structure of dextran, its degree of branching, and its structural properties vary widely depending on the specific dextran-producing microbial strain and the type of dextranase enzyme (Ninchan *et al.*, 2017). Dextran is responsible for many problems in the sugar industry, such as increased viscosity of sugarcane juice, deficiencies in heat transfer, and reduced sucrose crystallization, among others (Khalikova *et al.*, 2005; Ninchan *et al.*, 2017); hence, it is important to reduce its content in sugarcane juice.

Among the alternatives studied, the use of chemical treatments to reduce microbial concentration has been proposed (Misra *et al.*, 2020a; Misra *et al.*, 2020b), membrane processes (Li *et al.*, 2020; Lu *et al.*, 2017), and enzymatic hydrolysis processes with dextranases, which give satisfactory yields (Eggleston & Monge, 2005; Sufiate *et al.*, 2018). In this context, dextranases (EC 3.2.1.11; α -1,6-D-glucan-6-glucanohydrolase) are enzymes produced by microorganisms such as filamentous fungi, yeasts, and bacteria. Filamentous fungi are the major commercial sources of native and recombinant dextranases (Martinez *et al.*, 2021; Liu *et al.*, 2019; Li *et al.*, 2016). Dextranases hydrolyze the 1,6- α -D-glycosidic bond of the linear chain of the dextran structure at random endogenous sites and manage to break the high molecular weight structure into smaller and more manageable molecules and eventually into oligosaccharides such as isomaltotriose, isomaltose, and glucose as end products (Ninchan *et al.*, 2017).

However, such enzymatic processes require proper selection of the enzyme and its concentration, as well as control of the environmental conditions of biocatalysis, including pH, temperature, residence time, agitation, and substrate concentration (Eggleston & Monge, 2005; Sufiate *et al.*, 2018). Likewise, the application of dextranase in the sugar production process still needs to determine the type of enzyme and stage of the process to proceed with enzymatic hydrolysis, as each step of the sugar production process requires different conditions of temperature, pH, and sucrose concentration, among others, which affect enzyme activity and the ability to hydrolyze dextran molecules (Ninchan *et al.*, 2017). It has been reported that reducing the dextran content in the early stages of the process is the most efficient option; therefore, the hydrolysis of dextran present in sugarcane juice by enzymatic processes has been studied (Oropeza *et al.*, 2019).

The present investigation aimed to optimize the enzymatic hydrolysis of dextran process in sugarcane juice using response surface methodology.

Material and Methods

Raw material, enzyme, and dextran

Sugar cane (*S. officinarum*) of the Mexico-73 variety harvested in Caylan, Nepeña district, Santa province, Ancash region, Peru, was used. The commercial enzyme Dextranfree Xe was used in all the experiments (in non-viscous liquid form, amber or slightly yellow in color, 100 % soluble in water, with an enzymatic activity of 25,000 DU/g, produced and marketed by the

company Dextranfree Xe; Produced and marketed by Enmex S.A. in Mexico City, packaged in 25 kg), is a dextranase (1,6- α -D-glucan-6-glucanohydrolase) from *Chaetoniium erraticum*, with which enzymatic dilutions were prepared according to the conditions established in the experiments. Dextran (T-2000 Sigma Aldrich) from *Leuconostoc mesenteroides* NRRL B512F, which has an average molecular weight of 2,000,000 g/mol according to the supplier, was also used.

Enzymatic characterization

Linearity range

To determine the linearity range of the enzyme, the reaction rate of enzyme dilutions (1:2, 1:4, 1:5, and 1:10) was measured in a 0.1 M acetate buffer solution at pH 5.5 acting on a 500 ppm dextran solution. Test tubes containing 2.7 mL of dextran solution were incubated in a water bath at 45 °C for 3 minutes, 0.3 mL of enzyme dilution was added and allowed to react for 0, 10, 20, and 30 minutes. To stop the reaction, the tubes were placed in a water bath at 100 °C for 3 minutes. Then test tubes were removed from the water bath and placed on ice. An enzyme blank and a substrate blank were also used. Reducing sugar formation was determined by the dinitrosalicylic acid-DNS method (Miller, 1959).

Enzymatic activity

Enzyme activity was determined by measuring the initial reaction rate in triplicate, taking as reference the dilution and reaction time defined in the evaluation of the linearity range. For this purpose, 2.7 mL samples of a dextran solution (500 ppm) were incubated with 0.3 mL of an enzyme solution at 45 °C. Samples were taken at predetermined times and, to stop the enzymatic reaction, the samples were placed in a water bath at 100 °C for 3 minutes. The samples were then removed from the water bath and placed on ice. An enzyme blank and a substrate blank were also prepared. Finally, reducing sugar formation was measured using the dinitrosalicylic acid-DNS method (Miller, 1959). In this research, volumetric activity (DU/mL enzyme solution) and specific activity (DU/mg protein) were measured, where DU (dextranase unit) was defined as the amount of enzyme required to release 1 μ mol of reducing sugars per minute, and protein concentration was determined by the Bradford method (Bradford, 1976).

Determination of kinetic parameters

For the determination of the kinetic parameters, the Michaelis-Menten constant (K_m) and the maximum velocity (V_{max}), the initial enzymatic reaction velocities acting on different dilutions of dextran (200, 300, 400, 500, and 600 ppm) were evaluated at 45 °C, taking as reference the enzymatic dilution and the reaction time defined in the evaluation of the linearity range. The K_m and V_{max} constants were determined using the Lineweaver-Burk plot (Lira & Jasso, 2013).

Optimization of the enzymatic hydrolysis process of dextran

Mock solution sugar cane juice

A 3 L mock solution of sugarcane juice composition was prepared from a 0.1 M acetate buffer solution at pH 5.5 in which dextran and sucrose were dissolved to a concentration of 500 ppm and 15 °Brix, respectively. The mock solution was placed in a sterilized glass container and stored under refrigeration until further use. The obtained mock was characterized by determining °Brix (CNM, 2011), pH (ICUMSA2007), viscosity using an Ostwald capillary viscometer (SI Analytics) and reducing sugars using the dinitrosalicylic acid-DNS method (Miller, 1959).

Experimental Design

For the enzymatic hydrolysis process optimization, the response surface methodology was used with a Rotational Composite Central Design (RCCD) (Balakrishnan, Mohan, *et al.*, 2022) with three independent variables: enzyme concentration (ppm), reaction time (min), and reaction temperature (°C); and as response variable the reduction of dextran content (RDC) (%). A total of 17 experiments were designed, with 8 points corresponding to a 2^3 -factorial design, 3 central points, and 6 axial points, with $\alpha = \pm 1.68$. The ranges of independent variables were determined according to preliminary tests. The levels of the independent variables and the experimental design with coded variables are shown in Table 1. The results of the dependent variable were fitted to a full quadratic model, sampled in equation (1):

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j=1}^3 \beta_{ij} X_i X_j \quad (1)$$

Where Y is the response predicted by the model, β_0 , β_i , β_{ii} , and β_{ij} represent the model coefficients for the intercept and the linear, quadratic, and interaction terms between variables, and where X and X_j represent the independent variables. Significant terms in the model were evaluated by an analysis of variance (ANOVA) at a 5 % significance level. The model fit was determined by the values of the coefficient of multiple determination (R^2) and the adjusted coefficient of multiple determination (R^2_{adj}). Also, the value of the mean absolute deviation was determined following the methodology of Baş & Boyacı (2007).

All calculations and graphs were performed using Statgraphics Centurion XIX statistical software and Microsoft Excel 2016 software.

Table 1. Coded and uncoded values of the levels of the independent variables of the experimental design

Independent variables	Coded levels				
	$-\alpha^*$	-1	0	1	α
Enzyme concentration (ppm) (X_1)	120	165	230	295	340
Reaction time (min) (X_2)	15	21	30	39	45
Reaction temperature ($^{\circ}\text{C}$) (X_3)	30	36	45	54	60

Enzymatic hydrolysis of dextran in mock sugarcane juice solution

For the 17 RCCD experiments, a mini-reactor system with a total volume of 250 mL and a working volume of 100 mL containing the mock was used, which was kept under constant agitation and temperature control. The process was also carried out at a constant pH of 5.5. The procedure was as follows: once the volume of mock was added and the temperature of each run was reached, the appropriate amount of enzyme was added and allowed to react for the time specified for each experiment. At the end of the reaction time, the enzyme was inactivated by placing the mini-reactors in a boiling bath for 5 minutes, then the mini-reactors were placed in ice water for 3 minutes. The mini-reactors and their contents were kept refrigerated until further analysis.

Determination of dextran content reduction

The dextran content before and after enzymatic hydrolysis was determined by spectrophotometry (ICUMSA, 2011), in which 8 mL of the sample was taken, and 1.6 mL of trichloroacetic acid (10 %) was added and filtered. From the filtrate, two 2 mL samples were taken, 2 mL of distilled water (used as blank) was added to one and 2 mL of ethanol to the other, they were allowed to stand for 20 min and then the absorbance was read at 720 nm, once the measurement was made, the dextran was calculated using the formula determined from the calibration curve shown in Equation 2:

$$\text{Dextran (ppm)} = \left(\frac{\text{ABS} + 0.039}{0.0019} \right) * 1.2 * 2 * (\text{Dilution factor}) \quad (2)$$

The reduction in dextran content (RDC) was calculated using the formula shown in Equation 3 (Ninchan, *et al.*, 2017):

$$\text{Reduction in dextran content (\%)} = \left(\frac{\text{Initial dextran (ppm)} - \text{Final dextran (ppm)}}{\text{Initial dextran (ppm)}} \right) * 100\% \quad (3)$$

Validation of the optimization parameters in sugarcane juice

To validate the optimization parameters, the levels of the optimal independent variables that significantly influenced the responses of the factorial design and response surface analysis were determined. For this purpose, sugarcane juice was extracted 60 hours after harvesting through a sugarcane mill located at the Laboratorio de Operaciones Unitarias de la Escuela Profesional de Ingeniería Agroindustrial de la Universidad Nacional del Santa. The obtained sugarcane juice was filtered and characterized by determining the soluble solids content (ICUMSA, 2011), pH (ICUMSA 2007), viscosity using an Ostwald capillary viscometer (SI Analytics), and reducing sugars using the dinitrosalicylic acid-DNS method (Miller, 1959). Subsequently, 100 mL of cane juice was loaded into a mini-reactor for enzymatic hydrolysis according to the optimized conditions. This experiment was performed in duplicate.

Results and Discussion

Enzymatic characterization

Linearity range

Figure 1 shows the curve of reducing sugar formation (product) as a function of time for the 4 dilutions of DEXTRAFREE XE tested. It is known that the enzymatic hydrolysis of dextran is also influenced by the number of branches present in it; therefore, in the first phase of the enzymatic process, there is an irregular release of reducing sugars, for this reason, the results of the evaluation of the linearity range are shown from minute 25.

It is necessary to emphasize that in an enzymatic reaction, three phases can be distinguished: the lag phase, the linear phase, and the substrate depletion phase. The lag phase takes place immediately after mixing the reagents with the sample, it is a phase of encounter and coupling of substrate and enzyme, in the case of dextranase, in addition to its adaptation, it suffers variations with respect to the breaking of substrate bonds, this is due to the structure of the dextran since it originates from *Leuconostoc mesenteroides* NRRL-B512F, it has higher percentages of bonds 1-6, 95 % and 1-3, 5 % (Bourne *et al.*, 1962), where the bonds 1-6 are weaker and easier to break, but the bonds 1-3 take longer to break, which is the reason for the destabilization in the production of reducing sugars at the beginning, until finding the progressive break, which indicates the linear phase, in which the formation of products remains constant; finally, as the reaction progresses, the substrate is exhausted (depletion phase) and the reaction rate decreases (Tena & Jorin, 2000).

Figure 1 shows that the 1:5 dilution samples a greater linearity in a longer time, so it was determined to be the most favorable since it maintains the linearity range for 25 minutes, and this dilution was selected for this research. This confirmed what was stated by the supplier, who indicated this dilution as recommended. Oropeza *et al.* (2019), who performed a dextran hydrolysis analysis, also used the 1:5 dilution of the same dextran-free enzyme (ENMEX) and obtained favorable results.

On the other hand, for the determination of enzyme activity, it is necessary to perform it in the linear phase, where the only limiting factor is the concentration of the enzyme itself and the reaction conditions are optimal.

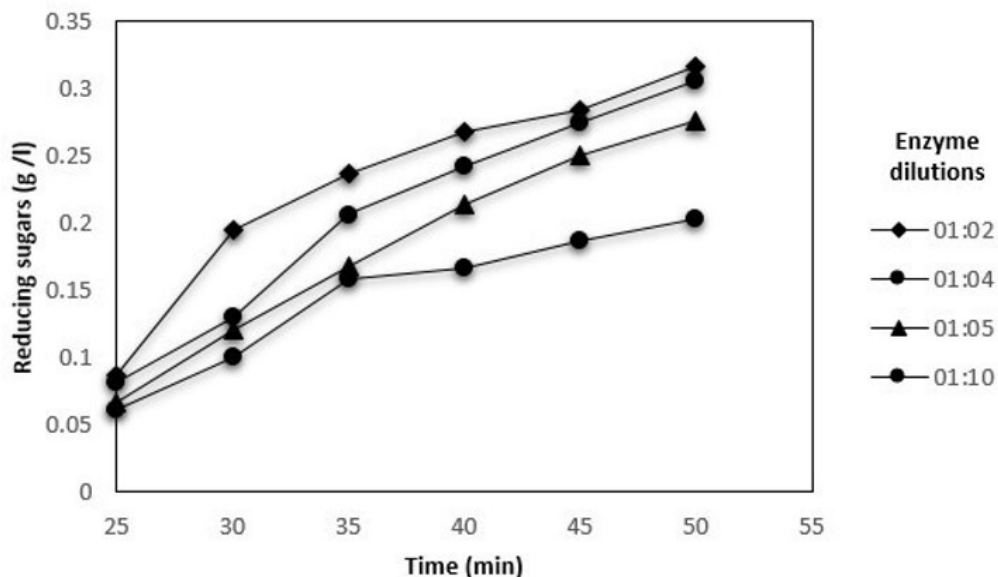


Figure 1. Formation of reducing sugars by Dextranfree Xe enzyme at different enzyme dilutions.

Enzymatic activity

The DEXTRAFREE XE enzyme had an activity of 0.42 ± 0.04 (DU/mL) and 4.7 ± 0.47 (DU/mg). Eggleston & Monge (2005) reported that dextranase produced by *Chaetomium erraticum* showed enzymatic activities of 4783.2 (DU/mL) and 6.4 (DU/mg), but these values were calculated on the basis of the release of reducing sugars equivalent to 1 mg of maltose per hour at 40 °C and therefore differ from the results obtained in the present study.

Kinetic Parameter Values

The kinetic parameter values obtained for dextranase were as follows Michaelis-Menten constant (K_m) of 0.215 (g/L) and maximum velocity (V_{max}) of 0.007 (g/L*min) for an enzyme dilution of 1:5 and a reaction time of 15 min.

Optimization of the Enzymatic Hydrolysis Process

Table 2 shows the results obtained for the RDC (%) for the 17 RCCD experiments of the present study as a function of the coded and uncoded variables. As can be observed, the RDC (%) values ranged between 40 % and 80 %, corresponding to the experiments with low and high levels for each dependent variable, respectively, highlighting that the central points presented a coefficient of variation of 0.8 %, indicating the good reproducibility of the process.

In the analysis of variance (ANOVA) (Table 3) of the experimental design of the present study, the data were fitted to a quadratic model. It was observed that the linear effects of the 3 independent variables (X_1 or A, X_2 or B, and X_3 or C) and the quadratic effect of the reaction temperature were significant ($p < 0.05$).

Likewise, Figure 2 shows the Pareto chart for standardized effects, where the significant effects are verified. Revealing that the linear effect of the variables reaction time (min) and enzyme concentration (ppm) was more significant than that of the variable reaction temperature ($^{\circ}\text{C}$).

Table 2. Reduction in dextran content (RDC) (%) of the experiments as a function of the coded levels of the independent variables

Experiment	X_1	X_2	X_3	RDC (%)
1	-1	-1	-1	40
2	-1	-1	1	46
3	-1	1	-1	52
4	-1	1	1	60
5	1	-1	-1	48
6	1	-1	1	59
7	1	1	-1	68
8	1	1	1	80
9	-1.68	0	0	42
10	1.68	0	0	75
11	0	-1.68	0	46
12	0	1.68	0	77
13	0	0	-1.68	46
14	0	0	1.68	51
15	0	0	0	59
16	0	0	0	59
17	0	0	0	59

The value of the coefficient of determination ($R^2 = 97.51$) and the adjusted coefficient of determination ($R^2_{\text{adj}} = 94.30$) indicate that the mathematical model adequately fits the experimental data. Similarly, the value of the mean absolute deviation was 2.81 %, which, together with the R^2 value, defines the true behavior of the system and can be used for interpolation in the experimental domain. The highest RDC values (%) were obtained at an enzyme concentration of 295 ppm, a reaction time of 39 min, and a reaction temperature of 54°C , which correspond to the high values of the independent variables

Likewise, the values of the non-significant effects were removed from the mathematical model obtained, thus obtaining equation (4):

$$RDC (\%) = -46.0194 - 0.0519 * X_1 - 0.7346 * X_2 + 3.7576 * X_3 - 0.0441 * X_3^2 \quad (4)$$

Correspondingly, no bibliographic references were found that specifically refer to the action under optimal conditions of the enzyme Dextranfree Xe (Enmex) on the dextran substrate to discuss these results.

Table 3. Analysis of variance for the reduction in dextran content (%)

Source	Sum of squares	Degrees of Freedom	Mean square	F-Ratio	p-value
X1: Enzyme concentration (ppm)	926.719	1	926.719	107.61	0.0000
X2: Reaction time (min)	1039.28	1	1039.28	120.68	0.0000
X3: Reaction temperature (°C)	150.985	1	150.985	17.53	0.0041
$X_1 X_1$	0.0153223	1	0.0153223	0.00	0.9675
$X_1 X_2$	28.125	1	28.125	3.27	0.1137
$X_1 X_3$	10.125	1	10.125	1.18	0.3142
$X_2 X_2$	11.8163	1	11.8163	1.37	0.2798
$X_2 X_3$	1.125	1	1.125	0.13	0.7284
$X_3 X_3$	143.872	1	143.872	16.71	0.0046
Total error	60.2828	7	8.61183		
Total (corr.)	2417.76	16			

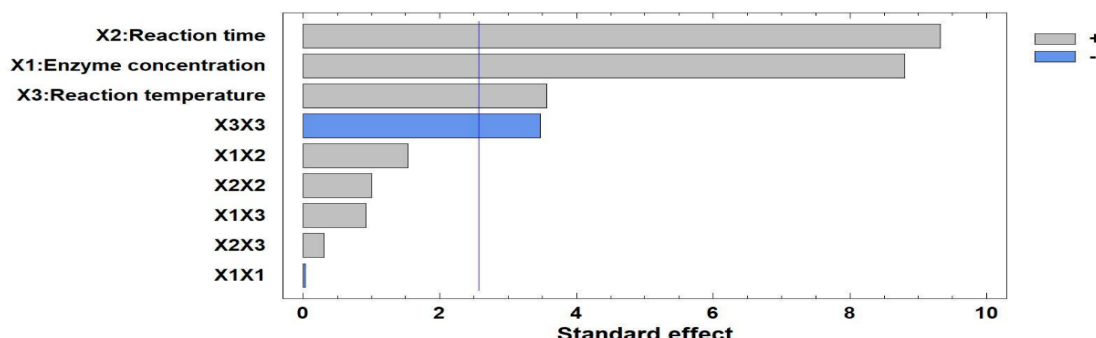


Figure 2. Pareto diagram for the reduction in dextran content (%).

However, Figure 3 shows the contour surfaces that allow the determination of the conditions that maximize the RDC value (%) obtained in a) an enzyme concentration between 340 and 350 ppm and a reaction time between 44 and 46 min; in b) an enzyme concentration between 340 and 350 ppm and a reaction temperature between 43 and 45 °C; and in c) a reaction temperature between 45 and 47 °C and a reaction time between 44 and 46 min. Likewise, Figure 4 shows the response surfaces obtained for the RCCD of the present study, which show that a) an increase in the enzyme concentration and the reaction time favors the RDC (%) within the limits studied; in b) an increase in the temperature favors the RDC (%) up to a certain point, from which it decreases, it is also confirmed that an increase in the reaction time favors the hydrolysis reaction; in c) the effect of the reaction temperature and the enzyme concentration is confirmed.

Also, using statistical software, the conditions that optimize the reduction of the dextran content (95.51 %) were determined to be an enzyme concentration of 340 ppm, a reaction time of 45 min, and a reaction temperature of 45 °C.

On the other hand, it has been reported that a higher enzyme loading and also longer residence times favor the catalytic process of dextranases (Eggleston & Monge, 2005; Ninchan *et al.*, 2017) since there is a greater number of active sites in contact with the substrate that operate for a longer time. It should also be considered that a lower enzyme concentration leads to a greater amount of water available to deactivate and denature the enzymatic protein structure and increase its conformational mobility, which can lead to a loss of enzymatic activity (Eggleston *et al.*, 2011).

Regarding the effect of temperature, Eggleston & Monge (2005) reported that a temperature range between 43.3 and 54.4 °C promotes the enzymatic activity of dextranases acting on dextran (500 ppm), and Ninchan *et al.* (2017) reported that the activity of a dextranase from *Chaetomium* sp. acting on dextran (1500 ppm) is efficient at a temperature range between

35 and 55 °C, and temperatures higher than these ranges have a negative effect on hydrolysis, which is consistent with that reported in the present investigation. Similarly, it has been reported that temperatures above 65 °C promote enzyme denaturation and the partial loss of its activity (Eggleston *et al.*, 2011).

Hence, the conditions that optimize the DRC (95.51 %) are 340 ppm enzyme concentration, 45 min reaction time, and 45 °C reaction temperature. Although in general, the response surface analysis gives a high value of dextran reduction, these conditions of a long time and high temperature are not favorable for the sugar elaboration process.

Validation of the Optimization Parameters

For the purposes of this study, the results of process optimization were validated by the response surface methodology, considering a target RDC of 70 % in sugarcane juice, for which the results obtained by the statistical software for this condition were enzyme concentration of 224 ppm, a reaction time of 32 min, and a reaction temperature of 36 °C.

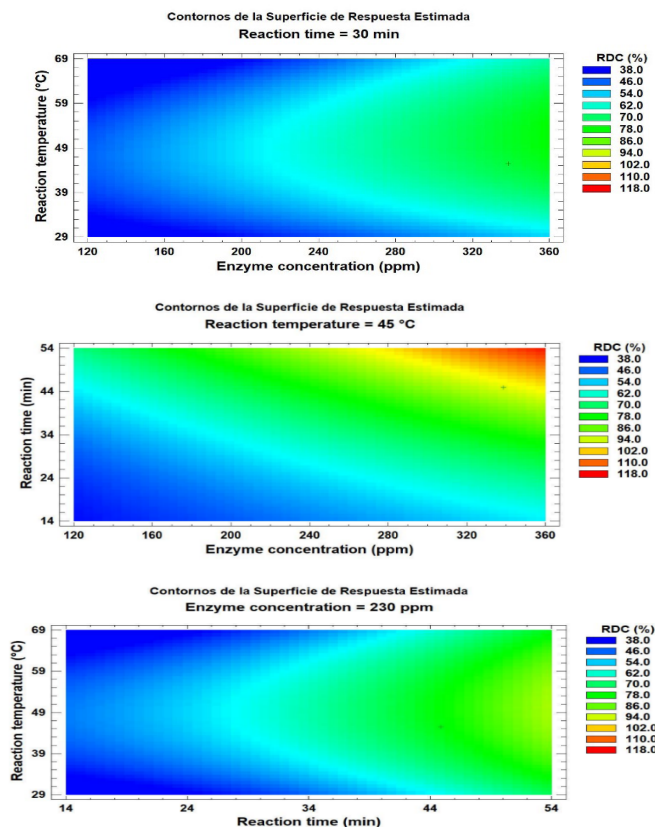


Figure 3. Contour surfaces for the reduction in the dextran content reduction (%).

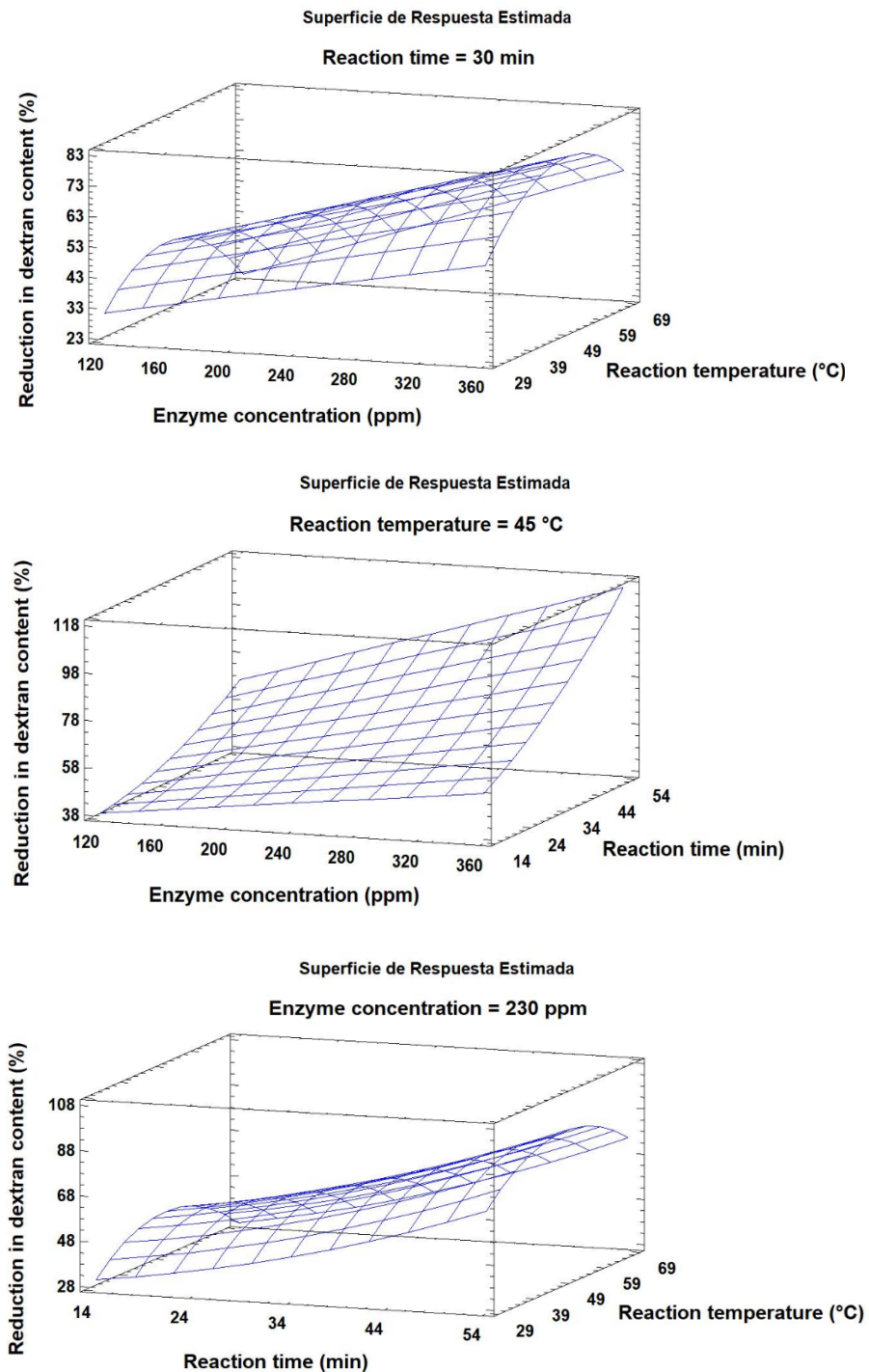


Figure 4. Response surfaces for the dextran content reduction (%).

Table 4 shows the composition of the sugarcane juice used before (base) and after enzymatic hydrolysis (hydrolysate). Also, the mean absolute deviation value was determined for the DRC value obtained according to the methodology of Baş & Boyaci (2007), which is 11.61 %, indicating the good fit of the model to the experimental data. As for the RDC, a value of 69.2 % was obtained, which is consistent with the value reported by Oropeza *et al.* (2019), who studied the enzymatic hydrolysis process of dextran in the fermented mash (with an initial dextran content of 353 ppm) using the Dextranfree enzyme with an enzyme:substrate ratio of 1:10 at 40 °C, pH 5.17, for 180 min. Thus, it is also stated that sugarcane juice is a better substrate than fermented mash since similar results were obtained with a shorter hydrolysis time. Likewise, it has been reported that dextranase activity is stable up to 25-30°Brix and values above this range negatively affect its activity (Oropeza *et al.*, 2019), so the 20.42°Brix condition of sugarcane juice used in the present study should not have inhibited dextranase during dextran hydrolysis. In addition, enzymatic dextran hydrolysis is known to increase the level of reducing sugars and cause a decrease in solution viscosity (Bashari *et al.*, 2013; Oropeza *et al.*, 2019). The above was confirmed in the present study because, as shown in Table 3, the levels of reducing sugars and the viscosity value in sugarcane juice increased by 30 % and decreased by 10 %, respectively, after hydrolysis.

Table 4. Physicochemical characteristics of sugar cane juice before (base) and after enzymatic hydrolysis (hydrolysate)

Parameter	Sugarcane juice	Hydrolyzed juice
Dextran (ppm)	227 ± 12	70 ± 4
°Brix	20.4 ± 0.5	20.5 ± 0.1
Reducing sugars (g/L)	0.23 ± 0.01	0.30 ± 0.01
Viscosity (cP)	0.01 ± 0	0.009 ± 0
pH	5.4 ± 0.5	5.4 ± 0.5

The increase in reduced sugar content is due to the formation of isomalto-oligosaccharides with two to five glycosidic units, products of hydrolysis (Eggleston *et al.*, 2011; Khalikova *et al.*, 2005); while the decrease in viscosity content is a consequence of molecular fractionation of dextran present in sugarcane juice (Eggleston *et al.*, 2011; Ninchan *et al.*, 2017).

Conclusions

The volumetric and specific activities of the Dextranfree Xe enzyme were 0.42 ± 0.04 (DU/mL) and 4.7 ± 0.47 (DU/mg), K_m was 0.215 g/L and V_{max} was 0.007 g/L*min. The pivotal factors were enzyme concentration, reaction time, and temperature were relevant to the enzymatic hydrolysis process of dextran, it is suggested for the reduction of dextran content optimization by 95.51 % are 340 ppm, 45 min, and 45 °C, respectively. Finally, it was determined that the conditions that achieved a reduction in dextran content with a statistically inferential target value of 70 % in sugarcane juice were: enzyme concentration 224 ppm, time 32 min, and reaction

temperature 36 °C. With these parameters, the validation of the hydrolysis in sugarcane juice resulted in a 69.2 % dextran reduction.

Contribución de los autores

“Work Conceptualization, C.C.C., K.S.G., A.C.C.; methodology development, C.C.C., K.S.G., A.C.C.; software management, J.D.B., C.C.C., K.S.G.; experimental validation, C.C.C., K.S.G., A.C.C.; results analysis, C.C.C., K.S.G., A.C.C., J.D.B.; data management, C.C.C., K.S.G., A.C.C., J.D.B.; writing and preparation of the manuscript, C.C.C., K.S.G., A.C.C., J.D.B.; writing, proofreading and editing, C.C.C., K.S.G., A.C.C., J.D.B.

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

- Balakishnan, R., Mohan, N., & Sivaprakasam, S. (2022). Application of design of experiments in bioprocessing: process analysis, optimization, and reliability. *Current Developments in Biotechnology and Bioengineering*, 289-319. <https://doi.org/10.1016/B978-0-323-91167-2.00013-7>
- Bashari, M., Lagnika, C., Ocen, D., Chen, H., Wang, J., Xu, X., & Jin, Z. (2013). Separation and characterization of dextran extracted from deteriorated sugarcane. *International Journal of Biological Macromolecules*, 59, 246-254. <https://doi.org/10.1016/j.ijbiomac.2013.04.046>
- Baş, D., & Boyacı, I.H. (2007). Modeling and optimization I: Usability of response surface methodology. *Journal of Food Engineering*, 78, 836–845. <http://dx.doi.org/10.1016/j.jfoodeng.2005.11.024>
- Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 48-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Bourne, E.J., Hutson, D. H., & Weigel, H. (1962). Studies on dextrans and dextranases 2. The action of mould dextranases on modified isomaltodextrins and the effect of anomalous linkages on dextran hydrolysis. *Biochemical Journal*, 85 (1), 158-163. <https://doi.org/10.1042/>

[bj0850158](#)

- Comisión Nacional de Normalización [CNM]. (2011). Industria azucarera y alcoholera - Determinación de grados brix en jugos de especies vegetales productoras de azúcar y materiales azucarados - Método del refractómetro (Norma Mexicana NMX-F-436-SCFI-2011). <http://www.economia-nmx.gob.mx/normas/nmx/2010/nmx-f-436-scfi-2011.pdf>
- Eggleston, G., Dilks, A., Blowers, M., & Winters, K. (2011). Successful application of dextranase in sugar beet factories. In: Proceedings of the American Society of Sugarbeet Technologists meeting, 1-16. <https://www.ars.usda.gov/research/publications/publication/?seqNo115=266696>
- Eggleston, G., & Monge, A. (2005). Optimization of sugarcane factory application of commercial dextranases. *Process Biochemistry*, 40(5), 1881–1894. <https://doi.org/10.1016/j.procbio.2004.06.025>
- International Commission for Uniform Methods of Sugar Analysis [ICUMSA]. (2011). The determination of dextran in sugars by a modified alcohol. Haze Method Official (Method GS1/2/9-15). <https://www.icumsa.org/index.php?id=835>
- International Commission for Uniform Methods of Sugar Analysis [ICUMSA]. (2007). The determination of pH by a direct method – in raw sugar, molasses, juices and syrups – Official – in white sugar, speciality sugars and plantation white sugar (Method GS1/2/3/4/7/8/9-23, 2007). <https://www.icumsa.org/index.php?id=935>
- Khalikova, E., Susi, P., & Korpela, T. (2005). Microbial dextran-hydrolyzing enzymes: fundamentals and applications. *Microbiology and Molecular Biology Reviews*, 69(2), 306–325. <https://doi.org/10.1128/MMBR.69.2.306-325.2005>
- Li, K., Lu, H., Hang, F., Li, S., & Liu, J. (2016). Improved dextranase production by *Chaetomium gracile* through optimization of carbon source and fermentation parameters. *Sugar Tech*, 19, 432-437. <https://doi.org/10.1007/s12355-016-0476-4>
- Li, Y., Luo, J., & Wan, Y. (2020). Biofouling in sugarcane juice refining by nanofiltration membrane: Fouling mechanism and cleaning. *Journal of Membrane Science*, 612, 118432. <https://doi.org/10.1016/j.memsci.2020.118432>
- Lira, E., & Jasso, R. (2013). Comparación de los diferentes métodos de análisis cinéticos para determinar el tipo de inhibición de dos compuestos. *Revista de Educación Bioquímica*, 32(1), 19-32. Recuperado en 29 de Noviembre de 2022, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1665-19952013000100004&lng=es&tlng=es
- Liu, J., Sun, Q., Yin, H., Wang, L., Wei, H., Li, K., & Hong, F. (2019). Optimal Fermentation of *Saccharomyces cerevisiae* Expressing a Dextranase from *Chaetomium gracile*. *Sugar Tech*, 22, 171-178. <https://doi.org/10.1007/s12355-019-00746-5>
- Lu, Y., Thomas, L., & Schmidt, S. (2017). Differences in the thermal behavior of beet and cane sucrose sources. *Journal of Food Engineering*, 201, 57-70. <https://doi.org/10.1016/j.jfoodeng.2017.01.005>
- Martínez, D., Menéndez, C., Chacón, O., Fuentes, A., Borges, D., Sobrino, A., Ramírez, R., Pérez, E., & Hernández, L. (2021). Removal of bacterial dextran in sugarcane juice by *Talaromyces maritimus* dextranase expressed constitutively in *Pichia pastoris*. *Journal of Biotechnology*, 333, 10-20. <https://doi.org/10.1016/j.jbiotec.2021.04.006>
- Miller, G. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31 (3), 426-428. <https://doi.org/10.1021/ac60147a030>
- Misra, V., Solomon, S., Hashem, A., Abd Allah, E.F., Al-Arjani, A.F., Mall, A.K., Prajapati, C.P., & Ansari, M.I. (2020a). Minimization of post-harvest sucrose losses in drought affected sugarcane using chemical formulation. *Saudi Journal of Biological Sciences*, 27(1), 309–317. <https://doi.org/10.1016/j.sjbs.2019.09.028>

- Misra, V., Solomon, S., Malla, A.K., Prajapati, C.P., & Ansari, M.I. (2020^b). Impact of chemical treatments on *Leuconostoc* bacteria from harvested stored cane/stale cane. *Biotechnology Reports*, 27. <https://doi.org/10.1016/j.btre.2020.e00501>
- 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 Industrial Biotechnology*, 1(1), 8-19. <https://www.alliedacademies.org/abstract/investigation-of-the-optimized-dextran-degrading-enzyme-conditions-on-the-decomposition-of-different-molecular-weights-of-pure-dext-6760.html>
- Oropeza, E., López, L., Luna, G., Urrea, G., & Cantú, D. (2019). Hidrolisis de dextrana y su reología en mostos provenientes del proceso de producción de bioetanol. *Revista Mexicana de Ingeniería Química*, 18(2), 543-554. <https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n2/Oropeza>
- Petit, H. (2021). Efecto del *Leuconostoc mesenteroides* en la caña de azúcar para la industria azucarera. *Revista de Ciencia y Tecnología Agrollanía*, 20, 23-30. <http://bdigital2.ula.ve:8080/xmlui/bitstream/handle/654321/5802/Art13.pdf?sequence=1&isAllowed=y>
- Sufiate, B.L., Soares, F.E.F., Gouveia, A.S., Moreira, S.S., Cardoso, E.F., Tavares, G.P., Braga, F.R., De Araujo, J.V., & De Queiroz, J.H. (2018). Statistical tools application on dextranase production from *Pochonia chlamydosporia* (VC4) and its application on dextran removal from sugarcane juice. *Annals of the Brazilian Academy of Sciences*, 90(1), 461-470. <https://doi.org/10.1590/0001-3765201820160333>
- Tena, M., & Jorrián, J. (2000). Estudio cinético de la actividad invertasa de levadura de panadería. Departamento de Bioquímica y Biología Molecular. Campus Universitario de Rabanales. Córdoba, España.