Kinetics of the hydrolysis of sorghum flour with two Amilaceouos enzymes

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Cinética de la hidrólisis de harina de sorgo con dos enzimas amiláceas

Cinètica de la hidròlisi de farina de sorgament amb dos enzims amilàcies

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ABSTRACT

In the production process of glucose syrup, the conversion of corn starches to sugars occurs through acid or enzymatic hydrolysis. Corn has been the traditional raw material in its preparation. The use of other cereals, such as sorghum, is reported for its similar properties to corn. The objective of the work is to study the kinetic behavior in the hydrolysis of sorghum flour, using two enzymes: Bialfa-T and Glucozyme 2X in the production of syrup.

The sorghum used was UDG 110. Two experimental screening designs 2^2 to study the effect of enzymes on sorghum flour were performed. The variables studied were: substrate concentration and enzyme concentration and as response variables reducing sugars (RS) (g/L), conversion (%) and enzymatic activity (UI).

The regression models for the three response variables were significant for the Bialfa-T enzyme, while for Glucozyme 2X, only the enzymatic activity model was significant, since this enzyme acts on already transformed starches and the variable with the highest influence turned out to be the substrate concentration, followed by the enzyme concentration.

Keywords: activity; glucose syrup; hydrolysis; reducing sugar.

RESUMEN

En el proceso productivo de los jarabes glucosados, ocurre la conversión de los almidones del maíz a azúcares, mediante hidrólisis acida o enzimática. El maíz ha sido la materia prima tradicional en su elaboración, aunque se reporta el empleo de otros cereales, como el sorgo, por sus propiedades similares al maíz; por lo que el objetivo del trabajo, es estudiar el comportamiento cinético en la hidrólisis de una harina de sorgo, empleando dos enzimas: Bialfa-T y Glucozyme 2X en la producción de jarabes.

El sorgo empleado fue el UDG 110. Se utilizaron dos diseños experimentales de cribado $2^{2,}$ para estudiar el efecto de las enzimas, sobre la harina de sorgo. Las variables estudiadas fueron: concentración de sustrato y concentración de enzima y como variables respuesta azúcares reductores AR (g/L), conversión (%) y actividad enzimática (UI).

Los modelos de regresión para las tres variables respuesta fueron significativos para la enzima Bialfa–T, mientras que para la Glucozyme 2X, solamente el modelo de actividad enzimática resultó significativo, pues esta enzima actúa, sobre los almidones ya transformados y la variable con mayor influencia resultó ser la concentración de sustrato, seguida por la concentración de la enzima.

Palabras clave: actividad; azúcares reductores; hidrólisis; jarabes glucosados.

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RESUM

En el procés productiu dels xarops glucosats, ocorre la conversió dels midons del blat de moro a sucres, mitjançant hidròlisi àcida o enzimàtica. El blat de moro ha estat la matèria primera tradicional en la seva elaboració, encara que es reporta l'ús d'altres cereals, com el melca, per les seves propietats similars al blat de moro; per tant, l'objectiu del treball és estudiar el comportament cinètic en la hidròlisi d'una farina de melca, emprant dos enzims: Bialfa-T i Glucozyme 2X en la producció de xarops.

El sorgo emprat va ser el UDG 110. Es van utilitzar dos dissenys experimentals de cribratge 2^2, per estudiar l'efecte dels enzims, sobre la farina de sorgo. Les variables estudiades van ser: concentració de substrat i concentració d'enzim i com a variables resposta sucres reductors AR (g/L), conversió (%) i activitat enzimàtica (UI).

Els models de regressió per a les tres variables resposta van ser significatius per a l'enzim Bialfa–T, mentre que per a la Glucozyme 2X, només el model d'activitat enzimàtica va resultar significatiu, ja que aquest enzim actua, sobre els midons ja transformats i la variable amb més influència va resultar ser la concentració de substrat, seguida per la concentració de l'enzim.

Paraules clau: activitat, sucres reductors, hidròlisi, xarops glucosats

IINTRODUCTION

Corn starch has always been the raw material par excellence in the production of glucose and fructose syrup, several authors[1,2] report the use of other starch-rich cereals in its production. Since the 1950s, sorghum has been seen as an alternative to replace corn in starch production, although it has several disadvantages, such as the presence of a peripheral endosperm that acts as a barrier against the penetration of the soaking solution, a matrix tougher, cross-linked protein that surrounds starch granules; however, it has a high potential for grain production, rich in antioxidants, with an acceptable protein value and highly assailable by the human body. It tolerates heat and salinity and can grow in a wide variety of soils with a limited supply of nutrients, as Dendy[3] points out, so it has good prospects for contributing to the development of agriculture. As it is gluten-free and a rich source of vitamins, minerals and carbohydrates, it offers a solution to the growing demand for food for people with celiac disease, a major health problem in many countries[4,5].

Sorghum starch can be used interchangeably with corn starch, because both have nearly identical viscoamylographic properties[6,7]. It is composed of 70-80% amylopectin and 20-30% amylose, this proportion being influenced by environmental and genetic factors that affect its physical-chemical and functional properties[8].

In the process of obtaining the syrup, the native starch is subjected to a modification of its structure, through physical, chemical or enzymatic methods, improving its properties and thus increasing its added value in the final product [9]. Currently, enzymes are mainly used in technical applications, for the catalysis of processes related to the food, cosmetic, pharmaceutical, chemical synthesis, research and development industries[9,10,11]. In syrup, the starch transformation process known as hydrolysis is applied to reactions where water performs a double decomposition with another compound[12]. Starch, through acid solutions or catalyzed by enzymes, gives rise to the progressive formation of different sugars[13]. Enzymes are effective catalysts in a process, but few are directly involved in the interaction with the substrate or in the catalysis of the reaction. Among its properties are stability, which translates into a total or partial loss of its activity, which is its essential property and which expresses the amount of substrate converted per unit of time, taking into account the reaction volume, the other more. The outstanding feature of enzymes is their high specificity with respect to the substrate, depending on whether the transformations to be achieved in it are of a physical or chemical type[14]. In starches, the hydrolysis reaction is endothermic and first order. The temperature must be controlled to avoid the formation of undesirable substances due to collateral reactions; although it is not the only variable of great weight in this process, but there are others such as pH, enzyme concentration, substrate concentration, reaction time[15]. Enzymatic kinetics studies the speed of reactions catalyzed by enzymes. This can be evaluated by measuring the appearance of the products or the disappearance of the reactants[16]. Having the possibility of using sorghum in research and taking into account the reported studies[12,17], using sorghum, other authors[18] studied the possibility of eliminating the first stage of starch extraction in the production of glucose syrup, starting from the hydrolysis of a white sorghum flour, obtaining a product with good acceptance, like the one traditionally obtained with corn, however, the kinetic studies of enzymatic hydrolysis had not been carried out, therefore, the main objective of this work is to study the kinetic behavior in the hydrolysis of a sorghum flour, using two enzymes: Bialfa-T and Glucozy 2X

MATERIALS AND METHODS

The fundamental raw materials to be used were white sorghum (UDG-110) in the form of flour, in solutions of 15g/L, 25g/L and 30g/L respectively, and the enzymes Bialfa T, and Glucozyme 2X, for hydrolysis, provided by the UEB Gydema of Cienfuegos. The enzyme/substrate ratios used were 0.6; 1.2 and 2.4 g enzyme/100 g substrate, respectively. In addition, Ca $(OH)_2$ was added at a ratio of 0.1% weight with respect to the mass of enzyme. The solutions were prepared and the pH was adjusted with acetate buffer solutions, it is prepared with equal concentrations of acetic acid and sodium acetate[19], for the optimal working conditions of the enzymes. We worked with an IKA RET brand stove for Bialfa T and a thermostatted bath for Glucozyme 2X to control temperatures. Once the operating conditions were set, the enzymes corresponding to

each experiment were added. Samples were taken at time intervals of 5; 10; twenty; 30 and 50 minutes respectively. Reducing Sugars (RS) were determined by the 3-5 Dinitro Salicylic method, to measure the glucose formed, the conversion and the activity of the enzyme on this substrate, was determined from the expression described in equation 1

$$A = \frac{r_0 * V}{MW} \; o \; A = \frac{RS * V}{MM * t} \qquad Eq.\ (1)$$

Where: A: is the activity of the enzyme against the substrate studied and is given in IU: 1 µmol converted substrate x min-1, ro: initial reaction rates expressed in g/L*min. (These values correspond to the slope of the line obtained by plotting RS Vs Time, taken for the first 5 minutes of reaction), V: volume of solution expressed in liters (L), Molecular weight of glucose MW:1,80*10^{-4} g/µmol, and t: time in minutes.

To see the influence of these two variables on the response variables in the experimental development, two screening designs 2^2 were carried out with a replica, one experiment for each enzyme, using two levels for each variable, substrate concentration [S] in levels of 15-30 g/L and enzyme concentration [E] in 0,6-2,4 g Enz/100 g subs and three response variables, ART, conversion and enzyme activity, were set against the studied substrate. The experimental matrix is shown in Table 1 and the results were processed using Statgraphics Centurion XV Software.

Table 1. *Experimental matrix used for the design.*

Experiments	[S](g/L)	[E]g_{enzyme}/100$g_{substrate}$
1	15	0,6
2	30	0,6
3	15	2,4
4	30	2,4
5	15	0,6
6	30	0,6
7	15	2,4
8	30	2,4

RESULTS AND DISCUSSION

Influence of substrate and enzyme concentrations on hydrolysis.

The behavior profile of the RS as a function of time for each enzyme is shown in Figures 1 and 2.

Table 2. *Initial rates as a function of substrate and enzyme concentrations*

[S]% w/w	[E] Bialfa-T% w/w			[E] Glucozyme 2X% w/w		
	0,6	1,2	2,4	0,6	1,2	2,4
15	0,86	0,86	1,15	0,104	0,188	0,527
25	1,15	1,214	1,80	0,260	0,322	0,618
30	1,61	1,46	2,2	0,335	0,409	0,726

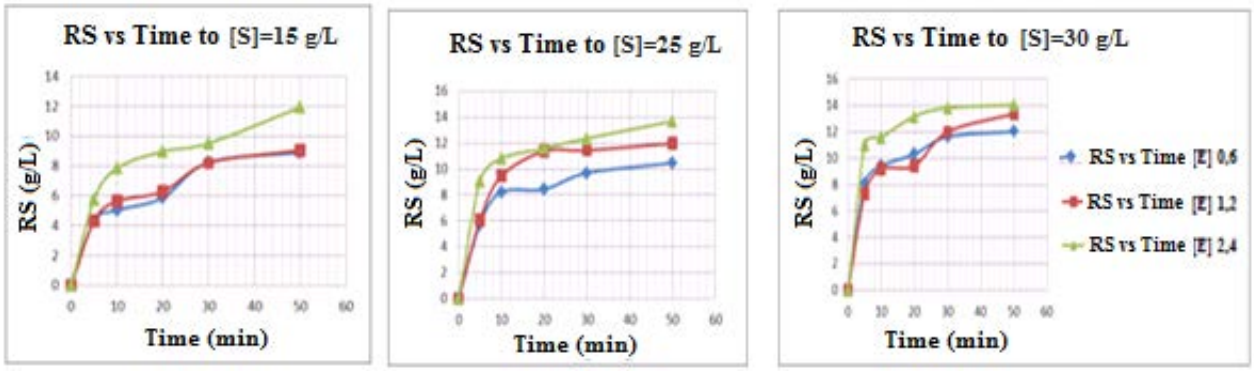

Figure 1 *Glucose production profile using Bialfa-T*

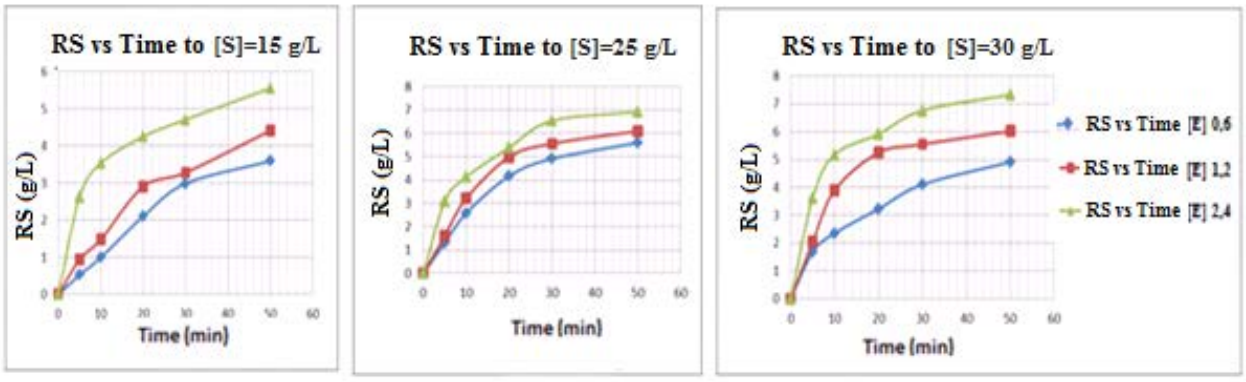

Figure 2 *Glucose production profile using Glucozyme 2X.*

Making a comparison between the two enzymes according to Table 2, it can be seen that the initial speeds obtained when hydrolyzing sorghum flour using Bialfa-T are higher than those obtained when using Glucozyme 2X. This is due, as some authors suggest[17,18], that glucoamylases, act on starches already transformed into maltoses or maltodextrins, acting directly on the α 1-6 bonds while alpha-amylases act only on the α 1-4 bonds, there could also be a synergism between the components of this enzyme, or a competitive inhibition of the product as proposed by these authors.

Results of the experimental design for hydrolysis taking the concentrations of substrate and enzymes as independent variables

Using the enzyme Bialpha-T.

Once each of the response variables, ART, conversion and enzymatic activity in IU have been determined, a summary of the results for the Bialfa-T enzyme is shown in Table 3, analyzing the influence of the two variables studied on the responses according to the design of experiments.

Table 3. *Hydrolysis results for Bialpha-T*

Experiments	[S] g/L	[E] % w/w	RS g/L	Conversion %	Enz Activity(UI)
1	15	0,6	8,89	59,29	476,69
2	30	0,6	12,04	80,27	896,53
3	15	2,4	11,92	79,48	638,11
4	30	2,4	14,06	93,73	1221,61
5	15	0,6	9,44	62,46	458,51
6	30	0,6	12,38	82,35	908,78
7	15	2,4	9,85	77,57	641,21
8	30	2,4	13,98	92,64	1221,6

Statistical analysis of the results using Bialfa-T.

With the results of Table 3, processing is done, where the influence of the independent variables on the response variables in the Pareto diagrams can be seen, figure 3, the models are represented in equations 2, 3 and 4, all with R2 greater than 90%

$$\mathbf{RS} = 11{,}57 + 1{,}54 \cdot \text{Subst Conc} \quad (2)$$

$$\mathbf{Conversion} = 78{,}47 + 8{,}77.\text{Subst Conc} + 7{,}38.\text{Enz Conc} \quad (3)$$

$$\mathbf{Enz\ Act.}\ 808{,}72 + 255{,}09.\text{Subst Conc} + 123{,}59.\text{Enz Conc} + 37{,}56.\text{Subst Conc.Enz Conc} \quad (4)$$

As can be seen in Pareto charts (figure 3) and in equations 2, equations 3 and equations 4, the most influential in RS variable was the substrate concentration and the only significant one; in conversion the two studied variables were significant as well as in enzyme activity, in this one, the interaction between the two variables is also significant. In results reported [18], who worked with different concentrations of flour and enzymes, a significant influence of the Bialfa T enzyme on the ART is observed, even for medium concentrations of the enzyme, so it could be said that for the studied substrate concentrations, the reaction behaves as a first-order kinetic process, where t rate depends only on the concentration of substrate, so there is free enzyme that is not part of the enzyme-substrate complex (ES), which is the one that gives rise to the formation of the product.

Results of the experimental design for hydrolysis using Glucozyme 2X

In the same way, it is proceeds for Glucozyme enzyme. The results of the response variables are shown in table 4.

Table 4. *Hydrolysis results for Glucozyme 2X.*

Experiments	[S] g/L	[E] % w/w	RS (g/L)	Conversion %	Enz Activity (UI)
1	15	0,6	5,556	37,04	58,19
2	30	0,6	3,592	23,94	292,85
3	15	2,4	4,909	32,73	186,58
4	30	2.4	7,331	48,87	403,30
5	15	0,6	7,93	49,87	59,22
6	30	0,6	5,009	34,73	295,74
7	15	2,4	3,672	22,95	184,13
8	30	2,4	5,356	36,04	409,16

Statistical analysis of hydrolysis results using Glucozyme 2X

With the results obtained in table 4, statistical processing was carried out. None of two independent variables were significant for RS and conversion. For the enzymatic activity the two variables and their interaction were significant, so only the model for this

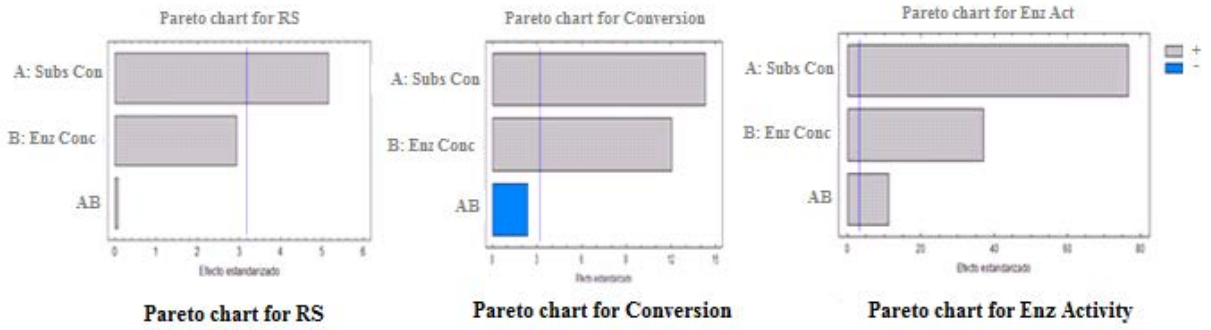

Figure 3 *Pareto chart for the response variables with Bialfa T.*

variable is reported in equation 5. The highest influence is also exerted by the substrate concentration.

$$\textbf{Enz Act} = 236{,}15 + 114{,}12\ .\ \text{Subst Conc} + 59{,}65.\ \text{Enz Conc} - 3{,}68.\ \text{Subst. Conc. Enz Conc}\quad R^2 = 99{,}97\% \qquad (5)$$

CONCLUSIONS

1. There is a marked influence of the variables enzyme concentration [E] and substrate concentration [S], on the initial rate of conversion, in the hydrolysis of sorghum starch flour (native), being higher for the higher concentration of both variables and for the enzyme Bialfa T.

2. Statistical models obtained for the response variables RS, conversion and enzymatic activity, were significant when Bialfa-T is used, while for Glucozyme 2X the only significant model was that of enzymatic activity and the greatest effects are due to the substrate concentration [S], for both enzymes.

3. Both enzymes have effective activity on sorghum flour, so they can be used to obtain dextrose syrup.

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