L-ARABINOSE PRODUCTION BY HYDROLYSIS OF MESQUITE GUM BY A CRUDE EXTRACT WITH α-L-ARABINOFURANOSIDASE ACTIVITY FROM *Aspergillus niger*

PRODUCCIÓN DE L-ARABINOSA A PARTIR DE LA HIDRÓLISIS DE LA GOMA DE MEZQUITE POR UN EXTRACTO CRUDO CON ACTIVIDAD α-L-ARABINOFURANOSIDASA DE *Aspergillus niger*

J. M. Loeza-Corte[1], J. R. Verde-Calvo[1], F. Cruz-Sosa[1], E. J. Vernon-Carter[2] and S. Huerta-Ochoa[1*]

[1]*Planta Piloto de Fermentaciones, Departamento de Biotecnología, Universidad Autónoma Metropolitana Iztapalapa. San Rafael Atlixco 186 Col. Vicentina, C.P. 09340, México D.F., México.*
[2]*Laboratorio de Bioprocesos, Departamento de Ingeniería de Procesos e Hidráulica, Universidad Autónoma Metropolitana Iztapalapa. San Rafael Atlixco 186 Col. Vicentina. C.P. 09340, México D.F., México.*

Received 25th, April 2007; Accepted 11th, October 2007

Abstract

A crude enzymatic extract from *Aspergillus niger* 10 with α-L-arabinofuranosidase activity (EC 3.2.1.55) was obtained and its effect on the hydrolysis of mesquite gum was determined and compared to that of a commercial α-L-arabinofuranosidase from *A. niger*. The growth parameters of *A. niger* 10 obtained were X_{max} = 3.03 g L^{-1} and μ_{max} = 0.07 h^{-1}. The maximum enzymatic activity obtained was 65.93 U L^{-1}. Optimum temperature and activation energy for the crude extract were 50°C and 46.15 KJ mol^{-1} and for the commercial enzyme 40 °C and 52.76 KJ mol^{-1}, respectively. The apparent kinetic parameters K_m and V_{max} for the crude extract were 4.87 g L^{-1} and 0.15 μmol min^{-1} g^{-1}, and for the commercial enzyme 76.45 g L^{-1} and 3.85 μmol min^{-1} g^{-1}, respectively. Yields of L-arabinose recovery for the crude extract and the commercial enzyme were 17.04 % and 2.78 %, respectively, based on the reported average content of L-arabinose in mesquite gum.

Keywords: α-L-arabinofuranosidase, *Aspergillus niger*, mesquite gum, L-arabinose, enzymatic hydrolysis.

Resumen

Se obtuvo un extracto enzimático crudo a partir de *Aspergillus niger* 10 con actividad α-L-arabinofuranosidasa (EC 3.2.1.55) y fue comparado con una α-L-arabinofuranosidasa comercial sobre la hidrólisis enzimática de la goma de mezquite. Los parámetros de crecimiento obtenidos para *A. niger* 10 fueron: X_{max} = 3.03 g L^{-1} y μ_{max} = 0.07 h^{-1}. La máxima actividad enzimática obtenida fue 65.93 U L^{-1}. La temperatura óptima y energía de activación para el extracto crudo fueron de 50°C y 46.15 KJ mol^{-1}, mientras que para la enzima comercial fueron de 40°C y 52.76 KJ mol^{-1}. Los parámetros cinéticos de la hidrólisis $K_{m\text{-}app}$ y $V_{max\text{-}app}$ con el extracto crudo fueron de 4.87 g L^{-1} y 0.15 μmol min^{-1} g^{-1}, y para la enzima comercial fueron de 76.45 g L^{-1} y 3.85 μmol min^{-1} g^{-1}, respectivamente. Los rendimientos de L-arabinosa con el extracto crudo y la enzima comercial fueron de 17.04 % y 2.78 %, respectivamente, basados en lo reportado para el contenido promedio de L-arabinosa en la goma de mezquite.

Palabras clave: α-L-arabinofuranosidasa, *Aspergillus niger*, goma de mezquite, L-arabinosa, hidrólisis enzimática.

1. Introduction

Mesquite gum is an exudate produced by the trunk and branches of *Prosopis* spp trees, which are extensively distributed in the arid and semi-arid lands of Mexico (Vernon-Carter *et al*., 2000). The genus is used for a variety of purposes and has a long tradition of use where it grows (Felker, 1993). Mesquite is of great economic importance to the inhabitants in this region as it is a source of food, forage, fuel, construction material, and even medicine. Furthermore, this species has great ecological value because it helps to control erosion and to improve soil fertility. However, many inhabitants where this resource grows live in conditions of extreme poverty, and in order to obtain a livelihood they deforest mesquite trees for forage, wood and charcoal, which on turn has caused an irreversible loss of genetic diversity (Buendía-González *et al*., 2007). Thus, an ongoing effort is

* *Corresponding author: E-mail: sho@xanum.uam.mx*
Phone (+52) 5558044999, fax: (+52) 5558044712

Publicado por la Academia Mexicana de Investigación y Docencia en Ingeniería Química A.C.

seeking for new technological alternatives that include the non-destructive exploitation of this resource in hope that it will provide the inhabitants of these regions with economical benefits, thus avoiding its deforestation (Vernon-Carter *et al*., 2000). One of the most important aspects of *P. laevigata*, which provides it with an added value, is that it produces a gum that has similar chemical composition (Orozco-Villafuerte *et al*., 2003) and physicochemical properties as those exhibited by gum Arabic. In general terms, it may be stated that mesquite gum is the neutral salt of a complex acidic branched polysaccharide formed by a core of β-D-galactose residues (43.3 %), comprising a (1-3)-linked backbone with (1-6)-linked branches (Vernon-Carter *et al*., 2000), bearing L-arabinose (40.4 %), L-rhamnose (1.3 %), β-D-glucuronate (16.2 %) (Anderson and Weiping, 1989; Vernon-Carter *et al*., 2000; Orozco-Villafuerte *et al*., 2003), and 4-O-methyl-β-D-glucuronate (Anderson and Weiping, 1989; Vernon-Carter *et al*., 2000) as single sugar or oligosaccharide side chains. Mesquite gum also contains a small amount of protein (0.7-5.8 %), which may be central to the overall primary structure branches (Vernon-Carter *et al*., 2000).

Purified L-arabinose has potential and current industrial applications. The sweet taste of L-arabinose is similar to that of sucrose, but approximately half the sweetness. Naturally occurring arabinose is the L-form, and it is not metabolized in animals; thus it is a non-caloric sugar. Furthermore, it strongly inhibits uncompetitively intestinal sucrase and consequently inhibits the absorption of sucrose from the small intestine. The addition of 2-3% L-arabinose to sucrose causes about a 60 % reduction of the digestion of sucrose in the small intestine (Susumu, 1999). L-arabinose is widely used in the development of antiviral agents such as nucleoside analogues, and is a raw material in the manufacture of L-ribose, which is used in for pharmaceutical applications such as synthetic oligonucleotides (Danisco, 2007). Simultaneous co-utilization of L-arabinose and D-xylose with recombinant strains of *S. cerevisae* improved bioethanol production from lignocellulosic biomass (Karhumaa *et al*., 2006).

L-arabinose has been obtained by mild acid hydrolysis of mesquite gum with 75.0 % (White, 1947) and 40.4 % yields (Orozco-Villafuerte *et al*., 2003) based on the maximum theoretical yield obtainable, estimated from the approximate chemical composition of the gum used in the experiments.

α-L-Arabinofuranosidase and other arabinases have been used to produce L-arabinose through enzymatic hydrolysis of complex polysaccharides. Apple juice ultrafiltration retentate arabinan was hydrolyzed by a commercial preparation of Pectinase 29 with a yield of 70.0 % (Rombouts *et al*., 1988). Deproteinated sugar beet pulp was hydrolyzed with a blend of commercial pure α-L-arabinofuranosidase and *endo*-arabinase obtaining yields as high as 88.8 % (Spagnuolo *et al*., 1999). Arabinoxylan from corn fiber was hydrolyzed with a commercial preparation of β-xylanase, β-xylosidase and α-L-arabinofuranosidase with 75.8 % yield (Park *et al*., 2001).

Enzymatic hydrolysis, as compared to acid hydrolysis, has the advantages of high specificity on the substrate, requires mild reaction conditions, and suffers no loss of saccharide due to side reactions. After the hydrolysis of complex polysaccharide, the resultant sugar mixture can be separated by chromatography (Park *et al*., 2001).

Thus, complex polysaccharide substrates like mesquite gum could be used to induce α-L-arabinofuranosidase activity to prepare hydrolysates such as L-arabinose. Therefore, the aim of this work was to obtain an enzymatic crude extract with α-L-arabinofuranosidase activity to release L-arabinose from mesquite gum, and compare these results with those obtained with a commercial enzyme.

2. Materials and methods.

2.1. Materials

Tear drops were collected from mesquite trees (*Prosopis laevigata*) located around the locality of Río Verde in the Mexican State of San Luis Potosi, during october 1999 – may 2000. The gum was purified by dispersing it in water, filtered through Whatman No. 1 filter paper, dialyzed in double distilled and deionized water using a cellulose membrane of 10,000 Da, with water rechanges every 4 h, during 24 h, and dried in a LABCONCO® LYPHLOCK 6 (Cole Parmer, Chicago, ILL) freeze-drier (Orozco-Villafuerte *et al*., 2003). A commercial α-L-arabinofuranosidase (EC 3.2.1.55) from *Aspergillus niger* was purchased from Megazyme International Ireland Ltd. (Bray Co., Wicklow, Ireland) with a 99.989 % of purity. Prior to usage the commercial enzyme was dialyzed in double distilled and deionized water using a cellulose membrane of 10,000 Da, with water rechanges every 4 h, during 24 h to eliminate the ammonium sulfate and sodium azide contained in the commercial preparation. Manufacturer reports 40°C as the optimum temperature for activity with this enzyme. Potato dextrose agar (PDA) was obtained from BD DIXON (BD Company, Sparks, MD). The chemicals $NaNO_3$, KH_2PO_4, KCl, $MgSO_4 \cdot 7H_2O$, NaOH and Na_2CO_3 were supplied by J.T. Baker (Xalostoc, State of Mexico, Mexico). Tween 80, *p*-nitrophenyl-α-L-arabinofuranoside, *p*-nitrophenol and L-arabinose were purchased from Sigma (St. Luis, MO).

2.2. Crude enzymatic extract

A crude α-L-arabinofuranosidase extract was obtained by filtration of the fermentation broth from

a strain of *A. niger* 10. The strain was kept at the Fermentations Pilot Plant, from the Biotechnology Department of the Universidad Autónoma Metropolitana-Iztapalapa (UAM-I). The microorganism was grown in 125 mL Erlenmeyer flasks containing 30 mL of PDA medium during 5 days at 30 ºC. After this time the spores produced were harvested with a tween 80 solution (0.01 %). Then a suspension of ca. $2x10^7$ spores, counted by Neubauer chamber, was inoculated into the slightly modified minimum medium proposed by van der Veen *et al*. (1993) that contained 10.0 g mesquite gum, 2.54 g $NaNO_3$, 1.5 g KH_2PO_4, 0.5 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$ and 1.0 L deionized water. pH was adjusted to 5.6 with 1N NaOH, temperature was maintained at 30 ºC and the broth was continually stirred at 150 rpm during 152 h. Samples were withdrawn at different time intervals, filtered, and activity of the crude extracts and pH were determined. Biomass of *A. niger* 10 growth was obtained after broth filtration by dry weight and modeled with the integrated form of the logistic model (Díaz-Godínez *et al*., 2001; Mirón *et al*., 2002; Liu *et al*., 2003):

$$X(t) = \frac{X_{\max}}{1 + \left(\left(\frac{(X_{\max} - X_0)}{X_0} \right) \cdot e^{(-\mu_{\max} \cdot t)} \right)} \qquad (1)$$

where X is the biomass concentration at any time = t, t is the fermentation time, $\mu_{\max}$ is the maximum specific growth rate, $X_{\max}$ is the maximum biomass concentration, and X_0 is the biomass concentration at t = 0.

The Soto-Cruz model (Soto-Cruz *et al*., 2002) was used for estimating the activity of α-L-arabinofuranosidase:

$$P(X) = P_0 + \alpha \cdot (X - X_0) + \frac{\beta \cdot X_{\max}}{\mu_{\max}} \cdot \ln\left(\frac{X_{\max} - X_0}{X_{\max} - X} \right) \qquad (2)$$

where P_0 is the enzyme activity at t = 0, α is the growth–associated coefficient for the enzyme and β is the non–growth–associated coefficient for enzyme. Estimation of the kinetic parameters in both equations was done using a non-linear least square fitting program called "Solver" contained in Microsoft Excel.

2.3. Enzymatic activity

The enzymatic activity of the commercial and crude extract of α-L-arabinofuranosidase was determined by reacting of 150 µL of the commercial and crude enzyme extract with 150 µL of 4 mM *p*-nitrophenyl-α-L-arabinofuranoside dissolved in citrate-phosphate (0.2 M and 0.1 M, respectively) buffer, pH 5.6 at 30 ºC for 10 min. The reaction was stopped by addition of 900 µL of 0.1 M Na_2CO_3. Activity of α-L-arabinofuranosidase was determined spectrophotometrically (Shimadzu UV–160A, Shimadzu Co., Kyoto, Japan) by measuring the absorbance of released *p*-nitrophenol at 400 nm (Tagawa and Kaji, 1988). One activity unit was defined as the amount of enzyme required to release 1 µmol of *p*-nitrophenol per minute under the assay conditions. Protein contents for the crude enzyme extract and the commercial enzyme were determined according to the Lowry method (Lowry *et al*., 1951), using bovine serum albumin as standard. Temperature for achieving optimal activity of the enzymes (commercial and crude extract) was established by performing the activity assay at 20, 30, 40, 50, 60 and 70 °C.

2.4. Activation energy

The activation energy required to initiate the hydrolysis reaction by the crude enzymatic extract and the commercial enzyme preparations was determined using an Arrhenius type expression:

$$A = A_0 \cdot e^{-Ea/RT} \qquad (3)$$

where A is the enzymatic activity, A_0 is the Arrhenius factor, Ea is the activation energy, R is the gas constant (8.314 KJ mol^{-1} K^{-1}), and T is the absolute temperature. A plot of Ln (A) versus $1/T$ generates a straight line of slope $-Ea/R$.

2.5. Enzymatic hydrolysis of mesquite gum

A volume of 150 µL of the crude extract (obtained after 84 h of fermentation and previously dried in a freeze-drier) or commercial enzyme preparations (both with 1 U of enzymatic activity) were mixed with 150 µL of mesquite gum aqueous solutions in citrate-phosphate buffer (0.2-0.1 M) at pH 5.6 with different gum concentrations (5, 10, 40, 70, 100, 130, 160 and 200 g L^{-1}). The hydrolysis kinetics was carried out at 30 °C. Samples were withdrawn every hour for evaluating the kinetics of the commercial or crude extract of α-L-arabinofuranosidase. L-arabinose concentrations could not de detected when mesquite gum concentration was less than 5 g L^{-1} in the case of the crude extract or 10 g L^{-1} in the case of the commercial enzyme. Enzymatic activity was determined as described above. The enzymatic reaction followed the Michaelis–Menten model:

$$v = \frac{V_{\max} \cdot S}{K_m + S} \qquad (4)$$

where v is the reaction rate, $V_{\max}$ is the maximum rate of L-arabinose released, K_m is the Michaelis–Menten constant and S is the mesquite gum concentration. The fit of K_m and $V_{\max}$ parameters was done using the non-linear least square fitting program "Solver".

The yield of L-arabinose was determined by hydrolyzing 300 µL of a 16 % (w/v) mesquite gum solution with 300 µL of crude extract or commercial enzyme with 8 U of enzymatic activity in citrate-

phosphate buffer (0.2-0.1 M) at pH 5.6 and 30 °C during 96 h. The reaction was stopped by adding 1.8 mL of Na_2CO_3 0.1 M. Quantification of L-arabinose was done as explained below.

2.6 Quantification of L-arabinose

The amount of L-arabinose produced was determined with an Agilent series 1100 high pressure liquid chromatograph (Palo Alto, CA), operated in the normal phase mode, fitted with a Rezex RHM monosaccharides column (300 x 7.8 mm, 8 μm; Phenomenex, Inc., Torrance, CA), and a refractive index detector (G1362A). Water was used as mobile phase at a flow rate of 0.4 mL·min^{-1} at 35 ºC. Peaks were integrated with the Agilent Chem Station software.

2.7 Statistical analysis

All experiments were done in triplicate and statistical analysis was carried out using the Duncan's test with a significance level set at 5 % with the statistical software package NCSS (2001, Kaysville, Utah).

3. Results and Discussion.

The experimental data of the growth kinetics for *A. niger* 10 fitted well (R^2 = 0.9412) the logistic model (Fig. 1), with values of μ_{max} of 0.07 h^{-1} and X_{max} of 3.03 g L^{-1}. The lag phase was estimated as 9.13 h from the fitted data. Comparison of our μ_{max} and X_{max} values obtained with *A. niger* 10 with those obtained with other strains of *A. niger* and other saccharides as substrates are given in Table 1. As can be seen, X_{max} is highly dependent on the complexity of the saccharide used as substrate. The simpler saccharide, glucose, had a higher was X_{max} (Favela-Torres *et al*., 1998). Pectin is a linear polysaccharide with a molecular weight ranging between 50 x 10^3 to 180 x 10^3 Da (Pedersen, 1980), so that it is not surprising that *A. niger* X_{max} for this polysaccharide (Díaz-Godínez *et al.*, 2001) was better than that calculated for mesquite gum, which has a highly branched structure and an average molecular weight greater than 2 x 10^6 Da (Vernon-Carter *et al*., 2000). The behavior of μ_{max} seems to be rather more complex than that of X_{max}. The estimated value for μ_{max} using mesquite gum as substrate was considerably lower than for pectin, but was practically the same as that for glucose. These results are difficult to explain in terms of the molecular structure of the substrates, and they seem to rather be a function of the microorganism employed which was *A. niger* 10 in the case of glucose and mesquite gum but was *A. niger* C28B25 in the case of pectin.

Fig. 1 shows the fit of the α-L-arabinofuranosidase crude extract activity data to the Soto-Cruz model (R^2 = 0.9738). Maximum activity was 65.93 U L^{-1} after 84 h of fermentation, which was used to carry out the hydrolysis of mesquite gum. The values for parameters α and β were 19.64 U g^{-1} and -0.01 U g^{-1} h^{-1}, respectively. While the value of parameter β was near zero, the relatively high value of parameter α suggests that the activity of the crude extract was closely associated to the kinetic growth parameters of the mold.

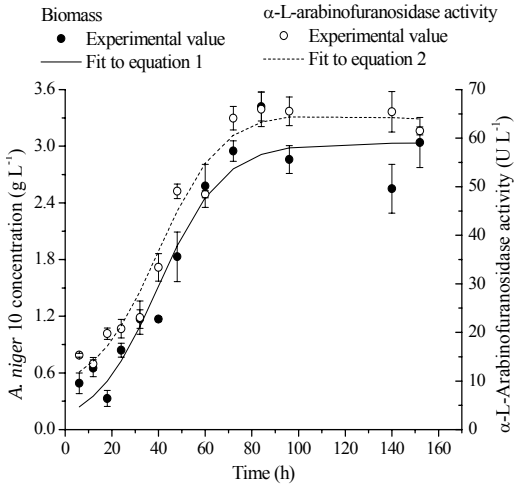

Fig. 1. Growth kinetics of *A. niger* 10 using mesquite gum like carbon source and α-L-arabinofuranosidase volumetric activity obtained from *A. niger* 10 with different fermentation times. Initial pH = 5.6 and temperature of 30 °C.

The specific activity obtained at 84 h of fermentation with the crude extract was 0.33 U mg^{-1} of protein, while that the commercial enzyme reported in the data sheet (40 U mg^{-1} of protein) was 121 times higher.

The effect of temperature on the crude extract enzymatic activity is shown in Fig. 2. The highest activity occurred at 50 °C. This temperature falls in between the 46 °C reported by van der Veen *et al*. (1991) and the 60 °C reported by Kaneko *et al.* (1993) and by Gunata *et al.* (1990) as being optimum for α-L-arabinofuranosidase from *A. niger* strains.

Table 1. Comparison of μ_{max} and X_{max} for *Aspergillus niger* using as substrate different saccharides

Substrate	X_{max} (g L^{-1})	μ_{max} (h^{-1})	Microorganism	Reference
Mesquite gum (10 g L^{-1})	3.03	0.07	*A. niger* 10	This work
Glucose (100 g L^{-1})	12.00	0.08	*A. niger* 10	Favela-Torres *et al*. (1998)
Pectin (15 g L^{-1})	4.38	0.22	*A. niger* C28B25	Díaz-Godínez *et al*. (2001)
Pectin + sucrose (15 + 40 g L^{-1})	11.00	0.19	*A. niger* C28B25	Díaz-Godínez *et al*. (2001)

Table 2. Comparison of K_m values for α-L-arabinofuranosidase on different substrates

Substrate	K_m (g L^{-1})	Enzyme	Reference
Mesquite gum	76.45	Commercial α-L-arabinofuranosidase	This work
1,5-L-arabinan	20.40	Purified α-L-arabinofuranosidase	Tagawa and Kaji (1988)
Mesquite gum	4.87	Enzymatic crude extract (apparent Km value)	This work
p-nitrophenyl-α-L-arabinofuranoside	1.36	Purified α-L-arabinofuranosidase	Tagawa and Kaji (1988)
L-arabinan	0.26	Purified α-L-arabinofuranosidase	Tagawa and Kaji (1988)

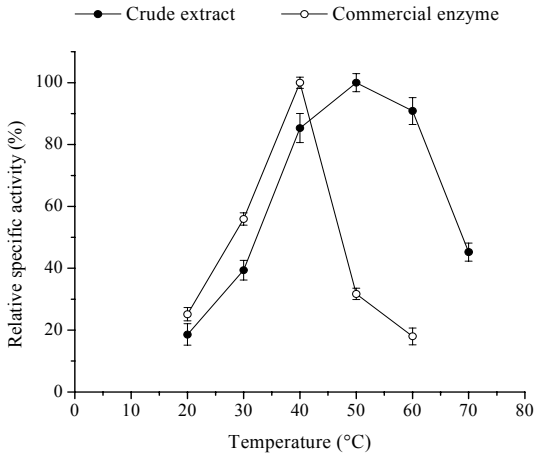

Fig. 2. Effect of temperature on α-L-arabinofuranosidase activity, at pH 5.6 with citrate-phosphate buffer (0.2-0.1 M). The y-axis represents the relative specific activity, i.e., the ratio of the maximum specific activity expressed as percentage.

The activation energy required for the mesquite gum hydrolysis was 46.15 KJ mol^{-1} with the crude extract estimated using a temperature interval between 20-50°C, and of 52.76 KJ mol^{-1} for the crude extract using a temperature range of 20-40°C. These results indicate that the hydrolysis reaction is probably more susceptible to temperature changes with the crude enzymatic extract than with the commercial enzyme possibly due to the synergistic enzyme action of diverse enzymes (e.g. glucosidase and rhamnosidase, induced possibly by the presences galactose and rhamnose residues in whole mesquite gum) contained in the crude extract.

The hydrolysis kinetics produced by the crude enzymatic extract and the commercial enzyme are shown in Fig. 3. These curves were modeled with the Michaelis-Menten equation (Eq. 4) with the following values calculated for the apparent K_m and apparent V_{max} for the crude enzymatic extract (4.87 g L^{-1} and 0.15 μmol min^{-1} g^{-1}, respectively) and K_m and V_{max} for the commercial enzyme (76.45 g L^{-1} and 3.85 μmol min^{-1} g^{-1}, respectively) (Table 2). The value of K_m is an indicator of the affinity of the enzyme for the substrate. Our results show that the crude extract had sixteen-fold times greater affinity for mesquite gum than the commercial enzyme.

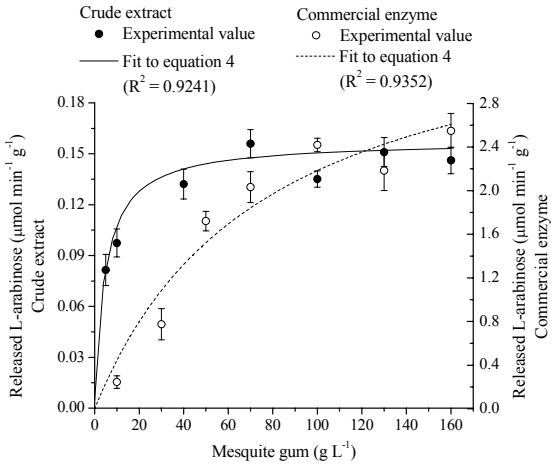

Fig. 3. Mesquite gum hydrolysis kinetics using the crude enzymatic extract and the commercial enzyme at pH 5.6 with citrate-phosphate buffer (0.2-0.1 M) and 30 °C.

These results affected V_{max} values, which were twenty five-fold times greater for the commercial enzyme than for the enzymatic crude extract. Tagawa and Kaji (1988) reported that L-arabinan hydrolysis by a purified α-L-arabinofuranosidase proceeded in two kinetic processes; a rapid process responsible for up to 30 % of the hydrolysis attributable to the cleavage of one unit L-arabinofuranose side chains which were attached along a main chain; and a slow process responsible for the cleavage of the main chain composed of α-L-1→5-linked arabinofuranose units. Saha (2000) mentioned that most α-L-arabinofuranosidases are exoenzimes, which act upon the (1→3) and (1→5)-arabinosyl bonds located on the side chains of the polysaccharides. Thus, it is probable that in the case of mesquite gum, the commercial enzyme acted mainly on these side chains, whereas the crude extract, which may contain other enzymes, appeared to have greater ability for cleaving the (1→3) and (1→5)-arabinosyl bonds contained within the macromolecular structure of mesquite gum, leading to greater release of L-arabinose. Rombouts *et al*. (1988) suspected that two α-L-arabinofuranosidases and an endo-1→5-α-L-arabinanase had considerable synergistic effects on the production of L-arabinose.

The amount of L-arabinose released by the crude enzymatic extract was 17.04 ± 1.08 %,

whereas for the commercial enzyme was 2.78 ± 0.18 %, based on the reported average value content of 40.4 % of the L-arabinose in whole mesquite gum (Orozco-Villafuerte *et al.*, 2003).

Conclusions

The obtention of L-arabinose from mesquite gum was more effective using an enzymatic crude extract with α-L-arabinofuranosidase activity than a purified enzyme preparation. These results are important in the economy of the process, as the production of crude extracts is lower than the purified enzymes. Procedures for increasing the growth kinetics of *A. niger* will probably result in higher α-L-arabinofuranosidase activity, and thus in higher L-arabinose yields. Higher L-arabinose yields represent the stepping stone for further potential products developments using this compound as an intermediate.

References

Anderson, D.M.W. and Weiping, W. (1989). The characterization of proteinaceous *Prosopis* (mesquite gums) which are not permitted food additives. *Food Hydrocolloids 3*, 235-242.

Buendía-González, L., Orozco-Villafuerte, J., Cruz-Sosa, F., Chávez-Ávila, V.M. and Vernon-Carter, E.J. (2007). Clonal propagation of mesquite tree (*Prosopis laevigata* Humb. & Bonpl. Ex Willd. M.C. Johnston). *In Vitro Cellular and Developmental Biology-Plant 43*, 260-266.

Danisco, A/S. (2007). Intermediates. L-arabinose CT99. Available at http://www.danisco.com/cms/connect/corporate/products%20and%20services/pharma%20and%20healthcare/pharma/intermediates/intermediates_en.htm.

Díaz-Godínez, G., Soriano-Santos, J., Augur, C. and Viniegra-González, G. (2001). Exopectinases produced by *Aspergillus niger* in solid-state and submerged fermentation: a comparative study. *Journal of Industrial Microbiology and Biotechnology 26*, 271-275.

Favela-Torres, E., Córdova-López, J., García-Rivero, M. and Gutiérrez-Rojas, M. (1998). Kinetics of growth of *Aspergillus niger* during submerged, agar surface and solid state fermentations. *Process Biochemistry 33*, 103-107.

Felker, P. (1993). Review of applied aspects of *Prosopis*. In: *Prosopis species in the arid and semi-arid zones of India*, (J.C. Temari, N.M. Pasecznik and P.C.C. Harris, eds.), Pp. 11-14. *Prosopis* Society of India, Jodhpur, Rajasthan, India.

Gunata, Z., Brillouet, J.M., Voirin, S., Baumes, R. and Cordonnier, R. (1990). Purification and some properties of an α-L-arabinofuranosidase from *Aspergillus niger*. Action on grape monoterpenyl arabinofuranosylglucosides. *Journal of Agricultural Food Chemistry 38*, 772-776.

Kaneko, S., Shimasaki, T. and Kusakabe, I. (1993). Purification and some properties of intracellular α-L-arabinofuranosidase from *Aspergillus niger* 5–16. *Bioscience Biotechnology and Biochemistry 57*, 1161-1165.

Karhumaa, K., Wiedemann, B., Hahn-Hägerdal, B., Boles, E. and Gorwa-Grauslund, M.F. (2006). Co-utilization of L-arabinose and D-xylose by laboratory and industrial *Saccharomyces cerevisiae* strains. *Microbial Cell Factories 5*, 18.

Liu, J.Z., Weng, L.P., Zhang, Q.L., Xu, H. and Ji, L.N. (2003). A mathematical model for gluconic acid fermentation by *Aspergillus niger*. *Biochemical Engineering Journal 14*, 137-41.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin fenol reagent. *Journal of Biological Chemistry 193*, 265-275.

Mirón, J., González, M.P., Pastrana, L. and Murado, M.A. (2002). Diauxic production of glucose oxidase by *Aspergillus niger* in submerged culture: A dynamic model. *Enzyme and Microbial Technology 31*, 615-620.

Orozco-Villafuerte, J., Cruz-Sosa, F., Ponce-Alquicira, E. and Vernon-Carter, E.J. (2003). Mesquite gum: fractionation and characterization of the gum exuded from *Prosopis laevigata* obtained from plant tissue culture and from wild trees. *Carbohydrate Polymers 54*, 327-333.

Park, N.H., Yoshida, S., Takakashi, A., Kawabata, Y., Sun, H.J. and Kusakabe, I. (2001). A new method for the preparation of crystalline L-arabinose from arabinoxylan by enzymatic hydrolysis and selective fermentation with yeast. *Biotechnology Letters 23*, 411-416.

Pedersen, J.K. (1980). Pectins. In: *Handbook of Water-Soluble Gums and Resins*, (R.L. Davidson, ed.), Pp. 15.1-15.21. McGraw Hill Book Company, New York.

Rombouts, F.M., Voragen, A.G.J., Searl-van, L.M.F., Geraeds, C.C.J.M., Schols, H.A. and Plink, W. (1988). The arabinanases of *Aspergillus niger* – purification and characterization of two α-L-arabinofuranosidases and an endo-1,5-α-L-arabinanase. *Carbohydrate Polymers 9*, 25-47.

Saha, B.C. (2000). α-L-Arabinofuranosidases: biochemistry, molecular biology and application in biotechnology. *Biotechnology Advances 18*, 403-423.

Soto-Cruz, O., Favela-Torres, E. and Saucedo-Castaneda, G. (2002). Modeling of growth, lactate consumption, and volatile fatty acid production by *Megasphaera elsdenii* cultivated in minimal and complex media. *Biotechnology Progress 18*, 193-200.

Spagnuolo, M., Crecchio, C. and Pizzigallo, M.D.R. (1999). Fractionation of sugar beet pulp into pectin, cellulose and arabinose by arabinases combined with ultrafiltration. *Biotechnology and Bioengineering 64*, 685-691.

Susumu, H. (1999). Nutritional and physiological functions and uses of L-arabinose. *Journal of Applied Glycoscience 46*, 159-165.

Tagawa, K. and Kaji, A. (1988). α-L-Arabinofuranosidase from *Aspergillus niger*. *Methods in Enzymology 160*, 707-712.

van der Veen, P., Flipphi, M.J.A., Voragen, A.G.J. and Visser, J. (1993). Induction of extracellular arabinases on monomeric substrates in *Aspergillus niger*. *Archives of Microbiology 159*, 66-71.

van der Veen, P., Flipphi, M.J.A., Voragen, A.G.J. and Visser, J. (1991). Induction, purification and characterisation of arabinases produced by *Aspergillus niger*. *Archives of Microbiology 157*, 23-28.

Vernon-Carter, E.J., Beristain, C.I. and Pedroza-Islas, R. (2000). Mesquite gum (*Prosopis* gum). In: *Novel Macromolecules in Food Systems*, *Developments in Food Science 41*, (G. Doxastakis and V. Kiosseoglou, eds.), Pp. 217-238. Elsevier Science Publishers, Amsterdam.

White, E. V. (1947). Isolation of L-arabinose. *Journal of the American Chemical Society 69*, 715-715.