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Conditioning and use of prickly pear peels for the production of lignocellulosic enzymes by *Aspergillus niger* sp. on solid-state cultures

Acondicionamiento y uso de cáscaras de tuna para la producción de enzimas lignocelulósicas por *Aspergillus niger* sp. en cultivos en estado sólido

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Abstract

Conditioning and use of prickly pear peel as a substrate to develop solid-state cultures has not been studied deeply. *Aspergillus niger* sp. produced laccases and xylanases when grown on a solid-state culture on prickly pear peels. Peels were sun-dried, or dried in an oven at different temperatures. The drying rate, the coloration of the dried material, and the degradation susceptibility depended on the drying mode and on temperature. Drying rate was 11.11% slower for sun-dried peels than oven-dried peels at 45°C, and at higher temperatures, were 2.4 times and 3.9 times higher than the sun-dried for 60 °C and 70 °C, respectively. peels showed differences in coloration, apparently associated with the release of different phenolic compounds. Fungal cultures grew twice faster on sun-dried peels than on oven-dried ones with a size of 1.84 mm, and reached highest enzymatic activities earlier, 48 hours prior to for xylanases and 24 for laccases. Oven drying caused hornification of peels, delaying both fungal growth and enzyme production. In our study laccases titers were higher than in previous studies using various agricultural residues. Prickly pear peels are a convenient substrate to produce a lignocelulolytic extract that can be used in several saccharification bioprocesses.

Keywords: laccases, xylanases, solid-state culture, particle characterization, hornification.

Resumen

El acondicionamiento y uso de la cáscara de tuna como sustrato para desarrollar cultivos en estado sólido no se ha estudiado en profundidad. *Aspergillus niger* sp. produjo lacasas y xilanasas al crecer en un cultivo sólido sobre cáscara de tuna. Las cáscaras se secaron al sol o en una estufa, utilizando diferentes temperaturas. La velocidad de secado, el color del material secado y la susceptibilidad a la degradación dependen del modo y la temperatura de secado. La velocidad de secado fue un 11,11% más lenta para las cáscaras secadas al sol que las cáscaras secadas al horno a 45 ° C, y a temperaturas más altas, fue 2,4 veces y 3,9 veces más alta que las secadas al sol a 60 ° C y 70 ° C, respectivamente. Las cáscaras mostraron diferencias en el color, aparentemente asociadas a la liberación de distintos compuestos fenólicos. Los cultivos fúngicos crecieron dos veces más rápido sobre las cáscaras secadas al sol que sobre las secadas en horno con tamaño de 1.84 mm, y produjeron más rápido las mayores actividades enzimáticas, 48 horas antes para xilanasas y 24 para lacasas. El secado en la estufa causó la hornificación de las cáscaras, retrasando tanto el crecimiento fúngico como la producción de las enzimas. En nuestro estudio, los títulos de lacasas fueron más altos que en estudios previos utilizando varios residuos agrícolas. Las cáscaras de tuna son un sustrato conveniente para producir un extracto lignocelulolítico que se puede utilizar en varios bioprocesos de sacarificación.

Palabras clave: lacasas, xilanasas, cultivo sólido, caracterización de partículas, hornificación.

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1 Introduction

Waste and by-products of different agro-industrial processes contain significant amounts of cellulose, hemicellulose, lignin, and pectin, which make them attractive as raw materials for the development of multiple bioprocesses (Magalhães *et al*., 2019). Because of its chemical composition, agro-industrial waste is also known as lignocellulosic material (Akanni *et al*., 2014; Ramírez-Carmona and Muñoz Blandón, 2016).

Peels of white prickly pears (*Opuntia amyclaea*) represent 40% of the weight of the fruit and are composed of lignin (2.4%), cellulose (27%), other polysaccharides as pectin, xylan, arabinan and galactan (35%) and appreciable amounts of bioactive compounds (Habibi *et al*., 2004; Dávila-Hernández *et al*., 2019). Although Mexico is the main producer of prickly pears in the world, with an average annual production of 180 thousand tons (SIACON, 2018); Opuntia fruit is an important and abundant raw material for the Moroccan industry (Habibi *et al*., 2004) this cactus is also cultivated in north-eastern Brazil for its fruits and fences (Santos *et al*, 2016). These characteristics make white prickly peels an attractive material for reuse, for biotransformation, and for use in biotechnological processes (Santos *et al*, 2016).

After conditioning, lignocellulosic materials can be used as raw supplies in biotechnological processes. If these materials are to be used as support and substrate for solid-state cultures it is essential to control particular characteristics of the material. For instance, porosity, water adsorption capacity, and initial moisture content, affect the metabolic behavior of microorganisms that use these materials as substrate during culture (Abu Yazid *et al*., 2017, Soccol *et al*., 2017).

Dried materials with well-defined particle size are useful as substrates; the type of drying to which the material is subjected to is decisive not only for the adjustment of humidity but also for the availability of polysaccharides for microorganisms consumption (Costa *et al*., 2018).

Materials with high absorption capacity are particularly suitable for solid-state processes, since the amount of water available to microorganisms (moisture) can be adjusted at the beginning of the culture depending on culture requirements. Solid-state culture is performed on porous substrates that do not have free water but have enough moisture (70% to 80%) to allow growth and reproduction of microorganisms (Khanahmadi *et al*., 2018; Grande-Tovar, 2016). On the other hand, porous spaces allow adequate gas exchange in aerobic processes, which is a sine qua non condition for the growth of filamentous fungi (Durán-Hinojosa *et al*., 2017).

Microorganisms can use lignocellulosic materials as primary carbon source if they can produce a specific set of degrading enzymes. Lignin degraders are typically bacteria and fungi. White rot fungi are the most common degraders of lignin, e.g. *Trametes versicolor* and *Phanerochaete chrysosporium*are two species of white-rot fungi that have been widely studied (Sista Kameshwar and Qin, 2018). Efficient degradation of lignocellulosic materials requires the activity of two kinds of microorganisms: those that produce oxidases (for degradation of lignin), and those that produce hydrolases (for degradation of polysaccharides) (Paramjeet *et al*., 2018). Hydrolases degrade polysaccharides of different lignocellulosic materials. Aspergillus species are the primary producers of several hydrolases, including esterases (Hamid *et al*., 2021), but they also produce enzymes that degrade lignin (Brink *et al*., 2019). The quantity and type of enzymes produced by fungi depend on the chemical composition of the material that they degrade (Costa *et al*., 2018, Martínez *et al*., 2017).

At the laboratory level, the combined action of oxidases and hydrolases produced by fungal species cultured separately can increase the degradation and the saccharification yield of lignocellulosic materials (Martínez-Trujillo *et al*., 2020). Production of all these enzymes in the same culture would be far more efficient, which would be possible by establishing mixed cultures were white-rot fungi and hydrolytic fungi species interact, thus producing both kinds of enzymes in parallel. In some cases, fungal co-cultures can cause antagonism between species, diminishing their ability to produce different enzymes (Daly *et al*., 2017).

Another way to produce these enzymes in the same culture is using fungal species that produce the full spectrum of enzymes required. *Aspergillus* species as *niger*, *oryzae*, *giganteus*, *awamori*, *fumigatus*, *wentii*, *flavus*, *terreus*, and *nidulans* are known producers of hydrolytic enzymes (Sohail *et al*., 2009; López-Gómez *et al*., 2020); and some reports indicate that this species can also produce oxidases (e.g., laccases, lignin, and manganese peroxidases) (Calle *et al*., 2007). In our knowledge, few studies report the production of laccases and hydrolases in the same culture with some species of *Aspergillus* by solid or semi-solid fermentation. Table 1 presents examples of these cultures. In this sense, this work is novel because it reports the simultaneous production of laccases and xylanases by *Aspergillus niger* sp. growing on a prickly pear peel, a material that is not widely exploited.

In summary, prickly pear peel is a lignocellulosic residue with potential as a substrate to develop solid-state cultures. However, the conditioning and use of prickly pear peel in this type of process has not been studied in deeply. On the other side, saprophytic fungi can use materials as prickly pear peels for producing a diversity of enzymes that have some industrial importance. These enzymes could act together in a concerted way, to develop processes as the saccharification of several agroindustrial residues.

Thus, this study evaluated the conditioning of peels of prickly pears for using it as support-substrate for *Aspergillus niger* sp to produce laccases and xylanases in solid-state culture.

Table 1. *Aspergillus* species producers of oxidases and hydrolases in the same culture.

Species	Substrate	Enzymes		Reference
		Oxidoreductases	Hydrolases	
A. niger	Wheat bran oats Straw, beetroot press	Laccases	FPases β-Glucosidases Amilases	Stoilova and Krastanov (2008)
A. niger	Winery and olive mill wastes	Laccases Peroxidases	Xylanases Cellulases	Salgado *et al*. (2014)
A. niger	Millet and sorghum pomace	Laccases	Amylases	Abu and Ado (2004)
A. tamarii *A. fumigatus*	Pu-erh tea	Laccases Vanillyl-alcohol oxidase	Endoglucanases Cellulases	Ma *et al*. (2021)
A. terricola *A. parasiticus*	Flax retting liquor	Laccases	Pectinases	Abd El-Rahim *et al*. (2020)

2 Materials and methods

2.1 Microorganism

The wild strain of *Aspergillus niger* sp. belongs to the Enzymatic Catalysis Laboratory of the Tecnológico de Estudios Superiores de Ecatepec. Cultures were maintained on potato dextrose agar at 4 °C by periodic subculturing. The *A. niger* sp. strain was activated in Erlenmeyer flasks with Czapek-Dox medium at 26±1 °C for seven days; this time is long enough for *A. niger* sp. to cover the spore medium. After the strain was activated, the inoculums were prepared on potato dextrose agar medium, and the resulting spores were harvested from 5-days cultures at 27 °C. Conidia were dispersed in a sterile 0.9% (w/v) NaCl solution, and the spores contained in the resulting suspension were counted in a Neubauer chamber (Membrillo-Venegas *et al*., 2013).

2.2 Conditioning of prickly pear peels

Having a well-characterized solid substrate is essential to control process variables. For this purpose, after collecting the prickly pear peels were cut into 20×40 mm rectangles to achieve homogeneous drying. The selection of this cut size was because homogeneous drying was not achieved with larger particles; on the other hand, grinding with smaller particles required a greater number of stages. Subsequently, cut peels were washed with tap water at room temperature and drained. Two types of drying were tested; the first type was drying in an oven at different temperatures (45, 60, and 70 °C). In the second type of drying, peels were placed under the sun (at 45 ºC on average). The drying temperatures were chosen considering other drying studies for prickly pear peels (Chaparro-Montoya *et al*., 2019) and other agricultural residues (Lahsasni *et al*., 2004). In both cases drying stopped after peels reached approximately 16% moisture; a gravimetric record of moisture loss was used for monitoring the drying every 30 minutes, the material was dried in an oven (Felisa E219). Rates of drying were calculated according to McCabe *et al*. (2002). The dried material was passed through a grain mill (Herremex, Bs101) in a single pass. The ground material was characterized by granulometric techniques using different Tyler-Series sieves, obtaining the particles retained between meshes 8 and 10 (1.84 mm average diameter) and between meshes 14 and 16 (1.095 mm average diameter). Hereafter, the resulting dried peels were named oven-dried peels (peels dried in the oven) or sun-dried peels (peels obtained after solar drying).

2.3 Solid-state culture

For analyzing the effect of drying mode and particle size on the behavior of solid-state culture, a factorial design was developed. In this case, four experimental conditions were proved: sun-dried peels with 1.095 mm diameter, sun-dried peels with 1.84 mm diameter, oven-dried peels with 1.095 mm diameter, and oven-dried peels with 1.84 mm diameter. The results were compared by means of an ANOVA analysis and the corresponding regression analysis was performed also. Both statistical analyses were done in Design Expert® version 12 software.

For solid-state culture, 2 g of the corresponding dry material (2.38 g of prickly pear peels with 16 % moisture) were mixed in 175 mL glass bottles with 3.62 mL of Mineral Medium. This Mineral Medium contained 2 g/L KH_2PO_4, 2 g/L K_2HPO_4, and 5 g/L $(NH_4)_2SO_4$; and its pH was adjusted to 5 with 2 M H_2SO_4. These bottles were sterilized for 15 min at 121 °C. Each bottle was inoculated with 4 mL of the spore suspension to obtain a substrate with 80% humidity (measured by gravimetry at 105°C, according to Villegas-Santiago, 2020) and 1×10^7 spores per gram of dry substrate. All experimental cultures were maintained at 37 °C for 10 days.

After the incubation period, the content of each bottle was suspended in 40 mL of sodium acetate buffer (100 mM, pH 5.0) and maintained under constant agitation (200 rpm)

for 30 min at 4°C. Solids were separated by centrifugation at room temperature (3500 rpm, 10 min) in a Onof CFG-02 centrifuge, and the supernatant was kept in the freezer until further analysis. The centrifuge cake was resuspended in 40 mL of water and centrifuged again for 10 minutes at 1780g at room temperature. The cake was then dried at 70 °C until constant weight was reached. The dried cake was used to estimate fungal mass and substrate consumption. Triplicates were performed for each experimental treatment, and the results were reported as the average ± standard deviation of these measurements.

2.4 Analytical procedures

Protein was determined according to Lowry *et al*. (1951), using Bovine Serum Albumin (1 g/L) as standard. Reducing sugars released after enzymatic catalysis were determined by the 3,5-dinitrosalicylic acid method (Miller, 1959). Total soluble sugars in the supernatant and hydrolyzed centrifuge cake were measured using the phenol-sulfuric method (Dubois *et al*., 1956; López-Legarda *et al*., 2017). For estimation of xylanase activity, samples were incubated with a 1.0 % (w/v) solution of Birchwood xylan dissolved in sodium acetate buffer (50 mM, pH 5), and released reducing sugars were then quantified (Membrillo-Venegas *et al*., 2013). For laccase activity, the oxidation level of guaiacol 10 mM in an acetate buffer (0.5 mM, pH 5) was registered at 470 nm every 20 s for 3 min, after adding the samples to the reaction mixture (Durán-Hinojosa *et al*., 2017). Enzymatic activities were expressed in units (U); one enzyme activity unit (U) is defined as the amount of enzyme required to release 1micromol of product per minute under the assay conditions given. Activities were reported based on one gram of the initial substrate?s dry weight (U/g dry wt).

2.5 Analysis of variance for the effect of conditioning of prickly pear peel on enzymatic activities

Maximum xylanases and laccases activities obtained on cultures, as well as the corresponding specific enzymatic activities produced by each gram of biomass, were analyzed using simple factorial designs. In these, the dependence of each response of the factors drying mode (X_1), particle diameter (X_2) and the interaction among both factors (X_1X_2) was analyzed. The experimental data were empirically fitted with polynomial regression, based on analysis of variance (ANOVA). The linear model that described the effect of each factor on the responses was:

$$Y = \beta_0 \pm \beta_1 X_1 \pm \beta_2 X_2 \pm \beta_{12} X_1 X_2$$

were Y is the maximum or specific enzymatic activities; β_0 is the intercept term, known as the average value of each activity; β_1 is the linear effect of variable X_1; β_2 is the linear effect of variable X_2; and β_{12} indicates the interaction effect between variables X_1X_2.

2.6 Estimation of fungal mass and residual substrate

In this work we propose a methodology that could be useful when the substrate in solid culture also acts as a support for growth. It is assumed that the composition of the material is identical throughout the process, and it is necessary to know the weight fraction of total carbohydrates (Xc) and protein (Xp) in the original substrate. According to Habibi *et al*. (2004), in prickly pear peels Xc is equal to 0.661, and Xp is equal to 0.086. On the other hand, mycelium cell wall is composed of polysaccharides and glycoproteins that determine the fibrillar structure of the wall (García-Reyes *et al*., 2017). The average protein content in the fungal mycelium is 32%, and the average carbohydrate content is 49% (Zobriskie, *et al*., 1980). It is possible to transform these percentages to the respective weigh fraction (Yp and Yc for protein and carbohydrates, respectively).

The known weight of the dried materials referred as “cake" in section 2.3 was recorded and assigned to the variable W_c.

$$W_c = RS + FM \tag{1}$$

In Equation (1) RS corresponds to residual substrate weight and FM to the weight of the fungal mass.

The cakes were placed in 70 mL test tubes with a cap, the apparent volume of each cake was calculated, and cakes were covered with the same volume of 1.5 M H_3PO_4 solution (Córdova *et al*., 1996). These mixtures were homogenized with a Hamilton Beach Mod.750 household homogenizer. Closed test tubes were placed in a water-bath at 80 °C for 20 minutes to promote the hydrolysis of the solid material. The mixture was then centrifuged for 15 minutes at 1,500 rpm, and the supernatant of the resulting suspension was neutralized and used to measure the protein content (P_c, g) and total carbohydrates in the cake (C_c, g), released after hydrolysis of the cake.

C_c included carbohydrates in the residual substrate (C_{rs}) and carbohydrates in fungal mass (C_{fm}), as shown in Equation (2):

$$C_c = C_{rs} + C_{fm} \tag{2}$$

Also, P_c included protein in the residual substrate (P_{rs}) and protein in the fungal mass (P_{fm}):

$$P_c = P_{rs} + P_{fm} \tag{3}$$

Finally, the initial composition of the substrate and biomass were used to establish the following set of equations (4)

$$C_{rs} = RS \cdot X_c \tag{4a}$$

$$C_{fm} = FM \cdot Y_c \tag{4b}$$

$$P_{rs} = RS \cdot X_p \tag{4c}$$

$$P_{fm} = FM \cdot Y_p \tag{4d}$$

Table 2. Batch drying summary.

Drying type	Temperature (°C)	Drying time (h)	Initial drying rate, Ro (kg water/ kg dry matter·min)*	Aspect	Average particle size (mm)
Oven-dried	70	20	0.0063 ± 4.84E-4^a	Greenish	1.566
Oven-dried	60	40	0.0039 ± 3.45E-4^b	Greenish	1.566
Oven-dried	45	140	0.0018 ± 1.37E-4^c	Greenish	1.566
Sun-dried	45	190	0.0016 ± 1.40E-4^c	Brown	1.275

*Differences between data in the same column higher than the corresponding LSD value are statistically different, as indicated by different lower-case letters.

After replacing Equations (4) in Equations (2) and (3), we obtained a system of two equations with two unknown variables (RS and FM):

$$C_c = RS \cdot X_c + FM \cdot Y_c \quad (5)$$

$$P_c = RS \cdot X_p + FM \cdot Y_p \quad (6)$$

In each sample, C_c and P_c were measured easily using the analytic techniques described above; we assumed that X_c, Y_c, X_p, and Y_p remained constant during the culture, so that RS and FM can be estimated after solving the equation systems.

For validate this technique, several Petri dishes that contained 1% (w/v) of the corresponding substrate (glucose, pectin, cellulose, or prickly pear peel) suspended in agar were prepared. Then, a sterilized 0.2 μm GTTP IsoporeTM membrane filter (Merck) was placed on, for covering all the surface of the agar plate, and each plate was inoculated on the top of the membrane with 1000 spores of *A. niger* sp., and incubated at 37 °C for 72 h. The mycelia grown on the surface of the membranes placed on the surface of the agar with different carbon sources were recovered with a spatula, for using dry-weight or acidic hydrolysis technique in biomass quantification.

The same volume used for estimating dry-weight of each mycelial suspension was used also for quantifying mycelium concentration by acidic hydrolysis. This volume was placed in a tube and a similar volume of 1.5 M H_3PO_4 solution was added, for submitting them in a water-bath at 80 °C for 20 minutes to promote the hydrolysis of the solid material. After centrifugation, protein and total carbohydrates were quantified in the supernatant; and using the equations described before, the mycelial concentration was estimated for each sample. Finally, the results obtained after analyzing the samples by means of dry-weight and the acidic hydrolysis technique, a similar behavior was observed, with a correlation coefficient of 0.9.

All the experiments and sample analyses were performed in triplicate, and the results were reported as the average ± standard deviation of these measurements. When necessary, results obtained from each experiment were compared by means of LSD technique.

3 Results and discussion

3.1 Conditioning of pear peels

Temperature and the drying mode of prickly pear peels affected the drying rate (R) and, consequently, the appearance of the dehydrated product in the dried material (DM). Table 2 summarizes the conditions and results for each drying type for the prickly pear peels. The drying rate of sun radiation (0.0016 kg/kg$_{DM-min}$) was similar to that of the oven set at 45 ºC (0.0018 kg/kg$_{DM-min}$); however, drying time in the oven set at 60 or 70 ºC was 100 and 120 hours, correspondingly shorter than the drying time at 45 ºC (Figure 1). Drying rates at 70 and 60 °C in the oven were 0.0063 and 0.0039 kg/kg$_{DM-min}$, respectively. Lahsasni *et al*. (2004) studied the drying kinetics of prickly pear peels in a convective solar dryer; they obtained initial drying rates ranging from 0.0189 to 0.0299 kg/ kg$_{DM-min}$ at 50°C, and drying rates ranging from 0.0298 to 0.0675 at 60 °C. From the results of Chaparro-Montoya *et al*. (2019), it is possible to calculate an initial rate of prickly pear peel drying of around 0.04 kg/ kg$_{DM-min}$ at 60 °C. Slama and Combarnous (2011) found initial drying rates between 0.032 and 0.044 kg/kg$_{DM-min}$ at 75°C in a study of orange peels dried in a solar dryer by forced convection. In this study, we did not use forced convection for solar or oven drying, which explains why the drying rates of prickly pear peels were an order of magnitude lower than in previous studies (Chaparro-Montoya *et al*., 2019).

The change in the material coloration seemed to be associated with the type of drying, rather than with drying rate or temperature. Drying rates were determined using three samples. Although drying rates at 45 °C differed by less than 13% between solar and oven drying, the material obtained in each case showed a different color: a brown color in sun-dried peels and greenish in oven-dried peels (Table 2). On the other hand, oven drying rates at 70 or 60 °C were 350% and 217% higher, respectively, than the oven drying rate at 45 °C; however, the peels dried under these three conditions had similar greenish appearance.

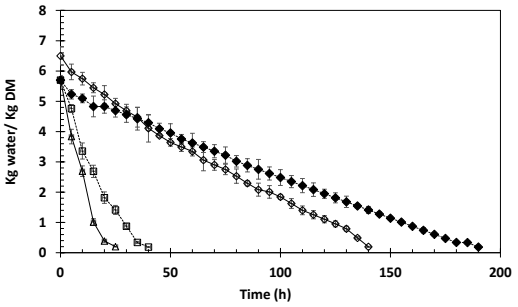

Figure 1. Water content of sun-dried prickly pear peel (□) or peels dried in an oven at 45 ºC (□), 60 ºC (□), and 70 ºC (Δ) as a function of time.

Sun-drying has been associated with tannin oxidation of pears and other fruits (Ferreira *et al*., 2008), with higher levels of total phenolic compounds in dates (Al-Farsi *et al*., 2005), and with increased flavonoid and proanthocyanidin in yellow figs (Kamiloglu and Capanoglu, 2015). Thus, the release of oxidized tannin, phenolic compounds, flavonoids, and proanthocyanidin could be the cause of color change in dried prickly pear peels. In fact, in our research group, a parallel study for the production of organic acids by *Aspergillus niger* sp. on sun-dried prickly pear peel during solid-state culture is being developed, until now, we have identified the presence of gallic acid by thin layer chromatography.

After grinding, grain size analysis indicated that the average diameter of sun-dried particles was about 18% smaller than the particles obtained by oven drying. It has been reported that the sun-drying processing of pears and other fruits, as persimmon fruit and pineapple, results in loss of cell wall integrity (Ferreira *et al*., 2008; Gouws *et al*., 2019), which can explain the differences between particles sizes. Regardless of the drying type, most of the particles were retained between sieves 9 and 10 (average diameter 1.84 mm), followed by particles retained between sieves 14 and 16 (1.095 mm). Both size fractions of the material obtained by oven-drying at 70 °C or sun-drying were chosen for substrate conditioning for use in solid-state cultures.

3.2 Growth and substrate consumption in solid-state culture

Conditioning can modify the chemical structure of materials such as prickly pears peels, causing an increase in the material's susceptibility to undergo degradation during solid-state culture. Both UV radiation of solar drying and the hornification effect caused by oven-drying explain why the drying method and the particle diameter determined the behavior of cultures grown on prickly pears peels. The drying method also determined the metabolic activity of *A. niger* sp. In the next paragraphs we describe the behavior of fungal growth on substrate and enzyme production in the different particles obtained after drying.

With respect to the fungal growth on sun-dried peels, all the growth process occurred during the first 100 h culture; while on oven-dried peels, the growth process lasted 150 h. Fungal mass reached the highest values (0.26-0.47 $g/g_{substrate}$) at 72 h on oven-dried peels, while on sun-dried peels the highest mass (0.20-0.35 $g/g_{substrate}$) was observed around 18-20 h of culture. Besides, the highest specific growth rate values (μ = 0.2483± 0.009 h^{-1}) were obtained with sun-dried peels, irrespective of particle size. In the oven-dried peels, the specific growth rates depended on particles size; μ was 0.1221 ± 0.0005 h^{-1} for particles with 1.84 mm diameter and 0.2231 ± 0.006 h^{-1} for particles with 1.095 mm diameter.

The drying method did not affect the total mass of peels consumed by *A. niger* sp. but affected the consumption rate. In both cases, the peels consumed were around 80% (Figures 2A and 2B). Consumption rates coincided with growth rate on sun-dried peels for both particle sizes and on the oven-dried peels only for 1.84 diameter particles. However, for the oven-dried peels with 1.095 mm diameter, the consumption was slower during the first 48 h (r_S = 0.016 ± 0.002 $g_{substrate}$ h^{-1}), even if almost all of the growth process occurred during this time. It is possible that during the first 48 h of culture on oven-dried peels with 1.095 cm diameter, microorganisms needed to adapt their metabolism to degrade the cell wall structure of the peels. After 48 h and until the end of the culture, the biomass consumed the peels at a higher rate (r_S = 0.0297 ± 0.006 $g_{substrate}$ h^{-1}) (Figure 2A).

The drying method used on prickly pear peels could have caused different effects on its structure, and on its degradation susceptibility. It is known that after breaking down lignin, the material becomes more porous and thus susceptible to degradation (Mäkelä *et al*., 2015; Martínez *et al*., 2005). Considering that with the hornification the material becomes harder and forms small flocks (Luo and Zhu, 2011), especially with the smallest particles, hornification could difficult the access the polymeric material for the fungus.

Fungal growth seemed to be sustained by the relationship between total sugar consumption, and the production of reducing sugars and xylanases. Total soluble sugar released from peels obtained by both drying methods was around 70 mg per gram of substrate, as measured at the beginning of the culture. These total sugars were consumed at a rate of rTS = 0.1709 ± 0.008 $g_{substrate}$ h^{-1} in all cases (Figures 2C and 2D). In this work it was not possible to identify the particular chemical species that were quantified as "total sugars". However, considering the effects of the drying method and of the sterilization process on peels structure, it is likely that conditioned peels released oligomers that served as initial substrates for priming the metabolism of *A. niger* sp. on this solid-state culture. These oligomers may activate the production of different hydrolases, generating the release of reducing sugars (Walia *et al*., 2017).

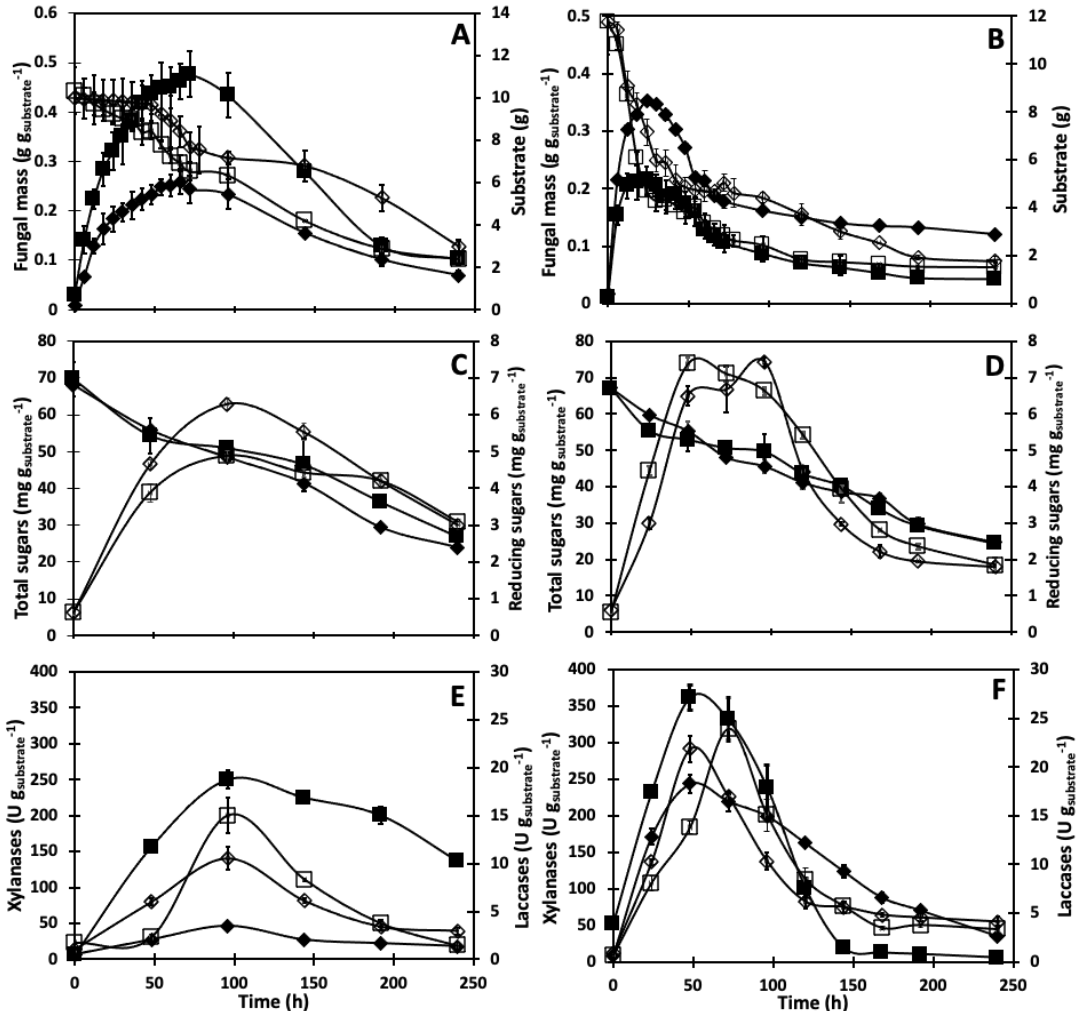

Figure 2. Solid-state culture of *Aspergillus niger* sp. growing on prickly pear peels. Oven-dried (left panels) or sun-dried (right panels), particles of 1.095 (diamonds) and 1.84 mm (squares). Fungal mass (closed figures) and prickly pear peels consumption (open figures) (A and B); total sugars (closed figures) and reducing sugars (open figures) (C and D); and production of xylanases (closed figures) and laccases (open figures) (E and F).

Fungal growth and the pattern of production of reducing sugars observed in *A. niger* sp. cultures growing on prickly pear peels are coincident with cultures in which an agro-industrial residue or a complex polysaccharide is used as the only carbon source. In a medium with wheat bran extract as the only carbon source, *Aspergillus* spp. showed the maximum production of reducing sugars during the first 36 h, which was caused by the hydrolysis of complex carbon chains in wheat bran; these reducing sugars decreased considerably after 36 h, and remained constant until the end of the experiment (Reginatto *et al*., 2017). Also, when *Aspergillus* spp. grew on ginkgo leaves on solid-state cultures at 35 ºC, there was a high hydrolytic activity and a consequent accumulation of resulting reducing sugars during the first 24 hours of culturing. Later, the consumption rate was higher than the hydrolytic activity, causing a decrease in the content of reducing sugars (Wang *et al*., 2018).

The behavior of the culture relative to fungal mass called for attention, because of a prominent drop in fungal mass after it reached the maximum level (Figure 2). The decrease in fungal mass coincided with the time of sporulation process, which was evident from the aspect of the cultures. Sporulation on solid-state cultures is common for this kind of fungi and has been reported as a consequence of the limitation of nutrients as the nitrogen source (García-Reyes *et al*., 2017), the reduction of moisture of the support (Wang *et al*., 2018) or of the low availability of ammonium sulfate in the medium (Swe *et al*., 2009). We did not record the respiration intensity of fungi in the culture, the moisture of the support, or the ammonium sulfate content during the culture; however, each experimental unit was a batch, in which water content cannot be controlled, neither

ammonium sulfate evolution registered. However, moisture of the support and nutrient content (including ammonium), could be lost during culturing because of metabolic activity, which explains the decrease in fungal mass and in enzymatic activities.

3.3 Enzyme activities in solid-state culture

Production of laccases and xylanases was related to fungal growth. Both enzymes reached their highest activity 24 h after the culture reached the highest fungal mass (96 h for oven-dried peels and 48 h for the sun-dried peels), and then decreased as fungal mass diminished, as can be seen on Figure 2.

Maximum activity of both enzymes was also observed at the same time, which suggests that production of xylanases and laccases was not sequential. The cultures developed on peels with particle size of 1.095 mm diameter showed the highest enzymatic activity levels, and enzymatic activities were higher when solar-dried peels were used as substrate (Figures 2 E and 2F).

Although enzymatic activities decreased as biomass decreased, xylanolytic activity in oven-dried particles decreased slower (0.3257 U $g^{-1}_{substrate}h^{-1}$ and 0.8142 $U/g^{-1}_{substrate}h^{-1}$ for 1.095 and 1.84 mm diameter, respectively) than the decrease observed in cultures developed on sun-dried peels (4 $U/g^{-1}_{substrate}h^{-1}$ and 3.56 $U/g^{-1}_{substrate}h^{-1}$ for 1.095 and 1.84 mm diameter, respectively), as can be seen on Figure 2. This phenomenon could be explained by taking into account that spore production occurs before carbon substrate exhaustion (Dorta *et al*., 1996), and the spores are obtained through a newly-synthesized wall (Larroche and Gross, 1992) and can germinate again later during culturing (Wang *et al*., 2018). It is thus possible that in oven-dried peels, fungi kept producing xylanases because of re-germination of spores formed after carbon exhaustion.

Although *A. niger* sp. produces hydrolytic enzymes, it can also produce oxidoreductases that degrade complex plant materials (Calle *et al*, 2007). Other wild strains of *Aspergillus* produce aromatic compounds, when cultivated in a liquid medium using residual lignin from wheat straw, with laccase titers close to 3 U g^{-1} of residual lignin for *A. fumigatus* after one week of culture and 2.4 U g^{-1} of residual lignin for *A. tubigensis* after three weeks (Baltierra-Trejo *et al*., 2016). Aromatic compounds or phenols are generally considered laccase inducers (Bertrand *et al*., 2013). A native strain of *A. terreus* isolated from sludge of carpet industry, showed laccase and manganese peroxidase activity that degraded and adsorbed azo dyes (Singh and Dwivedi, 2020).

Quinines, dimers, and oligomers released by UV radiation can act as inducers of oxidative activity, which could explain why laccase activity in *A. niger* sp. grown on sun-dried peels was higher than that produced on by *A. niger* sp. grown on oven-dried peels (Martínez *et al*., 2017). Our results suggest that using *A. niger* sp. to degrade plant cell walls is advantageous because its lignocellulosic enzyme system breaks down xylan and other polysaccharides, allowing lignin to be transformed into aromatic compounds.

We found that our *A. niger* sp. strain has low laccase activities and higher xylanases activities when growing on substrates, such as solid-state cultures made of combined sugar cane bagasse and wheat bran, probably because these materials contain lower lignin and higher hemicellulose levels than prickly pear peels. These observations suggest that specific enzymes are produced according to the composition of the substrate, as has been reported before (Silva *et al*., 2018). On the other hand, we have shown that the saccharification of agricultural residues can be improved by the joint action of laccases and hydrolytic activities that were produced by independent fungal cultures (Martínez-Trujillo *et al*., 2020). Thus, the finding that *A. niger* sp. can increase production of laccases when grown in a lignin-rich substrate suggests that all the ligninolytic activities could be produced in the same experimental unit, and that

Table 3 Regression coefficients of the models obtained after the ANOVA test for maximum and specific xylanase and laccase activities.

Parameter analyzed	β_0	β_1	β_2	β_{12}
Maximum xylanase activity ($U/g_{substrate}$)[a]	225.5	77.24***	80.3***	-21.21**
Maximum laccase activity ($U/g_{substrate}$)[b]	17.83	5.06***	1.62*	-0.59
Specific maximum xylanase activity ($U/g_{biomass}$)[c]	1417.31	1021.64***	567.91**	376.46*
Specific maximum laccase activity ($U/g_{biomass}$)[c]	126.94	86.13***	36.72**	42.49**

[a]Maximum xylanases activity was reached at 48 h on sun-dried peels and at 96 h on oven-dried peels.
[b]Maximum laccases activity was reached at 48 h on 1.095 mm diameter sun-dried peels, at 72 h on 1.84 mm diameter sun-dried peels, and at 96 h on oven-dried peels both particle sizes.
[c]Specific activities were calculated as the ratio of the maximum enzymatic activity and the biomass at the corresponding time.
***Highly significant for the corresponding enzyme production ($p < 0.0001$).
**Very significant for the corresponding enzyme production ($p < 0.005$)
*Significant for the corresponding enzyme production ($p < 0.05$)

the enzymatic extract obtained under these experimental conditions could have the same effect as mixed oxidative and hydrolytic enzymatic extracts used for saccharifying agricultural residues.

The results of the corresponding ANOVA, shown in Table 3, indicate that maximum xylanase activity had the same significant effect of both main factors, drying mode (X1) and particle diameter (X_2) as well as the interaction among them; while specific xylanolytic activity were influenced mainly by particle diameter. On the other side, maximum and specific laccase activities had the highest significant effect of drying type. These results strengthened our hypothesis about drying the peels under sun released several phenolic compounds that can favor the fungal growth and induce the laccase production.

Conclusions

This paper reflects the importance of the type of dried on prickly pear waste, an unusual and low-cost material, during its conditioning for been used as a substrate in solid cultures. At this respect, even when the drying at 45°C lasted more than 100 hours, drying rates were similar regardless the drying process. However, sun-dried peels yielded higher enzyme titles by *Aspergillus niger* sp. than oven-dried peels, which seemed to be affected by hornification phenomena. We also reported and analyzed the simultaneous production of laccases and xylanases in the conditioned material, finding that the maximum enzymatic production was achieved in less than 72 hours culture if the conditioning of the peel is done by sun-drying.

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