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PARTIAL CHARACTERIZATION AND IMMOBILIZATION OF CARBOXYMETHYLCELLULASE FROM *Aspergillus niger* PRODUCED BY SOLID-STATE FERMENTATION

CARACTERIZACIÓN PARCIAL E INMOBILIZACIÓN DE CARBOXI-METIL-CELULOSA DE *Aspergillus niger* PRODUCIDAS POR FERMENTACIÓN EN ESTADO SÓLIDO

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Abstract

The solid state fermentation is used in the bioprocess production of compounds of industrial applications. The objective of this paper is to characterize and immobilize carboxymethylcellulase enzymes produced by solid-state fermentation of the processing food wastes of acerola (*Malpighia glabra*), guava (*Psidium guajava*), cassava (*Manihot esculenta*), and passion fruit (*Passiflora edulis*) using the *Aspergillus niger*. Enzyme activities were determined at different temperatures (40-90 ℃) and pH (4.0-8.0). The enzymes were immobilized by adsorption onto silica gel and celite 545 supports. One could observe that the optimal carboxymethylcellulases temperatures of the crude enzymatic extracts from *A. niger* using acerola, guava, cassava and passion fruit residues ranged from 40-60 ℃ and that the optimal pH varied between 5.0-6.0. One it was found that the carboxymethylcellulases in these extracts presented high immobilization yields (85%, 93%, 46%, 34%) on silica gel and (91%, 87%, 100%, 38%) celite, respectively. The results are promising since it uses inexpensive solid supports and is a simple technique, thus providing optimal conditions for enzymatic recycling.

Keywords: solid-state fermentation, carboxymethylcellulase, physical adsorption, immobilization, agribusiness residues.

Resumen

La fermentación en estado sólido se utiliza en la producción de bioprocesos de compuestos de aplicaciones industriales. El objetivo de este trabajo es caracterizar e inmovilizar las enzimas carboximetilcelulasas producidas por fermentación en estado sólido del procesamiento de desechos alimenticios de acerola (*Malpighia glabra*), guayaba (*Psidium guajava*), yuca (*Manihot esculenta*) y maracuyá (*Passiflora edulis*) usando el *Aspergillus niger*. Las actividades enzimáticas se determinaron a diferentes temperaturas (40-90 °C) y pH (4.0-8.0). Las enzimas se inmovilizaron por adsorción en gel de sílice y se adhiere a celite 545. Se pudo observar que las temperaturas óptimas de carboximetilcelulasas de los extractos enzimáticos crudos de *A. niger* usando residuos de acerola, guayaba, yuca y maracuyá variaban entre 40-60 °C y que los pH óptimos variaban entre 5.0-6.0. Se encontró que las carboximetilcelulasas en estos extractos presentaban altos rendimientos de inmovilización (85%, 93%, 46%, 34%) en gel de sílice y (91%, 87% 129%, 38%) celita, respectivamente. Los resultados son prometedores ya que utiliza soportes sólidos de bajo costo y es una técnica simple, proporcionando así las condiciones óptimas para el reciclaje enzimático.

Palabras clave: fermentación en estado sólido, carboximetilcelulasa, adsorción física, inmovilización, residuos de agronegocios.

1 Introduction

Every year agribusiness activities generate millions of tons of waste. Some of these residues are harvested as animal feed or disposed of on field surfaces; however, although they present a high potential for biological and chemical conversion, most of them are still discharged without treatment, causing damage to the environment (Aggelopoulos *et al*., 2014).

About 40% of the products arising from fruit farming are considered to be agribusiness waste (Santos *et al*., 2013). The type of residue generated depends on the fruit being processed. The finding that agribusiness residues are rich sources of carbohydrates, proteins and minerals has contributed to expand scientific research and allow for their reutilization in a cost-effective and safe manner (Schieber *et al*., 2001; Ojumu *et al*., 2003). Biotechnological processes have become well-known worldwide, since their economic and operational characteristics give them advantages over conventional chemical processes (Chandra *et al*., 2010). Enzymes are one of the most relevant products in biotechnological processes. They are produced in the majority of the techniques, in which agriculture residues rich in lignocellulosic material are used as supports, which serve as a solid matrix and provide carbon and energy sources for the microorganism to grow (Pandey, 2003; Qu *et al*., 2006).

Among the techniques used in the production of enzymes using agribusiness residues are fermentation processes, such as solid-state fermentation (SSF), which is defined as the growth of microorganisms on solid substrates in the absence or near-absence of free water (Rahardjo *et al*., 2006). Fungi are the microorganisms most favorable for SSF since they develop well in environments presenting little water (Graminha *et al*., 2008). Several species of Aspergillus are used to produce enzymes and transform compounds and *A. niger* stands out as one of the most studied genera (Santos *et al*., 2016).

The production of enzymes by SSF using agribusiness residues enhances the economic feasibility of applying enzymes as biocatalysts in important industry segments, such as fuel, beverages, textiles and food (Díaz *et al*., 2007; Oberoi *et al*., 2010). For them the use of agribusiness residues has turned out to be a relevant alternative to reduce costs in the production of cellulases, an enzymatic complex capable of breaking down cellulose's glycosidic bonds, which results in the release of oligosaccharides, cellobiose, and glucose (Lynd *et al*., 2002). The enzyme technology for industrial-scale use has advanced with the immobilization of enzymes, which consists in confining said enzymes to a certain region of the space while preserving their catalase activity and promoting the recovery of products of a high degree of purity (Jose, 2006; Datta *et al*., 2013), which in turn results in an increased demand of the industry for immobilized biocatalysts. Physical adsorption is one of the simplest and most used techniques for enzyme immobilization. The enzyme is immobilized by means of weak interactions with the insoluble solid support, such as van der Waals forces (hydrophobic interactions), hydrogen bonds, and ionic bonds (Mendes *et al*., 2011; Romo-Sánchez *et al*., 2014). Adsorption immobilization presents such advantages as being a simple process and the fact that little is changed in the enzyme's conformational structure.

The objective of this paper is to characterize and immobilize carboxymethylcellulase (endoglucanase) enzymes produced by solid-state fermentation of the processing residues of acerola (*Malpighia glabra*), guava (*Psidium guajava*), cassava (*Manihot esculenta*), and passion fruit (*Passiflora edulis*) using the fungal species *A. niger* as inoculant.

2 Materials and methods

The enzymatic production was carried out by solid-state fermentation using Erlenmeyer flasks containing 10 g of food waste (acerola, guava, cassava and passion fruit) were autoclaved (121 ℃/1 atm/15 min) in Erlenmeyer flasks of 125 mL. Sterile water has added to the flasks until reaching 61% humidity, and the inoculation was performed with 108 spores.g^{-1} dry residue. The cultures were cultivated in a BOD (TE-317, TECNAL) with controlled temperature at 35 °C for 96 h (Santos *et al*., 2013; Do Santos *et al*., 2015). After fermentation, 50 mL of sodium phosphate buffer (0.1 M/pH 7.0) were added to the fermented substrate and the mixture was stirred (shake incubator, TECNAL) at 35 ℃ and 200 rpm for 20 min. The liquid phase was separated by mechanical pressing and permeation with gauze, followed by centrifugation (704.34 g) for 5 minutes to remove impurities (de Brito *et al*., 2015).

2.1 Enzyme activity evaluation

CMCase (endoglucanase) activity was assayed by determination of reducing sugar (glucose) released from carboxymethyl cellulose (CMC) as a substrate (Santos *et al*., 2011). The enzyme extract (crude extract) (0.25 ml) was incubated with 0.25 ml 2% CMC (Sigma) in 0.05 mM phosphate-citrate buffer solution, (pH 5.8) at 50ºC for 10 minutes. The resulted reducing sugars were determined according to Miller (1959) by dinitrosalicylic acid (DNS) using glucose as

standard. Enzyme activity was determined in terms of international unit (U) which is define as the amount of enzyme required to liberate one micromole of glucose per minute.

2.2 Determination of optimal pH and temperature of carboxymethylcellulase

The effect of temperature on carboxymethylcellulase activity was determined by enzyme activity assays in 10-minute reactions with different temperatures (40, 50, 60, 70, 80, and 90 ºC) in a 0.05M phosphate-citrate buffer, pH 5.8. The pH influence on carboxymethylcellulase enzyme activity was studied at 50ºC in a pH ranging from 4.0 to 8.0, being that the buffers used were a 0.05M phosphate-citrate one for the pH ranging from 4.0 to 7.0 and a 0.05M sodium-phosphate one for a pH of 8.0 (Mendels and Weber, 1969).

2.3 Carboxymethylcellulase enzyme immobilization in silica gel and Celite 545 by adsorption

Carboxymethylcellulase immobilization was done by contact using 0.4 mL of crude enzyme extract obtained by *A. niger* SSF using cassava, passion fruit, acerola, and guava residues and 0.1g of the solid support (silica gel or Celite 545), which were stirred at 80 rpm for 2 h at room temperature (25 ºC) (Sharma, *et al.*, 2008). After the immobilization period, the suspension was washed with a citrate-phosphate buffer (0.05M, pH 5.0), the supernatant was reserved for posterior total protein analysis and the immobilized enzyme was reserved for application in the CMC hydrolysis. The immobilization efficiency was evaluated subsequently. Each experiment was conducted in triplicates.

2.4 Determination of immobilized carboxymethylcellulase enzyme activity and immobilization rate

The activity was determined as previously described using carboxymethylcellulose (2%) substrate solution in a citrate-phosphate buffer (0.05M, pH 5.8). The immobilization rate describes the relationship between the activity of the immobilized enzyme and the amount of support used for immobilization, calculated by Eq. 1 (Ribeiro *et al.*, 2018):

$$Immobilization \ rate = \left(\frac{Enzyme \ activity \ (U/mL)}{Solid \ support \ (g)} \right) \quad (1)$$

2.5 Immobilization parameters

The immobilization yield (IY%) was calculated by using the concentration of initial protein and the concentration of protein present in the reaction medium after the immobilization process according to Eq. 2 (Ribeiro *et al.*, 2018):

$$IY(\%) = \frac{P_i - P_f}{P_i} \times 100 \quad (2)$$

where P_i and the protein concentration at initial time and P_f and the protein concentration at final time (mg mL^{-1}).

2.6 Free and immobilized enzyme's total protein dosage

The total protein dosage was performed by adding 100 μl of either the enzyme extract (for free enzymes) or immobilization supernatant (for immobilized enzymes) to 5.0 mL of Bradford reagent (Bradford 1976). The spectrophotometer read 595 nm and the total protein present in the supernatant was quantified. The amount of immobilized enzyme was evaluated by Eq. 3 (Ribeiro *et al.*, 2018):

$$PR(\%) = \left(1 - \frac{Protein \ in \ supernatant}{Free \ protein \ at \ crude \ enzyme \ extract} \right) \times 100 \quad (3)$$

where *PR* = Protein retention.

3 Results and discussion

3.1 Carboxymethylcellulase enzyme activity produced by A. niger using agribusiness residues

The results of carboxymethylcellulase activity determined (Figure 1) are similar with the activity studies that used similar SSF conditions. In the results found by Acharya *et al.*, (2008), cellulase maximum activity was 0.093 U mL^{-1} using Aspergillus as fungus genus and sawdust as substrate, an enzyme activity lower than that observed in the present study.
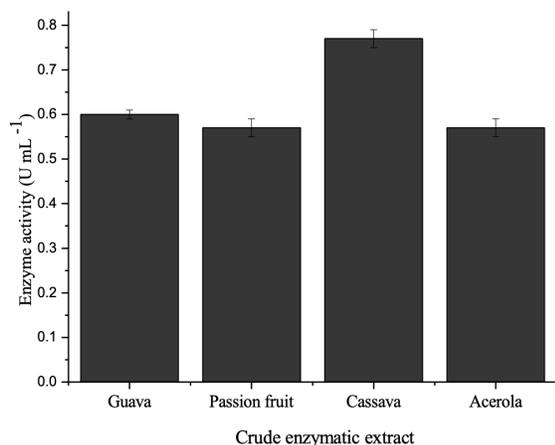
Fig. 1. Carboxymethylcellulase extract enzyme activity from *A. niger* using cassava, passion fruit, acerola, and guava residues.

Amaeze *et al.*, (2015), in turn, obtained similar enzyme activity values for cellulases: 0.83 U mL^{-1} using orange residues as support and *A. niger* as fungus. By using sugarcane bagasse as substrate and fungi *Aspergillus aculeatus* URM 4953 and *Aspergillus phoenicis* URM 4924 as inoculants, the carboxymethylcellulase activity ranged from 0.04 to 0.60 U mL^{-1} (Sales *et al.*, 2010).

According to the results obtained, *A. niger* fungus showed to be promising in the production of carboxymethylcellulases using cassava, passion fruit, acerola, and guava residues in the SSF. However, there were differences between the results of the enzyme extracts from different residues, which shows that not all substrates have the same potential for enzyme production. One factor that may have interfered with the amount of enzymes produced is the specific characteristic of each residue used, according to their partial chemical constitution (Pandey *et al.*, 2001).

3.2 Determination of optimal pH and temperature for carboxymethylcellulase activity

For the partial characterization of carboxymethylcellulase in crude extract, tests were performed to study the pH and temperature of the enzyme reaction.

The carboxymethylcellulase showed optimal activity at pH 5.0 for enzyme extracts from *A. niger* using cassava (0.47 ± 0.003 U mL^{-1}) and guava (0.16 ± 0.01 U mL^{-1}) residues, and at pH 6.0 for passion fruit (0.23 ± 0.009 U mL^{-1}) and acerola (0.20 ± 0.003 U mL^{-1}) residue extracts (Figure 2).
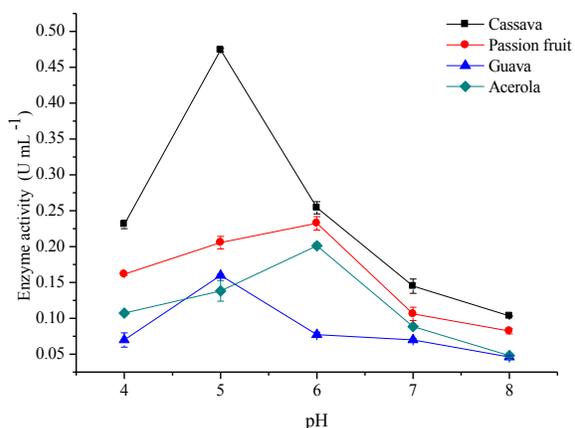
Fig. 2. Carboxymethylcellulase enzyme activity under different pH ranges (4.0 to 8.0).
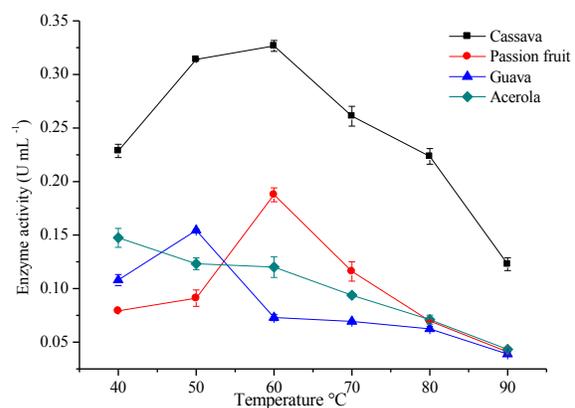
Fig. 3. Carboxymethylcellulase enzyme activity under different temperature ranges.

The optimal temperatures for cellulase enzyme activities were: 40 ℃ for acerola crude enzyme extract (0.15 ± 0.008 U mL^{-1}), 50 ℃ for guava enzyme extract (0.15 ± 0.001 U mL^{-1}), whereas cassava and passion fruit extracts showed greater activities at 60 ℃: 0.33 ± 0.005 U mL^{-1} and 0.19 ± 0.006 U mL^{-1}, respectively (Figure 3), which confirm the study that obtained a similar optimal temperature result, 65 ℃, using fungus enzymes of the same genus and wheat bran as substrate (Delabona *et al.*, 2012).

Several carboxymethylcellulases derived from fungi showed characteristics similar to those observed in this study regarding optimal pH and temperature. Dutt and Kumar (2014) obtained similar results of optimal pH 5.3 for cellulase activity using *A. niger* and rice straw as the carbon source. In the study of optimization of wheat straw saccharification conditions with cellulases produced by *Aspergillus heteromorphus*, the optimal enzyme activity occurred

at pH 5.0 (Singh *et al.*, 2009), confirming the results by Abubakar and Oloyede (2013), who verified 4.0 to be the optimal pH for carboxymethylcellulase enzyme activity.

Determining the optimal pH for carboxymethylce-llulases is relevant since variations in pH may result in changes in the ionic form of active site of the enzymes, hence interfering with their activity.

The 40-60 ℃ temperature range observed for the optimal carboxymethylcellulase activity is similar to that verified in other studies that found optimal temperature of 45 ℃ for endogluconases of *A. niger* using wheat bran and bark of orange as fermentation support (Delabona *et al.*, 2012; Amaeze *et al.*, 2015). At the optimal temperatures found in this study the protein denaturation rate was lower and the integrity level of the protein three-dimensional structure was higher.

At temperatures above 80 and 90 ℃ there was a 30% reduction in enzyme activity since, besides the likely thermal denaturation, the reaction displacement that occurs for product formation causes the cellulose mass to reduce, in addition to the likely production of inhibitors in the reaction, which contributes to its deceleration.

The thermophilicity of *A. niger* fungus is moderate its optimal production temperatures vary between 40 and 50℃ and it can be considered a good producer of thermostable enzymes (Polizeli *et al.*, 2005) since it can generate optimal activity enzymes at temperatures higher than the maximum growth temperature of the producer organism, as is the case of carboxymethylcellulases in this study.

3.3 Carboxymethylcellulase immobilization with silica gel and Celite

The immobilizations of carboxymethylcellulases produced by *A. niger* using agribusiness (cassava, passion fruit, acerola, and guava) residues were carried out using the adsorption technique of inorganic support (silica gel, Celite 545). Table 1 shows carboxymethylcellulase enzyme activity, demonstrating the mean ± standard deviation for the activity of both free and immobilized enzymes in each support.

Upon evaluation of the results found for carboxymethylcellulase immobilization by adsorption on silica gel and Celite supports, it was observed, when comparing the activity of the immobilized enzyme with that of the free enzyme, that on both supports there was a small loss of enzyme activity in most extracts. However, the enzyme from acerola residues presented better activity when immobilized (0.57 U mL^{-1}) than in its free state (0.73 U mL^{-1}).

The increased enzyme activity of the acerola extract occurs because cellulase adsorption on the support is ionic and has weak bonding. It does not involve strong chemical bonds that may cause distortions on the active site of the enzyme, which contributes to the conservation of a conformation that is suitable for the substrate to bind to the biocatalyst (Prado *et al.*, 2005).

The adsorption (immobilization) process occurs by electrostatic bonding between oppositely-charged groups in the molecules of both the enzyme and the support.

Table 1. Carboxymethylcellulase activity from *A. niger* immobilized by adsorption on silica gel and Celite 545.

Support		Free enzyme extract activity (U mL^{-1})	Immobilized enzyme activity (U mL^{-1})
Sílica gel	Crude enzyme extract (cassava)	0.773 ± 0.018	0.657 ± 0.006
	Crude enzyme extract (passion fruit)	0.567 ± 0.018	0.528 ± 0.009
	Crude enzyme extract (acerola)	0.567 ± 0.018	0.266 ± 0.011
	Crude enzyme extract (guava)	0.595 ± 0.012	0.208 ± 0.010
Celite 545	Crude enzyme extract (cassava)	0.773 ± 0.018	0.700 ± 0.007
	Crude enzyme extract (passion fruit)	0.567 ± 0.018	0.496 ± 0.024
	Crude enzyme extract (acerola)	0.567 ± 0.018	0.733 ± 0.011
	Crude enzyme extract (guava)	0.596 ± 0.011	0.226 ± 0.011

Table 2. Immobilization yields on silica gel and Celite 545 of enzyme extracts from *A. niger* using cassava, passion fruit, acerola, and guava residues.

Sample	Silica gel IY%	Celite 545 IY%
Crude enzyme extract (passion fruit)	93.0 ± 1.65	87.0 ± 0.70
Crude enzyme extract (cassava)	85.0 ± 0.76	90.5 ± 1.08
Crude enzyme extract (acerola)	46.0 ± 0.55	100.0 ± 1.46
Crude enzyme extract (guava)	35.0 ± 0.80	37.0 ± 0.07

IY: Calculated by using the concentration of initial protein and the concentration of protein present in the reaction medium after the immobilization process.

Such interactions occur between the residual amino groups in the enzyme molecule and the functional groups in the support molecules and are considered weak as they are ionic, dipole-dipole, hydrogen bonding, etc. (Jesionowski *et al*., 2014).

These supports were chosen because immobilization with Celite and silica gel by adsorption is a widely-used technique and the weak forces between the enzyme and the support may contribute to causing little or no interference in the enzyme activity (Luckarift *et al*., 2004). Moreover, Celite acts as a biosupport capable of improving both reaction rate and biocatalyst distribution (Villeneuve *et al*., 2000) whereas silica presents the silanol groups responsible for the chemical behavior on the silica surface and play an important role in adsorption processes (Satar *et al*., 2008).

The immobilization yields on silica and Celite 545 showed different results according to the enzyme extract employed (Table 2). The highest immobilization yields on silica gel (93%) and Celite (100%) were observed in *A. niger* crude enzyme extracts from cassava and acerola residues, respectively.
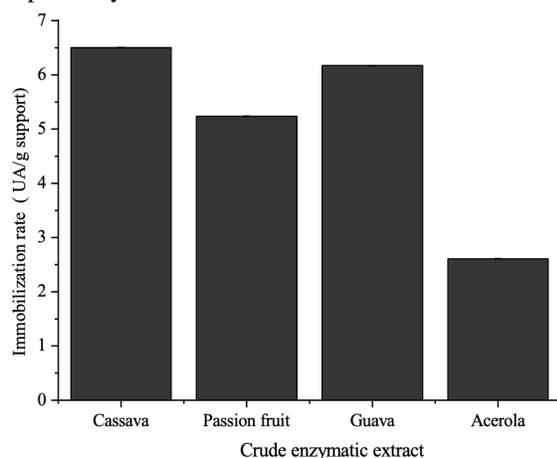
Fig. 4. Immobilization rate result with silica gel support .
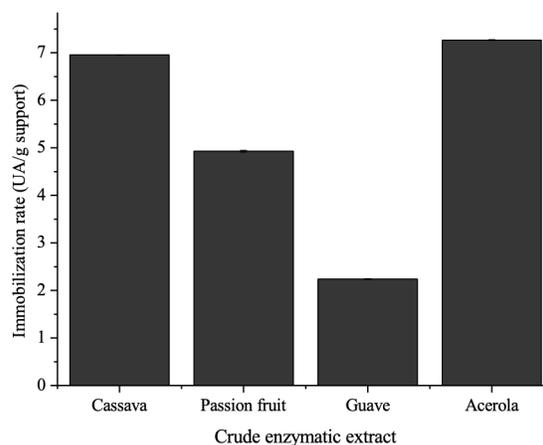
Fig. 5. Immobilization rate result with Celite 545 support.

Consequently, the highest immobilization rates per gram of support were in the cassava extract (6.50 AU/g of silica support) and in the acerola extract (7.27 AU/g of Celite support) (Figures 4 and 5).

The lowest immobilization rates with both silica (2.06 AU/g support) and Celite (2.24 AU/g support) supports were in the guava crude enzyme extract, although with silica gel there were not proteins found in the immobilization supernatant, which reveals that all the proteins in the extract were retained in the support, i.e. there was 100% of protein retention. Nonetheless, the enzyme activity of the immobilized guava crude enzyme extract decreased in about 2.5-fold when compared to that of the free enzyme extract.

The results obtained in Chang *et al*., (2011) showed that when cellulase is immobilized with silica nanoparticles it can achieve an effective cellulose-to-glucose conversion, excellent stability, and a yield of over 80%. Dalla-Vecchia *et al*., (2004) used the chitosan-alginate support activated with glycodol and obtained a yield of around 42.2%, which makes the yield rates of this present study more satisfactory, since the efficiency in the immobilization of cellulases in under 20 nm superparamagnetic nanoparticles for β-glucosidase A and cellobiohydrolase was 100% (Song *et al*., 2016).

Enzyme immobilization was performed using a simple, inexpensive, and safe adsorption method that has high carboxymethylcellulose hydrolysis yields, as observed in the study that used non-porous and porous silica as support and in which the immobilized enzyme maintained a catalase activity greater than 40% after its sixth reutilization (Ikeda *et al*., 2015).

Table 3. Total protein concentration of carboxymethylcellulase enzyme produced from *A. niger* with cassava, passion fruit, acerola, and guava residues immobilized by adsorption on silica gel and Celite 545.

Support	PntF	PntS	PntI	
Sílica gel	Crude enzyme extract (cassava)	0.120 ± 0.008	0.038 ± 0.001	0.081
	Crude enzyme extract (passion fruit)	0.102 ± 0.001	0.057 ± 0.003	0.045
	Crude enzyme extract (acerola)	0.074 ± 0.007	0.024 ± 0.001	0.050
	Crude enzyme extract (guava)	0.045 ± 0.003	0.000 ± 0000	0.045
Celite 545	Crude enzyme extract (cassava)	0.123 ± 0.002	0.041 ± 0.0001	0.074
	Crude enzyme extract (passion fruit)	0.125 ± 0.011	0.019 ± 0.0003	0.106
	Crude enzyme extract (acerola)	0.074 ± 0.007	0000 ± 0000	0.074
	Crude enzyme extract (guava)	0.046 ± 0.001	0.019 ± 0.0003	0.027

The total protein dosage was performed by the Bradford method. Total protein concentration (mg mL^{-1}).
PntF (Free protein); PntS (Protein in supernatant); PntI (Protein immobilized on silica gel and Celite).
Values/100

In another paper cellulase was immobilized on silica gel surfaces pretreated with (3-aminopropyl) triethoxysilane (3-APTES) and glutaraldehyde, and it showed specific activity $7 \pm 2\%$ when compared to the similar amount of free cellulose; furthermore, the immobilized cellulase maintained 48% of its initial activity after 4 days of reutilization (Zhang *et al.*, 2016). It was observed that there was a satisfactory binding of the enzyme to the silica gel support due to the low concentration of total protein found in the immobilization supernatant (Table 3). Of the 0.10 mg mL^{-1} of total protein in the passion fruit free extract, 0.04 mg mL^{-1} of the enzyme were immobilized; of the 0.12 mg mL^{-1} of free enzyme in the cassava extract, 0.08 mg mL^{-1} were immobilized; of the total 0.07 mg mL^{-1} of free enzyme in the acerola extract, 0.05 mg mL^{-1} were immobilized; however, no protein was found in the supernatant in the immobilization of the guava extract, which shows that of all free protein in said extract, 0.04 mg mL^{-1} were immobilized (Table 3). In Table 4, the amount of immobilized enzyme can be verified through the ratio that describes the protein retention according to the amount of proteins in the immobilization supernatant and in the free enzyme extract.

The total protein dosage immobilized on Celite 545 by adsorption was: 0.12 mg mL^{-1} for free carboxymethylcellulase enzyme in the crude enzyme extracts of passion fruit and cassava, 0.07 mg mL^{-1} in the acerola extract, and 0.05 mg mL^{-1} in the guava extract. The results found in the immobilization supernatant were: 0.02 mg mL^{-1} in the passion fruit extract, 0.04 mg mL^{-1} in the cassava extract, 0.0 mg mL^{-1} in the acerola extract, and 0.02 mg mL^{-1} in the guava extract, hence estimating that protein retention was 84.8% (passion fruit extract), 66.9% (cassava extract), 100.0% (acerola extract), and 59.1% (guava extract) (Table 4). A high binding of the extracts to the Celite support was observed as well as the fact that enzyme activity was better when immobilized, mainly for the acerola extract. The immobilized carboxymethylcellulase presented greater pH and temperature stability than the free one. Unlike conventional immobilization methods by covalent binding, which often require several complicated procedures, immobilization can be accomplished by the simply contacting between enzyme and support, as is the case with the adsorption technique tested in this study.

Conclusions

Carboxymethylcellulase immobilization is still not a widely used technique and the results of this type of immobilization are promising since carboxymethylcellulases immobilized on silica gel and Celite 545 have shown immobilization yields of up to 100.0%. The immobilized enzyme can be reused in sequential processes without losing its efficiency, and the studies on how to optimize carboxymethylcellulase immobilization have contributed to the overcoming of current limitations involving immobilization techniques and cost reduction with industrial bioprocesses for the hydrolysis of lignocellulose products. Therefore, since silica gel and celite are inexpensive supports and adsorption is a simple immobilization process, one intends to continue the research with the purpose of further improving the stability of the immobilized enzyme.

Table 4. Protein retention of carboxymethylcellulase in enzyme immobilization with silica gel and Celite 545.

Support	PntF	PntS	PntI	
Sílica gel	Crude enzyme extract (cassava)	11.95 ± 0.76	3.82 ± 0.19	68.0
	Crude enzyme extract (passion fruit)	10.19 ± 0.07	5.71 ± 0.35	44.0
	Crude enzyme extract (acerola)	7.35 ± 0.69	2.37 ± 0.08	67.7
	Crude enzyme extract (guava)	4.45 ± 0.29	0.00	100.0
Celite 545	Crude enzyme extract (cassava)	12.33 ± 0.24	4.08 ± 0.008	66.9
	Crude enzyme extract (passion fruit)	12.47 ± 1.06	1.90 ± 0.04	84.8
	Crude enzyme extract (acerola)	7.35 ± 0.69	0.00	100.0
	Crude enzyme extract (guava)	4.64 ± 0.03	1.90 ± 0.03	59.1

Protein retention as defined in Eq. 3. PntF (Free protein); PntS (Protein in supernatant); PR (Protein retention). Other details are described in Methods section.

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