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CAPTURE OF LECTINS FROM JACKFRUIT (*Artocarpus integrifolia*) SEEDS IN A SINGLE STEP USING A SUPERMACROPOROUS ION EXCHANGE CRYOGEL

CAPTURA DE LECTINAS DE SEMILLAS DE YACA (*Artocarpus integrifolia*) EN UN SOLO PASO USANDO UN CRIOGEL SUPERMACROPOROSO DE INTERCAMBIO IÓNICO

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Abstract

Lectins have potential for application in many fields because of their ability to selectively bind glycoconjugates. Jackfruit seeds are a source of various lectins, with several isoforms and isoelectric points. Therefore, ion exchange can be a viable alternative for lectin separation. In this work we developed a protocol to capture lectins from jackfruit seed extract in single-step using ion exchange cryogels. Supermacroporous cryogels were functionalized with cationic and anionic exchangers. The matrices produced showed high swelling capacity (12.4 and 14.7 kg/kg), expansion degree (12.70 to 18.0 L/kg), and total porosity around 90%. The performance of the ion exchanger cryogels in lectin purification were evaluated. A single elution peak was obtained in all assays. The purification factor (PF) was determined based on the agglutination of human blood ABO types, with a PF of 3.57 in the best condition, using the cryogel functionalized with 2-(Dimethylamino)ethyl methacrylate (DMAEMA) and blood type B agglutination, suggesting the jacalin purification. SDS-PAGE electrophoresis confirmed that the eluted samples showed two strong bands around 13.5 kDa and 16 kDa. The results demonstrate that ion-exchange monolithic cryogels showed potential use for the purification of lectins from jackfruit seeds in a rapid and single-step protocol.

Keywords: cryogels, polyacrylamide, functionalization, ion exchangers, jacalin.

Resumen

Las lectinas tienen potencial para aplicación en diversos campos por su habilidad de enlazar glicoconjugados selectivamente. Las semillas de yaca son una fuente de lectinas, con diferentes isoformas y puntos isoeléctricos. Así, el intercambio iónico es una alternativa para la separación de lectinas. En este trabajo, desarrollamos un protocolo de purificación para extracto de semillas de yaca en criogeles de intercambio iónico (catiónicos y aniónicos). Las matrices producidas demostraron alta capacidad de hinchamiento (12,4 a 14,7 kg/kg), grado de expansión (12,70 a 18,0 L/kg) y porosidad total de un 90%. Se evaluó el desempeño de las matrices en la purificación de lectina. Se obtuvo un único pico de elución en todos los ensayos. Se determinó el factor de purificación (FP) con base en la aglutinación de sangre humana de tipos ABO, con un FP de 3,57 bajo las mejores condiciones, usando un criogel funcionalizado con metacrilato de 2-dimetilaminoetilo y aglutinación de sangre tipo B, lo que sugirió la purificación de jacalina. La electroforesis SDS-PAGE confirmó que las muestras eluidas presentaron dos bandas fuertes de 13,5 kDa y 16 kDa. Los resultados demostraron que las matrices presentaron uso potencial para la purificación parcial de lectinas de semillas de yaca.

Palabras clave: criogeles, poliacrilamida, funcionalización, intercambiadores iónicos, jacalina.

1 Introduction

Lectins are proteins of non-immune origin that bind reversibly to carbohydrates or biomolecules that contain sugars. They have the ability to agglutinate cells and precipitate glycoconjugates without changing the structure of the glycosyl binder (Pandey *et al*., 2010). Because of these characteristics, lectins have a variety of biological effects, presenting insecticidal, fungicidal, bactericidal, and antitumor activities (Coelho *et al*., 2007; Sitohy *et al*., 2007; Santi-Gadelha *et al*., 2006; Petrossian, Banner and Oppenheimer, 2007).

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The main sources of lectins are plants, especially legume seeds. Lectins are also found in a variety of organisms, from bacteria to animals (Santana *et al*., 2008; Perçin and Aksöz, 2012). Jackfruit is a tropical tree of the family Moraceae that produces large-sized fruits Its potential for protein extraction has been reported in numerous studies, as is the case for lectins, proteases and protease inhibitors (Cecílio *et al*., 2016; Gangaraju *et al*., 2015; Lyu *et al*., 2015; Swami *et al*., 2012; Siritapetawee and Thammasirirak, 2011). Lectins are the main proteins found in jackfruit seeds and jacalin accounts for about 50% of their total protein content (Swami *et al*., 2012; Kabir, 1998). Jacalin is a galactose-selective lectin consisting of a glycosylated tetramer of about 66 kDa. Each unit is composed of a double polypeptide chain: one with 133 amino acid residues and the other with 20 residues (Swami *et al*., 2012; Sahasrabuddhe *et al*., 2004). It presented different isoforms with the isoeletric point (pI) ranging from 5 to 8.5 (Kabir, 1995). It has the ability to bind to human IgA, which allows its use for isolating plasma glycoproteins in investigating IgA nephropathies and detecting tumors (Ahmed *et al*., 2005). Jacalin also exhibits anti-HIV activity and insecticidal properties, in addition to being able to agglutinate erythrocytes from various animal species, including humans (Kabir, 1998).

ArtinM, formerly known as artocarpine or KM+, is another important lectin in jackfruit, but found in smaller amounts, is (Silva *et al*., 2008). It has a homotetramer structure, with a molar mass around 64 kDa (Cecílio *et al*., 2016). ArtinM has specificity for different sugars (Barre *et al*., 2004), but its highest affinity is for mannose and its derivatives (Cecílio *et al*., 2016). ArtinM has several functions, including immunomodulatory activity, aid in mucosal regeneration and action against intracellular pathogens (Cecílio *et al*., 2016; Barbosa-Lorenzi *et al*., 2011; Kim *et al*., 2013). Chromatographic techniques are the most important in developing biocompound purification processes, which have an average of two to three steps. To purify lectins, techniques such as affinity, ion exchange and gel filtration are used individually or combined (Jungbauer and Hahn, 2008; Lenhoff, 2011; Wu *et al*., 2016; Sharma *et al*., 2018). Affinity chromatography based on specific interactions is the most used lectin purification technique. Ion exchange also is used however because of its selectivity and separation capacity under near physiological conditions and maintaining the structural integrity of biomolecules. Ion exchange is based on differentiated adsorption of charged compounds (positively or negatively) on a surface with the opposite charge and has been used to purify proteins, peptides and enzymes (Chen *et al*., 2010; Machado *et al*., 2015; Khan *et al*., 2017; Miller *et al*., 2018).

Lectin purification from sea sponge was investigated by Marques *et al*. (2018), who used affinity chromatography to obtain a 21-fold purification and 34% recovery. Sharma *et al*. (2017) purified lectins from *Dioscorea bulbifera* by affinity techniques, also obtaining a 21-fold purification but a 16.8% recovery. Ion exchange was also used to purify lectins, with adequate results. Singh *et al*. (2018) obtained a 22.4-fold purification and 94% recovery isolating lectins from *Penicillium duclauxii* using anionic exchange. The authors improved lectin purity by gel filtration until a 60-fold purification. It is unusual to combine different techniques into a purification strategy. Wu *et al*. (2016) combined affinity, ion exchange and gel filtration techniques in sequence to purify lectins from *Phaseolus lunatus billb*, obtaining a 13-fold purification and recovery of 10.1%. Research is scarce on purifying jacalin by chromatographic techniques (To *et al*., 1995; Biewenga *et al*., 1988; Datta *et al*., 2016; Roy *et al*., 2005; Hagiwara *et al*., 1988; Kabir, 1995; Simone *et al*., 1994; Vijayakumar and Forrester, 1986), and involves the laborious multistep purification process and high cost of affinity ligands (To *et al*., 1995; Biewenga *et al*., 1988; Hagiwara *et al*., 1988; Kabir, 1995; Vijayakumar and Forrester, 1986).

Monolithic cryogels are fourth generation materials and present proven efficiency as a chromatographic matrix (Jungbauer and Hahn 2008; Veríssimo *et al*., 2017; Mól *et al*., 2017; Machado *et al*., 2015; Gonçalves *et al*., 2017; Gonçalves *et al*., 2016; Carvalho *et al*., 2014; Lozinsky *et al*., 2003). Cryogels are versatile and can be made as column or disc-shaped, or as membranes, in addition to having a low production cost when compared to traditional chromatography matrices (Guiochon, 2007, Veríssimo *et al*., 2018). These matrices also have other interesting characteristics such as low load loss and flow resistance. Their surface area however is significantly smaller than that of a packed bed, which is a disadvantage that may decrease their efficiency. For this reason, cryogel surface functionalization is a field in constant evolution, with the goal of increasing the efficiency of the separation processes (Mól *et al*., 2017; Machado *et al*., 2015; Gonçalves *et al*., 2016; Yao *et al*., 2007; Yun *et al*., 2007; Wang *et al*., 2008).

One of the modifications made on the cryogel surfaces is the immobilization of ion exchange binders, such as 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA) (Machado *et al*., 2015; Yao *et al*., 2007), 2-(Dimethylamino)ethyl methacrylate (DMAEMA) (Savina *et al*., 2005), [2-(Acryloyloxy) ethyl]trimethylammonium chloride (AETA-Q) (Savina *et al*., 2006) and acrylic acid (AAc) (Savina *et al*., 2005). Moreover, in these works, ion exchange chromatography was not used as a strategy to directly capture the jacalin in a single step.

In our previous works, lectins were purified on affinity cryogel functionalized with carbohydrates with about 44.5 mg of proteins adsorbed per gram of cryogel (Gonçalves *et al*., 2017; Gonçalves *et al*., 2016). In the present study we propose using ion exchange chromatography as a strategy for purifying lectins, especially jacalins, from crude jackfruit seed extract.

The functionalization of a polyacrylamide cryogel surface was assessed using cation (AMPSA or AAc) or anionic (AETA-Q or DMAEMA) exchangers, aiming to capture jackfruit seed lectins. The chemical, physical and morphological properties of the cryogels were characterized. Lectins were captured directly from crude extracts of jackfruit seed in a single-step protocol using a chromatography system and efficiency was evaluated as a function of purification factor and recovered hemagglutinating activity.

2 Materials and methods

2.1 Materials

AMPSA (99%), AAc (99%), DMAEMA (98%), AETA-Q (80% solution in water), N,N,N',N'-tetra-methyl-ethylenediamine (TEMED, 99%), acrylamide (AAm, 99%), N,N'-methylene-bis(acrylamide) (MBAAm, 99%), ammonium persulfate (APS, 98%) and potassium persulfate (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were purchased from Vetec (São Paulo, Brazil). All chemicals used were of analytical grade or higher. Deionized water was used in all experiments.

2.2 Synthesis of monolithic cryogels

The cryogels were synthesized using the methodology of Machado *et al*., 2015, with modifications. Initially, 100 mL of a solution containing 7.0% (w/v) AAm monomers (5.6 g) and BAAm (1.4 g) was prepared in an ice bath. Then, 120 μl of an APS solution (0.5 g/L) and 78 μl of TEMED were added. The solution was poured into plastic syringes and immersed in an ethanol bath at -20 °C for 24 h. The syringes were thawed at 4 °C for 4 h and the cryogels were dehydrated in an oven at 60 °C. The ends of the monoliths were then cut and the cryogels were washed with 100 ml deionized water, dried again and stored until use.

2.3 Functionalization of cryogels with ion exchanger radicals

Cryogel functionalization began with activating the diperiodate potassium cuprate, according to methodology of Machado *et al*. (2015), with modifications. Fifteen mL of the activating solution and a mixture of 1 mol/L NaOH and 0.0562 mol/L potassium diperiodate at the ratio of 1:3 were percolated by recirculation through a cryogel for 90 min at a flow rate of 1 mL min^{-1} at 45 °C. Afterwards, ionic radicals AMPSA, AAc, DMAEMA or AETA-Q were grafted. For the grafting purpose, 15 mL of a 2 mol/L solution of the selected ion exchanger was recirculated through each activated cryogel at a flow rate of 1 mL/min for 2 h at 45 °C. Then, each functionalized cryogel was washed with 100 ml of a 0.1 mol/L HCl solution at a flow rate of 2 ml/min and a temperature of 45 °C, followed by washing with 100 ml of deionized water. Finally, the cryogels were oven dried at 60 °C and stored until use. The same procedure was repeated for 32 experimental units (cryogel monoliths).

2.4 Characterization of cryogels

Swelling capacity (S), expansion degree (ED), porosity and constituent fractions were determined. The functionalization of the cryogel surfaces was assessed through Fourier-transform infrared (FTIR) spectroscopic analysis. The swelling capacity (S) and the expansion degree (ED) were determined following Savina *et al*. (2005) and Gonçalves *et al*. (2016). Six pure cryogels and cryogels functionalized with the different ion exchanger radicals were immersed for 24 h in containers with 50 mL of water at room temperature. The volume was subsequently determined in a 50 mL graduated cylinder containing 20 mL of distilled water. Excess water was removed and the masses were measured on an analytical balance. The cryogels were dried in a heating oven

at 60 °C and weighed again. Swelling capacity was calculated as the ratio between the amount of water removed from the cryogel and its dehydrated mass. The expansion degree was the ratio between its hydrated volume and its dehydrated mass. The macropore fractions (φ_M), with size ≥ 1 μm, meso and micropore fractions (φ_m), with size < 1 μm, water bound (φ_{wb}), dried polymer (φ_d) and total porosity fractions (φ_T) of pure and functionalized cryogels were determined following Plieva *et al*. (2004a, 2004b). Six samples of pure and functionalized cryogels were dehydrated and their masses were weighed. The samples were maintained for 15 days in a desiccator containing a saturated solution of potassium sulfate. Subsequently, the cryogels were saturated with deionized water for 24 h and their masses were weighed. The cryogels were gently squeezed and their masses were measured again. The parameters φ_M, φ_m, φ_{wb}, φ_d and φ_T were determined according to Eqs. (1-5).

$$\varphi_M = \frac{m_s - m_e}{m_s} \tag{1}$$

$$\varphi_m = \frac{m_e - m_{wb}}{m_s} \tag{2}$$

$$\varphi_{wb} = \frac{m_{wb} - m_d}{m_s} \tag{3}$$

$$\varphi_d = \frac{m_d}{m_s} \tag{4}$$

$$\varphi_T = \frac{m_s - m_{wb}}{m_s} = \varphi_M + \varphi_m \tag{5}$$

where ms is the mass of hydrated cryogel (kg), m_d is the mass of dehydrated cryogel (kg), m_e is the mass of squeezed cryogel (kg) and m_{wb} is the mass of the cryogel with bound water (kg).

The functional groups of the cryogels produced were evaluated by Fourier transformed infrared spectroscopy. The evaluated samples were dried, made into powder and read directly using the attenuated total reflectance technique (ATR) in the infrared region of 4000-500 cm^{-1} with a spectrophotometer FTIR Bruker, model Vertex 70.

2.5 Lectin capture assays

2.5.1 Preparation of jackfruit seed crude extract

Jackfruit seeds were manually removed from ripe fruits, washed and sun-dried. The dried seeds were ground in a knife mill and the meal was sieved in a 1 mm mesh. Then, 5 g of the sieved meal was weighed and mixed into 50 ml of 0.02 mol/L sodium phosphate buffer, pH 7.2, containing 0.15 mol/L NaCl. The mixture was gently stirred at 4 °C for 24 h. The mixture was centrifuged and the supernatant was filtered through a cellulose acetate membrane with 0.45 μm pores and dialyzed against an NaCl-free sodium phosphate buffer solution. The final extract solution was frozen and maintained at -18 °C until use.

2.5.2 Chromatographic capture assays

Capture assays were performed in an ÄKTA Pure 25L (GE Healthcare, Upsalla, Sweden) chromatograph system with constant flow of 1.5 mL/min at room temperature. Initially, the column (5 cm height and 1.1 cm diameter) of ion exchange cryogel (AMPSA, AAc, DMAEMA, or AETA-Q) was equilibrated with 10 mL (± 2 column volumes - CV) 0.02 mol/L of sodium phosphate buffer at pH 7.2. Afterwards, 7 mL (± 1.5 CV) of the crude solution (kept in an ice bath) was passed through the column forming a rupture curve. The column was washed with phosphate buffer until the absorbance at 280 nm was less than 5 mAU and was then eluted. A gradient from 0% to 100% of the elution solution (0.02 mol/L sodium phosphate buffer pH 7.2 added with 1.0 mol/L NaCl) in 15 mL (± 3 CV) was used, then levelled off at 15 mL (± 3 CV) with 100% elution solution. Finally, 10 mL (± 2 CV) of the equilibriation buffer was applied, restarting the process. The entire process was monitored at 280 nm. All chromatographic purification experiments were performed with three repetitions of each type of ligand tested and a non-activated matrix.

The elution peaks were collected manually after detection, for values greater than 70 mAU absorbance at 280 nm. The samples were dialyzed against phosphate buffer without NaCl, followed by protein quantification, qualitative analysis by SDS-PAGE electrophoresis, and determination of specific hemagglutinating activity.

2.5.3 Protein concentration, electrophoretic and hemagglutination analysis

The protein content of the samples eluted from the ion exchange cryogel columns was determined according to Bradford, 1976. The purity of the samples eluted from the columns was also assayed by sodium dodecyl sulfate polyacrylamide gel electrophoresis in reducing conditions (SDS-PAGE). The acrylamide gel was prepared as a 12% separating gel in 1.5 mol/L Tris-HCl (pH 8.8) and a 5% stacking gel in 1 mol/L Tris-

HCl (pH 6.8). All samples were treated with tris-HCl buffer containing SDS, bromophenol blue, glycerol, and β-mercaptoethanol and heated to boiling for 3 minutes. Twenty five μL aliquots of the samples were subjected to gel electrophoresis runs at a constant voltage of 250 V. Sample hemagglutinating activity was determined by the agglutination test of untreated human type A, B, and O erythrocytes in a 5% v/v suspension, according to the method modified from Santana *et al*., 2008. Sample hemagglutinating activity was expressed as hemagglutination units (HU), defined as the reciprocal of the highest dilution of sample promoting full erythrocyte agglutination. Specific hemagglutinating activity (HA) was also determined, defined as the ratio of hemagglutinating activity to protein concentration (mg/mL) in the sample. Recovery (R) and purification factor (PF) were determined from this information.

Recovery (R) was calculated as the ratio between the total protein mass in the samples eluted from the ion exchange column and total protein mass in the crude extract percolated in the column. The purification factor (PF) was determined from the ratio between the specific hemagglutination activity in the sample eluted and in the jackfruit seed extract solution (Samah *et al*., 2017).

2.6 *Experimental design and statistical analysis*

Cryogels were synthesized in a 100 mL batch of monomer solution and all non-activated monoliths were collected and randomized into 5 groups of 8 units. One group was maintained as original (control) and the others were functionalized with the different ion exchange binders (AAc, AMPSA, DMAEMA and AETA-Q). The physical characterization was arranged in a completely randomized design and the results of S, ED, φ_M, φ_m, φ_{wb}, φ_d and φ_T were examined by analysis of variance and the Tukey test, both at 5% probability. The purification assays were arranged in a completely randomized design with three replicates, with protein and hemagglutinating activity performed on each sample in duplicate.

3 Results and discussion

3.1 *Swelling capacity and degree of expansion*

When hydrated, the synthesized cryogels had a cylindrical and spongy structure, characteristics also reported by other authors (Mól *et al*., 2017; Gonçalves *et al*., 2016). Table 1 shows the values obtained for the swelling capacity and expansion degree of the pure and functionalized cryogels.

No significant difference ($p > 0.05$) was found for swelling capacity of pure and functionalized cryogels and grafting with ion exchange groups caused no changes in their hydration characteristics. The results for S obtained in this study were close to those found by Veríssimo *et al*., (2017) who reported 14.6 kg/kg for cryogels with immobilized tris (hydroxymethyl) aminomethane and lower than the results found by Gonçalves *et al*., (2016) who reported values between 16.4 kg/kg and 19.4 kg/kg for polyacrylamide cryogels with 6% monomers. Other authors reported values between 3 and 19.5 kg/kg for different types of functionalized cryogels (Mól *et al*., 2017; Çimen and Denizli 2012; Uygun *et al*., 2012). The expansion degree of the non-activated cryogels differed significantly ($p < 0.05$) from the ED of cryogels functionalized with AMPSA, AAc and DMAEMA, which in turn differed ($p < 0.05$) from the ED of cryogels functionalized with AETA-Q. It is likely that the functionalized cryogels enhanced the cross-interactions in their structure, making them less flexible and reducing ED values. The hydrophilic nature of the ion exchanger radicals however had little effect on their hydration capacity.

Table 1. Morphological parameters of pure and functionalized cryogels with different ion exchange groups.

Parameter	Non-activated cryogel	Cryogel functionalized with the ion exchanger			
		AAc	AMPSA	DMAEMA	AETA-Q
S (kg/kg)	$14.64^{a}\pm1.74$	$13.25^{a}\pm0.69$	$12.85^{a}\pm0.43$	$13.21^{a}\pm0.61$	$12.37^{a}\pm1.08$
ED (L/kg)	$18.03^{a}\pm2.32$	$15.12^{b}\pm2.78$	$14.07^{b}\pm1.97$	$14.65^{b}\pm1.46$	$12.70^{c}\pm1.16$

* Means followed by the same letters in the rows are not significantly different by the ANOVA Tukey test (p = 0.05).

Table 2. Physical parameters: pore fraction distribution and total porosity.

Parameter	Non-activated cryogel	Cryogel functionalized with the ion exchanger			
		AAc	AMPSA	DMAEMA	AETA-Q
φ_d	$0.065^a \pm 0.008$	$0.071^b \pm 0.004$	$0.072^b \pm 0.003$	$0.071^b \pm 0.003$	$0.075^b \pm 0.006$
φ_{wb}	$0.028^a \pm 0.005$	$0.029^a \pm 0.007$	$0.029^a \pm 0.004$	$0.035^b \pm 0.004$	$0.038^b \pm 0.005$
φ_M	$0.786^a \pm 0.043$	$0.690^c \pm 0.046$	$0.725^b \pm 0.040$	$0.755^b \pm 0.012$	$0.726^b \pm 0.045$
φ_m	$0.122^a \pm 0.044$	$0.210^c \pm 0.038$	$0.174^b \pm 0.040$	$0.140^a \pm 0.013$	$0.161^b \pm 0.039$
φ_T	$0.908^a \pm 0.010$	$0.901^a \pm 0.010$	$0.898^a \pm 0.004$	$0.895^a \pm 0.006$	$0.887^a \pm 0.010$

* Means followed by the same letters in the rows are not significantly different by Tukey test, at a significance level of 5%. φ_M: fraction of macropores; φ_m: fraction of meso and micropores; φ_{wb}: fraction of bound water; φ_d: fraction of the dry polymer; φ_T: Total porosity.

The results for constituent fractions and porosity of the pure and functionalized cryogels are shown (Table 2). Functionalization led to a larger fraction of dry polymer in the cryogels, confirming that the ionic radicals were immobilized and that cryogel solvation was little affected. Likewise, the total porosity of cryogels was little affected by the functionalization, although some of the macropores turned into smaller pores, which has been previously reported in the literature (Gonçalves *et al*., 2016). The increase in the dry fraction and the meso and micropores fraction in functionalized cryogels concurs with the reduction in ED values, indicating the matrices have become less flexible. The macropore fractions remained high, 69% and 75%, which is an interesting characteristic for the use of these matrices in purifying biomolecules from unclarified or viscous media.

3.2 FTIR analysis

Figure 1 shows the FTIR spectra of pure cryogels and cryogels functionalized with different ion exchangers. All spectra show a vibration band formed in the 1650 cm^{-1} region, a characteristic of the amide group present in the polyacrylamide cryogels produced (Figures 1 A and B). Several authors reported the occurrence of this band when using the monomers AAm and BAAm (Perçin and Aksöz, 2012; Gonçalves *et al*., 2016).

Functionalized cryogels showed a change in spectra in the 1750 to 700 cm^{-1} region (Figure 1 B) compared with the unmodified matrix. The AMPSA-functionalized cryogel had reduced transmittance at 1185 cm^{-1} and the appearance of a band at 1040 cm^{-1} associated with sulfur radicals (sulfonates) of AMPSA. The spectrum of the AAc-functionalized cryogel showed a slight increase at 1190 cm^{-1}, which may be associated with the carbon-nitrogen bond in the secondary amine arising from the grafting of acrylic acid onto the polyacrylamide matrix. The DMAEMA-functionalized cryogel spectrum showed a small change at 1160 cm^{-1}, which may be associated to the carbon-nitrogen bond in the tertiary amine of the exchanger group (Roy *et al*., 2008). The spectrum of the AETA-Q-functionalized cryogel showed a band at 956 cm^{-1} that is typical of the radical $N^+(CH_3)_3$ present in its structure (Zheng *et al*., 2014).

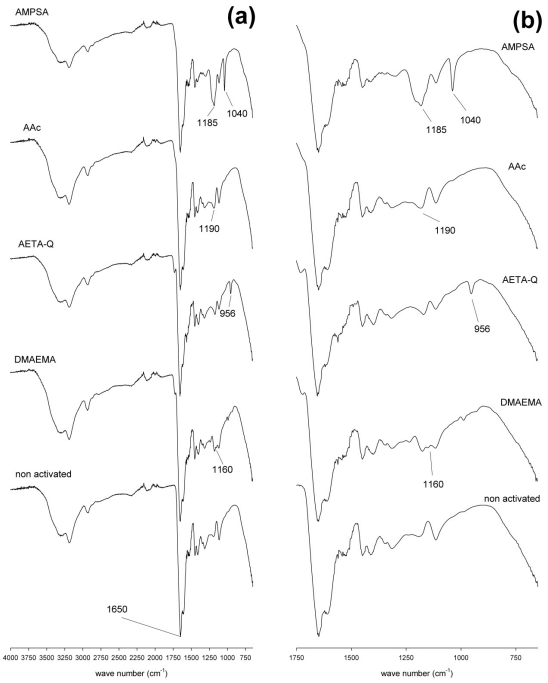

Fig. 1. FTIR spectra of pure and functionalized cryogels: (a) the entire wavelength range analyzed, (b) with highlight to the region between 1750 and 700 cm^{-1}.

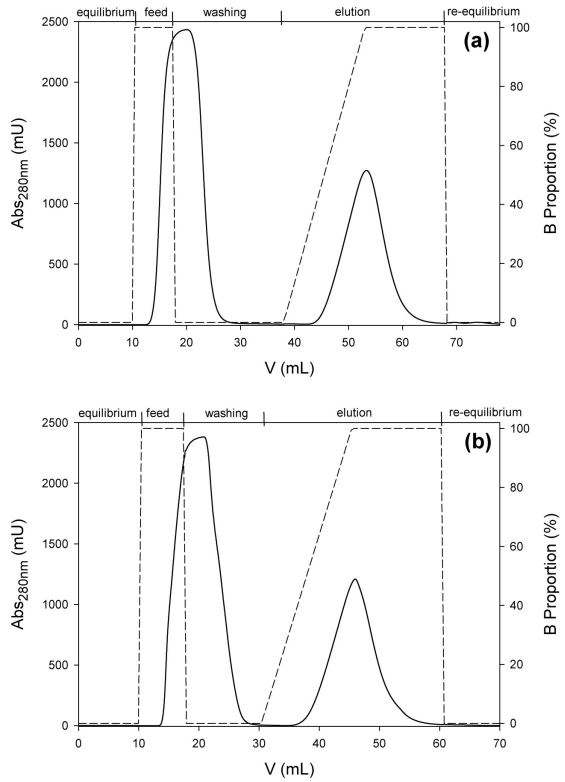

Fig. 2. Chromatographic profiles of purification assays obtained with the DMAEMA-functionalized cryogel column (a) and non-activated cryogel (b).

3.3 Capture of lectins

The crude protein extract obtained from the jackfruit seeds was purified using different ion-exchanger cryogels. The concentration of proteins in the crude solution was 1.54 ± 0.24 mg/mL and the chromatographic profiles obtained in the capture assays are shown in Figure 2. All samples eluted from the columns showed a single elution peak.

SDS-PAGE electrophoresis analysis under denaturing conditions (Figure 3) shows that the profiles of the samples eluted from the cryogel columns were very similar, displaying two stronger bands with molar masses close to 14.3 kDa, estimated at 13.5 kDa and 16 kDa, and two weaker bands with molar masses estimated at 23.5 kDa and 28.5 kDa. Results suggest the bands with lower molar masses are the non-glycosylated and glycosylated-heavy chains of jacalin (Kabir, 1998; Vijayakumar and Forrester, 1986). The band estimated at 16 kDa may also be from the ArtinM polypeptide chains (Cecílio *et al*., 2016; Pranchevicius *et al*., 2012).

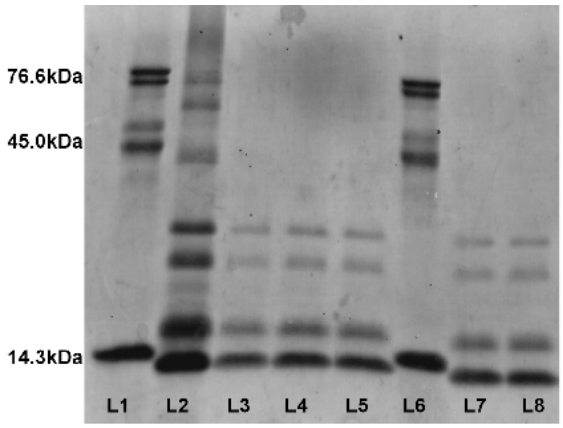

Fig. 3. SDS-PAGE analysis of chromatographic purification of jacalin extracted from jackfruit seeds by ion exchange cryogels with one-step elution. L1 and L6 - molecular markers (lysozyme 14.3 kDa, albumin 45 kDa and conalbumin 76.6 kDa, all from chicken egg white); L2 - raw extract. L3-AAc; L4-AMPSA; L5 - non-activacted cryogel; L7-DMAEMA; L8-AETA-Q.

The band with an estimated molar mass of 28.5 kDa is possibly the same that Lyu *et al*. (2015) reported in their work as a trypsin inhibitor purified from jackfruit seeds. The determined molecular masses agree with those found by Kabir (1995), Vijayakumar and Forrester (1986) and Moreira and Oliveira (1983) for the subunits of the lectins from jackfruit they isolated by ion-exchange, affinity and molecular sieve chromatography. The bandwidth estimated around 23.5 kDa has been reported in other studies, but has not been thoroughly discussed previously (Lyu *et al*., 2015; Ulloa *et al*., 2017).

Results for recovered protein (R), specific hemagglutinating activity (HA) and the purification factor (PF) are shown (Table 3). The presence of lectins in the eluted samples was confirmed by the erythrocyte agglutination for the blood groups tested. The results for the recovery factor confirmed that the type of exchanger used had an effect on the purified lectin fractions. These results, along with the electrophoresis analysis, suggest that most of the proteins recovered were jacalin, which is selective for recognition of D-galactose (Kabir *et al*., 1998; Roy *et al*., 2005), a sugar found in greater quantity in the membrane of red blood cells from group B compared with the others. It is also important to emphasize that the fractions collected from columns presented strong agglutination for human erythrocytes (group B).

Table 3. Results of hemagglutination tests performed with ABO groups.

	C (mg/mL)	MP (mg)	R (%)	Group A		Group B		Group O	
				HA (HU mL/mg)	PF	HA (HU mL/mg)	PF	HA (HU mL/mg)	PF
Crude Extract	1.54	11.54		676.83		676.83		1353.66	
AMPSA	0.23	4.21	36.48	1126.14	1.66	2252.28	3.33	2252.28	1.66
AAc	0.32	5.33	46.19	1652.37	2.44	1652.37	2.44	2815.05	2.08
DMAEMA	0.42	7.14	61.87	1207.69	1.78	2415.37	3.57	1207.69	0.89
AETA-Q	0.36	7.12	61.70	776.46	1.15	1552.91	2.29	3105.83	2.29
Pure cryogel	0.33 5.75	49.83	392.26	0.58	1584.97	2.34	1569.04	1.16	

C - protein concentration, MP - applied/recovered protein mass, R - recovery protein, HA - specific hemagglutinating activity, PF - purification factor.

This indicates a selective jacalin purification that is confirmed by PF values > 2.03. Vijayakumar and Forrester (1986) purified lectins from the jackfruit and obtained 50 mg of affinity-purified product from twenty-five grams of the defatted and dried seeds, representing a recovery in terms of the total mass of 0.2 wt%. The authors reported that the purified product retained more than 75% of the hemagglutinating activity found in the original extract. Simone *et al.* (1994), purified jacalin from *Artocarpus integrifolia* by preparative anion-exchange polyacrylate-DEAE column and obtained a recovery of 27-33% jacalin from a total soluble extract. In this study, the results achieved for the protein recovery were higher than 36.48% and retained more than 229% agglutination activity for group B and 58% of the hemagglutinating activity of the crude extract.

Results comparing functionalized ion exchanger matrices with pure cryogel columns showed that the total protein mass recovered was similar, but the PF value was higher in the ion exchangers, indicating their selectivity lectins.. In general, the anion exchangers had better results for PF values than the tested cation exchangers, indicating that changes in the working pH can lead to better results.

Jacalin was purified from jackfruit seeds using anion exchange chromatography at pH 7.4 and stepwise elution (Kabir, 1998). The presence of two bands with estimated molar masses around 12 kDa and 15.5 kDa in SDS-PAGE electrophoresis gels was confirmed, showing strong binding power of red blood cells of group B. Isoelectric focusing analysis revealed that at least 35 charged jacalin species could be identified over a pH range from 4 to 9, but most jacalin proteins were focused in the 5-8 range (Kabir, 1995).

The high value found for the recovery (61.87%) associated with the higher PF values (3.57) indicates that the DMAEMA-functionalized anion exchangers had the best results for jacalin purification and a significant part of the jacalin isoforms with greater affinity for the blood group B erythrocytes showed a basic character at pH 7.2. In addition, the results found in this work were superior to those reported by other authors for jacalin purification using a commercial anion exchange column (Kabir, 1995; Simone *et al.*, 1994).

The isoelectric properties of jacalin are complex. It is widely reported that it presents charge isomer species (Kabir, 1998; Kabir, 1995; Simone *et al.*, 1994; Vijayakumar and Forrester, 1986). Several authors observed that all charged isomers gave rise to only two peptides at 12 and 15.4 kDa, which is characteristic of jacalin (Kabir, 1998; Kabir, 1995; Simone *et al.*, 1994; Vijayakumar and Forrester, 1986) and was observed in this work (Figure 3). A more accurate analysis of the purification at different pH values will aid in understanding the interaction mechanisms of the jacalins with the matrices produced. Macroporous ion exchangers have potential for use in the purification or pre-purification of lectins and jacalins in particular, since the process results in an increase in hemagglutinating activity.

Conclusions

In this work, macroporous monolithic chromatographic matrices functionalized with AMPSA, AAc, DMAEMA and AETA-Q were produced and tested in the purification of jackfruit seed lectins by ion exchange chromatography. Matrix functionalization affected some properties such as pore distribution but did not affect matrix total porosity. The purified extracts obtained with the functionalized matrices showed higher binder capacity than the crude extract, demonstrating partial purification of lectins, in particular the jacalins. The matrix functionalized with

the anion radical (DMAEMA) had the best indicators, suggesting that at pH 7.2, the fraction of lectins of interest had a net negative charge. This study shows it is possible to use the matrices produced in purifying lectins from jackfruit seed crude extract.

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