



CHEMICAL-ACTIVATED CARBON FROM COCONUT (*Cocos nucifera*) ENDOCARP WASTE AND ITS APPLICATION IN THE ADSORPTION OF β -LACTOGLOBULIN PROTEIN

CARBÓN ACTIVADO QUÍMICAMENTE A PARTIR DE RESIDUO DE ENDOCARPIO DE COCO (*Cocos nucifera*) MODIFICADO Y SU APLICACIÓN EN LA ADSORCIÓN DE PROTEÍNA β -LACTOGLOBULINA

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Received October 4, 2017; Accepted December 26, 2018

Abstract

The aim of this study was to investigate the use of coconut (*Cocos nucifera*) endocarp, an agro-industrial by-product, as a precursor in the synthesis of activated carbon. The material was used as an adsorbent for whey protein purification, using β -lactoglobulin as the model molecule. The coconut endocarp was initially subjected to leaching in an alkaline medium. The activated carbon (AC) was prepared by the chemical activation method using $ZnCl_2$ as the activating agent. The process yields and the physical and chemical properties of the activated carbon were determined. The effects of pH, carbon mass, time, and temperature on the adsorption performance was also investigated. The activated carbon showed a pore size distribution in the mesoporous range and a high surface area. The adsorption assays showed a best adsorption performance of the AC at pH 3.0. The pseudo second-order kinetics model set better to the experimental data of β -lg adsorption onto AC, and the Langmuir model was more appropriate to represent the experimental data, with maximum adsorption capacity of 524.76 mg/g, evidencing the potential of the coconut endocarp waste for the production of activated carbon for whey protein purification.

Keywords: agro-industrial waste, alkaline leaching, activated carbon, adsorption of β -lactoglobulin.

Resumen

El objetivo de este trabajo fue estudiar el uso del endocarpio de coco, un subproducto agroindustrial, como precursor en la síntesis de carbón activado. El material obtenido fue aplicado como adsorbente para la proteína del suero, usando β -lactoglobulina como modelo molecular. El carbón activado (AC) fue preparado por el método de activación química usando $ZnCl_2$. El rendimiento del proceso y las propiedades físicas y químicas del carbón activado fueron determinadas. Además, se investigaron los efectos de varios factores en el rendimiento de la adsorción, como el pH, la masa de carbón, el tiempo y la temperatura. El carbón activado mostró un tamaño de poro en el rango de mesoporos con área de grande superficie. Las pruebas de adsorción mostraron que el AC tuvo el mejor rendimiento en pH 3.0. El modelo cinético de pseudo segundo-orden fue el mejor para la fase experimental de la adsorción de β -lg en AC y el modelo de Langmuir fue el más adecuado para representar los resultados experimentales, con una capacidad de adsorción máxima de 524.76 mg/g, mostrando el potencial del residuo de endocarpio de coco modificado para la producción de carbón activado para la purificación de la proteína del suero.

Palabras clave: residuo agro-industrial, alcalina lixiviación, carbón activado, adsorción, β -lactoglobulina.

1 Introduction

Activated carbons (AC) are porous materials with highly developed surface area and rich surface groups. They are widely used in adsorption, catalytic support, and purification and separation processes, in several areas including pharmaceutical, chemical, food and

fuel (Brito *et al.*, 2017; Pereira *et al.*, 2014; Hu *et al.*, 2008). They are produced from the dehydration and carbonization of raw materials followed by activation, and their characteristics are influenced mainly by the precursor material and the method used in its preparation (Mahamad *et al.*, 2015; Angin *et al.*, 2013).

The precursors used in the production of AC

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doi: 10.24275/10.24275/uam/izt/dcbi/revmexingquim/2018v17n2/Andrade

issn-e: 2395-8472

are materials with high carbon and low inorganic compounds contents, which are present in many agro-industrial wastes. Agro-industrial wastes are important raw materials for the preparation of activated carbon because they have a high content of volatile matter, which allows obtaining an adsorbent with a highly porous structure, and are low cost and renewable raw materials (Pezoti *et al.*, 2016; Mohanty *et al.*, 2008). Many studies have investigated the potential of these lignocellulosic materials as carbon precursors in the production of activated carbon, including red mombin fruit stones and cocoa shell (Pereira *et al.*, 2014), coffee residues (Pobletea *et al.*, 2017), olive stone (Elmouwahidi *et al.*, 2017), yellow mombin fruit stones (Brito *et al.*, 2017), among others.

The adsorption capacity of the activated carbon is determined by several factors, including its internal porosity, surface area, size distribution, pore volume, and the presence of functional groups on its surface (Hesas *et al.*, 2013; Deng *et al.*, 2010). The textural characteristics of the adsorbent are of paramount importance in the adsorption process, since the pore structure limits the size of the molecules that can be adsorbed, while the available surface area limits the amount of material absorbed by the matrix. On the other hand, the surface chemistry of the carbonaceous materials depends essentially on their content of heteroatoms, mainly in superficial oxygen complexes. These functional groups may affect the adsorption capacity and can be modified by thermal and chemical treatments (Moreno-Castilla, 2004).

A recent and little exploited application of activated carbon in whey protein adsorption has been reported (Alves *et al.*, 2016; Pereira *et al.*, 2014). Whey may be defined as the remaining aqueous phase of casein removal from milk. It has high nutritional value, due to its proteins with a high content of essential amino acids (Sgarbieri, 2005). However, for a long time, cheese whey has been considered a by-product of low or no commercial value, generally used for animal feed or discarded directly into rivers or public drains without previous treatments, causing serious environmental problems. Fortunately, this context has changed in recent years because of the bioactive and functional properties of the whey proteins, making cheese whey a high added value by-product in the dairy processing industry (Albrecht and Vovk, 2012).

Whey proteins have a globular structure containing some disulfide bonds, which confer a certain degree of structural stability. Whey fractions, or whey peptides, are composed of β -lactoglobulin, α -lactalbumin,

bovine serum albumin, immunoglobulins, and glycomacropptides. These fractions can vary in size, molecular weight, and function, providing special characteristics to the whey proteins (Haraguchi *et al.*, 2006). The β -lactoglobulin (β -lg), for example, is the main whey protein (McSweeney and Fox, 2013), and represents about 10% of total milk protein, or approximately 50% of whey protein. It is a globular protein consisting of 162 amino acid residues with a molecular mass of 18.4 kDa. Bovine β -lg has a particular lipocalin structure, with a hydrophobic character and cup-shaped conformation conferring functional properties with wide application in the food industry, such as foaming, gelling and emulsifying capacities, and flavor binding (Morr and Foegeding, 1990). Thus, the fractionation of these proteins has been investigated, once many of these characteristics are not found in protein concentrates due to interaction with other compounds (Chandan and Kilara, 2010).

In this context, several techniques have focused on the recovery of whey proteins, including ultrafiltration (Arunkumar and Etzel, 2014), aqueous biphasic systems (Kalaivani and Regupathi, 2015), chromatography (Lira *et al.*, 2009), adsorption (Sousa *et al.*, 2014), among others. Adsorption is often used in biomolecule separation processes, through various adsorbent-adsorbate interactions, including ionic, affinity, and hydrophobic bonding. The efficiency of the adsorption process is directly related to the type of adsorbent used. Although the synthetic adsorbents have been used to remove the biomolecules from the liquid phase, they have high cost, thus arousing interest for the application of alternative adsorbents such as the activated carbon (Pereira *et al.*, 2014).

The physicochemical characteristics of the activated carbon make this adsorbent as a promising matrix for whey protein adsorption. Thus, the production of activated carbon from the agro-industrial waste can be an effective alternative in the search for adsorbents of low cost and with characteristics that allow adsorbing whey proteins effectively. In this study, the coconut endocarp was chosen as the carbon precursor material for the synthesis of the activated carbon, once a significant intensification in the coconut cultivation and production has occurred in the recent years. This kind of crop generates a large amount of waste, which is improperly discarded in soils and/or natural waters, leading to the generation of chemical compounds and microorganisms that can contaminate the environment (Bitencourt *et al.*, 2014). The aim of this study was to synthesize and characterize the activated carbon

using the coconut endocarp subjected to alkaline leaching as a carbon precursor material, followed by its application on the adsorption of the whey protein β -lactoglobulin.

2 Materials and methods

2.1 Materials

Dried endocarp of coconut (*Cocos nucifera*) shell from Brazil was used as carbon precursor. β -lactoglobulin (purity \geq 90%, CAS number: 9045-23-2) was purchased from Sigma-Aldrich. All reagents were of analytical grade.

2.2 Preparation and characterization of the precursor material

The coconut endocarp was grounded and sieved to produce particles of 0.297 mm - 0.420 mm (40 mesh), which were subjected to alkaline leaching according to the methodology described by Liou (2010). The concentrations of neutral detergent fiber (NDF) and acid detergent fiber (FDA) were quantified according to the methodology described by Van Soest *et al.* (1991). The lignin content values were determined by the sulfuric acid method (72%) (Van Soest *et al.*, 1991). Cellulose, hemicellulose and ash were quantified following the protocols described by AOAC (1995), in which all chemical constituents are reported on a dry matter basis.

2.3 Synthesis and characterization of the activated carbon

Chemical activation was performed according to the method described by Prauchner and Rodriguez-Reinoso (2012) with some modifications. After leaching, the precursor material was impregnated with a $ZnCl_2$ solution at 4.3 g/g (2 mL/g precursor). Initially the mixture was heated at 80°C for 2 h under constant stirring. Then, the impregnated material was dried at 80°C for 72 h. The material was carbonized at 500°C (a heating rate of 5°C min⁻¹) for 2 h under a constant N_2 flow of 50 mL/min. After carbonization, 0.1 mol/L hydrochloric acid was added to remove the residues of the activating agent. The mixture was heated at 100°C for 1 h under reflux. Then, the acidic solution was drained and the activated carbon was

washed until reaching pH 7.0, and dried at 105°C for 24 h.

The ash content was determined according to the methodology described by AOAC (1995). The point of zero charge (pH_{PZC}) is defined as the pH at which the surface charge of the carbon is neutral. The methodology is called "11-point experiment" and was described by Regalbuto and Robles (2004). The functional groups of the activated carbon were identified by FTIR. The spectra were recorded on a Bruker spectrometer - VERTEX 70 in CETENE using ATR method. The pore structure was characterized by N_2 adsorption at 77K on a 2420 Micromeritics apparatus using approximately 0.15 g sample. Before the measurements, the sample was subjected to a pretreatment step. Later, the adsorption isotherm and the nitrogen desorption at 77K was determined. The specific surface area was determined by the BET equation. The pore size distribution was determined from the desorption isotherm using the BJH model, while the volume of the micropores was determined by t-plot analysis based on adsorption isotherm.

2.4 Adsorption experiments

2.4.1 Effect of pH, mass of activated carbon, and time on the adsorption process

To study the effect of pH (3.0, 7.0, and 9.0), mass of adsorbent (10 - 50 mg) and contact time on the adsorption process, the activated carbon was added to tubes containing 5 mL of β -lactoglobulin solution at a concentration of 500 mg/L. The pH was adjusted to 7.0 and 9.0 using potassium phosphate buffer (20 mM) and to pH 3.0 using monobasic potassium phosphate and phosphoric acid. The tubes were kept under constant stirring at 20 rpm (on an orbital shaker) for 24 h at room temperature followed by centrifugation (CENTRIBIO 80-2B).

2.4.2 Adsorption isotherms

To study the effect of the temperature on the adsorption process, the carbon mass chosen in the mass study was added to tubes containing 5 mL β -lactoglobulin solution at an initial concentration of 100 mg/L at 2250 mg/L pH 3.0. The tubes were kept under constant stirring (20 rpm) on an orbital shaker at 20°C, 30°C, and 40°C, using an incubator chamber (Scientific Logen LG340FT220-CBR) for temperature control. After the time necessary to achieve adsorption equilibrium, the tubes were centrifuged (CENTRIBIO 80-2B). The nonlinear models of Langmuir (Eq. 1)

Freundlich (Eq. 2) and Toth (Eq. 3) were adjusted to the experimental data, using the Gauss-Newton method, and evaluated according to the coefficient of determination (R^2) and root mean square error (RMSE).

$$q = q_s \frac{bC_{eq}}{1 + bC_{eq}} \quad (1)$$

$$q = aC_{eq}^{1/n} \quad (2)$$

$$q = q_s \frac{bC_{eq}}{[1 + (bC_{eq})^n]^{1/n}} \quad (3)$$

where, q is the mass of solute adsorbed at the equilibrium per unit mass of adsorbent (mg/g); C_{eq} (mg/L) is the protein equilibrium concentration in the liquid phase; q_s (mg/mL) is the saturation capacity; b (L/mg) is the adsorption equilibrium constant; a (L/mg) is the Freundlich constant, and n is a dimensionless constant.

2.4.3 Determination of protein adsorption capacity and adsorption efficiency

All supernatants obtained after centrifugation were filtered using a 25 mm syringe filter with hydrophilic PTFE membrane and pore size of 0.45 μ m. Protein quantification was performed by direct readings in a spectrophotometer (Quimis 0898UV2) at 280 nm. The experiments were conducted in triplicate with three replications. The adsorption capacity of the activated carbon was determined using Eq. (4) and the adsorption efficiency was obtained from Eq. (5).

$$q = (VC_{in} - VC_{eq})/m_{ads} \quad (4)$$

$$\varepsilon = [(VC_{in} - VC_{eq})/VC_{in}] \times 100 \quad (5)$$

where: q is the adsorption capacity after reaching equilibrium (mg/g), V is the volume of solution (mL), C_{in} is the initial concentration of the solution (mg/L), C_{eq} is the concentration of the solution (mg/L) at equilibrium; m_{ads} is the mass of adsorbent (g), and ε is the adsorption efficiency (%).

3 Results and discussion

3.1 Characterization of the carbon precursor before and after alkali leaching

Table 1 shows the results of the coconut endocarp characterization, before and after leaching with NaOH. It is observed that the alkaline leaching led to a reduction of ash content and lignin contents of the samples. The reduction of the inorganic compounds may be due to the removal of silica from the raw material, once it reacts with NaOH, forming sodium silicate, which can be easily removed during washing and filtration (Liou, 2010). On the other hand, the reduction of lignin content can be due to the effect of alkali leaching on the structure of the precursor material. During this process, the ether bonds between the phenylpropane units of the lignin structure were broken, forming phenolic groups that were responsible for the solubilization (Albinante et al., 2013). The pre-treatment of the carbon precursor material with the alkali solution tends to improve the properties of the carbon obtained. Liou (2010) studied the pre-treatment of the raw material with NaOH, and found positive effects of the alkali leaching on the surface area and mesoporosity of the synthesized activated carbon.

3.2 Yield and characterization of the activated carbon

An activated carbon yield of 20.94% was obtained, considering the initial mass of the precursor *in natura*. It is known that the synthesis yield is directly related to the nature of the precursor material and the different mechanisms of action of the chemical agents used in the impregnation. In the present study, the precursor material was leached with NaOH, leading to a reduction of 85% of lignin content. Once lignin is very resistant to thermal decomposition, it is directly associated with the yield of carbon (Zanzi et al., 2002). Another factor that influences yield is the type of activating agent employed.

Table 1. Characterization of the coconut endocarp *in natura* and coconut endocarp after the NaOH leaching.

Material	Ash (%)	Lignin (%)	Cellulose (%)	Hemicellulose (%)
<i>In natura</i>	2.61	15.20	10.35	33.70
NaOH leaching	1.54	3.8	10.85	33.91

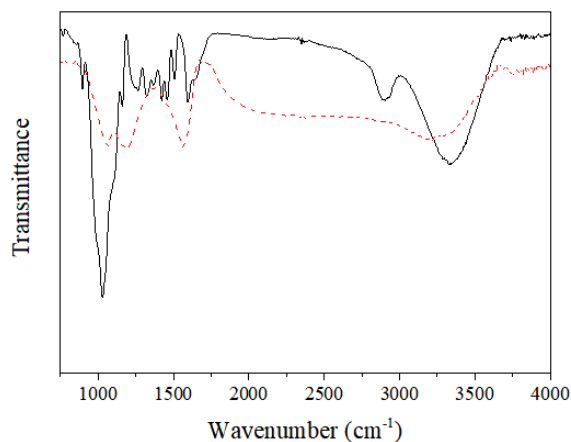


Fig. 1. FTIR spectra of coconut endocarp obtained after lixiviation (—) and CA (---).

When using ZnCl_2 , the passage of volatiles through the pores is not prevented, thus they are eliminated from the carbon surface during the activation, which affects the synthesis yield. The synthesized activated carbon presented an ash content of 9.78%, with an increase when compared to the precursor material. It is known that ZnCl_2 acts as a dehydrating agent during the activation process, whereas the carbonization results in the conversion of part of the carbonaceous materials into inorganic compounds, which are not effectively removed in the washing process, leading to an increase in ash content of the activated carbon (Mahamad *et al.*, 2015). In qualitative terms, low ash content is usually recommended for activated carbon since higher ash levels tend to reduce the adsorption by blocking the pores of the carbonaceous matrix and preferentially adsorb water due to its hydrophilic character (Saygili *et al.*, 2015).

The point of zero charge (pH_{pzc}) of the material provides a significant contribution to the adsorption study, because it is a useful information on the adsorbent surface behavior as a function of the pH of the medium and the degree of ionization of the adsorbate species. It was observed that the pH_{pzc} of the activated carbon was near to pH 7.1. At pH values below the pH_{pzc} , the surface of the activated carbon is protonated, favoring the adsorption of negatively charged compounds, while at pH above this value the activated carbon is deprotonated, favoring an opposite behavior (Vieira *et al.*, 2010).

Fig. 1 shows the FTIR spectra of both the activated carbon and the precursor material after leaching. For the precursor, a broad absorption band was observed at 3340 cm^{-1} , corresponding to the hydroxyl stretching

vibration of carboxylic acid, phenol or alcohol and water (López-García *et al.*, 2017; Silverstein *et al.*, 2007). The band located around 2905 cm^{-1} can be assigned to the stretching of C-H bonds of CH_2 and CH_3 groups. The bands between 1160 cm^{-1} and 1460 cm^{-1} corresponded to deformations of C-O group of carboxylic acids, ester, and alcohols, and are characteristic of coconut endocarp components, such as cellulose, lignin, phenolic compounds, among others. The intense band observed at 1267 cm^{-1} referred to the aromatic ring vibration band of lignin (guaiacyl rings) (Guilarduci *et al.*, 2006). In addition, it is worth mentioning the presence of bands in the region of 1032 cm^{-1} corresponding to stretching vibration of glycosidic linkages (COC) in cellulose and hemicellulose, while the stretching at 900 cm^{-1} is characteristic of the β -glycosidic linkages between sugar units in hemicelluloses. In the AC spectrum, a reduction of intensity and the disappearance of bands referring to functional groups of the precursor material was observed, indicating the breakage of weak bonds during the activation, as well as the appearance of new bands, referring to the incorporation of new functional groups resulting from the activation process. The band at 3260 cm^{-1} may be due to the OH group of water. Carbon skeleton vibrations, which are characteristic of activated carbons, were observed at 1562 cm^{-1} , corresponding to the C=C stretching vibration in the aromatic rings, typical of carbonaceous material (Corral-Escarcega *et al.*, 2017; Silverstein *et al.*, 2007). The bands observed at 1063 cm^{-1} and 1190 cm^{-1} are due to the presence of C=O stretching of esters and carboxylic groups. These groups can exert a significant effect on the surface properties of the activated carbons, thus affecting the adsorption characteristics (Li *et al.*, 2015; Song *et al.*, 2013).

The nitrogen adsorption-desorption isotherm and the pore size distribution of the activated carbon is shown in Fig. 2a and 2b. According to the shape, the isotherm can be identified as Type IV with a hysteresis cycle, when the adsorption and desorption curves do not coincide, as described in the IUPAC classification (Sing, 1985). Type IV isotherms are typical of mesoporous solids (Yocupicio *et al.*, 2017), in which the adsorption occurs in multilayers. The hysteresis cycle was of type H4, which is typical of complex materials containing both micropores and mesopores. Fig. 2b shows the pore size distribution according to the Barrett Joyner and Halenda method (BJH) (Barret, 1951). A concentrated pore distribution is observed between 3.0-4.0 nm, confirming the presence of micropores and mesopores.

Table 2. Textural properties of the activated carbon (AC).

Sample	Specific surface area (m ² /g)	Pore diameter (nm) ^a	Mesopore volume (cm ³ /g)	Micropore volume (cm ³ g ⁻¹)
AC	922	3.63	0.47	0.66

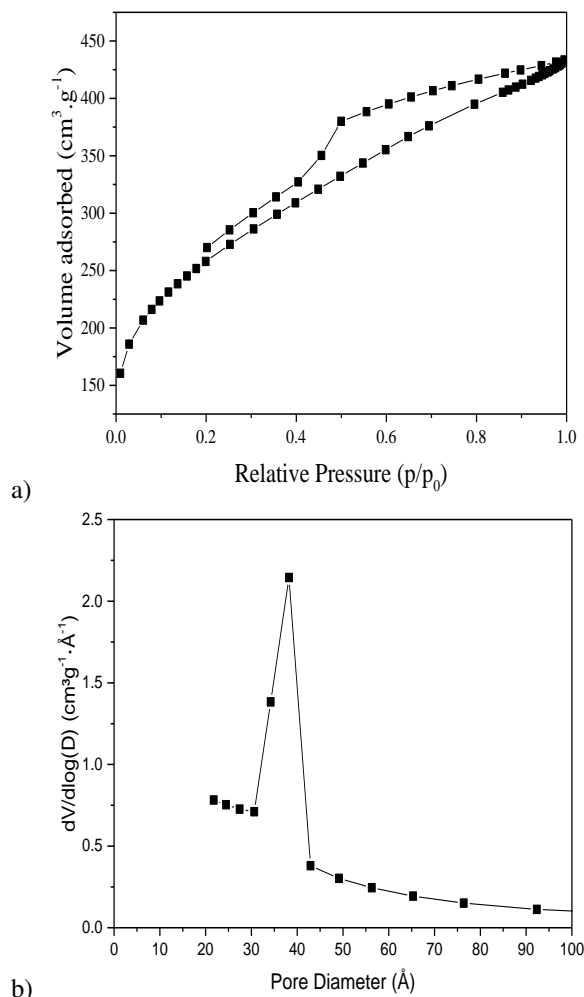
^aMaximum pore size distribution.

Fig. 2. Adsorption isotherm (a) and pore distribution (b) of activated carbon.

This characteristic is advantageous since the combination of micro and mesopores plays a significant role in the adsorption of large molecules such as proteins and dyes (Nuithitikul *et al.*, 2010). In general, the pores are directly responsible for the increase in the surface area of an adsorbent, and the pore size is of fundamental importance in relation to the adsorption capacity of certain compounds (Brito *et al.*, 2017).

Table 2 shows the specific surface area and pore volume of the activated carbon. An adsorbent with high surface area was obtained with activation with ZnCl₂. Studies have shown that ZnCl₂ promotes the dilation of the molecular structure of the cellulose molecules of the precursor during the activation process, leading to the breakdown of lateral bonds of the molecules, which results in an increase in inter and intra micelle spaces. As a consequence, ZnCl₂ leads to the development of pore structure of activated carbon. In addition, after the activation, when the carbon material is washed, the zinc salts are removed from the carbon by leaching, and the micelles cannot return to their original state, creating voids and leading to an increase in porosity (Angin *et al.*, 2013; Demiral and Demiral, 2008).

As shown in Table 2, the carbon exhibited a heterogeneous structure, with a mean pore diameter of 3.63 nm, being classified as a mesoporous material according to IUPAC (Sing, 1985). This behavior can be due to the alkaline leaching of the precursor material prior to the synthesis process, with consequent reduction of the lignin content. It is known that the precursor material is composed mainly of cellulose, hemicellulose, and lignin. The NaOH treatment has a strong effect of lignification and can effectively decrease the crystallinity of the biomass materials (Chosdu and Hilmy, 1993; Yang and Qiu, 2011). The ester linkages between lignin and carbohydrates undergo saponification when treated with NaOH, causing the destruction of the lignin structure, which can help activation, due to the great contact between the activating agent and the carbon precursor, thereby increasing the porosity of the material. Therefore, the pre-treatment with an alkali solution leads to the formation of a mesoporous material with a more uniform porous structure (Yang and Qiu, 2011), which can effectively promote the adsorption of larger molecules, such as proteins. The porous structure of the activated carbon is a parameter of great relevance in the process of mass transfer, since small molecules can be adsorbed in the micropores of the adsorbents, whereas the larger molecules, including proteins, are not adsorbed efficiently, being necessary the use of mesoporous or macroporous carbons.

Table 3. pH changes on the adsorption of β -lg at room temperature.

pH	C_{eq} (mg/L)	q (mg/g)	ε (%)
3.0	26.83	93.37	89.02
7.0	465.56	18.95	3.45
9.0	454.67	20.85	4.33

The present results demonstrated that the process allowed the synthesis of an adsorbent with a predominantly mesoporous structure, which can be used for whey protein adsorption.

3.3 Adsorption tests

3.3.1 Effect of pH on the adsorption process

The adsorption of the activated carbon was more effective at pH 3.0, thus further studies should be performed at this pH value (Table 3). Changes in pH affect the adsorption process by the dissociation of functional groups in the active sites of the adsorbent (Mall *et al.*, 2006). The pH of the solution, the isoelectric point of the biomolecule, and the point of zero charge of the adsorbent is extremely important for the adsorption of organic compounds. Whereas the maximum adsorption occurred at pH 3.0, which is below the point of zero charge (7.1) and the protein isoelectric point (5.2), when both have a positive charge density, it is believed that the energy of the electrostatic repulsion is small when compared to other favorable interactions such as hydrophobic interactions. Thus, the adsorption can occur even at pH values with similar net charges for proteins and carbon surface, once the proteins have amino acids with acidic, basic, or neutral character in their polypeptide chain (Wang *et al.*, 2012), which can interact with the adsorbent surface by the hydrophobic forces.

Whereas the interaction between proteins and carbon may be governed by the hydrophobic effect, a smaller adsorption is observed at pH 7.0, which is the more hydrophobic state of carbon. However, if the intra-particle adsorption is dominant, this behavior (higher adsorption at pH 3.0) can be explained based on the quaternary structure of β -lg, which varies with pH, as reported by Verheul *et al.* (1999). At pH values below 3.4 and above 8.0, the β -lg exists as a monomer. For pH values between 5.2 and 8.0, the β -lg is in the form of spherical dimers formed by non-covalent bonds, with a molecular mass of approximately 36

kDa and diameter of 18 Å (Sgarbieri, 2005; Verheul *et al.*, 1999). Thus, the increase in protein diameter at pH 7.0 may have limited the intra-particle diffusion, which can explain the poor adsorption at this pH value.

According to Damodaran *et al.* (2008), the difference in the carbon adsorption capacity at pH 3.0 and 9.0, in which the β -lg is a monomer, may be due to the strong intermolecular electrostatic repulsion at extreme pH values, caused by high net charge, leading to an expansion and unfolding of the protein molecule, which is greater in alkaline pH rather than acidic pH. Thus, the increased unfolding of β -lg at pH 9.0 led to an increase in protein diameter and a decrease in the intra-particle diffusion, resulting in lower amount of protein adsorbed. Similar results were reported by Norde and Giacomelli (2000), who observed that proteins of very low structural stability can adsorb even under electrostatically adverse conditions, provided that the adsorption is controlled by hydrophobic interactions.

3.3.2 Effect of the activated carbon mass

The results of the effect of the activated carbon mass on the adsorption of β -lactoglobulin showed that the adsorption efficiency increased from 89.02% to 98.63%, when using 0.01 g and 0.025 g of adsorbent, respectively, which, remained practically constant for higher mass values.

On the other hand, when evaluating the adsorptive capacity, the maximum q value was observed for a mass of 25 mg (98.30 mg/g), with a decrease in q with the increase in mass to 50 mg (49.37 mg/g). This phenomenon may be associated with two effects: first, the increase in the mass of the adsorbent at a fixed concentration and volume of the dye can lead to the saturation of the adsorption sites through the adsorptive process; second, the reduction of the adsorptive capacity may be due to the aggregation of particles resulting from a high mass of carbon, which can lead to a decrease in the surface available for adsorption and an increase in the diffusion resistance (Rovani *et al.*, 2014). Thus, the study on the amount of carbon used in the adsorption process is important to indicate the best mass for a better adsorption. A relatively small mass may not be sufficient to obtain a desirable efficiency. In contrast, a high mass of adsorbent can lead to a desirable efficiency, but with a significant increase in process costs. Therefore, the mass of 25 mg was chosen to be used in later tests.

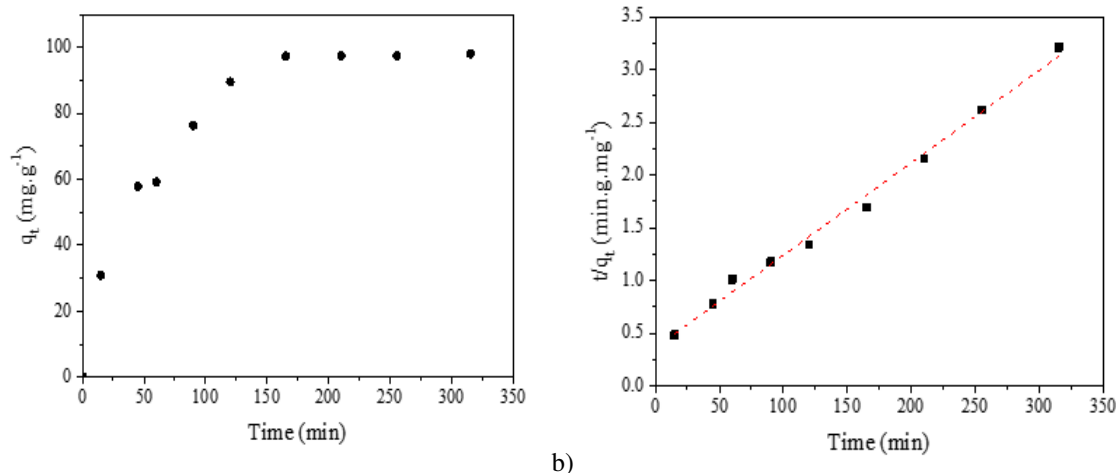


Fig. 3. (a) Effect of time on the β -lactoglobulin adsorption on activated carbon. (b) Plot of the pseudo-second order kinetics of β -lactoglobulin adsorption on activated carbon.

3.3.3 Adsorption equilibrium

Data from the study about the time effect on the adsorption equilibrium were used to develop suitable models to describe the kinetics of the adsorption process. The adsorption curve of β -lg on the activated carbon had two steps (Fig. 3a). The first step (time up to 100 min) may be due to the protein diffusion from the liquid phase to the outer surface of the carbon. The second step showed a tendency to reach the adsorption equilibrium, with smaller changes in adsorption capacity over time, with an intra-particle diffusion of the protein on the inner surface of carbon.

To better understand the kinetics adsorption mechanism, the rate constant was determined. The pseudo first-order and the pseudo second-order equations were used to make a more detailed study of the process (Eqs. 6 -7).

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (6)$$

$$t/q_t = (1 + k_2 q_e^2) + (1/q_e)t \quad (7)$$

where: q_e is the adsorption capacity after reaching equilibrium (mg/g), q_t is the adsorption capacity at a time t (mg/g), k_1 is the pseudo first-order constant rate (min⁻¹); k_2 is the pseudo second-order constant rate ((g/mg).min⁻¹), and t is time (min).

The first-order model did not fit the experimental data in the temperature under study, while the pseudo second-order model adjusted well to the experimental data (Fig. 3b).

The calculated q_e (114,0 mg/g; $R^2 = 0.99$ and RQEM = 0.04626) is in accordance to that found experimentally. It can also be seen that the time

required to reach equilibrium was approximately 165 min, which was lower than that reported by Sarvi *et al.* (2014) who studied the β -lg adsorption on mesoporous FDU-12 silica, and found that the equilibrium was reached at 25 h with a adsorption capacity of $q_e = 97$ mg/g.

The constant k_2 (2.1×10^{-4} g.mg⁻¹ min⁻¹) was used to calculate the initial adsorption rate h ((mg/g).min⁻¹) for $t \rightarrow 0$, according to Eq. (8).

$$h = k_2 q^2 \quad (8)$$

The initial adsorption rate was 2.73 min⁻¹, which refers to the protein diffusion rate from the liquid phase to the outer surface of the carbon. This value evidenced that the diffusion occurred relatively quickly from the solution, since the kinetic study was performed at pH 3.0, a condition in which the highest adsorption capacity of the protein was observed. In this value of pH the β -lactoglobulin is in the form of monomer with molar mass of 18.4 KDa, more easily penetrating into the pores of the adsorbent, thus characterizing the second step of the diffusion process.

In addition to the kinetic study, adsorption isotherms of the β -lg on the activated carbon from coconut endocarp were determined at 20°C, 30°C, and 40°C (Fig. 4), and the Langmuir, Freundlich and Toth non-linear models were adjusted to the experimental data (Table 4).

The Langmuir and Toth models were efficient to represent the experimental data since high R^2 values and low RQEM values were obtained. Similar results were observed for both the, Toth and Langmuir models at 30°C and 40°C.

Table 4. Adjusted models for the adsorption isotherms of (β -lg) on activated carbon.

Model	Temperature °C	Parameters				R^2	RQEM
		q_s (mg/g)	b (L/mg)	a [(mg/g) (L/mg) ^{1/n}]	n		
Langmuir	20	507.478	0.017	—	—	0.975	19.824
	30	524.763	0.069	—	—	0.995	9.528
	40	460.022	0.028	—	—	0.968	24.026
Freundlich	20	—	—	30.771	2.032	0.978	17.538
	30	—	—	93.654	2.668	0.962	21.777
	40	—	—	74.472	3.163	0.946	20.623
Toth	20	609.167	0.019	—	0.748	0.976	19.55
	30	524.763	0.069	—	1.000	0.995	9.616
	40	460.022	0.028	—	1.000	0.968	24.038

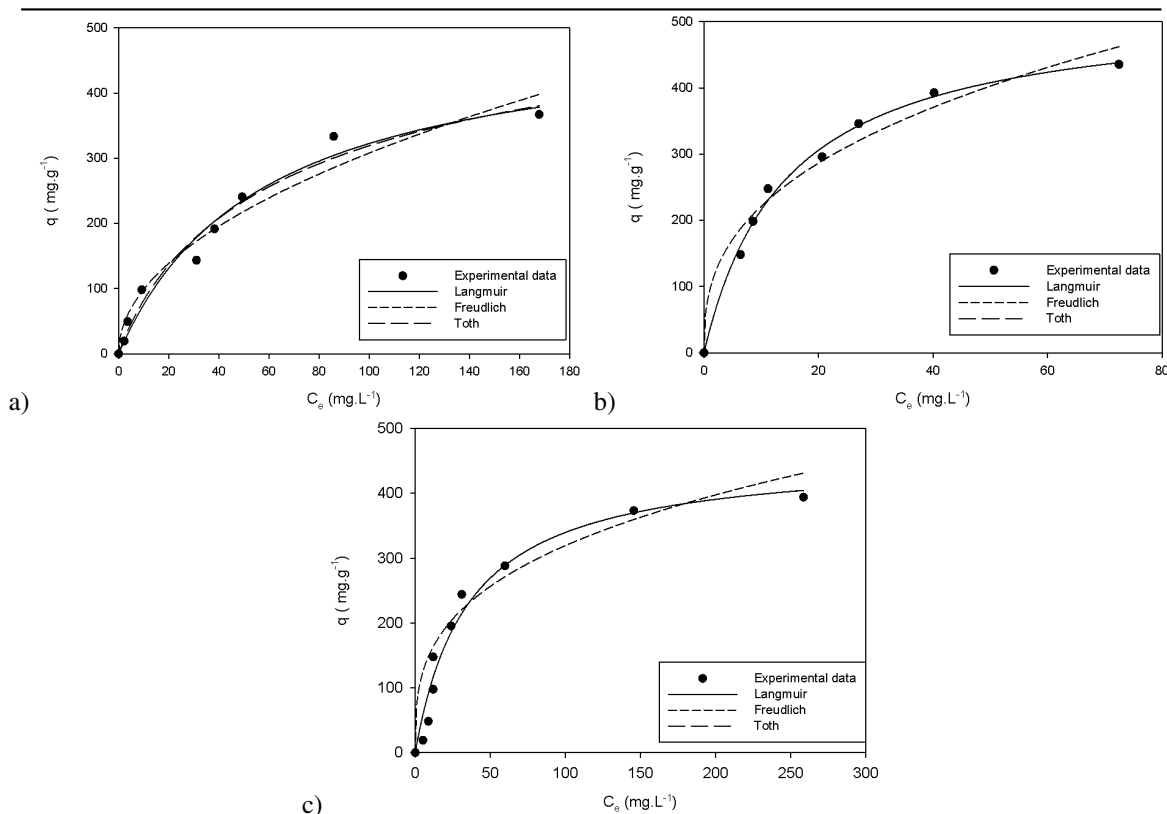


Fig. 4. Adsorption isotherms of β -lg on AC: (a) 20°C; (b) 30°C; and (c) 40°C.

This result may be due to the parameter n is approximately equal to 1. In this model, both q_s and b have the same meaning as in the Langmuir isotherm, and n is the heterogeneity parameter ($0 < n < 1$). When $n = 1$, the Toth isotherm becomes identical to the Langmuir isotherm. The Toth model has shown that the parameter q_s decreased with increasing

the temperature from 20°C to 40°C. Rüegg *et al.* (1977) used differential scanning calorimetry (DSC) to study the thermal transitions of whey proteins, including β -lg, and concluded that even the heat-resistant protein presented conformational changes at low temperatures, such as 30°C. Thus, it is assumed that the temperature increases from 30°C to 40°C has

contributed to a change in conformational structure of β -lg, suffering unfolding, which is characteristic of protein denaturation, providing greater coverage of the surface of the adsorbent and lower intra-particle diffusion, thereby reducing the number of available bonding sites. The Toth model was selected to represent the experimental data since most of the assumptions originally related to the Langmuir model (adsorption on homogeneous surfaces with a fixed number of identical adsorption sites, reversibility, same adsorption energy for all sites) is not valid for the heterogeneous surface such as activated carbon (Bueno and Carvalho, 2007).

When analyzing the parameters of the Langmuir model, it is observed that the constant k ($1/b$), which is the rate constant for the adsorption/desorption equilibrium, showed values below 1 at all temperatures studied, indicating that the adsorption phenomenon was favorable at those temperatures.

The maximum adsorption capacity of the activated carbon was observed at 20°C, which was higher than the result reported by El-Sayed and Chase (2009), who studied the ion exchange adsorption of the major whey proteins α -la and β -lg at pH 3.7, and found a maximum adsorption capacity of β -lg of 113.3 mg/mL through the Langmuir isotherm.

Conclusion

Alkaline leaching led to a reduction in ash, fiber, and lignin contents of the precursor, which directly affected the surface characteristics of the carbon obtained. The activated carbon showed carbonyl and lactones groups on its surface, and high-value surface area. The pre-treatment with basic solution led to the formation of a mesoporous material with more uniform porous structure, which can effectively promote the adsorption of larger molecules, such as proteins. At pH 3.0, the carbon had better β -lactoglobulin adsorption performance, with a high adsorption efficiency with a small mass balance in a short time. The adsorption equilibrium was reached quickly, and the protein diffusion occurred relatively quickly from the solution to the carbon surface, which can penetrate the pores. In the study of the equilibrium isotherms, the Langmuir adsorption isotherm model was most effective to represent the experimental data of the β -lg adsorption on the activated carbon, presenting a high adsorption capacity and a favorable adsorption process at the temperatures

studied. Therefore, the coconut endocarp has proven to be a promising agro-industrial waste for the production of activated carbon, and can be used as an adsorbent in the fractionation of whey proteins.

Acknowledgments

The authors thank the Center for Strategic Technologies of the Northeast (CETENE) for the determinations, and the National Council for Scientific and Technological Development (CNPq) for the financial support.

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