PHYSICOCHEMICAL CHARACTERISTICS OF THE COCONUT PULP (*Acrocomia aculeata*) FOR USE AS SUPPORT OF PROTEINS AND METAL MATERIAL

F.Yubero[1]; J. Ayala[1]; M. Lopez[1]; M. Monteiro[2]; W Thompson[3], J. Arguello[4], Y. Gonzalez[5], Valdovinos, V.[1]

[1] Dept. of Physical Chemistry. Faculty of Chemical Sciences. National University of Asunción, Paraguay CP 1055. E-mail: fyubero@qui.una.py

[2] Bio and Materials Laboratory. Polytechnic Faculty. National University of Asunción, Paraguay

[3] Dept. of Physics. State University of Londrinas, Brazil

[4] Institute of Chemistry. Federal University of Rio Grande do Sul, Brazil

[5] Dept. of Botany. Faculty of Chemical Sciences. National University of Asunción, Paraguay

ABSTRACT

The fruit of the Acrocomia aculeata, native palm tree typical of the tropical region, is exploited mainly for the manufacture of oils and animal consumption. This study was aimed to determine the physicochemical characteristics of the residue of the Acrocomia aculeata coconut pulp in order to apply it in the development of new materials. Therefore fruits collected for the production of pulp were drying and pulverized, the chemical and mineral compositions were studied by GAFTA standardized techniques for the analysis of crude fiber, protein, fat and calories and INAA respectively. Subsequently, the initial material was chemically modified and assessed the solubilities of the native material and the obtained modified material proving to be completely insoluble in solvents organic and aqueous (water, ethanol, terbutanol, isobutanol and hexane), the materials were analyzed by IR with Transformed Fourier and the results showed the existence of changes in the double link lengthening conjugated and aromatic; and link double nitrogen C=N/N=O. EPR analysis indicated that the materials obtained are not paramagnetic at room temperature, however can incorporate magnetite and Fe^0. These preliminary studies concluded that this ecomaterial could be applied as support of proteins and metals.

Key-words: support, coconut pulp (*Acrocomia aculeata*)

INTRODUCTION

Coconut is the fruit of the *Acrocomia aculeata* used since the previous century in Paraguay in order to obtain oils that can be extracted from the pulp and almond, among other uses. Production contributes oil almond, however, a higher percentage is used in the preparation of cosmetics. Oil extraction of the pulp is a process that is being conducted by pressing pulp remains finally discarded. However, the physicochemical and structural coconut pulp properties has been little studied in all this time and may have potential applications in the development of new materials that might retain metals or proteins.

Various organic and inorganic matrices have been used to confine the biocatalytic property of proteins (enzymes). These materials are chitin, chitosan, bovine bone powder, silica, magnetic particles, among others (1). Retention of enzymes on the surface is critical to improving its operational efficiency in a system that is stable and facilitates their reuse.

In the group of proteins, enzymes play a fundamental role catalyzing various reactions. In industry, all enzyme especially because of their high specificity, catalyzes the molecular changes that occur without undesirable side reactions that are common in the chemical synthesis (2).

While the adsorption of metals on certain surfaces such as adsorption of hexavalent chromium on the surface of rice husk chemically modified could be strong (3), the formation of magnetic particles synthesized by co-precipitation ensures the metal stability on the surface. In fact, Fe_3O_4 nanoparticles had attracted much attention due to their unique size and morphology dependent physical and chemical properties, biocompatibility and catalytic ability (4-7).Due to their magnetism they are integrated into a wide variety of materials, representing a convenient form of separation, through the aid of external magnetic field (8-10). In this work, a magnetic biocomposite (11) was synthesized by an in situ synthesis of Fe_3O_4 on grounded coconut pulp. This study aimed to determine the physicochemical characteristics of the residue of the coconut pulp *Acrocomia aculeata* in order to apply it in the development of new materials.

MATERIALS AND METHODS

The coconut pulp was obtained by a method (12) by which the fruits of freshly collected coconut are pre-dried for 48 hours in an oven at 60°C. For physicochemical characterization of coconut pulp powder we proceeded to perform physicochemical analysis following standardized GAFTA analysis methodology for the determination of crude fiber content, protein concentration, fat and calories. Subsequently, the presence of trace metals via the neutron activation analytical technique was evaluated. Also its solubility was tested in various kinds of solvents. For the characterization of the functional groups was used an Infrared spectrum with transformed of Fourier.

The studies of Electron Paramagnetic Resonance of the ecomaterial were performed on a JEOL (JES-PE-3X) operating in X-band spectrometer (9.5 GHz). The parameters of EPR spectra were obtained using the software Origin Pro 8. The original paramagnetic capacity of the ecomaterial under native and bleached conditions was evaluated and then the coconut pulp was transformed in a magnetic biocomposite.

The magnetic biocomposite was synthesized by in situ co-precipitation of $Fe^{+\,2}$ and $Fe^{+\,3}$ in a 1: 2 molar ratio in alkaline medium at room temperature, following a procedure described in (11). On this magnetic biocomposite subsequently was evaluated retention of pancreatic lipase, a versatile enzyme that can catalyze multiple reactions in aqueous conventional systems and also in unconventional systems with low or no water activity. All assays were performed in triplicate. Finally, the retention by protein concentration and activity of enzymes on different types of coconut pulp matrix was evaluated.

RESULTS AND DISCUSSION

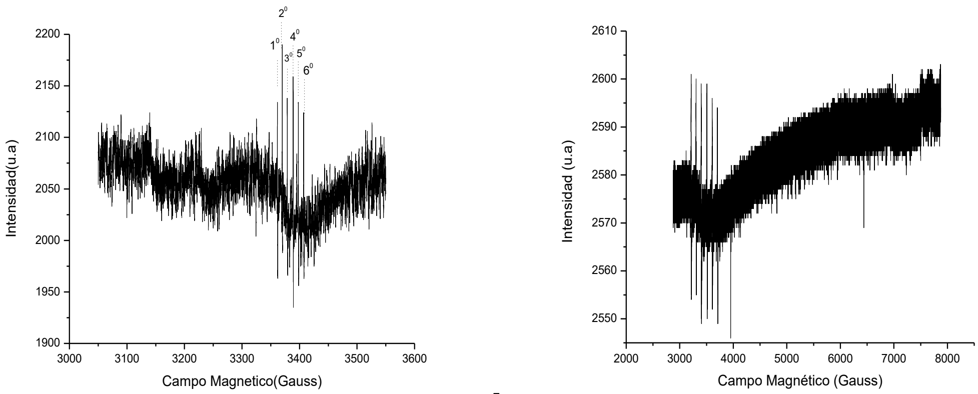
The physico-chemical studies performed under standardized GAFTA method obtained the following percentages expressed on a dry basis: moisture 7.81±0.5; fat 25.2±0.5, crude fiber 6.95 ± 0.5 and a heat capacity of 5262 ± 4 cal/ g. Due to the presence of β-carotene in coconut pulp (13) were performed a discoloration material using washed with isobutanol and *t*-butanol.

After the solubility test it was found that the coconut pulp was slightly soluble in water and insoluble in organic solvents assessed. The bleached ecomaterial mixed with 2% glutaraldehyde (W/V) resulted in a totally insoluble material in all solvents tested. The results of the solubilities are attached in Table 1 ;

Table 1: Solubility of *Acrocomia aculeata* coconut pulp in differents solvents

Solvent	*Solubility in native system*	*Solubility of bleached coconut pulp*	*Solubility of coconut pulp derivatized with glutaraldehyde*
Hexane	Insoluble	Insoluble	Insoluble
Isobuthanol	Insoluble	Insoluble	Insoluble
Terbuthanol	Insoluble	Insoluble	Insoluble
Ethanol	Insoluble	Insoluble	Insoluble
Water	Slightly soluble	Insoluble	Insoluble

In the chemical characterization of pulp was used Neutron Activation for finding traces of magnesium and potassium. The Electron Paramagnetic Resonance experiments were performed on a JEOL (JES-PE-3X) spectrometer operating at X-band (9.5 GHz). The magnetic field marker $MgO:Mn2^{+}$ was maintained in the cavity of RPE and data were obtained simultaneously with the sample due to that possible displacements of the magnetic field that could be corrected. The $MgO:Mn2^{+}$ present an EPR spectrum consists of six resonance lines, the value of *g* of the third line was equal to 2,034 and the fourth line was equal to 1,981.



A B

Figure 1- Spectrum of RPE at room temperature for coconut pulp native **(A)** and **(B)** bleached

In Figure 1A the EPR spectrum of the native coconut pulp, which has no resonance lines shown six lines of Mn^{2+} ion from magnetic field marker (MgO: Mn^{2+}) that appears in the spectrum. In Figure 1B shown EPR spectrum of the sample of coconut pulp chemically treated for discoloration which was no have resonance lines. Only six lines of Mn^{2+} ion from magnetic field marker (MgO:Mn^{2+}) appears in the spectrum . All of these experiments were performed at room temperature.

For studying the functional groups of the ecomaterial was used Infrared spectrometry with transformed of Fourier. For this propose was mixed 2 mg of native pulp or bleached pulp with 300 mg of KBr. In the complexity of molecular vibration, IR spectra can provide an unambiguous method of identifying functional groups present in a material and provide the possibility of recognizing the connection types found (14). For the native coconut pulp powder and bleached coconut pulp powder watched peaks in 2400 cm^{-1} and 3600 cm^{-1} that indicate the presence of elongations of links (+NH) and NH bonds elongations unassociated. In 1600 cm^{-1} watched peaks corresponding to elongations of double bonds C=N and N=O. The signals from bleached pulp showed a decrease in the intensity of the absorbance. However the signals appear much more defined.

The magnetic biocomposite based on coconut pulp was synthesized by in situ co-precipitation of Fe^{+2} and Fe^{+3} in a 1:2 molar ratio in alkaline media at room temperature. The reaction was conducted as follows: 40 mL of the iron solution was prepared by dissolving 1,53 g de $FeSO_4.7H_2O$ and 3,04 g $FeCl_3.6H_2O$ in deionized water. Subsequently, 1 g of grounded coconut pulp was placed into the solution, and then 20 mL of $NH_3.H_2O$ (25%) was added to the flask reaction under vigorous stirring. After the reaction, the dark product was washed with deionized water and dried in an oven at 70 ºC for three hours.
The reaction mechanism can be simplified as:

$$Fe^{+2} + 2Fe^{+3} + 8OH^{-} \leftrightarrows Fe(OH)_2 + 2Fe(OH)_3 \rightarrow Fe_3O_4 + 4H_2O$$

The magnetic biocomposite obtained above was suspended in 60 mL of 0,20 mol L^{-1} of $FeSO_4.7H_2O$ under vigorous stirring. Then, 20mL of 2,6 mol L^{-1} $NaBH_4$ was added drop by drop to reduce ferrous ion to zerovalent iron (ZVI). The final products were washed with deionized water and ethanol several times and dried in an oven at 70 ºC for three hours.

For the evaluation of protein retention rate (Table 3) an enzyme immobilization system was used (15-17) on different coconut pulp matrices; bleached coconut pulp, coconut pulp bleached with 2% glutaraldehyde and magnetized. An extract of 100 mg/L pancreatic lipase p.a. was prepared in 100 mM phosphate buffer, pH 7 at 25°C to generate the immobilized derivative. The biotechnological potential of lipases is related to the fact that not only catalyze the hydrolysis of triacylglycerides in an aqueous medium but also under low amount of water, in unconventional known systems. Lipases catalyze the synthesis of glycerol and alkyl esters by transesterification reactions (2) and if they are immobilized on supports ferromagnetic can be reused more easily.

Table 3: Retention rate of pancreatic lipase enzyme immobilized on three types of supports

Retention rate of pancreatic lipase immobilized on bleached coconut pulp	
Retained Protein concentration	77,53%
Retained lipase activity	14,24%
Retention rate of pancreatic lipase immobilized on bleached coconut pulp with glutaraldehyde	
Retained Protein concentration	63,61%
Retained lipase activity	13,87%
Retention rate of pancreatic lipase immobilized on magnetized coconut pulp	
Retained Protein concentration	80%
Retained lipase activity	14%

CONCLUSION

Due to the easy acquisition of the raw material and that its processing does not require high technology; the coconut pulp powder has a structure for use as a support of metals and proteins. The percentage of crude fiber, insolubility in solvents of varying degrees of polarity, functional groups of carbon-nitrogen and its versatile results in the immobilization of lipase generate an ecomaterial able to be a ferromagnetic support of enzymes.

ACKNOWLEDGEMENT

This project is funded by CONACYT through PROCIENCIA Program Fund resources for Excellence in Education and Research - FEEI the FONACIDE

REFERENCES

1. ARROYO, M. Inmovilización de enzimas. Fundamentos, métodos y aplicaciones. ***Ars Pharmaceutica*. 39:2; 23-29, 1998**
2. FARIA, L. A. ***Hidrólise do Óleo da Amêndoa da Macaúba com Lipase Extracelular de Colletotrichum gloesporioides Produzida por Fermentação em Substrato Líquido***. ; Junio – 2010. Tesis. Belo Horizonte – Brasil: Universidade Federal de Minas Gerais. Facultad de Farmacia, Programa de Pós-Graduação Ciencias de los Alimentos.
3. EGGS, N.; SALVAREZZA, S.;AZARIO, R.; FERNÁNDEZ, N.;GARCÍA, M.C.Adsorción de cromo hexavalente en la cascara de arroz modificada químicamente.***Av. cien. ing.: 3(3), 141-151 (Julio/Septiembre, 2012).***
4. CAMPOS, E.A.; et al. Characterization and Applications of Iron Oxide Nanoparticles – a Short Review. ***J Aerosp Technol Manag 7, 3, (2015) 267-276.***
5. LING, D.; LEE, N.; HYEON, T.Chemical Synthesis and Assembly of Uniformly Sized Iron Oxide Nanoparticles for Medical Applications. ***Acc Chem Res 48 (2015) 1276−1285***
6. HASANYS.F., ABDURAHMAN, N.H., SUNARTI, A.R., JOSER. Magnetic Iron Oxide Nanoparticles: ***Chemical Synthesis and Applications Review. Current Nanosci 9 (2013) 561-57***
7. WU, W.; WU, Z.; YU, T.; JIANG,C.; KIM, W.Recent progress on magnetic iron oxide nanoparticles: synthesis, surface functional strategies and biomedical applications. ***Sci Technol Adv Mater 16 (2015) 023501 (43pp)***
8. XIE, Y.; QIAN, D.; WU, D.; MA, X.Magnetic halloysite nanotubes/iron oxide composites for the adsorption of dyes, ***Chem Eng J 168 (2011) 959–963***
9. AZARI, A.; GHARIBI, H. KAKAVANDI, GHADER GHANIZADEH, B.; JAVID, A. MAHVI, A.H., SHARAFI, K.; KHOSRAVIA, T.Magnetic adsorption separation process: an alternative method of mercury extracting from aqueous solution using modified chitosan coated Fe_3O_4nanocomposites. ***J Chem Technol Biotechnol (2016)***
10. YANG, Q.; ZHU, Y.; YANG, M.; MA, S.; WU, Y.; LAN, F. GU, Z.; Ligand-Free Fe3O4/CMCS Nanoclusters with Negative Charges for Efficient Structure-Selective Protein Adsorption. ***Small 12, 17 (2016) 2344–2353***
11. LV, X.; XUE, X.; JIANG, G.; WU, D.; SHENG, T.; ZHOU, H.; XU, XNanoscale Zero-Valent Iron (nZVI) assembled on magnetic

Fe3O4/graphene for Chromium (VI) removal from aqueous solution. ***J Colloid Interface Sci 417 (2014) 51–59***

12. VALDOVINOS, MV y col. ***Evaluación de las propiedades fisicoquímicas y de procesamiento de la pulpa de coco (Acrocomia aculeata) con miras a su aplicación en la industria de alimentos.*** Tesis. 2016. (Lic. en Ciencia y Tecnología de Alimentos) - Facultad de Ciencias Químicas. Universidad Nacional de Asunción. Paraguay.
13. OBERLANDER, D.&BOHN E. *Acrocomia aculeata*: su potencial como cultivo para múltiples propósitos. Ed. 2009. Agroenergía S.R.L. Paraguay.
14. Chang, R. ***Fisicoquímica***. 3ra. Edición. Editorial Mc Graw-Hill Interamericana, México, 2000.
15. SORIA, F.; ELLENRIEDER, G.; BEZERRA OLIVEIRA, G.; CABRERA, M.; BEZERRA CARVALHO Jr., L. α-L-Rhamnosidae from *Aspergillus terréus* Immobilized on Ferromagnetic Supports. ***Appl Microbiol Biotechnol (2012) 93:1127–1134.DOI 10.1007/s00253-011-3469-y***
16. BETANCORT, L. y col. 2006. Different mechanism of protein inmobilization on glutaraldehyde activated supports: Effect of support activation and inmobilization conditions***. Enzyme and Microbial Technology 39(2006) 877-882.***
17. MATEO, C. y col. 2007. Improvement of enzyme activity, stability and selectivity via immobilization techniques. ***Enzyme and Microbial Technology 40:6, 1451-1463***