CHROMATOGRAPHIC AND ELECTROPHORETIC SEPERATION OF PROTEINS EXTRACTED AT pH 7.0 FROM THE HUSK OF CASHEW (*Anacardium occidentale L.*) NUT

Ogundolie, F A and Ajele, J O

Department of Biochemistry,
Federal University of Technology,
Akure, Ondo State, Nigeria
fa.ogundolie@futa.edu.ng

Abstract

Cashew (Anacardium occidental) is native to northeastern Brazil. It is a tree nut belonging to the plant flowering family of Anacardiaceae, they enjoy global acceptance and are valued for their sensory and nutritional attributes. The nuts are high in lipids (45-70%, w/w) and proteins (20-25%, w/w) and are therefore energy-rich foods. This research is aimed at the extraction, separation, and if possible, identification of the various proteins present in cashew nut shell/husk. The extraction of proteins from cashew nut shell/husk was carried out by isolating the cashew nut shell/ husk which is of interest from the cashew nut through manual method (knife and hammer). Extraction was done with 0.1 M sodium phosphate buffer at pH 7.0. The protein extract (17 ml) was applied on a sephacryl S-300 column. The molecular weight of the components of the crude extract was determined by 10 % Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The gel filtration chromatography separation of the extract showed three peaks, two minor and one major peak, Four bands were observed on the electrophoretic gel with molecular weights corresponding to 49, 200 Da; 24,045 Da; 18,400 Da and 16,206 Da respectively. This shows that the Cashew nut Husk contains at least three different types of proteins.

Keywords: sephacryl S-300, Cashew husk, molecular weight, SDS-PAGE electrophoresis, proteins, chromatography.

INTRODUCTION

The cashew tree (*Anarcardium Occidentale*) is a native of Brazil and the Lower Amazons. The cashew has been introduced and is a valuable cash crop in the Americas, the West Indies, Madagascar, India and Malaysia (Frankel, 1991; Akinhanmi *et al.,* 2008). Cashew is an important evergreen tropical crop. (Sengar *et al.,* 2012) The economic importance of this special tree is such that while the tree is native to Central and South America, it is now widely distributed throughout the tropics, particularly in many parts of Africa and Asia. The Cashew tree will tolerate a wide range of condition including drought and poor soil, but cannot withstand cold frost. Cashew has become one of the valuable commodities due to the marketing prospect for domestic and even for export (Mursalim *et al.,* 2002). World-wide trading of

cashew divided into two products which are Cashew Nut in Shells/husk (CNS) and cashew Nuts (kernel). Previous research works carried out on cashew nut husk popularly known as cashew nut shell were centred majorly on the cashew nut shell liquids and their proximate analysis (Amoo, 2005; Akinhanmi *et al.,* 2008; Ogunwolu *et al.,* 2010, Onilude *et al.,* 2010); industrialization of cashew juice (Da Silva *et al.,* 2008) and the various applications of CNSL (Dos Santos and de Magalhães, 1999; Ikeda *et al.,* 2002; Kumar *et al.,* 2002; De Lima *et al.,* 2008). With much neglect on the separation, identification and concentration of proteins from the cashew nut shell/husk unlike the numerous researches observed on the quantification of the protein content of cashew nut kernel/ seed. Hence, the objective of this project therefore is on the preparation of the protein extract from cashew nut shell/husk and separation by gel chromatography and the determination of the molecular weight of the proteins by SDS-polyacrylamide gel electrophoresis as a step towards identifying the various proteins present in.

MATERIALS AND METHOD

Cashew nuts were collected from a compound in Awule Street of Akure south local government in Ondo State, Nigeria. Identification of the nuts was done at the Crop soil and pest management department, CSP, Federal University of Technology, Akure. Other materials includes sephacryl S-300 (Phamacia F, Dihydrogen potassium phosphate salt (KH2PO4), Dipotassium hydrogen phosphate salt (K2HPO4) by sigma, Spectrophotometer (UNICO 2800 UV/VES), pH meter (jenway), Refrigerated centrifuge (centurion product), Top Loading Balance (Adventurer Pro AV8101 by Ohaus Corporation, Switzerland), Desiccator, Glass wool, Glass funnel, Volumetric flasks, Blender, Beakers, Micropipettes (pipetman), Wash-bottles, Laboratory drying cabinet, (Genlab widens England N18C), dialysis tubes, ependoff tubes, Vacuum pump (Rotavec valve tec by Heldolph Instruments, Germany), Analytical Weighing balance (by Adams Equipment), Magnetic stirrer and bar, and all other chemicals and reagents used were obtained from the Laboratory of the department of Biochemistry, Federal University of Technology, Akure, Ondo State.

METHODS

The Cashew nut shell/husk was manually removed from the seed and oven-dried for three days at 40 ^{o}C while their weight is being determined after each day until a constant weight was obtained.

Sample Extraction

Fifty grams of the sample was weighed and mixed with ice cold 600 mL of 0.1M potassium phosphate buffer pH 7.0 and was stirred for 5 h on cold ice using a magnetic stirrer. The mixture was filtered on four layers of cheese cloth and centrifuged at 6,000 rpm for 30 min at 4° C. The supernatant was stored in the freezer and used as crude extract.

Protein Determination

The protein concentration of the crude extract was determined by the Biuret method of Lowry *et al*., (1951) with bovine serum albumin (BSA) as the standard. The concentration of protein during purification studies was measured at an absorbance of 280nm.

Sample Concentration

The samples obtained from peaks (three peaks) obtained in the gel filtration calibration graph were pooled and then concentrated using 4M sucrose solution for 24 hrs.

Gel Filtration Chromatography

Crude extract was carried out to a column of sephacryl S-300 (2.5 x 75 cm) that had previously been equilibrated 0.1 M sodium phosphate buffer, pH 7.0. 17 ml of the crude protein extract was applied and elution was done with 0.1 M sodium phosphate buffer, pH 7.0 at a flow rate of 20 ml/hour. 5 ml fractions were collected. The protein content of the fractions was monitored at 280nm on a UNICO 2800 UV/VES Spectrophotometer.

Molecular Weight Determination / Separation of Components:

The molecular weight of the crude protein extract was determined by Sodium Dodecyl Sulphate Polyacrylamide Electrophoresis (SDS-PAGE) according to the method of Weber and Osborn (1969) in 10% gel using the SDS Tris-HCl buffer system pH 8.0. Protein markers used were Phosphorylase b (113,900 Da), Bovine Serum Albumin (72,000 Da), Ovalbumin (47,400 Da), Carbonic Anyhydrase (33,000 Da), Soyabean Trypsin Inhibitor (27,000 Da), α –Lactalbumin (17,400 Da). A volume of 0.01 ml of a solution of the electrophoresis low molecular weight calibration kit protein standards in sample buffer was boiled for 1 minute in a boiling water bath. A solution of the protein (20 µl) was laid on the gel and electrophoresis was run for 5 h at 4 mA/ gel during stacking and 8 mA/gel during separation. After electrophoresis, the lengths of the gel and the distance migrated by the tracking dye were measured before the gels were stained with 0.25%. Coomasie blue R-250, (10.25 g dissolved in 22.7 ml methanol 4.6 ml glacial acetic acid and distilled water to 100 ml) they were subsequently destined with the destaining solution (50 ml methanol, 7.5 ml glacial acetic acid plus distilled water to 100 ml). After destaining, the lengths of the gel and distances migrated by the various protein bands were measured, the relative mobility (Rm) of each of protein band was obtained according to the method of Weber and Osborn (1969).

RESULT AND DISCUSSION

From the results obtained on the various analysis carried out on the cashew nut husk, using the biuret reagent solution to determine the protein concentration, this result showed that the concentration of proteins generally in the cashew nut husk is very low compared to the concentrations of proteins in the cashew nuts kernel/seed as reported by Ogunwolu *et al.,* (2010); Amoo, (2005). It was observed also that the concentration of proteins in the samples A (0.206 g/ml) and B (0.125 g/ml) which were not passed through the activated charcoal were higher than samples C (0.066 g/ml) and D(0.106 g/ml) even though they were are the same fold dilutions respectively . The activated charcoal used was intended at decolorizing the extract but which later turned out to cause a reduction in the concentration of the overall protein. In the elution profile obtained on separation of the proteins in the sample extract in

figure 1, there is only one high peak which indicates a likely high molecular weight protein as well as two low molecular weight proteins (Ettre and Leslie, 2007).

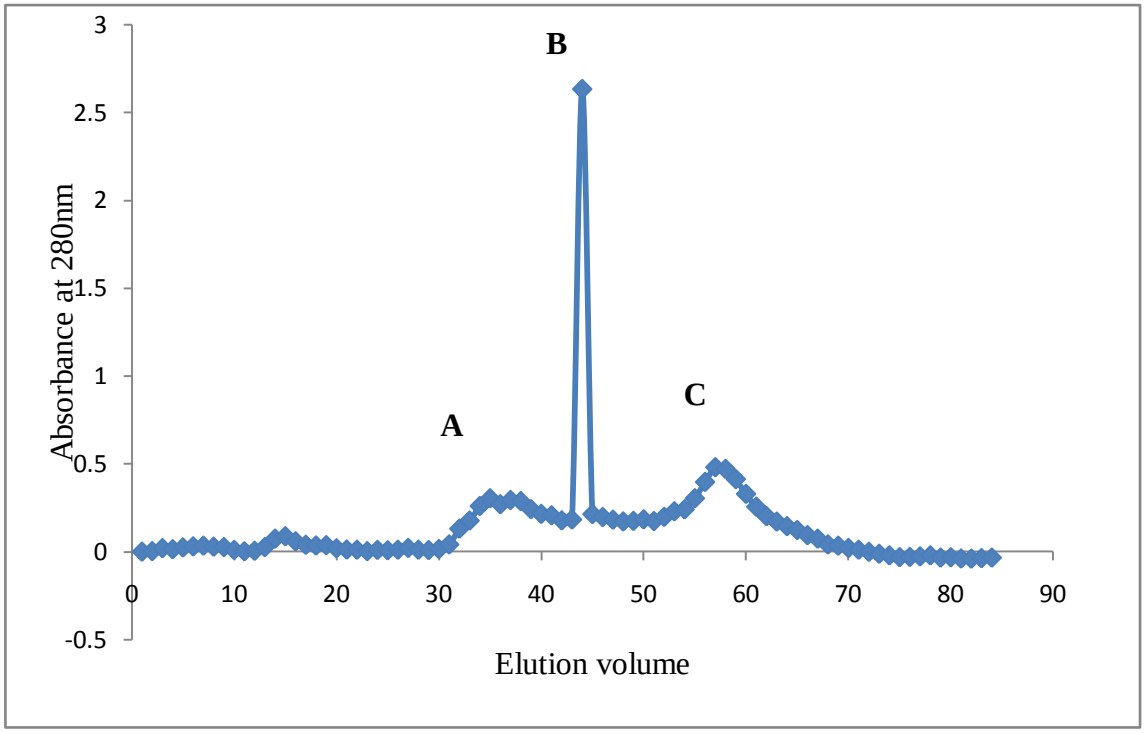

Figure 1: Elution profile of the crude cashew husk protein extract on a column of Sephacryl S-300.
The extract (17 ml) was eluted with 0.1 M sodium phosphate buffer, pH 7.0 at a flow rate of 20 ml/hr and 5ml fractions were collected. The fractions collected were monitor at 280 nm.

The peak obtained showed that there was retention in the sample as this shows interaction between the sample and the stationary phase as well as the sorbent bed. The result of the molecular weight determination by 10% SDS- polyacrylamide gel electrophoresis (PAGE) is illustrated in figure 2. Four protein bands were obtained after staining and destaining of the gels as shown in lane P. While the interpolation of the relative mobility values of the protein bands obtained observed while using 10 % Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS- PAGE) on plot of log molecular weight vs. mobility gave molecular weights 49, 200; 24,045; 18,400 and 16,206 for protein bands A, B, C and D respectively. These bands could be said to represent different proteins, moreover, the four protein bands represent subunits of a multi-subunit protein. One might not be able to conclusively say from the results obtained that the result showed that there are minimum of three proteins has shown by gel filtration chromatography using sephacryl S-300.

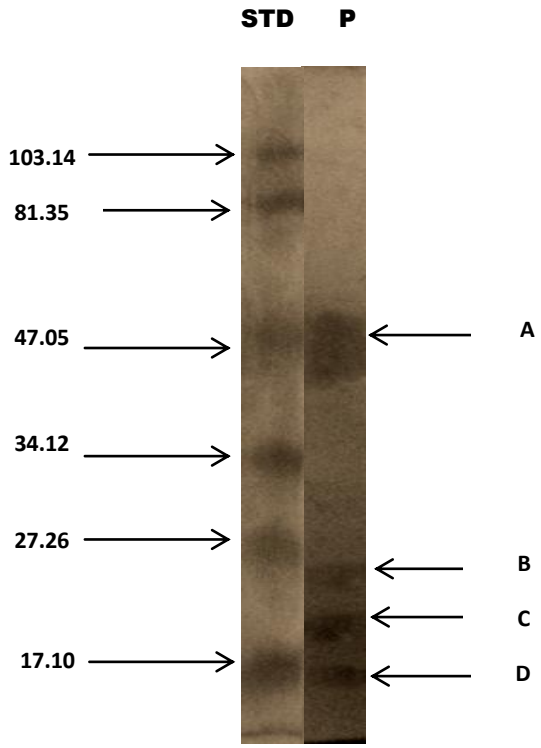

Figure 2: 10 % Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS- PAGE) of the crude cashew husk protein extract. STD contains the molecular weight standard while track P is the crude cashew husk extract.

One might not be able to conclusively say from the results obtained that the result showed that there are minimum of three proteins has shown by gel filtration chromatography using sephacryl S-300. It is not clear whether the numbers peaks and protein bands observed is determined by the method used in extracting the protein and the pH of the buffer used. This will be a subject of further investigation. Further research is expected to be carried out on these protein sample to determine their physicochemical properties and applications.

REFERENCES

Akinhanmi, T. F., Atasie, V. N., Akintokun, P. O. (2008). Chemical Composition and Physicochemical Properties of Cashew nut (*Anacardium occidentale*) Oil and Cashew nut Shell Liquid. J. Agricultural, Food and environmental sciences. 2 (1): 1-10

Amoo I. A. (2005). Proximate composition, minerals, and physico-chemical properties of cashew nut and oil. *J. Fd. Sci. and Tech. Mysore.*; 42 (5): 445-446.

Da Silva K.D.P, Collares F. and Finzer J.R.D (2000). A simple and rapid method for estimating the content of solids in industrialized cashew juice. *Food Chem.* 70: 247-250.

De Lima S.G., Feitosa1 C.M., Citó A.M.G.L., Moita Neto J.M., Lopes J.A.D., Leite A.S., Brito M.C., Dantas S.M.M. and Melo Cavalcant A.A.C (2008). Effects of immature cashew nut-shell liquid (*Anacardium occidentale*) against oxidative damage in *Saccharomyces cerevisiae* and inhibition of acetylcholinesterase activity. J. Genetics and molecular research, GMR. 7(3): 806-818

Dos Santos M.L and de Magalhães G.C (1999). Utilization of cashew nut shell liquid from *Anacardium occidentale* as starting material for organic synthesis: A novel route to lasiodiplodin from cardols. *J. Braz. Chem. Soc.* 10: 13-20.

Ettre, Leslie S. (2007). "The Rebirth of Chromatography 75 Years Ago." LG-CG North America **25**. Pp 15-23

Frankel, E. (1991). Poison ivy, Poison oak, Poison sumac and their relative pistachios: Mangoes and Cashew. *The Boxwood Press Pacific Grove CA* 2: 15-16

Ikeda R, Tanaka H, Uyama H and Kobayashi S (2002). Synthesis and curing behaviors of a crosslinkable polymer from cashew nut shell liquid. *Polymer* 43: 3475-3481.

Kumar, P.P.; Paramashivappa, P.J.;Vithayathil, P.J., Subra Rao, P.V.;Srinivasa Rao A. (2002) Process for isolation of cardanol from technical cashew (Anacardium occidentale.) nut shell liquid. *J. Agric. Food Chem., 50, 4705-4708.*

Lowry, O.H., Rosebrough N.J., Farr A.L. and Randall R.J., (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-75.

Mursalim, Supratomo, Yuliani Shinta Dewi (2002) Draying of Cashew Nut in Shell Using Solar Dryer *Science and Technology*, 3(2): 25-33

Ogunwolu S O, Henshaw F O, Mock H P and Matros A (2010). Production of Protein Concentrate and Isolate from Cashew (*Anacardium Occidentale L.*) Nut. African journal on food agriculture development; 10(5): 2501-2514.

Onilude A. A., Igbinadolor R. O. and Wakil S. M. (2010). Effect of varying relative humidity on the rancidity of cashew (*Anacardium occidentale* L.) kernel oil by lipolytic organisms: African Journal of Biotechnology Vol. 9(31), pp. 4890-4896

Sengar S. H., Mohod A. G., Khandetod Y. P. (2012). Performance Evaluation of Kiln for Cashew Nut Shell Carbonization and Liquid. *International Journal of Energy Engineering*, 2(3): 78-85

Weber, K. and M. Osborn, 1969. The relativity of molecular weight determination by Dodecyl sulphate polyacrylamide gel Electrophoresis. J. Biol. Chem., 244: 4406-4412.