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Determination of molecular weights from selected protein fractions obtained from Cashew (*Anacardium occidentale*) seeds

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Research Paper

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The Cashew (*Anacardium occidentale*) seed is considered to be a rich source of plant proteins that are essential for growth. Research attention has been directed towards increasing utilization of plant protein sources for food use, because of their unique functional properties, such as emulsification, fat and water absorption, texture modification, color control and whipping properties. Hence, this study aims to determine the molecular weights of purified soluble proteins from Cashew seed. The supernatant was purified by gel filtration column containing sephadex G-50. Five peaks were observed and concentrated by sucrose solution (4.0 M). The molecular weight of purified protein determined by SDS-PAGE showed five bands for fraction 3 with molecular weights 95,757.31 Da, 66,137.01 Da, 54,964.32 Da, 44,019.43 Da, and 27,208.13 Da respectively. Two out of the five possible proteins in fraction 3 was similar in weight to standard proteins Ovalbumin, 47.05 kDa; and Soybean trypsin inhibitor, 27.26 kDa respectively. Protein band of 44,019.43 kDa were observed in fractions 2, 3, 4 and 5 respectively.

Key words: Cashew nut, Chromatography, Electrophoresis, Proteins, Precipitation, Molecular weight

INTRODUCTION

The Cashew tree (*Anacardium occidentale*) originates from South and Central America to Africa, Asia and tropical Australia (Behrens, 1998). The major producing countries of Cashew are Tanzania, India, Mozambique, Srilanka, Kenya, Madagascar, Thailand, Malaysia, Indonesia, Nigeria, Senegal, Malawi and Angola. Cashew (*Anacardium occidentale*) belongs to the family Anacardiaceae, with about 75 genera and 700 species. Cashew tree tolerates a wide range of condition including drought and poor soil, but cannot withstand cold frost. (Ogundolie and Ajele, 2015) Cashew fruit is made up of the apple that bears the fruit, the Cashew apple is a pseudocarp, and in botanical terms, is the thickened stem of a fruit which the actual fruit, the Cashew nut, is attached. In its originating countries, the pear-shaped Cashew apple is eaten fresh. The nut is composed of the kernel and shell. The shell is about 3 mm thick and comprises roughly 45-50% of the total weight of the nut (Joseph, 1975). The kernel (seed) is slightly curved back on itself and is formed of two cotyledons, representing about 20-25% of the nut weight; it is surrounded by a fine, brown seed coat, called the testa, which contains antioxidants that protect the kernel (seed) from penetration by atmospheric oxygen so preventing it from becoming rancid (oxidative rancidity). The testa accounts for approximately 5% of the whole nut weight. Cashew

nut kernel is of high food value with about 40-57% oil, and 21% protein (Fetuga et al., 1975). Proteins utilized in food processing are of various origins, and can be roughly grouped into animal proteins (gelatine), vegetable proteins (e.g. Soya protein), and animal derived protein (milk proteins) (Penny, 1999). According to Moure *et al,* (2003), proteins that are essential to growth and health are currently required more in the developing countries of the world, because of the chronic problem of protein-energy malnutrition.

Shortages and high prices have recently caused restriction of animal proteins in the diets of many families in the developing countries of the world. However, vegetable proteins which are cheaper and available are of great potentials as a direct food for human consumption. Many of the vegetable proteins require processing to provide a food material that has acceptable organoleptic properties for human consumption (Kapur *et. al.,* 1975). Many plant proteins of which Cashew nut protein is a major example are being investigated and tested for new products such as low cost fabricated foods which are nutritious, attractive and acceptable to consumers just like conventional foods from meat, fish and dairy products (Mcwatters and Cherry, 1977) Research attention has been directed towards increasing utilization of plant protein sources for food use, because of their unique functional properties, such as emulsification, fat and water absorption, texture modification, colour control and whipping properties.

Proteins are made up of different amino acids, some which can be manufactured by the body and others that are found in high-protein foods. Cashew seed contain essential amino acids, making them a valuable source of protein and an alternative to animal protein. In addendum to the previous researches carried out on Cashew nut, this study aims to determine the molecular weights of soluble proteins from Cashew nut seed using gel filtration chromatography to purify the protein and SDS gel electrophoresis to determine the molecular weight of the proteins fractions obtained from gel filtration chromatography.

MATERIALS AND METHODS

Freshly gotten Cashew nuts, sephadex G-50 and column, Spectrophotometer (UNICO 2800 UV/VES), pH meter (model PHS-25), Analytical Weighing balance (by Adams Equipment), Top Loading Balance (Adventurer Pro AV8101 by Ohaus Corporation, Switzerland), Desiccator, Glass wool, Glass funnel, Volumetric flasks, Blender, Beakers, Micropipettes, Wash-bottles, Laboratory drying cabinet, (Genlab widens England N18C), Refrigerated centrifuge (centurion product), dialysis tubes, ependoff tubes, Vacuum pump (Rotavec valve tec by Heldolph Instruments, Germany), and all chemicals and reagents used were of analytical grades and were obtained from the Laboratory of the department of Biochemistry, Federal University of Technology, Akure, Ondo State.

Methods

The nut kernels (seed) were oven-dried for five days at 50°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 7.4 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℃. The supernatant was stored in the freezer and used as crude extract.

Protein determination

Protein was determined by the Biuret method of Lowry et al. (Bernfield, 1955) with bovine serum albumin (BSA) as the standard. The concentration of protein during purification studies was measured at an absorbance of 280 nm.

Concentration of separated Cashew nut seed proteins

The pooled samples were concentrated using the dialysis method. The samples were pipetted into a dialysis bag and placed into a beaker of 4 M sucrose. The process was carried out for about 36 h and the concentrated sample was subjected to electrophoresis.

Gel filtration chromatography (using Sephadex G-50)

Sephadex G-50 was swollen in 0.1M potassium phosphate buffer pH 7.4 for 3 days before it was packed into column. 20 mL fraction was applied to a Sephadex G-50 (Pharmacia) column (1.5×75) which had been previously equilibrated with 0.1M potassium phosphate buffer pH 7.4. The column was eluted with the same buffer at a flow rate of 15 ml/hr. A fraction of 5.0 ml were collected at interval of 30 min and the absorbance at 280 nm was read using spectrophotometer (Jenway, 6305).

Determination of molecular weight

The apparent molecular weight of the beta amylase was

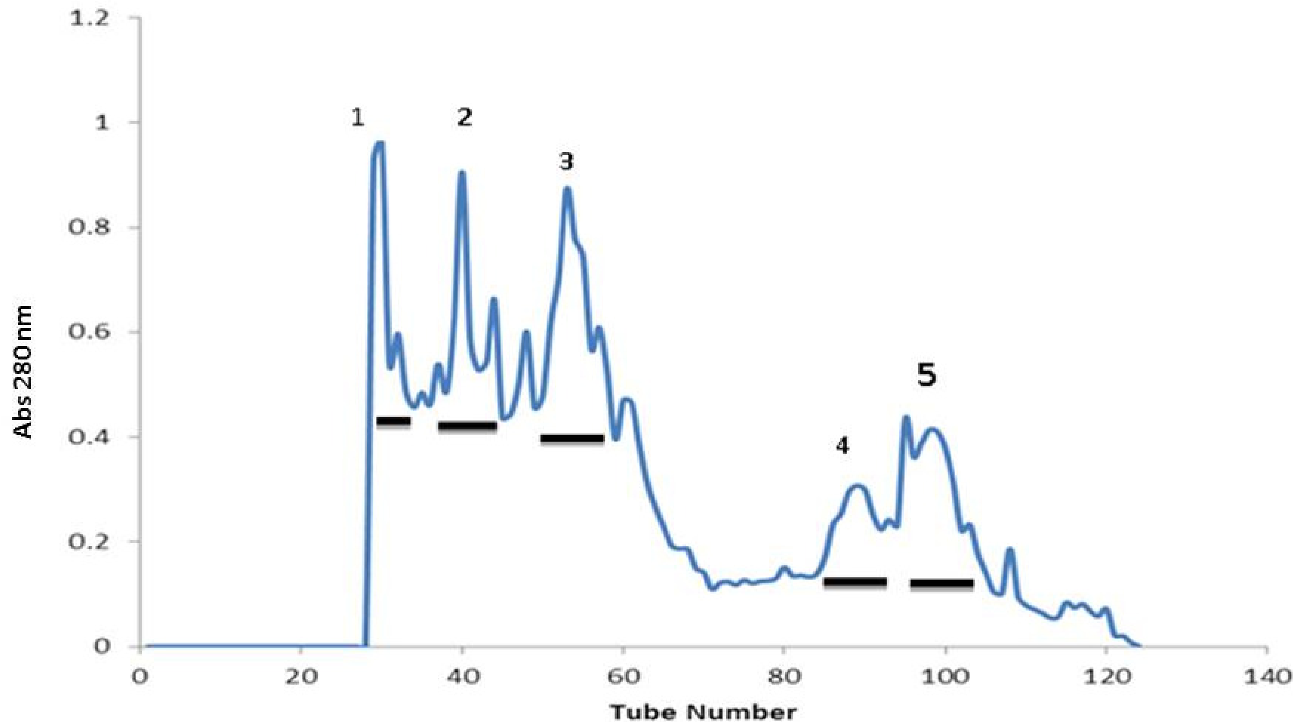

Figure 1. Chromatographic elution fraction graph at absorbance 280 nm.

PEAK 1A:Pooled fractions 29-35,PEAK 2:Pooled fractions 38-46,PEAK 3:Pooled fractions 49-63,PEAK 4:Pooled fractions 85-92,PEAK 5:Pooled fractions 94-104.

estimated from the gel filtration column using Phosphorylase b, 103.14 kD; Bovine serum albumin, 81.35 kD; Ovalbumin, 47.05 kD; Carbonic anhydrase, 34.17 kD; Soybean trypsin inhibitor, 27.26 kD and Lysozyme, 17.67 kD. (Sigma, UK) as reference proteins.

RESULTS AND DISCUSSION

The biuret reaction method was used to determine the concentration of proteins because peptide bonds occur with the same frequency per amino acid in the peptide. The intensity of the color, and hence the absorption at 540 nm, is directly proportional to the protein concentration, according to the Beer-Lambert law (Lowry, 1951). The biuret reaction gave protein concentrations of 79.0 g/ml, 100.65 g/ml and 106.06 g/ml showing the presence of proteins in the sample. From (Figure 1), the purified pooled protein fraction (2,3,4,5) extracts, were concentrated with sucrose to remove the water and buffer content from the extract through dialysis tube pores which function by allowing materials with smaller size through it, thus increasing the concentration of the extract. Figure 2 represents the electrophoretic pattern of the proteins obtained with SDS- PAGE a technique where electromotive force (EMF) is used to move molecules through gel matrix (Weber and Osborn, 1969). By placing the molecules in wells in the gel and applying an electric field, the molecules moved through the matrix at different rates, determined largely by their mass when the charge to mass ratio (Z) of all species is uniform, toward the anode if negatively charged or toward the cathode if positively charged. This principle was applied during the electrophoresis process which revealed the presence of 5 protein bands in pooled protein fraction (2), 5 protein bands in pooled protein fraction (3), 3 protein bands in pooled protein fraction (4), and 2 protein bands in pooled protein fraction (5).

The molecular weight of the protein band varies from 26.22KDa to 95.76KDa. According to the results, the molecular weights of the proteins present in the pooled fraction (2, 3, 4, 5) extract were identified. The proteins present in the pooled fraction (2) extract were (Protein 2_{I}, 2_{II}, 2_{III}, 2_{IV} and 2_{V}, 95.76, 66.14, 44.02, 37.96, and 26.22 KDa respectively). Three out of this five different possible proteins (that is, 2_{III}, 2_{IV}, 2_{V}) were similar in molecular weight to standard proteins Ovalbumin; 47.05 kDa Carbonic anhydrase, 34.17 kDa; and Soybean trypsin inhibitor, 27.26 kDa respectively. The proteins present in the pooled fraction (3) extract were (Protein 3_{I}, 3_{II}, 3_{III}, 3_{IV} and 3_{V} (95.76, 66.14, 54.96, 44.02, and 27.21 KDa respectively) Two out of these five possible proteins (that is. 3_{IV}, 3_{V}) is similar in weight to standard proteins Ovalbumin, 47.05 kDa and soybean trypsin inhibitor,

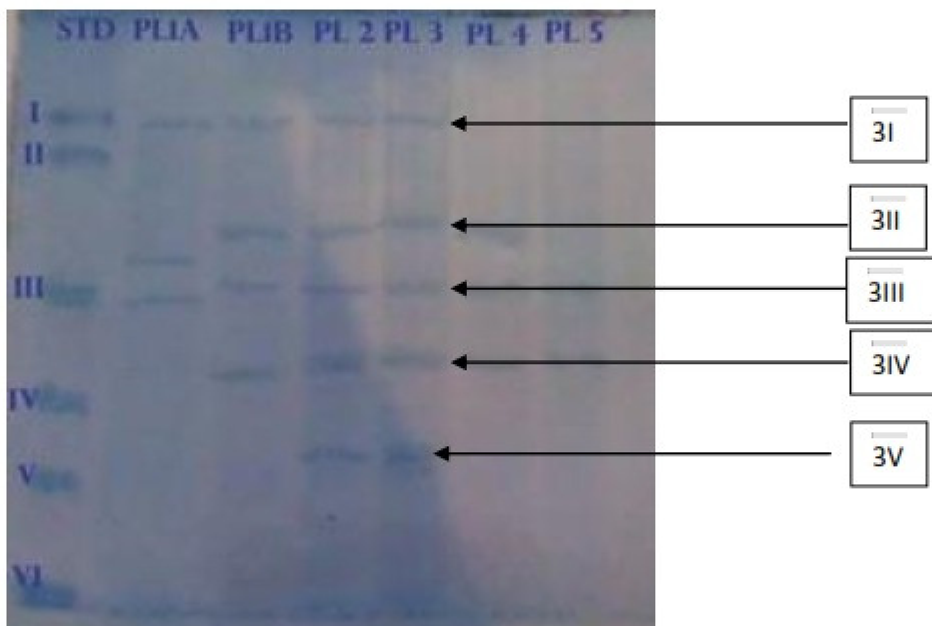

Figure 2. Electrophorectogram of protein pools (1A, 1B, 2, 3, 4 and 5) of crude protein of Cashew seed purified by gel filtration on sephadex G-50 column.

27.26 kDa respectively. The proteins present in the pooled fraction (4) extract were (Protein 4_{I}, $4_{II,}$ 4_{III} (57.04, 44.02, 27.21 KDa respectively) Two out of these three possible proteins (i.e. $4_{II,}$ 4_{III}) is similar in weight to standard proteins Ovalbumin, 47.05 kDa; and Soybean trypsin inhibitor, 27.26 kDa respectively.) The proteins present in the pooled fraction (5) extract were (Protein 5_{I}, $5_{II,}$ (57.04, 44.02 KDa respectively) one out of these two possible proteins (that is, $5_{II,}$) is similar in weight to standard proteins Ovalbumin, 47.05 kDa.

The protein of the Cashew (*Anacardium occidentale*) seed presented molecular weights similar to the proteins of various plant species. In Lupinus mutabilis, Sathe, *et al.,* (1982) reported the existence of proteins with 85.0, and 97.3 kDa. In Cashew nut protein, the presence of proteins of molecular weights of 24.83, and 31.99 kDa (Sathe, 1994) have been reported. In black mucuna pruriens proteins of 21.0, 30.0 (Machuka, 2000), and 97.0 kDa (Adebowale & Lawal, 2003) have been reported. Mnembuka and Eggum (1993) identified in the groundnut the presence of proteins with molecular weights 12.0, 13.2, 18.6, 33.1, and 39.8 kDa. In winged bean protein, of weights 67.0, and 82.0 kDa (Meng and Ma, 2001) have also been reported.

CONCLUSION

The result obtained from the Biuret reaction method for protein assay together with the result from the separation process carried out on the protein extract using sephadex G-50 and the absorbance reading of eluted fractions at 280 nm characterizes the kernel as a rich source of protein. The SDS-PAGE revealed the presence of 5 protein bands in pooled protein fraction (2), 5 protein bands in pooled protein fraction (3), 3 protein bands in pooled protein fraction (4), and 2 protein bands in pooled protein fraction (5).The molecular weight of the proteins in pooled protein fraction (2 and 3) ranges between 26.22KDa and 95.76KDa, and the molecular weight of the proteins in pooled protein fraction (4 and 5) ranges between 27.21KDa and 57.04KDa, which are also reported in various other plant proteins. One common molecular weight that cut across all the pooled protein fractions is 44.02KDa which is similar in molecular weight to standard proteins Ovalbumin, 47.05 kDa. Considering all these factors, it could be concluded that the Cashew (*Anacardium occidentale*) seed can probably be used in human nutrition. However, more research is needed to be carried out to ascertain the specific properties and amino acid sequence of these proteins.

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