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Effect of acid and alkaline hydrolysis on the concentrations of albumin and globulin in *Thevetia peruviana* seed cake protein extract

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Abstract

Thevetia peruviana seeds cake were defatted and then treated with varying concentrations each of hydrochloric acid, sodium hydroxide and calcium hydroxide solutions. Each product of hydrolysis was extracted with chloroform to isolate aglycones, the toxins of the seed. Various concentrations of hydrochloric acid and sodium hydroxide solution effected complete detoxification. Only 0.4M and 0.5M of calcium hydroxide solution detoxified the seeds completely. Albumin and globulin determination by biuret method confirmed that various concentrations of the hydrolyzing agents increased the quantity of extractable albumin and globulin in the cake. Each solution used for the detoxification had closely related trend on the total albumin and globulin value of the treated cake. Higher quantities of albumin and globulin were recorded in the samples treated with various concentrations of calcium hydroxide solutions. The study suggests that calcium hydroxide at high concentrations appear to be the best detoxicant.

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Keywords: Thevetia peruviana; detoxification, albumin, globulin

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INTRODUCTION

The presence of antinutrients in some oil seeds has adverse effects on their nutritional quality hence limit their use by man. To optimize their utility, several detoxification techniques have been used. The efficacy of the adopted techniques depends on the nature and the properties of the antinutrients. Some antinutrients are sensitive to heat while some can readily be removed from the seed by solvent extraction. For instance, the polar nature of gossypol has enhanced its removal from cottonseed with different polar solvent[1]. Acidic butanol was used by Canella and Sodini[2], to remove the gossypol without altering the protein quality. However, the use of dichloromethane by Cherry and Gray[1], allowed a larger reduction of gossypol.

Castor oil seed contain at least three toxic substances; ricin, an extremely toxic alkaloid, allergin and a toxic polysaccharide. Puttaraj *et al*[3] detoxified the seeds by boiling, hydrolysis with lime and heat treatment[3]. Systematic studies by Mendel *et al*[4] of the factors that inactivate soybean trypsin showed that thiols facilitate inactivation.

It has been established by Yinglang and Chunsu[5] that *Thevetia peruviana* seed is rich in protein and oil but it is poisonous due to the presence of cardiac glycosides that are bitter. The polar nature of the toxins enhances their removal from the seed by solvent extraction, using suitable solvents. However, preliminary studies have revealed that solvent extraction is not very effective, as the seed cake still retains some of its bitter taste. Hydrolysis of the toxins to less toxic products prior to solvent extraction becomes inevitable. However, other components of the seed, such as carbohydrates and proteins are equally susceptible to hydrolysis. It is for this reason that, we set out to investigate the effect of detoxification by hydrolysis with some selected hydrolyzing agents (NaOH, $Ca(OH)_2$ and HCl) on the quantity of extractable albumin and globulin (protein fractions) in the seed cake.

MATERIALS AND METHOD

Ripe fruits of *Thevetia peruviana* plant were collected in large quantity from different locations in Ilorin, Kwara State in October 2002. The fruits were processed to obtain the seed. All the chemicals used for this work were of analytical grade.

Defatting of Seed Cake

Standard official and tentative method of oil chemists' society[6] was used to defat the seed cake. In the method, light naphtha was distilled at 80^{o}C. *Thevetia* seed was crushed in a mortal with pestle. The distilled light naphtha was added to the crushed seeds in an aspirator bottle to extract the oil by refluxing over water bath, for 8hrs using soxhlet method. The extraction was repeated several times until the cake was free from oil. The defatted seed cake was dried and kept for further analysis.

Acid Hydrolysis of Defatted Seed cake

Method similar to that of Ojoawo[7] was adopted for the hydrolysis. 10 grams of the defatted seed was weighed separately into five separate boiling tubes. Each was moistened with 20ml of 0.1M to 0.5M HCl solutions (Equivalent to 0.002 mole 0.004mole, 0.006mole, 0.008mole and 0.01mole of the prepared solutions). They were placed in a water bath and maintained at 80^{o}C for an hour, which has been established as an ideal time for hydrolysis in preliminary investigations. The products were then washed with water, dried and extracted with chloroform for 2hrs using the soxhlet method. The residue was dried and tasted.

Alkaline Hydrolysis of Defatted Seed Cake

Method similar to that to Alegbemi[8] was adopted for the hydrolysis. 10grams of the defatted seed cake was weighed separately into ten separate boiling tubes five out of which were moistened with 20ml of 0.1M to 0.5M NaOH (Equivalent to 0.002mole, 0.004mole, 0.006mole, 0.008mole and 0.01 of the prepared solution). The remaining five were moistened with 20ml of 0.1M to 0.5M calcium hydroxide solutions, equivalent to the number of moles of sodium hydroxide solutions. They were then placed in a water bath and maintained at 80^0C for an hour. The resulting samples were washed with water, dried and extracted with chloroform for 2 hours, using soxhlet method. The residue was dried and tasted.

Isolation of Albumin and Globulin

Albumin was isolated using an adapted method of Marcone *et al*[9]. The method involves extracting 2grams of the cake with 0.4M NaCl containing 0.1M KH_2PO_4 and 0.1M NaOH buffer (pH 7.5) at ratio of 1:1 (w/v) for 12hours. After centrifugation for 30min, the supernatant fraction was dialyzed against distilled water for 72hours. The mixture was centrifuged as described above. After centrifugation the precipitated debris was removed and supernatant containing albumin retained.

The method of Hall *et al*[10] was adopted for the isolation of globulin. The protein fraction was extracted from 2grams of cake with 0.5M NaCl containing 0.0025M HCl at a ratio of 1:10 (w/v) at room temperature for 2 hours with constant magnetic stirring. The slurry was centrifuged, filtered through a Whatman filter paper to remove any debris. 5ml of cold, distilled, deionized water were added to precipitate the globulin. The precipitate was collected after centrifuging the dilute filtrate. The precipitate was dissolved in 10ml of 0.5M NaCl and further subjected to cold precipitation and centrifugation as above three more times. The final precipitate was dissolved in 10ml of 0.5M NaCl and dialyzed.

Determination of Albumin and Globulin Fraction of Protein in treated and untreated Seed Cake.

The albumin and globulin fractions of protein in the samples were determined by biuret method of Gornal *et al*[11]. A calibration curve was prepared using standard solutions of egg albumin. The absorbance of the extracts was read after 30min against the blank on spectrophotometer. The absorbance was determined in triplicates. Ducan multiple range test was used for the analysis of the results.

RESULTS AND DISCUSSION

Residues obtained from sodium hydroxide and hydrochloric acid hydrolysis, irrespective of the strength of the solution used was found to have lost its bitter taste. This establishes complete removal of all available bitter principles, which account for the toxicity of the seed. However, in comparison with that of calcium hydroxide hydrolysis, it was found that only the samples hydrolyzed with 0.4M and 0.5M $Ca(OH)_2$ solution lost their bitter taste. The bitter taste was retained by other sample treated with lower concentration of calcium hydroxide solution.

Table 1 shows a steady increase in the quantity of albumin and globulin as the concentration of detoxification solution increases. The increase is attributable to the liberation of more albumin and globulin from their conjugated form, in form of glycoprotein, which consequently increases

Table 1: Albumin and Globulin Of Seed Cake Residue Obtained From HCl Hydrolysis

HCl – Hydrolyzed Extracts	Concentration (Mg/ml)		Percentage Composition (%)	
	Albumin	Globulin	Albumin	Globulin
0.1M HCl hydrolyzed extract	0.588±0.002	0.58±0.002	0.29	0.29
0.2M HCl hydrolyzed extract	0.713±0.001	0.630±0.002	0.36	0.31
0.3M HCl hydrolyzed extract	0.838±0.02	0.710±0.002	0.42	0.36
0.4M HCl hydrolyzed extract	0.950±0.02	0.850±0.002	0.48	0.43
0.5M HCl hydrolyzed extract	1.008±0.02	1.00±0.001	0.60	0.50

The globulin content of untreated seed cake = 0.11. The albumin content of untreated seed cake = 0.29. Each value is a mean of data from 3 determinations

Table 2: Albumin and Globulin Content of Seed Cake Residue Obtained from NaOH Hydrolysis

NaOH – Hydrolyzed extracts	Concentration (Mg/ml)		Percentage Composition (%)	
	Albumin	Globulin	Albumin	Globulin
0.1M NaOH hydrolyzed extract	0.20±0.007	0.10±0.002	0.12	0.05
0.2M NaOH hydrolyzed extract	0.53±0.007	0.630±0.002	0.26	0.21
0.3M NaCl hydrolyzed extract	0.79±0.02	0.61±0.001	0.39	0.31
0.4M NaCl hydrolyzed extract	0.10±0.002	0.90±0.02	0.55	0.45
0.5M NaCl hydrolyzed extract	1.95±0.002	1.53±0.01	0.98	0.76

The globulin content of untreated seed cake = 0.11. The albumin content of untreated seed cake = 0.29. Each value is a mean of data from 3 determinations.

Table 3: Albumin and Globulin Content of Seed Cake Residue Obtained from $Ca(OH)_2$ Hydrolysis

$Ca(OH)_2$ – Hydrolyzed extracts	Concentration (Mg/ml)		Percentage Composition (%)	
	Albumin	Globulin	Albumin	Globulin
0.1M $Ca(OH)_2$ Hydrolyzed extracts	4.15±0.02	4.73±0.10	2.08	2.36
0.2M $Ca(OH)_2$ Hydrolyzed extracts	4.54±0.07	4.89±0.06	2.27	2.41
0.3M $Ca(OH)_2$ Hydrolyzed extracts	4.94±0.03	5.03±0.04	2.47	2.51
0.4M $Ca(OH)_2$ Hydrolyzed extracts	5.13±0.01	5.13±0.04	2.56	2.56
0.5M $Ca(OH)_2$ Hydrolyzed extracts	5.25±0.01	5.23±0.10	2.63	2.61

The globulin content of untreated seed cake = 0.11. The albumin content of untreated seed cake = 0.29. Each value is a mean of data from 3 determinations.

the quantity of albumin and globulin in the seed cake.

Native polyacrylamide gel electrophoresis analysis of albumin oligomer by Marcone and co workers[9] showed that the fraction was susceptible to and did undergo alkaline pH – induced hydrolysis. The same type of hydrolysis was reported for Amaranth globulin by Marcone and Yada[12] and Konishi *et al*[13]. The hydrolysis is also characteristic of other storage proteins like soybean globulin as reported by Kitamura *et al*[14] and Marcone *et al*[15].

The results presented in Table 2 followed the same pattern as reported by the above named workers. There is a steady increase in the quantity of albumin and globulin, but the quantities are not as high as those of hydrochloric acid detoxification. This might be due to alkaline pH – induced hydrolysis of the fraction. Hydrolytic products are low molecular weight peptides and amino acids that are lost through washing of the residue with water and dialysis of the fraction[13]. Higher quantities of albumin and globulin in the residues obtained through 0.4M and 0.5M sodium hydroxide hydrolysis showed that the conjugated fractions undergo hydrolysis at those concentrations. The hydrolysis of the conjugated fractions produced albumin and globulin, which consequently compensated for the loss of free albumin and globulin through alkaline pH induced hydrolysis.

Alkaline pH induced hydrolysis is not shown in the results presented in Table 3. The quantities of albumin and globulin in the residues are higher than those of the other residues. From the quantity of albumin and globulin recorded in the samples treated with $Ca(OH)_2$ solutions, there is an indication that there is acidic pH – induced hydrolysis of albumin and globulin in the residue treated with hydrochloric acid solutions. High values of albumin and globulin recorded in the samples treated with calcium hydroxide solutions justified their mild hydrolytic effect on the protein fraction (albumin and globulin).

Mean values of various quantities of albumin and globulin isolates were analyzed by Ducan multiple range test. The results revealed that, the mean values of albumin and globulin from the residue got through hydrochloric acid and sodium hydroxide hydrolysis are not significantly different. It can therefore be established that both reagents effect hydrolysis of the conjugated fraction, which compensated for the loss of free albumin and globulin through acid and alkaline hydrolysis. The results also showed that, the mean values of the quantities of albumin and globulin isolated from the residue obtained through calcium hydroxide hydrolysis is significantly different from the mean values of the fraction got through hydrochloric acid and sodium hydroxide hydrolysis. This signifies the mild hydrolytic effect of calcium hydroxide solution on the peptide bonds of both fractions, when compared with hydrochloric acid and sodium hydroxide solutions.

Various concentrations of sodium hydroxide and hydrochloric acid solutions effect detoxification, but 0.1M, 0.2M and 0.3M of calcium hydroxide solution do not effect detoxifications. Only 0.4M and 0.5M of the solution detoxified the *Thevetia* seed. Various concentrations of detoxicants increased the quantities of albumin and globulin in the seed cake. Higher quantities of albumin and globulin in the cake hydrolyzed with calcium hydroxide solution showed its mild hydrolytic effect in the fraction. Judging from the quantities of albumin and globulin in the sample treated with calcium hydroxide solution, the solution, at higher concentration, is adjudged to be the best detoxicant.

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