African Journal of Biotechnology Vol. 8 (22), pp. 6374-6378, 16 November, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Effect of alkaline hydrolysis on the quantity of extractable protein fractions (prolamin, albumin, globulin and glutelin) in *Jatropha curcas* seed cake

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Accepted 18 June, 2008

Jatropha curcas **seeds were processed and defatted to obtain the seed cake. 60 g of them were separately moistened in 0.1 – 0.5 M NaOH and $Ca(OH)_2$ solutions and autoclaved. Each of the autoclaved samples was separately divided into three portions. A portion was washed with ethanol, another with water and the third was left unwashed. Prolamin, albumin, globulin and glutelin fractions were extracted from the treated and untreated seed cakes and subsequently quantified. Untreated seed cake sample express 0.11 g/Kg prolamin, 4.06 g/Kg albumin, 4.23 g/Kg globulin and 10.91 g/Kg glutelin of seed proteins. In $Ca(OH)_2$ treated cake, albumin and globulin had their maximum yield in ethanol washed cake samples. The quantities obtained were 3.95 and 5.11 g/Kg of the seed protein, respectively. Prolamin and glutelin had their maximum yield in unwashed and water washed cake samples. The yields were 4.84 and 9.27 g/Kg of the seed protein, respectively. In NaOH treated seed cake, the four fractions had their maximum yield in water washed cake. The quantities were 3.37, and 3.64 g/Kg of seed protein for prolamin and albumin. The quantities of globulin and glutelin were 4.90 and 9.26 g/Kg of seed proteins. Quantity of prolamin increased in all the treated cake while the quantities of albumin, globulin and glutelin were reduced by the treatments.**

Key words: Prolamin, albumin, globulin, glutelin, alkaline hydrolysis.

INTRODUCTION

Oil seeds are major source of vegetable proteins. The presence of toxins and antinutrients in some of them has limited their use as food and animal feed source. Such antinutrients include glucosinolate in rape seed, thevetin in *Thevetia peruviana* seed, gossypol in cotton seed, and nimbin, nimbidin and nimbosterol in neem seed (Timothy and Michael, 1988; Yignlang and Chunsu, 1965; Canella and Sodini, 1977; Karkar, 1976).

Several efforts have been made by nutritionists and food chemist to convert the seed to consumables. The effort was either to remove the toxins from the seeds or extract protein from them. The adopted detoxification techniques were guided by the nature of the toxins, for instance susceptibility of cardiac glycosides (thevetin, thevebioside, perusitin, nerifolin) in *T. peruviana* seed to hydrolysis and dissolution of their hydrolytic products in ethanol had enhance their removal from the seed by hydrolysis and solvent extraction (Usman et al., 2003; Oluwaniyi et al., 2007). Bitter principles (nimbin, nimbidin, nimbosterol) in neem seed have been extracted with n-hexane by Udayashekara (1987). Glucosinolate in rape seed was extracted with ethanol by Timothy and Michael (1988).

It has been established that *Jatropha curcas* seed is oily and proteinous, (Aderibigbe et al., 1997). Both attribute qualified the seed as oil source and meal provider that could serve as highly nutritious and economic protein supplement in animal feed, if the seed is detoxified (Becker and Makkar, 1988). Initially lectin was thought to be responsible for the toxicity of the seed (Cano-Asseleih, 1986; Cano-Asseleih et al., 1989); subsequent findings reveal that lectin was not the major toxic principle of the seed (Aderibigbe et al., 1997; Aregheore et al., 1998) but phorbolester (Markkar and Becker, 1997). However, lectin is susceptible to heat and can be removed by heat treatment. The major toxin phorbolester is not vulnerable to heat, but can be hydrolyzed to less toxic substances extractable by either water or ethanol.

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It is on the basis of the above attribute of lectin and phorbolester that Aregheore et al. (2003) employed heat treatment, alkaline hydrolysis and solvent extraction for the detoxification of the *Jatropha curcas* seed cake.

Meanwhile some valuable protein fractions (prolamin, albumin, globulin and glutelin) are also prone to hydrolysis. It is for this reason that we set out to investigate the effect of alkaline hydrolysis by 0.1 – 0.5 M concentrations of NaOH and $Ca(OH)_2$ solutions on the quantity of extractable prolamin, albumin, globulin and glutelin in *J. curcas* seed cake.

MATERIALS AND METHODS

Preparation of seed cake

The seeds were obtained from ripe fruits harvested from different locations in Ilorin, Nigeria. The seeds were dehulled and milled with magnetic blender (SHB – 515 model, made by Sorex Company Limited, Seoul, Japan). Standard Official and Tentative Method of Oil Chemists Society procedure was used to defat the seed cake (AOCS, 1979). The defatted seed cake was dried and kept for analysis.

Alkaline hydrolysis of the seed cake

Method similar to that of Aregheore et al. (2003) was adopted for the hydrolysis of the seed cake. In the method, 60 g of the seed cake were separately moistened with 0.1 – 0.5 M NaOH and $Ca(OH)_2$ solutions in separate beaker. Each mixture was milled into a paste using glass rod, and was subsequently covered with aluminum foil, placed in an autoclave at 121°C for 30 min. The autoclaved samples were removed and allowed to cool to room temperature. Each of the samples was then divided into three portions; a set of portions were left unwashed, another set were washed with water and the other set were washed with ethanol. The samples were subsequently dried at room temperature.

Extraction of protein fractions from the seed cake

Seed protein fractions were extracted from the treated and untreated seed cake. Albumin and globulin were extracted from the cake using the method of Murray (1979). The extraction technique used for prolamin and glutelin were similar to those used by Vijayakumari et al. (1994). In the method the residual pellets obtained after the extraction of albumin and globulin were separately extracted with 80% (v/v) ethanol 1:10 (w/v) overnight and centrifuged at 20, 000 X g for 20 min. The supernantants thus obtained were designated as prolamin. From the above residual pellets, the glutellin fraction was extracted from each sample with 0.2 M NaOH 1:15 (w/v) overnight and centrifuged at 20, 000 X g for 20 min. The supernantants thus obtained were designated as glutellin. The four fractions obtained were kept for analysis.

Analysis

The samples were analyzed for crude protein using the method of AOAC (1990). The protein fractions were estimated by biuret of Gornall et al. (1949). All data collected were subjected to analysis of variance (ANOVA), means were compared using Duncan multiple range test.

RESULTS AND DISCUSSION

The untreated cake sample afforded 0.11 g/Kg prolamin, 4.06 g/Kg albumin, 4.23 g/Kg globulin and 10.91 g/Kg glutellin of the seed proteins. Native polyacylamide gel electrophoresis analysis of albumin oligomer by Macrone et al. (1994) 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 (1992) and Konishi et al. (1985). The hydrolysis is also characteristics of other storage proteins like soybean globulin as reported by Kitamura et al. (1975) and Marcone et al. (1994). This same type of hydrolysis was also reported by Usman et al. (2003) for *Thevetia* albumin and globulin.

The results presented in Tables 1 – 6 followed the same pattern by the aforementioned workers for albumin, globulin and glutelin in all the treated cake. For prolamin, the treatments increased the quantity of the fraction which implied that prolamin is either not prone to alkaline pH-induced hydrolysis or prone to hydrolysis but more of the fraction must have been liberated from their conjugated form which subsequently compensated for the quantity loss through hydrolysis.

Tables 1 – 3 showed the effect of $Ca(OH)_2$ treatments on the quantity of the four fractions. For the unwashed sample in Table 1, the quantity of albumin and globulin increase steadily as the concentration of $Ca(OH)_2$ solution increases. For prolamin and glutelin, their quantities increased steadily from the cake treated with 0.1 M $Ca(OH)_2$ solution to the cake treated with 0.4 M $Ca(OH)_2$ solution. However their quantities reduced in the cake treated with 0.5 M $Ca(OH)_2$ solution. The reduction is attributable to strong hydrolytic effect of $Ca(OH)_2$ solution on the two fractions at 0.5 M concentration. Hydrolytic products are peptides and amino acids. The results in Table 2 showed the effects of $Ca(OH)_2$ solutions in water washed samples. The results followed the same pattern as presented in Table 1 for albumin and globulin. The quantities of prolamin and glutelin were not reduced by the treatment. Non-reduction in the quantities of these fractions by the treatments could be attributed to the removal of non-proteinous water soluble constituents of the cake which subsequently increased the quantity of both fractions at 0.5 M concentrations. The results in Table 3 showed the effect of $Ca(OH)_2$ treatments on the fraction in ethanol washed cake samples. The result followed the same pattern as the result presented in Table 2 for prolamin, albumin and globulin. For glutelin, its quantity reduced in the cake treated with 0.5 M $Ca(OH)_2$ solution which indicate strong hydrolytic effect of $Ca(OH)_2$ at 0.5 M concentration.

Tables 4 – 6 showed the effect of NaOH treatments on the quantity of the four fractions. In Table 4, the quantities of the four fraction increase with increase in the concentration of NaOH solution in unwashed cake samples. The results presented in Tables 5 and 6 for the water washed

Table 1. Seed protein fractions in $Ca(OH)_2$ treated *Jatropha curcas* seed cake (unwashed).

Conc. (mol/dm^3)	Quantity of extractable protein fractions in (g/Kg) of the seed protein			
	Prolamin	Albumin	Globulin	Glutelin
0.1	3.04 ± 0.01	1.13 ± 0.01	1.36 ± 0.03	3.83 ± 0.02
0.2	4.42 ± 0.03	1.84 ± 0.01	2.39 ± 0.02	3.89 ± 0.01
0.3	4.79 ± 0.02	2.67± 0.03	2.46± 0.01	3.90± 0.04
0.4	4.84 ± 0.01	3.96± 0.02	3.79 ± 0.01	4.09 ± 0.02
0.5	4.08 ± 0.01	3.81± 0.01	3.87 ± 0.01	3.87 ± 0.01

Values are means of 3 determinations ± SD

Table 2. Seed protein fractions in $Ca(OH)_2$ treated *Jatropha curcas* seed cake (water washed).

Conc. (mol/dm^3)	Quantity of extractable protein fractions in (g/Kg) of the seed protein			
	Prolamin	Albumin	Globulin	Glutelin
0.1	1.23 ± 0.01	0.49 ± 0.01	0.82 ± 0.01	2.36 ± 0.01
0.2	1.41 ± 0.02	0.91 ± 0.01	0.91 ± 0.03	2.96 ± 0.02
0.3	1.52 ± 0.16	1.05 ± 0.02	1.25 ± 0.02	3.72 ± 0.01
0.4	1.72 ± 0.01	1.07 ± 0.01	1.58 ± 0.04	3.75 ± 0.03
0.5	2.50 ± 0.01	1.67 ± 0.01	2.17 ± 0.01	5.14 ± 0.01

Values are means of 3 determinations ± SD

Table 3. Seed protein fractions in $Ca(OH)_2$ treated *Jatropha curcas* seed cake (Ethanol washed).

Conc. (mol/dm^3)	Quantity of extractable protein fractions in (g/Kg) of the seed protein			
	Prolamin	Albumin	Globulin	Glutelin
0.1	0.36 ± 0.01	2.21 ± 0.01	1.12 ± 0.01	4.70 ± 0.02
0.2	0.44 ± 0.02	2.23 ± 0.01	2.24 ± 0.01	5.28 ± 0.01
0.3	0.69 ± 0.01	2.21± 0.02	2.87 ± 0.02	6.17 ± 0.03
0.4	0.79 ± 0.03	3.77 ± 0.02	3.79 ± 0.03	8.13 ± 0.01
0.5	1.37 ± 0.03	4.95 ± 0.01	5.11 ± 0.01	6.73 ± 0.01

Values are means of 3 determinations ± SD

Table 4. Seed protein fractions in NaOH treated *Jatropha curcas* seed cake (unwashed).

Conc. (mol/dm^3)	Quantity of extractable protein fractions in (g/Kg) of the seed protein			
	Prolamin	Albumin	Globulin	Glutelin
0.1	0.17 ± 0.03	0.51 ± 0.04	0.95 ± 0.01	3.22 ± 0.01
0.2	1.56 ± 0.01	1.71 ± 0.02	1.71 ± 0.01	3.48 ± 0.01
0.3	1.60 ± 0.02	2.29 ± 0.01	2.29 ± 0.02	4.60 ± 0.01
0.4	1.63 ± 0.01	2.70 ± 0.01	2.70 ± 0.03	5.91 ± 0.03
0.5	2.95 ± 0.02	3.13 ± 0.01	3.13 ± 0.01	7.62 ± 0.01

Values are means of 3 determinations ± SD.

and ethanol washed cake samples followed the same pattern as Table 4. The pattern is attributable to strong hydrolytic effect of NaOH which readily liberates the fraction from their conjugated form, thus compensated for the loss of the free fractions through hydrolysis. In the NaOH treated cake, prolamin afforded 3.37 g/Kg of seed protein as its maximum yield in water washed sample; this may be because it is sparingly soluble in water. Despite the fact that albumin and globulin are readily soluble in water they had their maximum yield of 3.64 and

Table 5. Seed protein fractions in NaOH treated *Jatropha curcas* seed cake (Water washed).

Conc. (mol/dm^3)	Quantity of extractable protein fractions in (g/Kg) of the seed protein			
	Prolamin	Albumin	Globulin	Glutelin
0.1	0.20 ± 0.01	0.24 ± 0.03	1.42 ± 0.01	4.41 ± 0.02
0.2	1.64 ± 0.02	1.67 ± 0.01	1.83 ± 0.01	5.47 ± 0.01
0.3	3.14 ± 0.01	3.23 ± 0.02	3.66 ± 0.01	6.28 ± 0.01
0.4	3.21 ± 0.01	3.63 ± 0.01	3.95 ± 0.03	8.99 ± 0.04
0.5	3.37 ± 0.03	3.64 ± 0.01	4.90 ± 0.01	9.27 ± 0.01

Values are means of 3 determinations ± SD.

Table 6. Seed protein fractions in NaOH treated *Jatropha curcas* seed cake (Ethanol washed).

Conc. (mol/dm^3)	Quantity of extractable protein fractions in (g/Kg) of the seed protein			
	Prolamin	Albumin	Globulin	Glutelin
0.1	0.43 ± 0.01	0.53 ± 0.01	1.01 ± 0.01	3.59 ± 0.01
0.2	0.96 ± 0.01	1.90 ± 0.02	2.02 ± 0.01	6.20 ± 0.03
0.3	1.76 ± 0.03	2.16 ± 0.01	2.36 ± 0.02	7.48 ± 0.02
0.4	1.88 ± 0.01	2.34 ± 0.01	2.39 ± 0.01	7.53 ± 0.01
0.5	2.75 ± 0.01	3.36 ± 0.02	3.64 ± 0.03	8.22 ± 0.01

Values are means of 3 determinations ± SD.

4.90 g/Kg of seed proteins, respectively, in water washed cake samples after treatment with NaOH solutions. This could be attributed to the abundance of the conjugated fractions which are susceptible to strong hydrolytic effect of NaOH solutions. Hence increase their quantities as the concentration of NaOH solution increases. Glutelin also has its maximum yield of 9.27 g/Kg in water washed cake after treatment with NaOH solution.

Mean values of various quantities of the four fractions obtained from $Ca(OH)_2$ and NaOH treated cake samples were compared by the Duncan Multiple Range test. The result revealed that, the mean value of the four fractions obtained from unwashed, water washed and ethanol washed $Ca(OH)_2$ and NaOH treated cake are significantly different ($P<0.05$). The difference may be due to variation in the hydrolytic strength of the two solutions. NaOH solution is a stronger hydrolyzing agent than $Ca(OH)_2$ solutions. From these results we conclude that the quantity of prolamin increase in all the treated cake, while the treatments reduced the quantity of globulin, albumin and glutelin. Effects of the two hydrolyzing agents on lectin and phorbolester are currently being investigated.

REFERENCES

Aderibigbe AO Johnson COLE, Makkar HPS, Becker K, Foidl N (1997). Chemical composition and effect of heat on organic matter and nitrogen degradability and some anti – nutritional components of *Jatropha* meal. Anim. Feed Sci. Technol., 67: 223-243.

AOAC, (1984). Official methods of analysis (14th edn.) Arlington, VA: Association of Official Analytical Chemists.

Aregheore EM, Makkar HPS, Becker K (1998). Assessment of lectinactivity in a toxic and a non – toxic variety of of *Jatropha curcas* using latex agglutination and haemagglutination methods and inactivation of lectin by heat treatments. J. Sci. Food Agric. 77: 349-352.

Aregheore EM, Becker K, Makkar HPS (2003). Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats. S. Pac. J. Nat. Sci., 21: 50-56.

Becker K, Makkar HPS (1988). Toxic effects of phorbolester in carp (*Cyprinus carpio* L.), Vet. Hum Toxicol. 40: 82-86.

Canella M, Sodini G (1977). Extraction of gossypol and oligosaccharide from oil seed meals, J. Food Sci., 42: 1219-1219.

Cano–Asseleih LM (1986). Chemical investigation of *Jatropha curcass* L. seeds. Ph. D. Thesis, University of London, U. K., p. 290.

Cano–Asseleih LM, Plumbly RA, Hylands PJ (1989). Purification and partial characterization of the hemagglutination from seeds of *Jatropha carcass*. J. Food Biochem. 13: 1-20.

Gornall AG, Bardwill CJ, David MM (1949). Determination of serum protein by means of the biuret reaction. J. Biol. Chem. 177: 751-756.

Karkar CN (1976). Utilization of neem (*A. indica*) and it's by – products: Report of the modified neem cake manorial project 1969 – 76. Directorate of no – edible oils soap industry Khadi and village industries commission, 3, Iria Road, Vile Park (West), Bombay, India.

Kitamura K, Okudo K, Shibasaki O (1975). The purification of soybean vs globulin with ConA – sepharose 4B and sepharose 6B. Agric. Biol. Chem. 38: 1083–1085.

Konishi Y, Fumita Y, Ikeda K, Okuno K, Fuwa H (1985). Isolation and characterization of globulin from seeds of *Amaranthus hypochondriacus* L. Agric. Biol. Chem. 49: 1453-1459.

Marcone MF, Francis KN, Marc LM, Rickey VY (1994). Purification and characterization of the physicochemical properties of albumin fraction from the seeds of *Amaranthus hypochondriacus*. Food Chem. 51: 287-294.

Marcone MF, Yada RY (1992). Study of the change profile and covalent subunit association of oligomeric seed globulin from *Amaranthus hypochondriacus* J. Agric. Food Chem. 40: 385-389.

Markkar HPS, Becker K (1997). *Jatropha curcas* toxicity: identification of toxic principle(s). in: 5th International symposium on poisonous plants. May 19 – 23, 1997, San Angelo, Texas, USA.

Murray DR (1979). The seed proteinsof Kowhai, *Sophora microphylla*.

AIT. *Z.* Pflanzeuphysiogyl. 93: 423-428.
Oluwaniyi OO, Ibiyemi AS, Usman LA (2007). Effect of detoxification on the nutrient content of *Thevetia peruviana* seed cake, Res. J. Appl. Sci. 2(2): 188-191.
Official and Tentative methods of the American Oil Chemicals Society (AOCS) (1979) vol. 1, AOCS, Champaign, IL.
Timothy JA, Michael JL (1988). Ethanolic extraction of commercial rape seed meal in the production of protein concentrate of reduced glucosinate content, J. Sci. Food Agric, 45(2): 155-163.
Udayashekara RP (1987). Chemical composition and biological evaluation of debetterised and defatted neem (*Azadrachta indica*) seed kernel cake. J. AOCS, 64: 1348-1352.
Usman LA, Ibiyemi SA, Oluwaniyi OO, Ameen OM (2003). Effect of acid and alkaline hydrolysis on the concentrations of albumin and globulin in *Thevetia peruviana* seed cake protein extract, BIOKEMISTRI, 15(1): 16-21.
Vijayakumari K, Siddhurju P, Janardhanam K (1994). Nutritional assessment and chemical composition of the lesser known tree legume *Aeoeia leucophloea*. (Roxb), Food Chem. 50: 285-288.
Yignlang H, Chunsu N (1965). The cardiac glycosides of *Thevetia peruviana* seeds: isolation and identification of ceberin, ruvoside and a new glycoside perusitin, Chemical Abstr. 62: 946-959.