

## Effect of Alkaline Hydrolysis on the Quantity of Extractable Protein Fractions (Prolamin, Albumin, Globulin and Glutelin) in *Jatropha curcas* Seed Cake

L.A. Usman, O.M. Ameen, A. Lawal and G.V. Awolola

Department of Chemistry, University of Ilorin, Ilorin, Kwara State, Nigeria

**Abstract:** *Jatropha curcas* seeds were processed and defatted to obtain the seed cake. About 60 g of the were separately moistened in 0.1-0.5 M NaOH and Ca (OH)<sub>2</sub> solutions and were autoclaved. Each of the autoclaved samples was separately divided into 3 portions. A portion was washed with ethanol, another with water and the 3rd 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 afforded; 0.11 g kg<sup>-1</sup> prolamin, 4.06 g kg<sup>-1</sup> albumin, 4.23 g kg<sup>-1</sup> globulin and 10.91 g kg<sup>-1</sup> glutelin of seed proteins. In Ca (OH)<sub>2</sub> 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<sup>-1</sup> 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<sup>-1</sup> of the seed protein, respectively. In NaOH treated seed cake, the 4 fractions had their maximum yield in water washed cake. The quantities were 3.37 and 3.64 g kg<sup>-1</sup> of seed protein for prolamin and albumin. The quantities of globulin and glutelin were 4.90 and 9.26 g kg<sup>-1</sup> 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 presences of toxins/antinutrients in some of them have 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, nimbin, nimbidin and nimboesterol 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 *Thevetia 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, nimboesterol) 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 phorbolster (Markkar and Becker, 1997). However, lectin is susceptible to heat hence, can be removed by heat treatment. The major toxin phorbolster is not vulnerable to heat, but can be hydrolyzed to less toxic substances extractable by either water or ethanol.

It is on the basis of the above attribute of lectin and phorbolster that Aregheore *et al.* (2003) employed heat treatment, alkaline hydrolysis and solvent extraction for the detoxification of the *Jatropha curcas* seed cake. Mean while, 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)<sub>2</sub> solutions on the quantity of extractable prolamin, albumin, globulin and glutelin in *Jatropha 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)<sub>2</sub> solutions in separate beaker. Each mixture was milled in to 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 3 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<sup>-1</sup>) ethanol 1:10 (w v<sup>-1</sup>) overnight and centrifuged at 20,000×g for 20 min. The supernatants thus obtained were designated as prolamin. From the above residual pellets, the glutelin fraction was extracted from each sample with 0.2 M NaOH 1:15 (w v<sup>-1</sup>) overnight and centrifuged at 20,000×g for 20 min. The supernatants thus obtained were designated as glutelin. The four fractions obtained were kept for analysis.

**Analysis:** The samples were analyzed for crude protein using the method of AOAC (1984). The protein fractions were estimated in other study 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<sup>-1</sup> prolamin, 4.06 g kg<sup>-1</sup> albumin, 4.23 g kg<sup>-1</sup> globulin and 10.91 g kg<sup>-1</sup> glutelin of the seed proteins.

Native polyacrylamide 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 Table 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.

Table 1-3 showed the effect of Ca (OH)<sub>2</sub> treatments on the quantity of the 4 fractions. For the unwashed sample in Table 1, the quantity of albumin and globulin increase steadily as the concentration of Ca (OH)<sub>2</sub> solution increases. For prolamin and glutelin, their quantities increased steadily from the cake treated with 0.1 M Ca (OH)<sub>2</sub> solution to the cake treated with 0.4 M Ca (OH)<sub>2</sub> solution. However, their quantities reduced in the cake treated with 0.5 M Ca (OH)<sub>2</sub> solution. The reduction is attributable to strong hydrolytic effect of Ca (OH)<sub>2</sub> solution on the 4 fractions at 0.5 M concentration. Hydrolytic products are peptides and amino acids.

The study in Table 2 showed the effects of Ca (OH)<sub>2</sub> solutions in water washed samples. The study 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 study in Table 3, showed the effect of Ca (OH)<sub>2</sub> treatments on the fraction in ethanol washed cake samples. The study 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)<sub>2</sub> solution which indicate strong hydrolytic effect of Ca (OH)<sub>2</sub> at 0.5 M concentration.

Table 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

Table 1: Seed protein fractions in Ca (OH)<sub>2</sub> treated *Jatropha curcas* seed cake (unwashed)

Conc. (mol dm <sup>-3</sup> )	Quantity of extractable protein fractions in (g kg <sup>-1</sup> ) 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)<sub>2</sub> treated *Jatropha curcas* seed cake (water washed)

Conc. (mol dm <sup>-3</sup> )	Quantity of extractable protein fractions in (g kg <sup>-1</sup> ) 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)<sub>2</sub> treated *Jatropha curcas* seed cake (Ethanol washed)

Conc. (mol dm <sup>-3</sup> )	Quantity of extractable protein fractions in (g kg <sup>-1</sup> ) 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

concentration of NaOH solution in unwashed cake samples. The results presented in Table 5 and 6 for the water washed and ethanol washed cake samples followed the same pattern as Table 4. The pattern is attributable to strong hydrolytic effect of NaOH which readily liberate 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<sup>-1</sup> 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 4.90 g kg<sup>-1</sup> of seed proteins 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<sup>-1</sup> in water washed cake after treatment with NaOH solution.

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

Conc. (mol dm <sup>-3</sup> )	Quantity of extractable protein fractions in (g kg <sup>-1</sup> ) 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

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

Conc. (mol dm <sup>-3</sup> )	Quantity of extractable protein fractions in (g kg <sup>-1</sup> ) 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 <sup>-3</sup> )	Quantity of extractable protein fractions in (g kg <sup>-1</sup> ) 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

Mean values of various quantities of the 4 fractions obtained from Ca (OH)<sub>2</sub> 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)<sub>2</sub> and NaOH treated cake are significantly different (p<0.05). The difference may be due to variation in the hydrolytic strength of the 2 solutions. NaOH solution is a stronger hydrolyzing agent than Ca (OH)<sub>2</sub> solutions.

## CONCLUSION

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 phorbol ester are currently being investigated.

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