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Short Communication

EFFECT OF HEAT AND ALKALINE HYDROLYSIS ON THE AMINO ACID PROFILE OF *JATROPHA CURCAS* SEED CAKE

Ameen OM*[1]; Usman LA[2]; Muhammed NO[1]; Okeola OF[1]; Boluwarin EO[1] and OO Fadeyi[1]

Mubarak Ameen

*Corresponding author's email: aommohd@yahoo.com; moameen@unilorin.edu.ng

[1]Chemistry Department, University of Ilorin, Ilorin, Nigeria

[2]Biochemistry Department, University of Ilorin, Ilorin, Nigeria

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ABSTRACT

In recent times, *Jatropha curcas* has attracted attention of various research organizations, governments, public and international developmental agencies and industries in the tropics and subtropics due to its adaptability to semi-arid marginal sites, the possibility of using its oil as a diesel fuel substitute and its role in erosion control. In tropical countries it is well known for its medicinal properties and as an oilseed. The seeds of *J. curcas* are a good source of oil, yielding between 40 – 80 % oil. Although the seed cake meal is rich in protein, it is toxic to rats, mice, ruminants and humans due to the presence of antinutritional factors; thus, its use as food or feed source has not been encouraging. However, recent findings indicate that after a proper detoxification process the seed meal can serve as a protein substitute in feed meals of animal feeds. The seeds of *J. curcas* were collected, dehulled, grounded and defatted to obtain the seed cake. The seed cake was divided into five portions, 60 g of each of the cake portion was separately moistened with 1 M, 2 M, 3 M, 4 M and 5 M NaOH solutions, respectively and autoclaved. Each of the autoclaved samples was washed with water and later with ethanol. A 60 g of the seed cake which was not treated with NaOH, water and ethanol was labeled untreated. The treated and untreated seed cakes were thus analyzed for their crude protein contents as well as amino acid profile. The untreated seed cake afforded 63.02 % yield of crude protein, while the crude protein content of the treated seed cake was 70.53, 71.46, 67.76, 60.82 and 56.19 % for the 1 – 5 M NaOH treated seed cake, respectively with the 2 M NaOH treated seed cake having the highest yield. The amino acid profile of the treated seed cake was similar and comparable to the values of WHO/FAO standard and those of soybeans. The seed can thus be used as an alternative protein source in animal feed formulation after it has been properly detoxified. If well processed, it would reduce competition between man and livestock for the conventional sources of proteins.

Key words: Amino acid, *Jatropha curcas*, protein detoxification

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INTRODUCTION

One of the major challenges to increased and sustainable livestock production in Africa and Nigeria in particular is lack of affordable protein in the animal feeds. The protein sources such as soybean, cotton, rape, sunflower and peanut seed cakes are either not readily available or costly for subsistence farmers. Fortunately *Jatropha curcas,* which is a drought-resistant shrub belonging to the Family Euphorbiaceae, is a good substitute as a protein source for livestock feeds. This is because besides being easy to grow, the crop seeds are oily and highly proteineous thereby making it a cheap protein source. Nevertheless, *J. curcas* seeds are toxic, and thus have to be detoxified prior to supplementing as a protein source in animal feeds [1 - 3]. Recently, *J. curcas* has attracted attention of various research organizations, governments, public and international developmental agencies and industries in the tropics and subtropics due to its adaptability to semi-arid marginal sites, the possibility of using its oil as a diesel fuel substitute and its role in erosion control [4]. The seeds of *J. curcas* are a good source of oil, yielding between 40 – 80 % oil [5 – 9]. Although the seed cake meal is rich in protein, it is toxic to rats, mice, ruminants and humans due to the presence of antinutritional factors such as phorbol esters, curcin, trypsin inhibitors, lectin, [5, 6, 8, 10 – 12], .thus its use as food or feed source has not been encouraging. Recent findings indicate that after proper detoxification process the seed meal can serve as a protein substitute in feed meals of animal feeds [5, 13].

Consequently, several works has been carried out on the *J. curcas* seed so that it can be used as a source of protein in animal feed. For example Oladele and Oshodi [8] attempted the detoxification of the seeds using local fermentation process while Martı´nez – Herrera *et al*. [13] also used chemical such $NaHCO_3$, ethanol as well as irradiation as a method of detoxification. However, Aregheore *et al.* [10] reported that heat and chemical (ethanol) treatments were able to reduce the antinutrient factors in *J. curcas* seed to a tolerable minimum while solid state fermentation employed by Belewu and Sam [14] was able to detoxify and inactivate almost 100 % of the antinutrient contents of *Aspergillus niger* treated sample of *Jatropha* kernel cake to a tolerable level [10, 14]. Similarly Usman *et al.* reported the effect of alkaline hydrolysis on the quantity of extractable protein fractions in *Jatropha* treated seed cakes [15]. In the work reported by Usman *et al.* [15], it was concluded that the treatment increase the quantity of prolamin in all the treated cake, while the quantity of globulin, albumin and glutelin were reduced.

Since the quality of any protein source relates to its amino acid composition, digestibility, bioavailability and ability to supply the essential amino acid in the amount required by the organism consuming it. Based on these facts there are two methods of assessing the quality of proteinous species, amino acid analysis and feed test. In view of the foregoing, the amino acid profile of heat and chemically treated *Jatropha cucas* seed cake in comparison to the untreated cake was investigated.

MATERIALS AND METHODS

Preparation of seed cake

Jatropha curcas seeds were obtained from ripe fruits harvested from different locations in Ilorin, North – Central, Nigeria. The seeds were dehulled and milled with magnetic blender (SHB – 515 Model, Sorex Company Limited, Seoul, Japan). Standard Official and Tentative Method of oil Chemists Society procedure was used to defat the seed cake [16]. The defatted seed cake was dried and kept for analysis.

Alkaline hydrolysis and chemical treatment of the seed cake

A method similar to that of Aregheore *et al*. [10] was adopted for the hydrolysis of the seed cake. In the method, five portions of 60 g of the seed cake were separately moistened with 10 ml 1 – 5 M NaOH solutions in separate beakers and left to stand for 24 hrs. Each mixture was milled into a paste using glass rod, and was subsequently covered with aluminum foil, placed in an autoclave at 121 ^{o}C for 30 min. The autoclaved samples were removed and allowed to cool to room temperature. Each of the samples was then put into different white clothes, immersed in a jar of distilled water and mixed thoroughly. This was followed by squeezing and subsequent immersing in another jar containing ethanol for 30 minutes, the sample was squeezed and spread to dry at room temperature. A 60 g of the seed cake which was not treated with NaOH, water and ethanol was also kept and labeled as untreated cake.

Determination of crude protein

The crude protein (CP) of the untreated, defatted and chemically treated *Jatropha* meals was determined in accordance to AOAC procedure [17].

Determination of Amino Acid Profile

The amino acid composition of the treated and untreated *J. curcas* seed was determined using Technicon Amino Acid Analyzer (TSM–1 Technicon Instrument, Basingstoke, Hampshire, UK) using Norleucine as internal standard. This procedure follows the method described by Bassler and Buchholz [18]. The contents of different amino acids recovered were presented in g/100 g of protein. The amino acid contents of the total seed proteins were compared with the FAO/WHO reference pattern, soya bean and reported literature values for *J. curcas* [4, 11, 19 – 21].

Statistical Analysis

All data collected for the concentrates were subjected to analysis of variance, ANOVA. Means were compared using Duncan's multiple range tests at $P<0.05$ confidence level.

RESULTS

The defatted seed cake of *J. curcas* has been subjected to chemical and heat treatment in order to improve its nutritional quality. The percentage crude protein in the treated and untreated seed cake is presented in Table 1. From Table 1, it is observed that the chemical and heat treatment has improved the quantity of crude protein in the seed

cake compared to the untreated sample. The crude protein content of the untreated sample was 63.02 % while seed cake treated with 1 M, 2 M and 3 M NaOH gave crude protein contents of 70.53, 71.46 and 67.76 %, respectively Table 1. The data presented in Table 1 also revealed that the percentage crude protein content for the seed cake treated with 4 M and 5 M NaOH were lower than that of the untreated seed cake, the reduction in the quantity of the crude protein content may be due to high concentration of NaOH leading to loss of some amino acids in the sample.

The amino acid composition of the treated and untreated seed cake is presented in Table 2. The table data presented in Table 2 revealed that the amino acid profiles of the treated seed cakes were similar and comparable to the values reported for different provenances of *J. curcas*, (Cape Verde and Nicaragua) [21]. The trend for the non – essential amino acids is similar to that recorded for the essential amino acids (Table 3). The values increase from 1 M to 2 M and decrease steadily from 3 M to 5 M NaOH treated seed cakes, the exception being the values for histidine and aspartic acid whose values do not follow a regular pattern. From the global perspective of the data presented, the values for the treated seed cakes were higher than those of the untreated cakes**.** Table 4 presents the leucine to lysine values for the treated and untreated *J. curcas* seed cakes, soybean and the WHO/FAO recommended standards. The leucine to lysine ratio of the untreated and treated *J. curcas* seed cakes are lower than the marginal value and compare favourably with the WHO/FAO standard and the soybean values.

DISCUSSION

The observation that the heat and chemical treatment does not adversely affect the crude protein contents of treated *J. curcas* indicates that this method may be successfully used to detoxify *J. curcas* seed cake. However, the highest crude protein value of 71.46 % was obtained with the cake treated with 2 M NaOH, thereby suggesting that this concentration might be a better method of treating *Jatropha* seed cake. This value is comparable to the one obtained by Martinez-Herrera ***et al*** [4] in which four different methods were employed for the detoxification of *Jatropha* seed cake from four different locations and found that the chemical and heat treatment method gave the best of 70.9 % crude protein content in the seed from Mexican city of Yautepec, Morelos state [4].

It has been reported that protein solubility in neutral salt solutions depends on ionic strength and pH of the medium used for the extraction [22]. Increase in ionic strength of any salts solution may reduce the solubility of protein fractions. The effect of pH on the solubility of protein depends on whether the desired protein is at its isoelectric pH. Some protein fractions are at their minimum solubility at isoelectric pH, while some are completely insoluble at this pH. This fact may explain the low crude protein content obtained at high concentration of NaOH.

Results present in Table 2 indicate that there was increase in the essential amino acids of the treated *J. curcas* seed cake with 2 M NaOH treated seed cake recording the

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highest values. The only exception being cystine whose value was the same for both the treated (at 1 M and 2M NaOH) and untreated seed cakes. In all, the value increases from 1 M to 2 M NaOH and then decreases steadily from 3 M to 5 M NaOH treated seed cakes. However, a steady decrease from 1 M to 5 M NaOH treated cake was recorded for cystine.

Generally, levels of essential amino acids for the *J. curcas* treated seed cakes were higher than the WHO/FAO recommended standards with the exception of cystine and methionine in which lower values were obtained (Table 2). In comparison with the amino acid profile of soybean, it is observed that the values obtained for the *J. curcas* treated seed cake were higher than those for soybeans. The exceptions being the values for isoleucine, tyrosine and cystine in which the data reported for soybeans were higher than those obtained for the *J. curcas* treated seed cake (Table 2). This observation indicates that the nutrition value of J. curcas treated cake compare favourably with that of soybeans. This observation has already been reported by Makkar and Becker [11].

It has been established that utilization of lysine and isoleucine in protein is affected by the amount of leucine present [20]. A leucine to lysine ratio greater than 4.6 will hinder the utilization of lysine. Results present in Figure 1 indicate that the value is not only lower than 4.6 but it is also lower than those of some Nigerian grown cereals [23]. Hence the lower ratio will no doubt make the treated *J. curcas* seed cake to be nutritionally superior to commonly grown Nigerian cereals.

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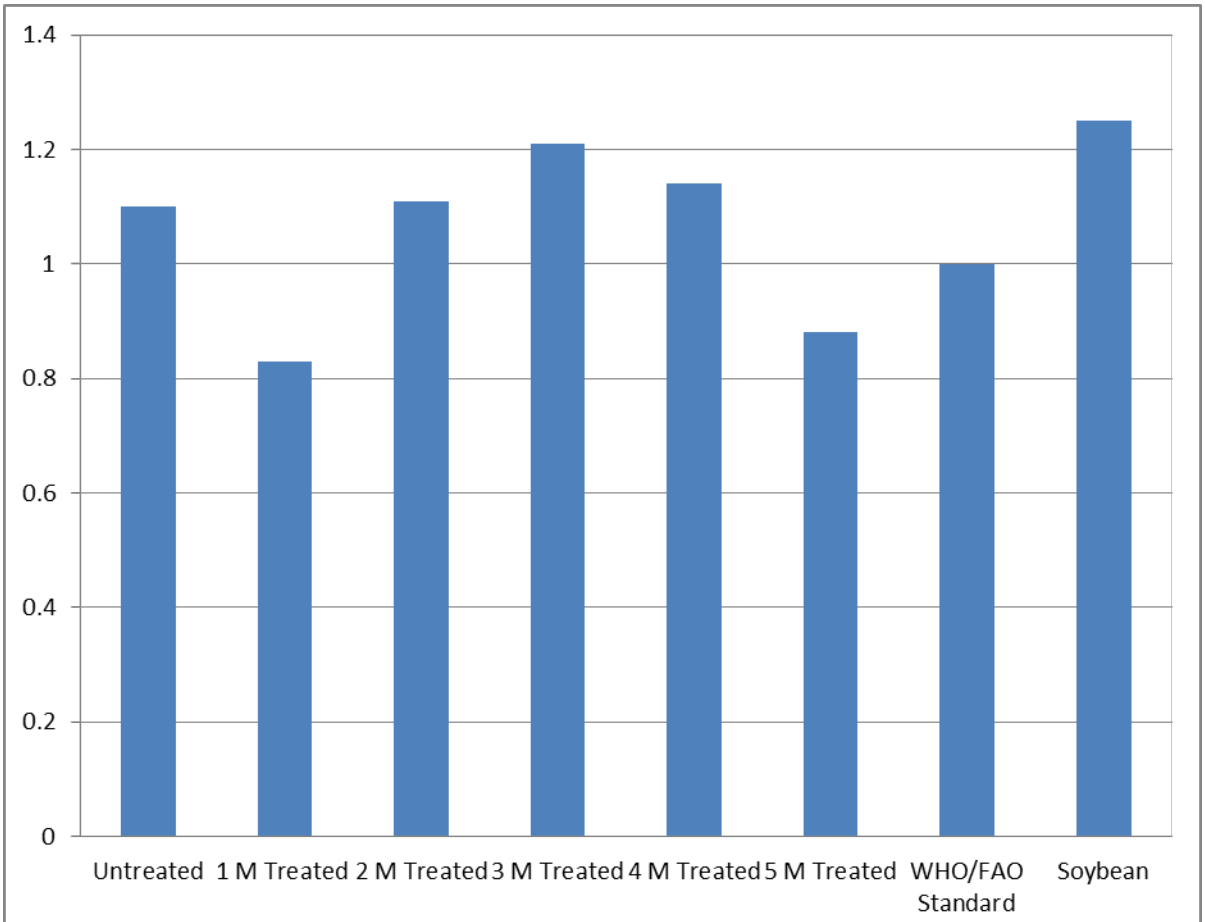

Figure 1: The leucine to lysine ratio for the treated and untreated *J. curcas* seed cakes, soybean and the WHO/FAO recommended standard.

CONCLUSION

The world has shifted sourcing protein for animal diets and oils for industrial raw material to oil seeds. Notable among the oil seeds is *Jatropha curcas*, from the researches carried out so far on *J. curcas*, the seed has nutritional potential that compares favorably to conventional oil seeds and protein source (soybean). The levels of essential amino acids except cystine and methionine were comparable with that for the FAO reference protein. The seed can thus be used as an alternative protein source in animal feed formulation after it has been properly detoxified. If well processed, it would reduce competition between man and livestock for the conventional sources of proteins. Thus, cultivation of this potentially rich plant is encouraged in order to reduce over-dependence on the currently limited sources of protein and oil.

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Table 1: Percentage crude protein in the treated and untreated *Jatropha curcas* seed cake

Sample		% Crude Protein
Untreated seed cake		63.02±0.07
Treated caketreated samples	1 M	70.53±0.06
	2 M	71.46±0.06
	3 M	67.76±0.08
	4 M	60.82±0.07
	5 M	56.19±0.05

Values are means of 3 determinations ± S. D

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Table 2: Essential amino acid compositions of treated and untreated of *J. curcas* seed cake, soybean and essential amino acid pattern suggested by FAO/WHO (g/100 g protein)

Amino acid	Samples						WHO/FAO standard	Soybean
	Untreated seed cake	Treated seed cake						
		1 M	2 M	3 M	4 M	5 M		
Isoleucine	3.74	4.12	4.69	3.30	3.23	3.04	4.2	4.8
Leucine	7.54	6.12	8.46	7.59	5.77	4.26	4.2	8.0
Lysine	6.86	7.35	7.62	6.27	5.08	4.86	4.2	6.4
Phenyalanine	5.14	5.66	5.66	4.71	4.62	3.08	2.8	4.8
Tyrosine	2.86	2.86	3.17	2.54	2.22	1.90	2.8	3.2
Cystine	0.66	0.66	0.66	0.53	0.40	0.26	2.0	0.8
Methionine	1.30	1.09	1.30	0.89	0.83	0.63	2.2	0.9
Threonine	4.06	4.58	4.36	4.02	3.86	3.37	2.8	4.0
Tryptophan	ND	ND	ND	ND	ND	ND	1.4	1.3
Valine	5.60	5.27	6.24	5.27	4.33	3.05	4.2	4.8

ND = Not Determined

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Table 3: Non – essential amino acid compositions of treated and untreated of *J. curcas* seed cake

Amino acid	**Samples**					
	Untreated seed cake	**Treated seed cake**				
		1 M	**2 M**	**3 M**	**4 M**	**5 M**
Histidine	2.14	2.27	1.89	2.25	1.64	1.20
Arginine	5.87	4.66	6.73	4.31	4.14	3.62
Aspartic acid	9.74	10.05	9.80	10.20	10.17	9.99
Serine	3.80	4.07	4.61	3.80	3.36	3.15
Glutamic acid	13.63	13.93	14.8	14.69	11.51	10.15
Proline	3.26	3.05	3.66	3.15	3.46	2.44
Glycine	3.65	3.56	3.70	3.70	3.07	1.68
Alanine	3.49	3.65	4.18	3.42	3.04	2.35

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