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CHANGES IN CHEMICAL COMPOSITION OF *JATROPHA CURCAS* KERNEL CAKE AFTER SOLID-STATE FERMENTATION USING SOME SELECTED FUNGI

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Abstract

This study was conducted to compare the proximate composition and some antinutritional factors of treated and untreated *Jatropha Curcas* Kernel Cake(JKC).Standard procedures were employed to analyse the samples of dried seeds of *Jatropha curcas* purchased from open market. Results showed that after solid state fermentation with *Absidia spinosa, the* treated JKC contained 52.50% crude protein, *Mucor rouxii* treated JKC contained 52.10% crude protein. Treatment of JKC with the mixed culture of *Absidia spinosa* and *Mucor rouxii* yielded 53.20% crude protein. Tannin content of the raw, toasted, *Absidia spinosa* treated, *Mucor rouxii* treated and those treated with the mixed culture of *Absidia spinosa* and *Mucor rouxii* were 4.80g, 3.81g, 3.53g, 3.40g and 3.34g respectively. Saponin and phytate contents also decreased when treated with various methods employed in this study. This preliminary study has shown that the methods of detoxification employed in this study have potentials to improve the nutritive value of JKC and also to reduce appreciably some of the Antinutritional factors present in the raw, unprocessed cake.

Key Words: *Jatropha, fermentation, proximate, anti-nutritional factors.*

1. Introduction

Jatropha curcas meal which typically contains 1-2% residual oil has a crude protein of 58-64%. It thus has a high potential to complement and/or substitute the expensive and scarce soybean meal and groundnut meal which constitute the major portion of conventional protein sources used in composite livestock feed.. However, Phorbolester, Tannin, Alkaloid, Saponin and Phytate are the major causes of toxicity in *Jatropha curcas* which has to be lowered to levels that do not elicit toxic response for *Jatropha curcas* meal to be used as an ingredient in livestock feed (Heller, 1996; Makkar and Becker, 1997). Phytate constitutes a major heat-resistant, antinutritive component in Jatropha meals, the consumption of which can decrease the bio-availability of minerals, particularly of calcium and zinc ions. Phytates have also been implicated in decreasing protein digestibility by forming complexes and also by interacting with enzymes such as trypsin and pepsin (Reddy and Pierson, 1994). Saponins are glycosides containing a polycyclic aglycone moiety of either C_{27} steriod or C_{30} triterpenoid (sapogenins). They are characterized by bitter taste and foamy characteristics. Saponins from some plants produce adverse effects. The oily seeds are processed into oil, which may be directly used to fuel combustion engines or may be subjected to trans-esterification to produce biodiesel. Jatropha oil is not suitable for human consumption, as it induces strong vomiting and diarrhoea.

Belewu (2008) showed reduction in some antinutritional factors such as phytate, saponin, and tannin when treated with *Rhizopus oligosporus*. Belewu *et al.*,(2010) deduced that *Aspergillus niger* was effective in lowering the level of antinutrients in the kernel cake to level that do not elicit toxic response in the West African Dwarf goats while *Trichoderma longibrachiatum* was not effective in detoxifying the antinutrients as the toxic responses was noticed (persistent diarrhoea, dehydration and sudden deaths). This present study seeks to explore the use of other available beneficial fungi that will safely detoxify *Jatropha curcas* kernel cake. The fungi used include *Absidia spinosa, Mucor rouxii* and a mixed culture of *Absidia spinosa* and *Mucor rouxii*. The Jatropha curcas seeds were also toasted as a means of reducing or eliminating these inherent antinutritioinal factors known to be present in this age-long multipurpose plant.

2. Materials and Methods

2.1. Seed Collection

Mature and sun dried seeds of *Jatropha curcas* were collected around Ilorin metropolis, while some were purchased from open market in the same city which in situated in Kwara State, North Central region of Nigeria.

2.2. Toasting and Milling

The seeds were decorticated, and the kernels were toasted and milled using the mechanical grinder.

2.2.1. Mechanical Oil Extraction

The Jatropha kernel meal was packed in a sieving cloth which was sown into bags. They were packed on a specially designed wooden oil extractor. The oil was extracted mechanically using hand-driven screw press for about 3 days. A small bowl was placed underneath for easy collection of oil.

2.2.2.Cold Solvent Extraction

The compacted cake was crutched using pestle and mortar. Petroleum spirit was added just above the level of the cake (1:1 v/w) in 5litres white plastics. They were left for 24 hours. The oil layer was discounted, the cake was squeezed in a sieving cloth and sun dried.

2.3. Solid State Fermentation

30mls of distilled water was added to 20g of Jatropha kernel cake (3:2 v/w) in a covered bowl, which was earlier disinfected using ethanol. It was kept in a dark room for 5 days at room temperature to allow natural solid-state fermentation to occur. Different colonies of fungi were observed to have grown on the substrate.

2.4. Media and Culture Conditions

The pin mouth was used to pick from different colonies and spread on the sarbroud agar in 5 bottles. They were left for 5 days to allow proper growth of the micro-organisms. Stock cultures of fungi were later maintained at 4^{o}C on commercially prepared Potato Dextrose Agar.

2.5. Identification and Isolation of Microorganisms

Lactophenol blue was used to stain a slide. Pin mouth was used to pick from each colony and placed on the slide. Then, slide cover was placed on each slide. This was observed under the microscope (Olympus CX21) at a magnification of X40. After several observations, *Rhizopus Sp. , Absidia spinosa. . Aspergillus Sp. , Mucor rouxii and Klebsilla oxytoca* were identified.

2.5.1. Pure Culture

Mucor rouxii and *Absidia spinosa* were tissue cultured to obtain homogenous samples of fungal mycelia. The pure culture obtained was maintained on plate of potato dextrose agar (PDA).

2.5.2. Sub-culturing Method

20g of potato dextrose agar was weighed into two 250mls conical flasks. 250mls of distilled water and 0.3 mls on streptomycin were added to each flask. They were immediately covered with cotton wool and aluminium foil and autoclaved at 121^{0}C for about 15 minutes. They were placed in cold water. About 10mls of the prepared agar medium was poured into each 40 petri dishes and were allowed to solidify. Inoculating pin was used to place part of the colony on the medium. It was flamed when switching over to the second fungus. Lab coat, hand gloves and nose mask were put on. The inoculated media were kept in the dark cupboard in the laboratory for 5 days at 30^{o}C and 100% RH (Relative humidity).

2.5.3. Inoculation and Incubation of Substrate

Spores from 5 days old agar slants were collected by adding sterile distilled water. Three big black bowls were disinfected with ethanol inside which the autoclaved *Jatropha curcas* kernel cake was inoculated separately in layers with the fungi (*Absidia spinosa* and *Mucor rouxii*) using the dilution and later incubated at room temperature. *Absidia spinosa* and *Mucor rouxii* were harvested from thirteen petri dishes each to inoculate two bowls while 7 plates of each fungi culture (plate size 10cm diameter) were mixed in the suspension to inoculate the third bowl. They were moistened with distilled water to aid proper growth of the fungi. On the seventh day, the fungi were observed to have enveloped the substrate. white and dark-grey growth was observed to have covered the substrate inoculated with *Mucor rouxii while* greyish growth was noted on the substrate inoculated with *Absidia spinosa*. They were mixed properly and left for another 7 days. The growth was terminated by autoclaving in a foil paper .

2.6. Chemical Analysis

The proximate composition of fungi treated and untreated *Jatropha curcas* kernel cake samples were determined according to the method described by AOAC, (1995). Dry matter was determined by oven drying the milled samples to a constant weight at 105^{0}C for 8 hours. Crude protein was determined as Kjedhal nitrogen x 6.25. Ether extracts and ash were determined according to (AOAC, 1995) method. Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) were determined using the method described by Van Soest *et al.,* (1991). Hemicellulose was calculated as the difference between NDF and ADF while cellulose is the difference between ADF and ADL. Saponin content was evaluated by the method of Edeoga *et al*. (2005). Tannin was determined by the method of Josyln (1970) while phytate was determined using the method Wheeler and Ferrel (1971).

3. Results and Discussion

Crude protein (CP) value of 38.5% was observed for *Jatropha cathartica* seeds (Ebun-Oluwa *et al.,* 2007), 50.80% for untreated Jatropha curcas (Belewu *et al.,* 2010) of which the latter is similar to the one obtained in this study. This value is obviously much higher than most legumes/grains consumed in Nigeria, eg guinea pig

has a crude protein value of 5.25 (Amoo, 1998), *B. Eurycoma* "Achi" has a protein content of 3.35% (Eze and Ibe, 2005). The high crude protein and ash content of the fungi treated Jatropha seed cakes was due to the addition of microbial single cell protein during the process of fermentation(Martinez-Herrera *et al*., 2006; Belewu, 2008). CP increase could also be as a result of hydrolysis of starch to glucose and its subsequent use by same organism as a carbon source to synthesise fungal biomass rich in protein (Hammond and Wood,1985). Also, increase in crude fat as reported in this work is considered as an improvement in the ether extract content of fungus treated Jatropha seed cake. This shows that the fungi have more lipogenic ability than lipolytic ability thus increasing the ether extract content of the fungi treated sample (Belewu and Popoola, 2007).

Table 1: Proximate composition of the treated and untreated *Jatropha curcas* kernel cake on dry matter basis

Parameters	Untreated JSC	*Absidia spinosa* treated JSC	*Mucor rouxii* treated JSC	*Absidia spinosa* and *Mucor rouxii* treated JSC	±SEM	P-VALUE
Dry Matter	85.00^{b}	86.77^{ab}	87.71^{ab}	87.24^{a}	0.97^{ns}	0.899
Crude Protein	49.00	52.50	52.10	53.20	1.04^{ns}	0.600
Ether Extract	8.60	9.30	9.10	10.20	0.65^{ns}	0.924
Ash	8.10	12.31	13.00	11.48	1.01^{ns}	0.393
Crude Fibre	6.50	4.2	3.95	2.55	0.61^{ns}	0.091
Nitrogen Free Extract	12.80	8.46	9.56	9.81	0.83^{ns}	0.316
Neutral Detergent Fibre	61.74^{b}	55.60^{ab}	59.76^{b}	47.50^{a}	2.3	0.087
Acid Detergent Fibre	5.00^{c}	3.10^{b}	3.24^{b}	1.36^{a}	0.49	0.007
Hemicellulose	56.74^{b}	52.50^{ab}	56.52^{b}	46.14^{a}	1.85	0.095
Lignin	0.2^{c}	0.13^{bc}	0.10^{ab}	0.05^{a}	0.02	0.018
Cellulose	3.66^{b}	0.51^{a}	0.53^{a}	0.51^{a}	0.54	0.015
Silica	1.14^{a}	2.46^{b}	2.61^{b}	1.16^{b}	0.25	0.002

a,b,c, Means within a row with different superscripts are significantly different ($p < 0.05$) ns = not significantly different ($p>0.05$).

Crude fibre is the sum total of all those organic compounds of the plant cell membranes and supporting structures which in chemical analysis of plants food stuff remain after removal of the crude protein, fat and nitrogen-free extract. Thus the crude fibre in diet consists mostly of plant polysaccharides that cannot be digested by human dietary enzymes such as cellulose, hemicellulose, lignin and some materials that encrust the cell walls (Melon, 1980). Fibre content is a significant component of the diet. It increases stool bulk and decreases the time that waste materials spend in the gastrointestinal tract. It is commonly used as an index of value in poultry and feeding stocks feeds (Eze and Ibe, 2005).

The decrease in the value of neutral detergent fibre (hemicellulose, cellulose and lignin) and acid detergent fibre (lignin and cellulose) for the fungal treated Jatropha kernel cake could be indicative of the degradation of the cell wall component of the substrates produced by extra cellular enzymes. Previous authors concluded that lignifications of structural polysaccharides not only inhibit ruminal microbial digestion of polysaccharide by forming 3-D matrix, but also that the presence of highly lignified tissues form a physical barrier preventing accessibility of the otherwise highly digestible tissue to the action of hydrolytic enzymes of the rumen micro-organism (Karunanandaa *et al.*, 1995), and have shown that increased digestibility was associated with the degradation of structural carbohydrates. The synergistic enzymic reaction of *Absidia spinosa* and *Mucor rouxii* produced higher amounts of xylanase , carboxymethyl cellulase and ligninase such that plant residues such as crude fibre, cellulose, neutral detergent fibre, and acid detergent fibre decreased more when compared with other fungi treated samples. Thus, these fungi had fibrilolytic effects on the kernel cake (Belewu ,2008). Other constituents of the feed had ability to provide fermentable energy to the rumen microbes in the form of available cellulose and hemicellulose which stimulates fibre digestion. The acid detergent fibre fraction of the neutral detergent fibre was very low which is indicative of high levels of hemicellulose. The low proportion of both lignin and cellulose is based on the fact that the analysed part of the plant were the seeds. The fibre fraction (ADF and lignin) was higher in the untreated Jatropha kernel cake (5% and 0.2% respectively) than in the fungus treated diets. This is expected, since there was no treatment of the kernel cake by any of the fungus.

Table 2: Toxins in treated and untreated J*atropha curcas* kernel cake

Parameters	Untreated Jatropha Kernel Cake	Toasted Jatropha Kernel Cake	*Absidia spinosa* treated Jatropha Kernel Cake	*Mucor rouxii* treated Jatropha Kernel Cake	*Absidia spinosa* and *Mucor rouxii* treated Jatropha Kernel Cake	±SEM	P-Value
Tannin (g)	**4.80**	**3.81**	**3.53**	**3.40**	**3.34**	**0.28^{ns}**	**0.545**

Alkaloid (g)	**2.85^{c}**	**2.62^{bc}**	**0.92^{ab}**	**0.84^{ab}**	**0.41^{a}**	**0.38**	**0.047**
Saponin (g)	**7.69^{c}**	**3.34^{b}**	**2.27^{ab}**	**1.34^{a}**	**1.10^{a}**	**0.82**	**0.001**
Phytate (g)	**0.10**	**0.09**	**0.08**	**0.07**	**0.05**	**0.10^{ns}**	**0.703**

a,b,c, Means within a row with different superscripts are significantly different ($p < 0.05$) ns = not significantly different ($p>0.05$).

The antinutritional factors measured were reduced to a level that could impact some qualities of ruminal undegradable protein thereby enhancing the utilization of its protein. The antinutrient, phytate, observed in untreated *Jatropha curcas* kernel cake is similar to that of *Jatropha cathartica* of 2945.96mg/100g which is comparable to those of some commonly consumed tropical legumes-cowpea (2.0-2.9%), pigeon pea (2.0-2.4%) and African yam beans (2.4%), (Oboh, 2006). Phytate is not only a very stable and potent chelating food component that is considered to be an antinutrients by virtue of its ability to chelate divalent minerals and prevent their absorption (Oboh and Akindahunsi, 2003), but it has also been shown to have anticancer and antioxidant activity. It forms an iron chelate that suppresses lipid peroxidation by blocking iron-driven hydroxyl radical generation (Sudheer *et al.,* 2004). Tannins are complex polyphenolics found widely in the plant kingdom. The tannins bring about antinutritional influence largely by forming complexes and thus precipitating dietary proteins and digestive enzymes (Oboh and Akindahunsi, 2003; Wanapat *et al*., 1997).

Tannin could form protein complexes with which they are by-passed in the rumen and are digested at the lower segment of the gastrointestinal tract. The tannin content was in consonance with that of *Jatropha cathartica* which was 0.41%. This value is far lower than what Agbede and Aletor, (2004) reported for the leaves of *Leuceania leucocephala* and *Glyricidia sepium* and that reported for *Struchium sparganophora* Leaves (0.6%) by Oboh, 2006. Moreover, the level of tannins present in this untreated *Jatropha curcas* kernel cake is far below the recommended deleterious dose of 0.75-0.95 % (Aletor, 1993). There have been recent reports of lower methane emissions by ruminants consuming forages containing low levels of condensed tannins (CT), determined in vitro and in vivo with cattle (Roth *et al*., 2001; Woodward *et al.,* 2001, 2002). Goats are relatively less affected by antinutritional factors such as saponins and alkaloids that are present in many plants because of factors such as differences in salivary proteins (Foley *et al*., 1999).

4. Conclusion

From the results of this study, the fungi treated Jatropha kernel cake is nutritionally promising as a good source of carbohydrate, protein, crude fibre and minerals. The resultant effects biological detoxification methods are the pre-digestion of the fibrous materials, the availability of fungal protein and the addition and release of bound minerals. The synergetic enzymatic effects of mixed culture of *Absidia spinosa* and *Mucor rouxii* lowered the antinutritional factors to bearable level. It follows that they are cheap sources in detoxifying most antinutritional factors against inorganic means.

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