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Effect of Varying Pretreatment Techniques on Nutrient Composition of *Indigofera Arrecta* Seeds

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Abstract

This study was conducted to determine the effect of different pretreatment methods on the nutrient composition of pretreated *Indigofera arrecta* seeds. Five different pretreatment methods which include steam (StPS), acid (AcPS), alkaline (AlPS), combined alkaline and steam (CoPS), biological pretreatment (BiPS) and the ground sample (GdPS) as control were carried out on the seeds of *I. arrecta*. Results show significant ($p<0.05$) changes in the proximate composition, antinutrient, mineral, lignin, hemicellulose and cellulose contents of the pretreated sample when compared with the control (ground sample). Significant reduction in ($p<0.05$) in lignin, cellulose content was observed in all the pretreatments. The BiPS resulted in highest percentage reduction in lignin and cellulose content by 71% and 59% respectively when compared with other samples. The BiPS with values of 0.103 ± 0.03, 0.017 ± 0.01, 0.017 ± 0.01 and 2.157 ± 0.41 resulted in highest significant ($p<0.05$) reduction in tannin, lectin, trypsin and saponin content of the seeds respectively and also significantly ($p<0.05$) increased the calcium, magnesium, iron, zinc, potassium, and phosphorus with values 21.813 ± 0.01, 20.03 ± 0.02, 10.557 ± 0.00, 50.641 ± 0.01, 10,200 ± 0.01 and 4,333 ± 0.02 respectively. This report indicates that pretreatment is an efficient method in improving the nutritional and mineral content and also in the reduction of antinutrients present in the seeds of *Indigofera arrecta*.

Keywords: Pretreatment; *Indigofera arrecta*; Proximate composition; antinutrients; Minerals; Lignin; Cellulose; Hemicellulose

Introduction

All essential experimental phases engaged in making fermentable substrates are referred to as pretreatment. Several investigations have reported very promising results for processes such as alkaline pretreatment [1,2] aqueous ammonia-soaking pretreatment [3], low acid pretreatment [4], steam pretreatment [5] and sequential pretreatment with diluted acid and then alkali [6]. Pretreatments change the structure of cell walls and polymers by disrupting intermolecular forces holding them together, allowing greater access by enzymes and water. The presence of lignin also impedes enzymatic hydrolysis, as enzymes bind onto the surface of lignin and hence do not act on the cellulose chains [7]. Pretreatment has to overcome the resistance of the plant cell walls to deconstruction in order to separate the cellulose from lignin and hemicelluloses to make it more accessible to microbes or enzyme hydrolysis [8]. The digestibility of plant material in the rumen is related to the proportion and lignification of plant cell wall. Presence of lignin and hemicellulose makes the access of enzymes to cellulose fibers difficult. Therefore, the removal of lignin and hemicellulose as well as the increase of porosity during the pretreatment process increases the hydrolysis rate significantly [9].

The genus Indigofera Linn. is a large genus of about 700 species of flowering plants belonging to the sub-family Papilionoideae in the family Fabaceae/ *Leguminosae.* They occur throughout the tropical and subtropical regions of the world. *Indigofera* in Greek means indigo dye which is famous for the natural blue colors obtained from the leaflets and branches of this herb. The fruits are oval shaped and elongated, 4- angled or flattened and often curved with many seeds [10]. Burkill [11] recognized 60 species while Soladoye and Lewis [12] recorded 60 species in Nigeria with over 60% abundance in the Northern region of the country with 27 species distributed across the South Western area of the country. *Indigofera tinctoria* is regarded as much superior to *Indigofera tinctoria* and farmers have largely supplanted in India [13]. The most important of the species are *Indigofera arrecta* and *Indigofera tinctoria*. *Indigofera* spp. possesses diverse morphological and agronomic attributes, significant to their use as forage and cover crops [14]. Investigation of *Indigofera* species shows that they are rich in organic and fatty acids, flavonoids such as carotenoids and coumarin [15]. Some of these species, *Indigofera tinctoria* and *Indigofera suffruticosa* are used to produced indigo dyes while some have medicinal values such as *Indigofera* articulate used for the treatment of toothache, *Indigofera* oblongifolia, *Indigofera suffruticosa* and *Indigofera* aspalthoides are used as

anti–inflammatories for treatment of insect stings, snake bites and swellings [16]; and *Indigofera arrecta* extract is used to relieve ulcer pain. The stem of *Indigofera tinctoria* is chewed to cure cough and decoction of leaves is used to cure chest pains, epilepsy, nervous disorders, asthma, bronchitis, fever and complaints of stomach, liver, kidney and spleen- especially in Cameroon [17]. The twine paste cures dislocation. Also the warm leaves dismiss bruises [18].

Legumes are the most important sources of proteins, carbohydrates and vitamins in the diet of many populations, especially in developing countries [19]. They range from the highly utilized legumes such as *Vigna unguiculata*, Glycine max and Arachishypogaea to the lesser known ones such as *Sphenostylis stenocarpa*, *Mucuna flagellipes* and *Vigna racemosa* [20]. As a result of the high demand of the common legumes, there is an upsurge in their prices which has compelled the need to look for replacements such as the wild legumes. The seed of *Indigofera arrecta* is known for its hardness which is due to the presence of a hard seed coat which is the seed's primary defense against adverse environmental conditions. Phenolic compounds in the seed coat contribute to seed hardness and inhibition of microorganism growth hence the need for pretreatment in other to increase the accessibility to enzyme hydrolysis or fermentation. This study is therefore led to evaluate the effect of pretreatment on nutrient composition of *Indigofera arrecta* seeds.

Materials and Methods

Collection and preparation of samples

Indigofera arrecta fruits were collected from Zaria metropolis with identification done at the Herbarium of the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria. The voucher number 663 was assigned to *Indigofera arrecta*. The fruits were sun-dried for 3-4 days, threshed and winnowed. Dust and other foreign materials were removed to obtain clean seeds. The whole seeds were then stored in plastic containers for subsequent analysis.

Pretreatments

Mechanical pretreatment: One kilogram of the sample was dried in an oven at 55oC for 4hr. After drying, the sample was ground in a bench mill and sieved to obtain a particle size of 0.05-0.250 mm fraction which was stored in clean polythene bags until required.

Steam pretreatment: Two hundred grams of the sample was mixed with 1L of distilled water and autoclaved for 90 min at 121°C. The residues was washed thoroughly with distilled water and dried in an oven to a constant weight at 80°C [21].

Acid pretreatment: Two hundred grams of the ground sample was mixed with 1L of 1M HCl for 1 hour after which the mixture was neutralized with 1M NaOH [22]. The residue was then washed with distilled water and dried to constant weight at 80°C in the oven.

Alkaline pretreatment: Two hundred grams of the ground sample was mixed with 1L of 0.25 M NaOH solutions for 1 hour after which the mixture was neutralized with 0.1M HCl. The residue was washed with distilled water and dried to constant weight at 80°C in the oven [23].

Combined alkaline and steam pretreatment: Two hundred grams of the ground sample was mixed with 1L of 5% NaOH solution and autoclaved for 60 min at 121°C. The residue was washed with distilled water and dried to constant weight at 600C in an oven [24].

Biological pretreatment: Forty grams of the sample was moistened with 200 ml of distilled water to obtain 70% w/v of moisture in 1L Erlenmeyer conical flask. The mixture was autoclaved for 30 min at 121°C. After cooling, it was inoculated with spore suspension of Ustilago maydis and incubated at 28°C for 21 days.

Biochemical and mineral analysis

The pretreated samples after drying will be used for biochemical and mineral analysis with the ground sample as control. Ash, crude lipid, crude fibre, and crude protein were determined following the methods of AOAC [25]. Lignin, cellulose and hemicellulose content were estimated according to Georing and Van Soest [26].

The anti-nutritional factors in both samples and control were examined as follows: Hydrogen cyanide content was determined according to the method of AOAC [27], tannin content was estimated spectrophotometrically by Folin-Denis method [28], phytic acid was determined using the procedure described by Lucas and Markakas [29]. Saponins was determined by the gravimetric method of AOAC [27], trypsin inhibitor was analysed by using the spectrophotometric method, described by Amtfield [30]; lectin content was determined using the spectrophotometric method of Onwuka [31], alkaloid content was determined by the gravimetric method of Harbone [32] and oxalate was determined by using the method of Oke [33] .

The following minerals: magnesium, calcium, zinc, iron, potassium, phosphorus and sodium were determined using atomic absorption spectrophotometry as described by AOAC [25].

Statistical Analysis

Means and standard deviation (SD) of factors examined were calculated. The effects of alkaline, acid, steam, combined steam and alkaline and biological pretreatment on the nutritional, antinutritional factors, lignin, cellulose, hemicellulose and mineral content of I. arrecta seeds were resolved by Analysis of variance (ANOVA) using SPSS version 20. Duncan's Multiple Range Test (DMRT) was applied to separate and show means that differed significantly. Significance was accepted at $p \leq 0.05$.

Results and Discussion

Changes in the proximal composition after pretreatment

The effect of pretreatment conditions on the proximate composition of the control and pretreated samples are shown in **Table 1**. The significant decrease ($p<0.05$) in the protein content of the steam pretreated sample could be as a result of the effective elimination of much of the heat-sensitive indigenous micro flora [34]. The increase in crude protein may be due to the addition of fungal protein or the bioconversion of carbohydrates in the colonized substrates into mycelia protein or single cell protein (SCP) by the growing fungus during the fermentation process [35].

Table 1: The Effect of Pretreatment on the Proximate Composition of *Indigofera arrecta*(%).

Pretreatment method	Moisture (%)	Crude Protein (%)	Crude fat (%)	Ash (%)	Fibre (%)	Carbohydrate (%)
GdPS	6.150 ± 0.02^{a}	22.277 ± 0.02^{b}	8.150 ± 0.02^{b}	3.867 ± 0.02^{b}	10.730 ± 0.03^{c}	50.627 ± 0.58^{c}
AlPS	2.007 ± 0.01^{d}	26.027 ± 0.05a^{b}	7.113 ± 0.04^{d}	3.067 ± 0.02^{c}	7.347 ± 0.02^{e}	54.467 ± 0.02^{b}
AcPS	3.023 ± 0.02^{d}	24.097 ± 0.08a^{b}	7.040 ± 0.07^{e}	2.983 ± 0.01^{e}	12.703 ± 0.05^{a}	50.060 ± 0.15^{d}
StPS	3.633 ± 0.12^{c}	16.267 ± 0.02^{c}	7.827 ± 0.12^{c}	2.650 ± 0.01^{f}	11.383 ± 0.05^{b}	42.317 ± 0.02^{f}
BiPS	2.247 ± 0.01^{e}	28.050 ± 0.06^{a}	8.867 ± 0.15^{a}	7.145 ± 0.01^{a}	9.856 ± 0.14^{c}	44.010 ± 0.10^{e}
CoPS	4.167 ± 0.05^{b}	22.980 ± 5.23^{b}	7.373 ± 0.03^{d}	3.060 ± 0.06^{d}	5.597 ± 0.02^{f}	60.117 ± 0.29^{a}

Values are mean ± SD of triplicate determinations. Mean values with different superscripts down the column are significantly different ($p \leq 0.05$). Where; GdPS=Grinded sample, AlPS=Alkaline pretreated sample, AcPS=Acid pretreated sample, StPS=Steam pretreated sample, BiPS=Biologically pretreated sample and CoPS=combined steam and alkaline pretreatment.

It may also be partly due to the secretion of some extracellular enzymes such as cellulases and amylases by the fungus in an attempt to use cellulose and starch as sources of carbon [36,37].

The observed increase in the ash content of BiPS pretreated sample indicates an increase in the inorganic mineral elements of the sample. This confirmed the report of Oyeleke [38] that the ash content of a sample indicates the inorganic element in the sample. Same was observed when *C. albidum* seed meal was fermented with *A. niger* [39].

Decrease in the lipid content of all pretreated sample apart from the biologically pretreated sample could be as a result of the solubilization of the lipid content by the action of heat and chemicals during pretreatment.

Also, there is need for the pretreated biomass to be washed with water and this could also lead to washing off of the soluble lipid component of the sample. Significant increase in the lipid content of the biologically pretreated sample could be as a result of solubilization of some component of the sample.

The pretreated fungal sample had significant reduction in carbohydrate content. Carbohydrates are used through different biochemical processes by microorganisms to produce simple sugars during bioconversion of lignocelluloses [40,41]. Lignin is removed only to a limited extent during steam pretreatment but is redistributed on the fiber surfaces as a result of melting and depolymerization/repolymerization reactions [42].

The removal and redistribution of hemicellulose and lignin increase the volume of the pretreated sample and this could be responsible for the high fiber content of the steam pretreated sample.

Table 2: Effect of pretreatment on the lignin, hemicellulose and cellulose content of *Indigofera arrecta* seeds (%).

Pretreatment method	Lignin	Hemicelluloses	Celluloses
GdPS	26.32 ± 0.21^{a}	22.11 ± 0.03^{a}	32.12 ± 0.02^{a}
AlPS	9.12 ± 0.12^{d}	18.51 ± 0.44^{b}	19.62 ± 0.74^{b}
AcPS	18.3 ± 0.13^{b}	5.58 ± 0.42^{e}	20.17 ± 0.32^{b}
StPS	10.63 ± 0.35^{c}	11.48 ± 0.24^{d}	13.45 ± 0.38^{d}
BiPS	7.54 ± 0.10^{e}	10.96 ± 0.08^{d}	13.13 ± 0.16^{d}
CoPS	8.93 ± 0.13^{d}	17.63 ± 0.44^{c}	15.06 ± 0.07^{c}

Values are mean ± SD of triplicate determinations. Mean values with different superscripts down the column are significantly different ($p \leq 0.05$). Where; GdPS=Ground sample, AlPS=Alkaline pretreated sample, AcPS=Acid pretreated sample, StPS=Steam pretreated sample, BiPS=Biologically pretreated sample and CoPS=combined steam and alkaline pretreatment.

Degradation of lignin, cellulose and hemicelluloses

While comparing the lignin, cellulose and hemicellulose content of the entire pretreated sample as against the ground sample, a significant ($p<0.05$) decrease of all these content were observed. The percentage of lignin content was decreased by 66.07% (**Table 2**) for the BiPS sample. The finding was in accordance with the previous reports of Lechner and Papinutti [43] and Sherief [44] where lignolytic activities of fermenting microorganisms were found during biodegradation of rice straw, saw dust, wheat straw, coffee pulp and banana leaves. From the result on **Table 2**, the most promising pretreatment option for

hemicellulose breakdown is dilute acid with a decrease of 74.76%, which significantly increases susceptibility to hydrolysis for cellulose.

The cellulose content was found to decrease to 53.11% for the BiPS sample when compared with the ground sample. Cellulose degradation is a usual phenomenon during SSF of lignocelluloses as reported by Bisaria [45], Sherief [44] and Jahromi [46]. The alkaline pretreatment (AlPS) and combined steam and alkaline pretreatment (CoPS) of *Indigofera arrecta* seeds also significantly reduced the amount of lignin composition in the sample. The major action of NaOH is to degrade the lignin by breaking ester bond cross linking, thus creating porosity in biomass [47,48].

Effect of antinutritional content

Significant reduction ($P<0.05$) in the anti-nutrient composition was also observed after pretreatment (**Table 3**). BiPS had the highest percentage reduction in tannin, saponin, trypsin, lectin and hydrogen cyanide by 57%, 48%, 64%, 49% and 70% respectively when compared with the ground sample.

Table 3: The Effect of Pretreatment on the Antinutritional Content of *Indigofera arrecta*.

Pretreatment Method	Tannin (%)	Phytate (%)	Alkaloid (%)	Saponin (%)	Oxalate (%)	Trypsin inhibitors (TIU/g)	Lectin (%)	Hydrogen cyanide (mg/100 g)
GdPS	0.237 ± 0.04^{b}	0.303 ± 0.05^{a}	9.040 ± 0.11^{a}	4.173 ± 0.20^{a}	0.760 ± 0.20^{a}	0.047 ± 0.01^{ab}	0.033 ± 0.01^{b}	0.090 ± 0.02^{a}
AlPS	0.193 ± 0.02^{cd}	0.137 ± 0.03^{c}	2.313 ± 0.10^{b}	2.180 ± 0.25^{c}	0.063 ± 0.02^{d}	0.037 ± 0.02^{b}	0.020 ± 0.01^{c}	0.029 ± 0.02^{bc}
AcPS	0.267 ± 0.04^{a}	0.163 ± 0.01^{c}	0.670 ± 0.03^{e}	2.333 ± 0.07^{c}	0.137 ± 0.03^{c}	0.050 ± 0.03^{a}	0.040 ± 0.02^{a}	0.060 ± 0.03^{ab}
StPS	0.257 ± 0.01^{ab}	0.297 ± 0.15^{a}	0.817± 0.06^{d}	2.757 ± 0.01^{b}	0.447 ± 0.06^{b}	0.023 ± 0.01^{cd}	0.023 ± 0.01^{bc}	0.057 ± 0.02^{bc}
BiPS	0.103 ± 0.03^{e}	0.150 ± 0.03^{bc}	1.207 ± 0.10^{c}	2.157 ± 0.41^{d}	0.140 ± 0.04^{c}	0.017 ± 0.01^{d}	0.017 ± 0.01^{d}	0.027 ± 0.01^{c}
CoPS	0.143 ± 0.04^{d}	0.227 ± 0.04^{b}	0.157 ± 0.01^{f}	2.203 ± 0.21^{c}	0.073 ± 0.02^{cd}	0.033 ± 0.02^{bc}	0.027 ± 0.01^{bc}	0.027 ± 0.01^{c}

Values are mean ± SD of triplicate determinations. Mean values with different superscripts down the column are significantly different ($p \leq 0.05$). Where; GdPS=Grinded sample, AlPS=Alkaline pretreated sample, AcPS=Acid pretreated sample, StPS=Steam pretreated sample, BiPS=Biologically pretreated sample and CoPS=Combined steam and alkaline pretreatment

Table 4: The effect of pretreatment on the mineral composition of *Indigofera arrecta*.

Pretreatment Method	Ca (ppm)	Mg(ppm)	Fe (ppm)	Zn (ppm)	K(ppm)	P(ppm)	Na(ppm)
GdPS	18.657 ± 0.00^{c}	11.609 ± 0.02^{b}	1.481 ± 0.00^{e}	1.665 ± 0.01^{e}	6600 ± 0.01^{b}	2200 ± 0.01^{b}	233± 0.01^{d}
AlPS	16.389 ± 0.00^{d}	9.347 ± 0.02^{c}	2.638 ± 0.00^{b}	2.311 ± 0.01^{d}	2767 ± 0.03^{d}	2300 ± 0.03^{b}	4000 ± 0.01^{a}
AcPS	11.154 ± 0.00^{f}	5.075 ± 0.02^{f}	1.566 ± 0.04^{d}	2.829 ± 0.01^{b}	2433 ± 0.03^{e}	2000 ± 0.02^{b}	1500 ± 0.02^{c}
StPS	15.962 ± 0.00^{e}	8.258 ± 0.05^{d}	2.452 ± 0.04^{c}	1.631 ± 0.03^{f}	4333 ± 0.01^{c}	2100 ± 0.02^{b}	567 ± 0.01^{d}
BiPS	21.813 ± 0.01^{a}	20.03 ± 0.02^{a}	10.557 ± 0.00^{a}	50.641 ± 0.01^{a}	10200 ± 0.01^{a}	4333 ± 0.02^{a}	2500 ± 0.05^{b}
CoPS	19.320 ± 0.00^{b}	6.230 ± 0.02^{e}	1.259 ± 0.00^{f}	2.343 ± 0.01^{c}	500 ± 0.01^{f}	1267 ± 0.01^{c}	1467 ± 0.01^{c}

Values are mean ± SD of triplicate determinations. Mean values with different superscripts down the column are significantly different ($p \leq 0.05$). Where; GdPS=Grinded sample, AlPS=Alkaline pretreated sample, AcPS=Acid pretreated sample, StPS=Steam pretreated sample, BiPS=Biologically pretreated sample and CoPS=Combined steam and alkaline pretreatment.

Mineral composition

The mineral content was evaluated after pretreatment (**Table 4**). The high mineral content of the BiPS sample could be as a result of the ability of the white-rot fungi to degrade recalcitrant molecules like lignin. This increase could also be attributed to the ability of the fungi to degrade the antinutrients or the complexes they form with these ions leading to the significant increases in their concentrations. There was significant reduction in the calcium content of the acid and steam pretreated sample. This could be as a result of calcium ions to extensively cross-linked lignin molecules and prevented lignin solubilization thereby decreasing the amount of available calcium ion in the sample. The reduction in mineral content for most of the pretreated sample could be as a result of the washing process of the pretreated seeds to remove materials that are inhibitory to microbial growth and fermentation.

Conclusion

Pretreatment is an efficient method in enhancing the protein content, mineral composition and in reducing tannins, phytates, alkaloids, saponins, hydrogen cyanide, trypsin inhibitors, lectins and oxalate in the seeds of *Indigofera arrecta*. This present research work has shown that fermentation using Ustilago maydis is more effective compared to the other pretreatment methods, although there is still need for further studies on the effect of BiPS on other antinutrients not studied. Furthermore, other pretreatment methods needs to be employed in other to further study the effect of pretreatment on the protein content, mineral composition and antinutrients in the seeds of *Indigofera arrecta*.

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