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Improvement of the Nutrimental Status of *Albizia lebbeck* Seed through Solid State Fermentation by Some Fungal Species

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Abstract

Fungal fermentation is a promising method of converting under-utilized non-conventional feed materials into edible food to combat hunger and malnutrition in the growing world population and provide a suitable alternative to conventional food materials. The nutritional status of Albizia lebbeck seed was improved through fungal solid state fermentation in this research. The pulverized seed was subjected to spontaneous fermentation to isolate resident fungi. Isolates were characterized and identified using DNA amplification and sequencing and were used in induced, monoculture solid-state fermentation of A. lebbeck seed for five days. Effects of fermentation on the seed were assessed on proximate, phytochemical, anti-oxidant, mineral contents and amino acid profile using standard methods. Isolated fungi were Aspergillus niger (OR879114), Penicillium citrinum (OR879113), and Cladosporium cladosporioides (OR879115). Generally, there was significant difference (P≤0.05) in the proximate composition of the post-fermented seed; the crude protein and total ash content increased from 10.79±0.53 to 22.69±1.83, and 5.16 ± 0.02 to 9.29 ± 0.12 respectively while the total carbohydrate decreased from 45.99±3.01 to 25.95±1.03, phytochemical content reduced significantly; tannins and alkaloids reduced from 12.14±0.20 to 0.34±0.01, and 20.70±2.18 to 0.79±0.08 respectively in sample fermented with Penicillum citrinum (OR879113). Anti-oxidant, mineral contents, and the amino acid profile were also remarkably improved. Fermentation with Penicillium citrinum strain OR879113 has the best products, while the least was in products fermented with Aspergillus niger OR879114. The fermentation with the isolated fungi has a significant desirable effect on the nutritional status of the seed of Albizia lebbeck.

Keywords: Albizia lebbeck, Fermentation, Fungi, Nutritional status, Aspergillus niger, Penicillium citrinum, Cladosporium cladosporioides

INTRODUCTION

Hunger and malnutrition are likely to result from the current global exponential population growth (Yafetto *et al.*, 2023), poor agricultural harvests brought on by climate change, and a lack of interest among younger people in farming, particularly in many developing nations. Foods high in protein are in constant demand for both human and animal consumption (FAO, 2018; Senanayake *et al.*, 2023). The loss of arable land and the emission of greenhouse gases by farm animals limit the amount of food that can be produced. Man was, therefore, more compelled than ever to look for new food sources.

One of the main causes of food shortage has also been the struggle between humans and their livestock for edible food (Pesante *et al.*, 2022). Without looking into other food sources, it may be exceedingly difficult for humans and their animals to achieve food sustainability (Yafetto *et al.*, 2023; Senanayake *et al.*, 2023). Man must thus look for new food sources to keep up with the rapid population expansion in order to reduce hunger, malnutrition, and poverty (Kapilan *et al.*, 2018; Reihani and Khosravi-Darani, 2019).

A most likely substitute is to think about using microbial fermentation to transform less palatable plant components into edible ones. It's fascinating to note that turning waste from unusual plants into useful items is becoming more appealing and feasible from an economic standpoint. The accessible basic foods are preserved for human consumption since such plants could be employed as feed supplements for farm animals. Foods high in protein are in constant demand for both human and animal consumption (FAO, 2018; Senanayake *et al.*, 2023). The loss of arable land and the emission of green gas by farm animals limit the amount of food that can be feasible.

The accessible basic foods are preserved for human consumption since such plants could be employed as feed supplements for farm animals. This kind of feed is referred to as "non-conventional food" sources, which are feeds that have not historically been utilized as animal feed. For humans and their animals, legumes constitute a significant source of plant protein (Stagnari *et al*., 2017). Many leguminous plants have been domesticated for food, their edible seeds, which humans can eat, and their leaves, which are the main source of protein for animals.

In many diets, they make up as much as 80 % of the protein, and in many foods, they are the sole source of protein (Senanayake *et al*., 2023). The presence of phytochemicals and poisonous metabolites in certain plants may make them non-edible and, as a result, less used. Since ancient times, many legume plants have been a component of human diets, although some have not been used to their full potential. The chemical and sensory qualities of such leguminous plants must be improved in order for them to be considered edible food; therefore, efforts must be directed toward enhancing their organoleptic status and lowering the levels of anti-nutritional factors that have restricted their acceptability as food. Underutilized legumes can be processed using microbial activity to create products that are suitable for consumption as food and include nutrients that are available to the body (Abdullahi *et al*., 2021).

The food and beverage processing sectors have a long history of using fermentation processes. It has been applied in a variety of ways to both produce food and lengthen its shelf life. According to Senanayake *et al*. (2023), fermentation is a long-standing process that transforms less edible plant material into edible form for human consumption or cattle. Fermentation has the benefit of improving a plant's nutrition and edibility by reducing or eliminating harmful metabolites. Fermentation has a long history of turning inedible plant material into edible forms. For example, the non-edible seed of *Parkia biglobossa* has been fermented by microbes to create a condiment that is widely used in Nigerian cuisine and many other West African countries (Achi, 2005).

Through the actions of microorganisms like bacteria and fungi, fermentation is a metabolic process that breaks down complex organic substances into simpler molecules. Temperature, pH, moisture content, and oxygen presence or absence all have a direct impact on the fermentation product (Mensah and Twumasi, 2017). During the fermentation process, microorganisms break down complex bindings in the chemical constituents of plant-based raw materials, improving the biological availability of nutrients, enhancing food's sensory quality, improving food safety, imparting bio-preservative effects, degrading toxic and anti-nutritive factors, generating anti-oxidant and antimicrobial compounds, promoting probiotic functions, and fortifying with certain health-promoting bioactive compounds (Thapa and Tamang, 2015). Additionally, fermented foods have been linked with health several health and nutritional enhancement of food properties (Obafemi *et al*., 2022). This is mostly credited to the activities of the microbial community and chemical reactions (Şanlier *et al*., 2019).

A traditional technique for extending food's shelf life and improving its nutritional value is fermentation (Obafemi *et al*., 2022). It has been employed to maintain and fortify nutriment in plant and animal-based food material. Fermentation creates new biological molecules in food, modifies the anti-oxidative status, plus increases the effectiveness of chemical extraction. Recently, there has been a growing recognition that precision fermentation is a significant advancement in the upcoming fourth industrial revolution of the food business. Precision fermentation is gaining popularity as a way to modernize food production. Due to its capacity to lower waste boosts the creation of proteins, fats, and carbohydrates (Obafemi *et al*., 2022). Food processing has employed and continues to use a variety of *Aspergillus, Penicillium, Rhizopus, Saccharomyces*, and other fungal species, either in solid state or submerged fermentation under spontaneous or induced system (Garrido-Galand *et al*., 2021).

Certain plants that grow in the tropics yearly are thought to be less useful mostly because of their phytochemical composition, which may make them inedible. Such plants, when subjected to fermentation found to be used as food, condiments, or feed supplements. One of such underutilized plants of the tropic is *Albizia lebbeck*. *Albizia lebbeck* is among the underutilized legumes, and it belongs to the class Magnoliopsida and the family Fabaceae. It is a perennial plant that grows widely as well as domesticated in the tropics and subtropics. *Albizia lebbeck* can be called a multipurpose tree. Various classes of cattle can be fed its leaves, twigs, blooms, and immature pods; every part of the tree has been reported to have antimicrobial and pharmaceutical potential (Zia-Ul-Haq *et al*., 2013).

Despite its enormous uses in the plants, including the seeds, there is limited information on the effect of fermentation on the nutrient contents of the seed as well as the microorganisms that are responsible for the fermentation (Orwa *et al.*, 2019).

MATERIALS AND METHODS

Sample Collection

Albizia lebbeck dried pods were obtained along Akerebiata road, Ilorin, Kwara State, Nigeria. They were identified and assigned a voucher number (UILH/001/814/2023) at the Plant Biology (Botany) Department of the University of Ilorin, Ilorin, Kwara State, Nigeria.

Preparation of *Albizia lebbeck* Seeds Samples for Natural Fermentation

Dried pods of *Albizia lebbeck* were manually processed to remove the dried seeds. The husk from the seed was pulverized (50g) and homogenized with 20 ml sterile peptone water solution and left for 5 days to enhance maximum fungal growth and full sporulation on the decomposing husk (Adedayo and Sani, 2019).

Isolation of Fungi from Fermenting *Albizia lebbeck* Seeds

Enumeration of the total fungi was carried out on the pre-fermented sample after 5 days (Adedayo and Sani, 2019). Ten fold serial dilutions were performed using 10 g of sample in 90 ml of sterile distilled water, and 1 ml of the aliquot from the third dilution was plated on Potato dextrose agar using the pour plate method. Plates were incubated at laboratory conditions for 72 hours. Distinct colonies observed were counted, purified, and stored for further analysis (Olutiola *et al.*, 2000).

Characterization and Identification of Fungal Isolates

The cultural characteristics of the isolates on the PDA plates were observed by staining the cultures with lactophenol blue on a sterile grease-free glass slide and placed under the microscope to view at x10 and x 40 (Olutiola *et al.*, 2000).

Molecular Identification of Fungal Isolates

Genomic DNA was extracted from the Samples received using the Quick-DNA™ Fungal/Bacterial kit (Zymo Research, C Atalogue No.D6005). The ITS target region was amplified using OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486). The PCR products were run on a gel and cleaned up enzymatically using the EXOSAP method. The extracted fragments were sequenced in the forward and reverse direction (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050). The purified fragments were analyzed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction for every sample, as listed in Section 1. DNASTAR was used to examine and assess the Ab1 files produced by the ABI 3500XL (Victor *et al.*, 2021)

Phylogenetic Tree Construction

A phylogenetic tree was generated using MEGA X (M11) software to determine the similarity and analyze the evolutionary relationship between the identified fungi and selected fungi strains obtained from Gene Bank of The National Center for Biotechnology Information (NCBI) based on the consensus sequence of ITS1 and ITS4 gene regions. The evolutionary relatedness was confirmed by using the Maximum Likelihood method and the Tamura-Nei model (Tamura and Nei, 1993). The bootstrap consensus tree inferred from 10 replicates is taken to represent the evolutionary history of the taxa analyzed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test 10 replicates) are shown next to the branches (Tamura *et al.*, 2021). This analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 117 positions in the final dataset. Evolutionary relatedness was determined using MEGA11 (Tamura *et al.*, 2021). Genetic Analyzer and results were obtained by a BLAST Search (NCBI).

Induced Fermentation Process of *Albizia lebbeck* Seed

Preparation of Inoculum

Fully sporulated Petri dishes containing pure cultures of the isolated fungi were covered with 10 ml of sterile deionised water (Abarna and Vishnupriya, 2022). The colony was twirled to dislodge the spores (Balogun *et al.*, 2022).

Solid-State Fermentation of *Albizia lebbeck* Seed

To initiate the fermentation, 30 g of substrate was weighed and transferred into a plastic container (fermenter) using the procedures described by Zhang *et al.* (2023). Prior to autoclaving the fermenting medium at 121^{0}C for 15 minutes (Celik *et al.*, 2020), the substrate was moistened with 10 ml of deionised water. Following sterilization, the fermenting medium was inoculated with 3 ml of the prepared and standardized inoculum containing the fungal spores and distilled water; at standard, 1 ml of spore inoculum contains about 1.5x10^8 CFU/ml (Audu *et al.*, 2023).

To ensure that the inoculum was evenly distributed throughout the substrate, the fermenter was covered with a lid and vigorously shaken. Fermentation lasted for a period of 5 days at laboratory conditions of temperature, humidity, and aerobic. After that, fermentation was arrested by altering the temperature of the medium, in this case by drying for 24 hours at 40 ^{0}C in an oven until constant weight was achieved.

Proximate Analysis of Pre-fermented and Post-Fermented *A. lebbeck* Seed Samples

The proximate composition of the pre and post-fermentation samples of *A. lebbeck* seed was determined according to AOAC (2020), Osman *et al.* (2019), and Tesfaye *et al.* (2024). The parameters that were monitored include crude protein, crude fats, crude fibre, moisture, and carbohydrate contents.

Determination of Moisture content

A predetermined amount of the sample was weighed and placed in a crucible. The crucible was heated in an oven at 105 ^{0}C for 1 hour. The crucible was cooled in a desiccator and then weighed. The heating and cooling cycle was carried out until a constant weight was reached. The moisture (%) content was calculated using the difference in weight (AOAC, 2020; Osman *et al.*, 2019; Tesfaye *et al.*, 2024).

Determination of Crude Protein Content

The Kjeldahl wet digestion method was adopted to determine the total nitrogen content of the fermented samples. The samples were digested and distilled. The percentage of nitrogen and the corresponding crude protein (CP) content was calculated (AOAC, 2020; Osman *et al.*, 2019; Tesfaye *et al.*, 2024).

Estimation of Crude Fat Content

The Soxhlet extraction technique was used to determine the crude fat content using the ether extract of the samples (AOAC, 2020; Osman *et al.*, 2019; Tesfaye *et al.*, 2024).

Determination of Ash Content

The ash content was determined using a known quantity of sample in a crucible that had weighed. The sample was then incinerated in a muffle furnace at 550 ˚C for 8 hours. The sample was then left to cool in a desiccator, and its weight was measured. The ash content of the samples (%) was calculated using the difference in the weight of the sample and the ash generated (AOAC, 2020; Osman *et al.*, 2019; Tesfaye *et al.*, 2024).

Estimation of Crude Fiber Content

The crude fiber content was estimated through digestion, filtration, washing, drying, and incineration (AOAC, 2020; Osman *et al.*, 2019; Tesfaye *et al.*, 2024).

Determination of Carbohydrate Content

The digestible carbohydrate was calculated by subtracting the combined values of moisture, ash, crude fat, crude fiber, and crude protein from 100, as described in the formula (Tsefaye *et al.*, 2020):

Carbohydrate (%) = 100 − [(M (%) + A(%) + CFa (%) + CFb (%) + Cp (%)]

where: M = moisture; A = ash; CFa = crude fat; CFb = crude fiber; and Cp = crude protein.

Phytochemical Analysis of Unfermented and Fermented *Albizia lebbeck* seeds

Fermented and unfermented seed of *Albizia lebbeck* seeds were subjected to qualitative and quantitative phytochemical screening to confirm the presence and the amount of phytochemical compounds such as alkaloid, flavonoid, glycosides, tannins, saponin, phytate, oxalate, steroids, flavonoids, terpenoid, triterpenes, coumarins, phlobatamins, anthocyanins, polyphenol, and glycoside and others were done according to AOAC (2020). Steroids, terpenoids, triterpenes, coumarins, phlobatamins, and anthocyanins were not determined in the post-fermentation samples.

Determination of Tryptophan Content (DPPH Radical Scavenging assay)

The total free radical scavenging capacity of the extracts from the samples was estimated according to a previously reported method (Brand-Williams *et al.*, 1995) with slight modification using the stable DPPH radical, which has an absorption maximum of 515 nm. A solution of the radical is prepared by dissolving 2.4 mg DPPH in 100 ml methanol. A test solution (5 µl) was added to 3.995 ml of methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 30 min in the dark. The absorbance of the reaction mixture was measured at 515 nm spectrophotometrically. The absorbance of the DPPH radical without anti-oxidant, i.e., blank, was also measured. All the determinations were performed in triplicate. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH Scavenged (%)= ((AB-AA)/AB)×100…..(1),

where, AB is absorbance of blank at t= 0 min; AA is absorbance of the antioxidant at t= 30 min. A calibration curve was plotted with % DPPH scavenged versus concentration of standard anti-oxidant (Trolox).

Determination of Amino Acid Profile

The Amino Acid profile in the known sample was determined using the methods described by Benitez (1989). The sample was dried to

constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator, and loaded into the Applied Biosystems PTH Amino Acid Analyzer. The sample was defatted using a chloroform/methanol mixture of a ratio of 2:1. About 500mg of the sample was put in an extraction thimble and extracted for 15 hours in the soxhlet extraction apparatus (AOAC, 2020).

Mineral Content Analysis

The mineral composition was measured using the "Buck Scientific Atomic Absorption Spectrophotometer Model 210A" AOAC (2020) after the samples had been digested using concentrated Trioxo-nitrate (V) acid. Both macro and micro mineral contents were analyzed.

The samples after acid digestion were used for elemental analysis. Sodium and potassium were estimated by a flame photometer (Flame Photometer Model-EEL). An atomic absorption spectrophotometer (Perkin Elmer Model 5000) was used to measure calcium, manganese, magnesium, zinc, copper, and iron, while the phosphovanado method was used to determined phosphorus–molybdate (Zia-Ul-haq *et al.*, 2012). The samples were quantified against standard solutions of known concentration that were analyzed concurrently (Kirtikar and Basu, 2000).

Statistical Analysis

All analyses were carried out in triplicates. Analysis of variance (ANOVA) plus Duncan's multiple range test was used for the comparison of means using SPSS software (version 16.0 for Windows, SPSS Inc. Statistical significance was recorded at P≤0.05.

RESULTS

Total Fungal Count and Morphological Identification of Fungal Isolates

The total fungal count from the pre-fermented sample was 1.3 x 10^5 CFU/g (Table 1). Three (3) distinct fungal colonies were purified and identified based on characteristics such as hyphae type, thickness, presence or absence of conidia, types of conidia, the colour of conidia, and others. These are presented in Table 2 and Figures 1 and 2

Molecular Identification of Fungal Isolates

The molecular identification is presented in Table 3 with the gel electrophoresis image presented in Figure 1. The isolated fungi were confirmed as *Aspergillus niger* strain (OR879114), *Penicillium citrinum* strain (OR879113), and *Cladosporium cladosporioides* strain (OR879115). The isolates had 90 and 100 % homologies with related taxa, as shown in Figure 2.

Proximate composition of samples prior to and after fermentation

Fermentation by the fungi affected the proximate content of the seed of *A. lebbeck* (Table 4). The effect of fermentation was significantly different (P ≤ 0.05) in the fermented sample compared with the pre-fermented sample. The sample fermented with *P. citrinum* has the highest improvement in proximate content when compared to other samples. There was an increase in crude protein from 10.79±0.53 to 22.69±1.83, Total ash from 5.16±0.02 to 6.84±0.03, crude fat from 0.98±0.05 to 5.03±0.22 while crude fibre reduced from 30.19± 2.03 to 28.96±1.42, and carbohydrate from 45.99±3.01 to 25.95±1.03 in sample fermented by *P. citrinum*.

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Table 1: Total Fungal Count during Spontaneous Fermentation of *A. lebbeck*

Sample	Total fungal count (CFU/g X10^5)
Spontaneously-fermented *A. lebbeck*	1.3

Table 2: Morphological Identification of Isolated Fungi from Fermented *A. lebbeck*

Isolates	Cellular and Morphological Characteristics	Probable Fungi
Z1	They are initially white to yellow and then turn black with time, appearing cottony with black spores. The reverse is white to cream. Made up of conidiophores. In microscopy, the conidial heads are globose; hyphae are septate.	*Aspergillus niger*
Z3	Fast growing, grey but sometimes whitish, mostly consisting of dense conidiophore. Chains of single conidia were formed from conidiophores on a phialide.	*Penicillum* sp.
Z4	Species produce black colonies, with dark reverse and have dark-pigmented conidia that are formed in simple or branching chains. Septate dark hyphae, erect and pigmented conidiophores, and conidia.	*Cladosporium* sp.

Table 3: Molecular Identification of Fungal Isolates

Isolates	Percentage ID (%)	Gen Bank Accession Number	Names of Fungal Organisms
Z1	90.00	OR879114	*Aspergillus niger* (OR879114)
Z3	90.00	OR879113	*Penicillium citrinum* (OR879113)
Z4	100.00	OR879115	*Cladosporium cladosporioides* (OR879115)

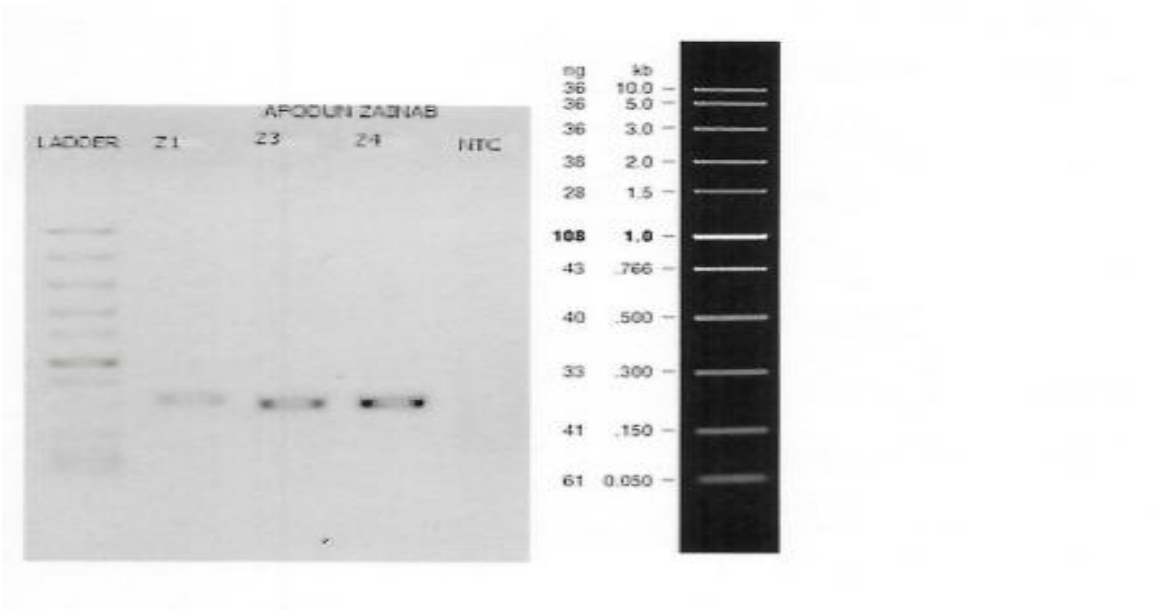

Figure 1: Visualization of extracted DNA by gel electrophoresis. Patterns in the gel confirmed the presence of extracted DNA.
Key: Z1=*Aspergillus niger* (strain OR879114), Z3= *Penicillium citrinum* (strain OR879113) and Z4=*Cladosporium cladosporioides* (strain OR879115).

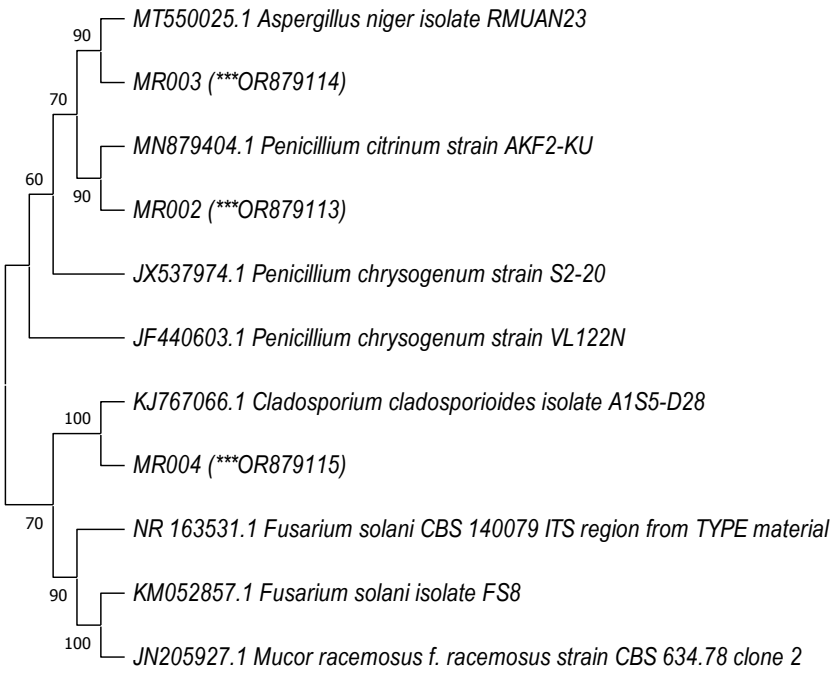

Figure 2: Phylogenetic Tree Constructed by Maximum Likelihood method. The Isolates (Accession Numbers: OR879113, OR879114 and OR879115**) designated with asterisks*****

Table 4: Proximate Composition of Pre and Post-Fermented Samples

Parameters (%)	Samples			
	Unfermented	Fermented		
		A. niger	*P. citrinum*	*C.cladosporioides*
Moisture content	6.90±0.08^{a}	9.57±0.39^{d}	9.38±0.33^{c}	8.46±0.13^{b}
Crude protein	10.79±0.53^{a}	20.92±1.32^{c}	22.69±1.83^{d}	19.11±1.03^{b}
Total ash	5.16±0.02^{a}	8.52±0.07^{c}	9.29±0.12^{d}	6.84±0.03^{b}
Crude fibre	30.19± 2.03^{c}	29.11±1.70^{b}	28.96±1.42^{a}	31.90±2.18^{d}
Crude fat	0.98±0.05^{a}	4.64±0.18^{c}	3.75±0.12^{b}	5.03±0.22^{d}
Carbohydrate	45.99±3.01^{d}	27.25±1.13^{b}	25.95±1.03^{a}	28.68±1.23^{c}
Calorific value (Kj/100g)	985.13±16.07^{c}	995.42±16.09^{d}	993.98±15.91^{a}	995.08±16.03^{b}

Values are means of triplicates ± SD
Mean values with the same superscript across the row are not significantly different (P≤0.05).

Qualitative and Quantitative Phytochemical Composition and Anti-oxidant of Sample Prior Fermentation and after fermentation

The phytochemicals content detected qualitatively in the sample were saponin, tannins, alkaloid, flavonoids, steroids, phenolics, glycoside, terpenoids, triterpenes coumarins, while Phlobatanin and Antholyarin were not detected (Table 4). The tannins, glycosides, phytate, oxalate, alkaloids, and trypsin inhibitors contents were significantly reduced after fermentation by the fungi. The tannin content was significantly reduced from 12.14±0.20 to 0.341±0.01 in the sample fermented by *P. citrinum*, and alkaloids were significantly reduced from 20.70±2.18 to 0.799±0.08 in the sample fermented by *A. niger*. There were similar trends in all the fermented samples, as all the phytochemicals screened were significantly reduced in concentrations. Results of qualitative and quantitative phytochemical content of samples pre and post-fermentation are presented in Tables 5 and 6.

Anti-oxidant Concentrations Pre and Post Fermentation of *A. lebbeck*

DPPH concentration after fermentation by the fungi was the highest concentration with the sample fermented with *Cladosporium* species (75.70±3.87mgGAE/g), followed by the sample fermented with *Penicillium citrinum* (74.70±3.20mgGAE/g), while sample fermented with *Aspergillus niger* recorded the lowest concentration (73.40±3.01mgGAE/g). The result is presented in Table 7

Table 5: Qualitative Phytochemical Composition of Unfermented and Fermented Samples

Phytochemicals	Fermented Samples			
	Pre-fermented	*A.niger*	*P. citrinum*	*C. cladosporioides*
Saponin	+	++	++	++
Tannin	++	+	+	+
Alkaloids	++	+	+	++
Flavonoid	++	++	+	++
Steroids	+	ND	ND	ND
Phenolics	++	+	ND	+
Glycoside	+	+	+	++
Terpenoids	+	ND	ND	ND
Triterpenes	++	ND	ND	ND
Coumarins	+	ND	ND	ND
Phlobatanin	ND	ND	ND	ND
Anthocyanin	ND	ND	ND	ND
Phytate	ND	+	+	++
Trypsin	+	+	+	++

Key: ND = not detected + = mildly detected ++ = moderately detected

Table 6: Quantitative Phytochemical Composition of Unfermented and Fermented Samples

Phytochemicals	Fermented samples			
	Pre-fermented	*A. niger*	*P. citrinum*	*C. cladosporioides*
Saponin	0.059±0.005^{a}	72.99±3.22^{b}	88.04±4.31^{c}	89.95±4.72^{d}
Tannin	12.14±0.20^{d}	0.355±0.02^{ab}	0.341±0.01^{a}	0.598±0.08^{c}
Alkaloids	20.70±2.18^{d}	0.799±0.08^{a}	1.046±0.10^{b}	1.268±0.13^{c}
Flavonoid	292.00±11.23^{d}	0.100±0.05^{b}	0.066±0.01^{a}	0.174±0.07^{c}
Steroids	1.52±0.002	ND	ND	ND
Oxalate	-	0.106±0.03^{a}	0.140±0.05^{b}	0.181±0.12^{c}
Phenolics	18.90±2.03^{d}	0.035±0.02^{b}	0.024±0.01^{a}	0.081±0.05^{c}
Glycoside	0.19±0.05^{b}	0.121±0.03^{a}	0.201±0.06^{b}	0.475±0.12^{c}
Trypsin	-	0.0095±0.001^{a}	0.0161±0.005^{c}	0.0155±0.003^{b}
Terpenoids	3.12±0.07	-	-	-
Triterpenes	11.16±0.13	-	-	-
Coumarins	0.71±0.009	-	-	-
Phlobatanin	0.00±0.00	-	-	-
Anthocyanin	0.00±0.00	-	-	-
Phytate	0.059±0.005^{a}	1.038±0.01^{b}	1.079±0.03^{b}	2.964±0.10^{c}

Key: **- = not detected**
Values are means of triplicates ± SD
Values within the same row having different superscripts are significantly different (P≤0.05)

Table 7: Anti-oxidant Concentrations Pre and Post Fermentation of *A. lebbeck*

Phytochemicals	Fermented Samples			
	Pre-fermented	*A. niger*	*P. citrinum*	*C. cladosporioides*
DPPH (mgGAE/g)	+	73.4±3.01^{a}	74.7±3.20^{b}	75.7±3.87^{c}

Key: +=Detected
Values are means of triplicates ± SD.
Values within the same row having different superscripts are significantly different (P≤0.05)

Mineral Contents of Post-Fermented Samples of *A. lebbeck*

Table 4 revealed the results of mineral composition samples after fermentation with fungal species. There was a significant difference in the values of mineral content in samples fermented by the isolated fungi. The results showed that sodium concentration was highest with the sample fermented with *Cladosporium* species (70.54±3.83mg/100g), followed by the sample fermented with *Aspergillus niger* (69.59±3.41mg/100g) while the least (65.99±2.33mg/100g) was recorded in sample fermented with *Penicillium citrinum*. Calcium concentration was highest in the sample fermented with *Penicillium citrinum* (325.10±4.18mg/100g), next was in the sample fermented by *Aspergillus niger* (319.12±3.77mg/100g) while the lowest (319.10±3.72mg/100g) was recorded in sample fermented with *Cladosporium* species (Table 8).

Amino acids composition of samples after fermentation

Fungal fermentation of the seed has effects on the amino acid profile. There was a significant difference in values of amino acid after fermentation with each of the isolates. Aspartic acid was found to be the major component in the sample (18.61, 19.31, and 18.01 100 g^{-1} dry weight) for samples fermented with *Aspergillus niger*, *Penicillium citrinum*, and *Cladosporium* species, respectively, followed by glutamic acid (14.31, 15.00 and 13.59g 100 g^{-1} dry weight) for samples fermented with *Aspergillus niger*, *Penicillium citrinum*, and *Cladosporium* species respectively while the least amino acid component was Tryptophan (1.00, 0.94 and 0.80 100 g^{-1} dry weight) in the same trend (Table 9).

Table 8: Mineral Composition of *A. lebbeck* Samples after Fermentation

Mineral Contents (Mg/100g)	Fermented Samples		
	A. niger	*P. citrinum*	*C. cladosporioides*
Sodium (Mg/100g)	69.59±3.41^{b}	65.99±2.33^{a}	70.54±3.83^{c}
Calcium (Mg/100g)	319.12±3.77^{a}	325.10±4.18^{b}	319.10±3.72^{a}
Potassium(Mg/100g)	497.68±4.30^{b}	498.81±4.51^{c}	496.76±4.21^{a}
Magnesium(Mg/100g)	375.34±3.35^{c}	356.58±2.53^{a}	365.26±3.03^{b}
Phosphorus(Mg/100g)	418.42±3.32^{a}	418.99±3.51^{c}	418.90±3.44^{b}
Copper (mg/kg)	49.53±1.02^{a}	56.11±1.13^{b}	49.63±1.07^{ab}
Iron (mg/kg)	0.144.34±1.33^{a}	144.35±1.35^{b}	144.34±1.33^{a}
Zinc (mg/kg)	29.15±1.01^{c}	27.36±0.71^{b}	23.10±0.53^{a}
Manganese (mg/kg)	42.30±2.13^{b}	42.96±2.37^{c}	30.70±1.23^{a}

Values are means of triplicates ± SD.

Values within the same row having different superscripts are significantly different (P≤0.05)

Table 9: Amino acid Profile of *A. lebbeck* Samples after Fermentation

Amino Acid	Fermented Samples		
	A. niger	*P. citrinum*	*C. cladosporioides*
% N (fat-free)	4.09	3.37	3.96
Wt of hydrolysate	0.338	0.337	0.431
Leucine	6.12	6.30	5.66
Lysine	5.66	5.41	5.16
Isoleucine	4.21	4.59	4.06
Phenylalanine	3.82	4.01	3.55
Tryptophan	1.00	0.94	0.80
Valine	4.41	4.61	4.18
Methionine	1.22	1.30	1.10
Proline	5.01	4.90	4.65
Arginine	4.65	4.85	4.21
Tyrosine	3.06	3.45	2.91
Histidine	2.20	2.29	1.88
Cystine	1.99	2.25	1.76
Alanine	4.66	4.31	4.09
Glutamic acid	14.31	15.00	13.59
Glycine	3.96	4.11	3.64
Threonine	3.11	3.01	2.81
Serine	4.21	4.55	3.96
Aspartic acid	18.61	19.31	18.01

DISCUSSION

The fungi that were identified (*Aspergillus niger* (OR879114), *Penicillium citrinum* (OR879113), and *Cladosporium cladosporioides* (OR879115)) belonged to the saprobitic group and had a special capacity for breaking down organic materials. Their capacity to release extracellular enzymes to predigest the substrates they are growing upon improves this feeding strategy. The fungal mycelium absorbs the basic nutrients from digestion for metabolism and development. This mold's interwoven network of hyphae makes it easier for extracellularly digested substrates to be absorbed and transported. The presence in the soil of conidia and hyphae of these fungi in the soil and air environment make them available for the decomposition of the A. lebbeck seed.

Previous authors have isolated similar fungi from naturally decomposing organic materials (Adedayo and Sani, 2019; Kawata *et al.*, 2024). The molecular study further confirmed the identity of the *Aspergillus niger* strain, *Penicillium citrinum* strain, and *Cladosporium cladosporioides* strain. The observed homologies of 90 and 100 % with related taxa further support the accurate identification of these isolates. The high homologies suggest a close evolutionary relationship with the referenced strains, supporting the reliability of the molecular identification. The fungal isolates have a previous history of relevance in fermentation processes (Adedayo and Sani, 2019; Kawata *et al.*, 2024).

There was a significant increase in the proximate content of the fermented samples. Microbial fermentation has been reported to have various desirable impacts on the proximate content of organic substrates (Garrido-Galand *et al.*, 2021; Toor *et al.*, 2022; Deveci *et al.*, 2023). This observation is mostly due to the breaking down of complex molecules into simpler units through fungal metabolism, thereby making the nutrients in the molecules biologically available. The improvement in protein contents of the fermented sample added to the nutritional quality of the processed seeds. This enhancement in protein contents was similar to the findings of several authors while fermenting grain legumes, which had been attributed to the ability of the fermenting microbiota in substrates to utilise the carbohydrate contents, thereby resulting in their proliferation (Adegbehingbe *et al.*, 2017; Ifesan *et al.*, 2017). The observed increase in ash content of the fermented seeds, traceable to hydrolysis of some of the anti-nutrient contents, which naturally bound some of the mineral elements (Ifesan *et al.*, 2017), is desirable as this enhances the availability of the minerals. The process of fermentation has been reported to have a significant effect on the crude fibre content of seeds (Bankefa and Oyedeji, 2015) while fermenting date fruits with *Bacillus* species. The observation in this research is in line with this report. The relative decrease in crude fibre content due to fungal predigestion is of benefit, and the residual crude fibre may infer that the product will maintain the health of the gastrointestinal tract when consumed (Audu and Aremu, 2011). The significant increase in fat content may be due to the residual fungal mycelium in the fermented samples. It could also be due to poor leaching during fermentation. Furthermore, it might as well be adduced to lipolytic enzymes produced by the natural microflora, which hydrolyzed the fats in the sample to glycerol and fatty acids. The carbohydrate and calorific values of the products of fermentation are desirable for substances to be used as food (Andeta *et al.*, 2018). These significant changes in nutritional contents suggest that the fermentation process has the potential to enhance the nutritional value of *Albizia lebbeck* seeds, making them a more valuable resource in various applications, such as animal feed and supplements (Bosha *et al.*, 2016). Phytochemicals are useful in food at certain concentrations beyond which they become detrimental. There was notable statistical significance in the phytochemical content of the fermented products, similar to earlier reports (Vashishth *et al.*, 2017). It is, however, noteworthy that all the phytochemicals did not respond in the same manner to fermentation as noticed with tannins, alkaloids, phenolics, and flavonoids, which experienced reduction against saponin and phytate where it was an increase (Zhang *et al.*, 2015). These findings highlight the specificity of the fermentation process, indicating that certain compounds remain relatively stable, maintaining their original concentrations (Fowomola and Akindahunsi, 2008). Tannins have a stringent taste, which affects palatability and decreases feed intake. The anti-nutritive attributes of tannins in foods include impaired utilization of minerals and vitamins and inhibition of digestive enzymes. Phytates and oxalates form insoluble complexes with proteins and minerals such as zinc, iron, magnesium, and calcium, which can lead to a decrease in protein solubility and minerals availability, rendering them unavailable for absorption into the bloodstream (D'souza, 2013).

Tannins in legumes are known to inhibit the activities of digestive enzymes and nutritional effects are mainly related to their interaction with protein and minerals. They also reduce the absorption of nutrients such as vitamin B_{12}. Tannin-protein complexes are insoluble and this decreases the protein digestibility. Oxalate forms complexes with minerals example is the calcium-oxalate complex. This can result in kidney stones and could impair renal functions. The general reduction observed in some phytochemicals could be due to leaching or they are being metabolized by the microorganisms involved during the fermentation, while hydrolyses of the constituent anti-nutrient contents could be responsible for the increases in some of them (Adegbehingbe, 2014). The better rating of the fermented samples could be due to the activity of microorganisms on the fermented seeds. The observation on anti-nutrients after fermentation indicated that fermentation is an effective technique for enhancing the utilisation of the seeds. Metabolism by bacteria and fungi have been reported to be effective in degrading anti-nutritive factors and natural toxins. Trypsin inhibitors impair the digestion of protein, thereby reducing the nutritional value of seeds that contain them in excesses, limiting their usefulness as food (Adegbehingbe, 2014). Saponins and phytate content were observed to increase after fermentation. Saponins are known for their bitter taste and potential properties to lyse blood cells at very high concentrations, it has a bitter taste and affects palatability of (Adebowale *et al.*, 2005). Phytates and oxalates form insoluble complexes with proteins and minerals such as zinc, iron, magnesium, and calcium, which can lead to a decrease in protein solubility and minerals availability, rendering them unavailable for absorption into the bloodstream. The reduction in phytic acid during fermentation has been attributed to the action of the microbial enzyme phatase (D'souza, 2013). Their presence affects the bioavailability of minerals (Duffus and Duffus, 1991).

Fermentation generally improves the availability and accessibility of mineral content in the food samples/seed. Fluctuations observed in the mineral contents of the samples after fermentation could be due to several factors. The reduction observed in some of them could be due to leaching, or they are being metabolized by the microorganisms involved during the fermentation, while hydrolyses of the constituent anti-nutrient contents could be responsible for the increases in some of them (Adegbehingbe, 2014). The breaking down of complexes formed by phytochemicals with the minerals could also contribute to the changes recorded. The better rating of the fermented samples could be due to the activity of microorganisms on the fermented seeds.

The observed trends in the results of the mineral composition provided valuable insights into the nutritional changes induced by solid-state monoculture fungal fermentation in *Albizia lebbeck* seed. While potassium showed a significant increase, other minerals did not exhibit marked alterations. These findings contribute to our understanding of the nutritional implications of the fermentation process and lay the groundwork for further exploration into the broader impact on seed composition and potential health benefits. DPPH concentration after fermentation using species of fungi recorded the highest concentration in sample fermented with *Cladosporium cladosporioides*. Fungi play important roles in lignocellulose degradation and have been exploited for the production of fermented lignocellulose-based feed materials. They grow on solid substrates with low moisture content. A complex set of enzymes is involved in lignocellulose degradation such as ligninases, cellulases, and hemicellulases.

Further results revealed the detection of 18 amino acid components in the *Albizia lebbeck* seed sample analyzed. The amino acid profiles in the fermented samples are significant in daily diet, and the microbial degradation has further enhanced their ability (Adedayo and Sani, 2015; Pesante *et al.*, 2023). Fermented *A. lebbeck* could be considered a good source of essential amino acids. Fermentation with *Penicillium citrinum* strain OR879113 has the best products, while the least was observed in products fermented with *Aspergillus niger* OR879114.

CONCLUSION

The findings from this study show that solid-state fermentation could significantly improve the nutriment value of *Albizia lebbeck* seed and could also reduce the anti-nutrient contents of the seeds. Generally, there was a significant difference ($P \leq 0.05$) in the proximate composition of the post-fermented seed; the crude protein and total ash content increased while the total carbohydrate decreased, and some of the phytochemical content reduced significantly (tannins and alkaloids) sample fermented with *Penicillum citrinum* (OR879113). Anti-oxidant, mineral contents, and the amino acid profile were also remarkably improved. Thus suggesting that fermented *Albizia lebbeck* seed could be considered as a

potential condiment as well as a protein supplement in animal feed. It could invariably add to the existing sources of plant protein, cheaper and readily available than other conventional protein sources. This will ameliorate the competition between man and his livestock for the existing protein food.

RECOMMENDATIONS

Further work on fermented *Albizia lebbeck* seed is recommended for the possible use as a condiment as well as a protein supplement in animal feed; the fermentation could be scaled up and improved upon to an industrial scale that can contribute to the national economic development through the use of the fermented seed in feed formulation and as a supplement to support livestock husbandry. Acid or alkali pre-treatment of seed prior to fermentation could improve product formation and hasten, hence reducing the fermentation period; the culture parameters for fermentation could be studied for fungal enzyme production, and the dominant isolates could be studied as starter cultures for future fermentation.

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