Traditional Sonru production as a bioprocess for detoxification and nutritional enhancement of *Mucuna pruriens*seeds.

Abstract

Mucuna pruriens is a protein-rich underutilized legume with considerable potential for food and nutrition security. However, its utilization for human consumption is limited by the presence of high concentrations of antinutritional compounds, particularly L-3,4-dihydroxyphenylalanine (L-DOPA). This study evaluated the effectiveness of traditional Sonru production as a bioprocess for detoxification and nutritional enhancement of *Mucuna pruriens* seeds. Seeds were subjected to soaking, dehulling, cooking and fermentation using *yanyanku*, a traditional starter culture, for 72 h under ambient conditions. Samples collected at 0, 24, 48 and 72 h were analyzed for physicochemical characteristics, antinutritional factors and microbial populations. Pearson correlation analysis, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to elucidate relationships among fermentation variables. During fermentation, pH increased significantly from 5.61 to 6.24, whereas moisture, crude protein and crude fat decreased from 75.2 to 63.7%, 38.4 to 33.3 g/100 g, and 4.64 to 2.31 g/100 g, respectively. Ash content increased from 3.87 to 5.78 g/100 g. Fermentation markedly reduced L-DOPA (1905 to 30.3 mg/100 g; 98.4%), phytates (12.3 to 2.67 mg/100 g; 78.3%), oxalates (155 to 41.9 mg/100 g; 73.0%) and trypsin inhibitor activity (6.26 to 3.32 TIU/mg protein; 46.9%), resulting in concentrations below recommended safety thresholds. Total viable counts, *Bacillus cereus* and *Staphylococcus* spp. increased significantly and were strongly negatively correlated with L-DOPA concentration ($r = -0.995$, -0.987 and -0.983, respectively; $p < 0.001$). PCA and HCA confirmed a close association between microbial proliferation, increased pH and antinutrient degradation. These findings demonstrate that traditional Sonru production is an effective strategy for detoxifying

M. pruriens and improving its nutritional quality, thereby supporting its utilization as a sustainable alternative protein source.

Keywords:*Mucunapruriens*;Sonru;fermentation;detoxification; L-DOPA;antinutritionalfactors;*Bacillus*spp.;sustainableprotein source.

1. Introduction

The growing demand for sustainable protein sources has intensified interest in underutilized legumes that can contribute to food and nutrition security, particularly in developing countries. Among these alternative protein crops, *Mucuna pruriens* (velvet bean) has attracted considerable attention due to its high protein content, adaptability to marginal environments, soil-improving properties, and resilience to climatic stresses. The seeds contain between 23 and 35% protein, appreciable amounts of carbohydrates, minerals, dietary fiber, and several bioactive compounds, making them a promising resource for both human food and animal feed applications (Adebowale & Lawal, 2003; Sridhar & Bhat, 2007). Despite these nutritional advantages, the widespread utilization of *Mucuna pruriens* remains limited.

The major obstacle to the exploitation of *Mucuna* seeds is the presence of high levels of antinutritional and toxic compounds. Among these, L-3,4-dihydroxyphenylalanine (L-DOPA) is considered the most critical because concentrations can reach 3-10% of seed dry weight depending on the genotype and growing conditions (Siddhuraju et al., 1996; Vadivel & Janardhanan, 2000). Although L-DOPA is a therapeutic precursor used in the treatment of Parkinson's disease, excessive dietary intake may cause adverse physiological effects, including nausea, vomiting, gastrointestinal discomfort, and neurological disturbances (Sridhar & Bhat, 2007). In addition to L-DOPA, *Mucuna* seeds contain phytates, oxalates, tannins and protease inhibitors that reduce nutrient digestibility and mineral bioavailability (Samtiya et al., 2020; Vadivel & Janardhanan, 2000). Consequently, the development of

efficient processing techniques capable of reducing these antinutritional factors is essential before the seeds can be safely incorporated into human diets.

Traditional food-processing technologies such as soaking, dehulling, cooking, germination and fermentation have been widely reported as effective strategies for the detoxification of legumes. These processes reduce antinutrients through mechanisms including leaching, enzymatic degradation, thermal destruction and microbial metabolism (Samtiya et al., 2020; Shimelis & Rakshit, 2007). Among them, fermentation represents one of the most promising approaches because it simultaneously improves food safety, nutritional quality, sensory properties and shelf life. During fermentation, microorganisms produce diverse hydrolytic enzymes capable of degrading phytates, tannins, oxalates and proteinaceous inhibitors while promoting the formation of desirable flavour compounds and increasing nutrient bioavailability (Marco et al., 2021; Tamang et al., 2016).

In West Africa, several traditional alkaline-fermented condiments are produced from protein-rich legumes and oilseeds. Products such as Soumbala, Dawadawa, Afitin, Iru and Sonru are characterized by the predominance of *Bacillus* species, which drive extensive proteolysis and contribute to flavour development and detoxification (Azokpota et al., 2010; Hongbété et al., 2017; Oladeji & Ogidi, 2025; Ouoba, Cantor, et al., 2003; Parkouda et al., 2009). Sonru is a traditional fermented condiment consumed in Benin and produced using indigenous starter cultures known locally as *yanyanku*. Although Sonru production has been traditionally applied to various legumes, scientific information regarding its application to *Mucuna pruriens* remains scarce. In particular, little is known about the dynamics of microbial populations, the evolution of physicochemical characteristics, and the extent of antinutrient degradation during fermentation of *Mucuna*-based Sonru.

Understanding the interactions between microbial growth, biochemical transformations and detoxification processes is essential for the valorization of *Mucuna pruriens* as a sustainable

food resource. Multivariate statistical approaches, including correlation analysis, principal component analysis (PCA) and hierarchical cluster analysis (HCA), provide valuable tools for identifying the key factors associated with successful fermentation and detoxification. Such information is necessary for optimizing traditional fermentation processes and supporting the development of safe and nutritionally improved products from underutilized legumes.

Despite the recognized nutritional potential of *Mucuna pruriens* and the numerous studies reporting the effectiveness of conventional processing methods such as soaking, cooking, germination, and fermentation in reducing antinutritional factors, information on the application of traditional Sonruproduction to this legume remains extremely limited. Previous research has mainly focused on the nutritional composition of *M. pruriens*, the occurrence of L-DOPA and other antinutritional compounds, or the microbiological characteristics of alkaline-fermented condiments such as Sonru, Afitin, Iru, and Soumbala. However, little is known about the microbial succession, physicochemical changes, and detoxification dynamics that occur when *M. pruriens* is processed into Sonru. In particular, no study has comprehensively examined the relationships among microbial growth, physicochemical transformations, and the degradation of major antinutritional factors, especially L-DOPA, during traditional Sonru production. Furthermore, the potential contribution of fermentation-associated microorganisms to the detoxification of *M. pruriens* has not been sufficiently investigated. Therefore, the present study aimed to fill these knowledge gaps by monitoring changes in physicochemical characteristics, microbial populations, and antinutritional factors throughout the fermentation process and by applying multivariate statistical approaches to identify the key factors associated with detoxification and nutritional improvement during the production of Sonru from *Mucuna pruriens*.

2. Materials and Methods

2.1.Sonru based Mucuna seeds production

Mucuna seeds were purchased from agri-livestock farmers inTchaourou, Benin, and were used to produce Sonru according to traditional process described inFigure 1. First, the *Mucuna* seeds were sorted and cleaned to remove all foreign materials, broken seeds, and damaged seeds. After cleaning, the seeds were soaked for 48 hours at ambient temperature. The soaking water was replacedevery 6 hours to facilitate the removal of water soluble antinutritional compounds. Following soaking, the seeds were dehulled to remove the seed coats and minimize attrition loss. The cotyledons were washed with clean water. The dehulled seeds were then boiled at 100°C for 2 hours, with the water being changed three timesat 30 minutes intervals. After cooking, the seeds were cooled for 2 hours at room temperature under hygienic conditions.The cooked seeds were mixed with *yanyanku* (a traditional starter culture). The mixture was then transferred into sterile fermentation containers for 72 hours at ambient temperature. Samples of the fermented product were collected at 0, 24, 48, and 72 hours for physicochemical, microbiological, and antinutritional analyses in triplicate. The study was conducted using two independent processing batches.

2.2.Physicochemical analysis

Moisture, ash, crude fat, crude proteincontents were determined according to standard AOAC methods (AOAC, 1995).

2.3.Antinutrient analysis

L-DOPA content was determined using a modified LC-MS/MS method based on Hasegawa et al (2013) and Yumoto et al (2022). Briefly, 50 mg of flour from each *Mucuna* samples were extracted with 10 ml of 80% methanol (MeOH) containing L-DOPA-d3 (10 mg/100 mL MeOH) as an internal standard. The mixture was homogenized and centrifuged at 3500 rpm

for 10 min. The resulting supernatant was collected, diluted 10-to 1000-fold as required, and filtered through a 0.22 µm nylon membrane filter. The filtrate was subsequently analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for L-DOPA quantification. Phytate content was determined using ferric ion precipitation and colorimetric quantification methods (Raboy et al., 2020). Tannins were determined using the Folin-Denis method described by Elgailani e al. (2014). Total oxalates were determined by acid extraction following titrimetric analysis (Ezegbe 2023). Trypsin inhibitor activity (TIA) was determined using ISO 1492 standard method(Animal Feeding Stuffs — Determination of Trypsin Inhibitor Activity of Soya Products (ISO Standard N°. 14902:2001), 2001).

2.4 Microbiological analysis

Microbiological assessments were performed on fermented cotyledons before drying and on the final powder. Ten grams of each sample were homogenized in 90 ml of sterile peptone-salt solution, followed by serial dilutions. Total Viable Count (TVC) was determined on Plate Count Agar (PCA, Oxoid CM0325, Basingstoke, Hampshire, England), after incubation at 30 °C for 72 hours (ISO-4833, 2003). Yeasts and moulds were enumerated using Yeast Extract Agar (Oxoid CM0019, Basingstoke, Hampshire, England) supplemented with chloramphenicol (Oxoid SR0078E, Basingstoke, Hampshire, England) and the inoculated plates were incubated at 25°C for 3-5 days (ISO-7954, 1988). *Bacillus* spp. was enumerated on *Bacillus cereus* agar base (Oxoid, CM0617, Basingstoke, Hampshire, England) and incubated at 37 °C for 48 hours (ISO-7932, 2004). *Staphylococcus* spp. was enumerated on Baird-Parker (BP) agar base (Oxoid CM0275, Basingstoke, Hampshire, England) supplemented with egg yolk tellurite emulsion (SR54, Basingstoke, Hampshire, England) and incubated at 37 °C for 48 hours (ISO-6888, 1999)

2.5. Statistical analysis

Data were expressed as mean ± standard error from two independent production batches. Changes occurring during processing and fermentation were evaluated using descriptive statistics analyses. Relations among nutritional, antinutritional and microbiological variables were explored using Pearson correlation analysis. Principle component analysis (PCA) was performed to identify major sources of variables. Hierarchical cluster analysis (HCA) using Euclidean distances and Wards linkage method was applied to classify samples according to their compositional characteristics. Statistical analyses were carried out using Statistica version 7.1 (StatSoft, France) and Jamovi software version 2.7.12.

3. Results and discussion

3.1. Changes in physicochemical characteristics during Sonru production

The physicochemical characteristics of *Mucuna pruriens* cotyledons changed significantly during Sonru production (Table *1*). The pH increased progressively from 5.61 at the beginning of fermentation to 6.24 after 72 h, indicating a gradual alkalinization of the fermenting substrate. The absence of a marked acidification phase suggests that Sonru processing is predominantly driven by proteolytic rather than acidogenic microorganisms. The increase in pH may result from microbial proteolysis of storage proteins and the subsequent release of amino acids and ammonia, a hallmark of alkaline fermentations dominated by *Bacillus* spp. in traditional African fermented condiments (Ouoba, Rechinger, et al., 2003; Parkouda et al., 2009).

A significant reduction in moisture content was observed after 48 h, decreasing from 75.2% to 63.7% at the end of fermentation. This decline could be attributed to metabolic heat generation, surface evaporation, and structural modifications of cotyledon tissues during microbial activity.

Crude protein content gradually decreased from 38.4 to 33.3 g/100 g throughout fermentation. Different superscript letters indicate that all fermentation stages differed significantly (Tukey test, $p < 0.05$), suggesting continuous protein degradation during the process. The decrease may be explained by microbial proteolysis, production of soluble nitrogenous compounds and utilization of amino acids as energy sources. The reduction in protein and lipid contents further supports the occurrence of intense proteolytic and lipolytic activities, which are characteristic biochemical events during *Bacillus*-mediated alkaline fermentations (Ouoba, Cantor, et al., 2003; Ouoba, Rechinger, et al., 2003).

Similarly, crude fat content showed a significant decline from 4.64 to 2.31 g/100 g, with no significant difference between 48 and 72 h ($p > 0.05$). This pattern indicates that lipid hydrolysis occurred mainly during the first 48 h of fermentation, after which fat degradation reached a plateau. Lipolytic activity is likely associated with the development of fermentative *Bacillus* populations.

Conversely, ash content increased significantly from 3.87 to 5.78 g/100 g. The relative increase in ash concentration may be attributed to the loss of organic matter through microbial metabolism and moisture reduction, leading to concentration of mineral constituents. The stabilization observed between 48 and 72 h suggests that most compositional transformations were completed before the final fermentation stage.

These results indicate that Sonruproduction induced substantial biochemical modifications characterized by moisture reduction, degradation of proteins and lipids, and a relative enrichment in mineral matter. These changes are consistent with active microbial fermentation and contribute to the development of the final product quality.

3.2. Changes in antinutritional factors during Sonru production

The concentrations of major antinutritional factors decreased significantly during Sonru production (Table 2), demonstrating the effectiveness of the process as a detoxification strategy for *Mucuna pruriens*.

L-DOPA showed the most pronounced reduction, decreasing from 1905 mg/100 g at the beginning of fermentation to only 30.3 mg/100 g after 72 h, corresponding to a reduction of approximately 98.4%. The Tukey test revealed significant differences among all fermentation times ($p< 0.05$), indicating continuous degradation throughout the process. Importantly, the final concentration was well below the recommended safety threshold of 100 mg/100 g, confirming the effectiveness of Sonru production as a detoxification process for *Mucuna pruriens*, whose utilization is largely constrained by its high L-DOPA content (Sridhar & Bhat, 2007; Vadivel & Janardhanan, 2000).

Phytate concentration decreased steadily from 12.3 to 2.67 mg/100 g, representing a reduction of about 78%. The progressive decline suggests activation of endogenous and/or microbial phytases during fermentation. Since phytates are known to chelate divalent minerals, their degradation may improve mineral bioaccessibility in the fermented product. The final phytate level was below the recommended threshold of 5 mg/100 g. The marked reduction in phytate content is consistent with the activation of endogenous and microbial phytases during fermentation, which improve mineral bioavailability by hydrolyzing phytate-mineral complexes (Gupta et al., 2013).

Oxalate content followed a similar trend, decreasing significantly from 155 to 41.9 mg/100 g after 72 h, corresponding to approximately 73% reduction. The final concentration was lower than the recommended value of 50 mg/100 g, indicating substantial improvement in product safety and mineral utilization potential.

Trypsin inhibitor activity initially increased during the first 24 h of fermentation before decreasing sharply to 3.32 TIU/mg protein at 72 h. This transient increase may result from the release of inhibitor proteins from cellular structures during early fermentation, while the subsequent decline likely reflects microbial proteolysis and thermal instability of inhibitor molecules. The final activity was below the recommended limit of 5 TIU/mg protein.

Tannin content remained very low throughout fermentation. Although a temporary increase was observed at 48 h, tannins became undetectable after 72 h. Given their already low initial levels, tannins likely contributed little to the antinutritional burden of the raw material compared with L-DOPA, phytates and oxalates.

Collectively, these findings demonstrate that Sonruproduction efficiently reduces the major antinutritional constraints associated with *Mucuna pruriens*, producing a fermented ingredient whose antinutrient concentrations fall within recommended safety limits.

3.3. Changes in microbial populations and L-DOPA during fermentation

Figure *2* illustrates the dynamic evolution of microbial populations and L-DOPA content during Sonruproduction. Total viable counts increased continuously from approximately 4.5 to more than 7.5 Log CFU/g over 72 h, indicating active microbial colonization and successful establishment of the fermentative microbiota. Simultaneously, *Bacillus cereus*increased from less than 1 to approximately 5 Log CFU/g, suggesting that Bacillus species were among the dominant microorganisms responsible for substrate transformation, in agreement with previous studies on Sonru and related African alkaline-fermented condiments where *Bacillus* spp. constitute the predominant functional microbiota (Azokpota et al., 2006; Parkouda et al., 2009).

Lactic acid bacteria increased during the first 48 h, reaching their highest population at approximately 48 h before declining at 72 h. This pattern suggests that LAB contributed

primarily during the early and intermediate stages of fermentation. The subsequent decrease may be related to increasing pH and competition from alkaliphilic microorganisms such as *Bacillus* spp.

Staphylococcal populations also increased gradually throughout fermentation, although at lower levels than total viable counts. Their persistence may contribute to proteolytic and flavour-forming activities commonly associated with traditional African alkaline fermentations.

Notably, the dramatic decline in L-DOPA content occurred simultaneously with microbial growth. L-DOPA concentrations decreased from approximately 1900 mg/100 g to near-zero levels by 72 h, suggesting an active microbial involvement in detoxification. The inverse relationship between microbial proliferation and L-DOPA concentration strongly supports the hypothesis that fermentative microorganisms metabolize or transform L-DOPA during fermentation. Although the exact metabolic pathways responsible for L-DOPA degradation were not assessed, the strong inverse association between microbial growth and L-DOPA concentration suggests possible microbial biotransformation during fermentation

3.4.Correlation between physicochemical properties, antinutritional factors and microbial populations during Sonru production

Pearson correlation analysis revealed strong relationships between physicochemical characteristics, antinutritional compounds and microbial populations during Sonruproduction, highlighting the interconnected mechanisms responsible for detoxification and biochemical transformation of *Mucuna pruriens*(Table 3).

The increase in pH was strongly and positively correlated with dry matter ($r = 0.870$, $p < 0.001$), ash content ($r = 0.919$, $p < 0.001$), total viable counts ($r = 0.981$, $p < 0.001$), *Bacillus cereus* ($r = 0.966$, $p < 0.001$), and *Staphylococcus* populations ($r = 0.959$, $p < 0.001$).

Conversely, pH exhibited strong negative correlations with moisture (r =-0.870, $p< 0.001$), crude protein (r = -0.974, $p< 0.001$), crude fat (r = -0.961, $p< 0.001$), L-DOPA (r = -0.969, $p< 0.001$), phytate (r = -0.969, $p< 0.001$), oxalates (r = -0.881, $p< 0.001$) and trypsin inhibitor activity (r = -0.624, $p< 0.05$).

These relationships indicate that alkalinization constituted a central feature of the fermentation process and occurred simultaneously with microbial proliferation and antinutrient degradation. The strong association between pH and Bacillus growth supports the occurrence of an alkaline fermentation driven mainly by proteolytic microorganisms.Similar relationships between increasing pH, *Bacillus* proliferation and substrate proteolysis have been reported in fermented locust bean condiments such as Soumbala, Afitin, Iru and Sonru(Azokpota et al., 2006; Parkouda et al., 2009).

Crude protein was highly positively correlated with L-DOPA (r = 0.997, $p< 0.001$), phytate (r = 0.967, $p< 0.001$), crude fat (r = 0.946, $p< 0.001$), and oxalates (r = 0.854, $p< 0.001$). Similarly, crude fat exhibited strong positive correlations with phytate (r = 0.965, $p< 0.001$) and oxalates (r = 0.902, $p< 0.001$).

These results suggest that the depletion of storage macromolecules and the reduction of antinutritional compounds occurred concomitantly throughout fermentation. The progressive utilization of proteins and lipids by microorganisms was therefore closely linked to the detoxification process**.**

Strong positive correlations were observed among the principal antinutritional compounds. L-DOPA was positively associated with phytate (r = 0.953, $p< 0.001$), oxalates (r = 0.823, $p = 0.001$) and crude protein (r = 0.997, $p< 0.001$). Likewise, phytate exhibited a strong correlation with oxalates (r = 0.954, $p< 0.001$), while oxalates were positively correlated with trypsin inhibitor activity (r = 0.894, $p< 0.001$).

The simultaneous decline of these compounds strongly suggests that common processing factors, including microbial enzymatic activities and prolonged fermentation, contributed to their degradation.

Total viable counts showed extremely strong negative correlations with L-DOPA ($r = -0.995$, $p < 0.001$), crude protein ($r = -0.996$, $p < 0.001$), phytate ($r = -0.965$, $p < 0.001$), and oxalates ($r = -0.848$, $p < 0.001$). Similar trends were observed for *Bacillus cereus*, which was negatively correlated with L-DOPA ($r = -0.987$, $p < 0.001$), phytate ($r = -0.937$, $p < 0.001$), oxalates ($r = -0.800$, $p = 0.002$), and crude protein ($r = -0.983$, $p < 0.001$).

Furthermore, *Staphylococcus* counts displayed strong negative associations with L-DOPA ($r = -0.983$, $p < 0.001$), phytate ($r = -0.967$, $p < 0.001$), oxalates ($r = -0.862$, $p < 0.001$), and crude protein ($r = -0.984$, $p < 0.001$).

These findings provide compelling evidence that microbial growth was the main driving force behind the detoxification of *Mucuna* seeds. The exceptionally high negative correlation between TVC and L-DOPA ($r = -0.995$) suggests that microbial metabolism played a key role in the degradation or biotransformation of this toxic compound.

Unlike the dominant bacterial populations, lactic acid bacteria exhibited no significant correlations with physicochemical parameters or antinutritional factors ($p > 0.05$). This result suggests that LAB participated only marginally in the detoxification process.

Yeasts and moulds were positively correlated with tannins ($r = 0.804$, $p = 0.002$) and LAB populations ($r = 0.701$, $p = 0.011$), whereas Enterobacteriaceae were strongly associated with tannins ($r = 0.891$, $p < 0.001$), yeasts and moulds ($r = 0.909$, $p < 0.001$), and LAB ($r = 0.720$, $p = 0.008$).

The absence of significant correlations between these microbial groups and L-DOPA reduction suggests that they contributed less to detoxification than the dominant Bacillus-related microbiota.

Overall, correlation analysis demonstrated that Sonruproduction is characterized by a strong coupling between microbial proliferation, alkalinization and antinutrient degradation. The near-perfect negative correlations observed between microbial counts and L-DOPA, phytate and oxalate concentrations indicate that detoxification of *Mucuna pruriens* is largely microbiologically driven. Among the microbial groups monitored, *Bacillus cereus* emerged as the microorganisms most closely associated with biochemical modifications and detoxification efficiency, highlighting their potential role as key functional organisms during traditional Sonruproduction.

However, correlation analysis does not establish causality; therefore, the observed associations between microbial populations and antinutrient reductions should be interpreted as evidence of co-occurrence rather than direct proof of degradation activity.

3.5. Multivariate relationships among nutritional, microbiological and antinutritional variables

Principal component analysis (PCA) explained 96.3% of the total variance, with Dim1 and Dim2 accounting for 71.1% and 25.2%, respectively (Figure 3). Such a high cumulative variance indicates that the PCA adequately summarizes the major transformations occurring during Sonru production.

Positive values of Dim1 were associated mainly with pH, ash, total viable counts, *Bacillus cereus*, *Staphylococcus* spp. and LAB, whereas negative Dim1 values were associated with L-DOPA, phytates, oxalates, crude protein, crude fat and trypsin inhibitor activity. This

distribution indicates that microbial development and increasing alkalinity occurred concomitantly with antinutrient degradation.

The strong proximity between TVC, Bacillus populations and pH suggests a positive correlation among these variables, reinforcing the role of Bacillus-driven alkaline fermentation. Conversely, L-DOPA, phytates and oxalates grouped together on the opposite side of the biplot, indicating that these compounds followed similar degradation patterns during fermentation.

Dim2 appeared to be primarily associated with tannins, Enterobacteriaceae, yeasts and moulds, and LAB, reflecting secondary microbial and compositional variations. The PCA therefore highlights detoxification and microbial proliferation as the two dominant processes governing the fermentation of *Mucuna* into Sonru.

3.6.Hierarchical cluster analysis of fermentation characteristics

The hierarchical cluster analysis (Figure 4) complements the PCA by grouping variables according to their similarity patterns throughout fermentation. Variables associated with microbial proliferation, including TVC, Bacillus spp., LAB and pH, clustered together, indicating strong positive correlations among these parameters. In contrast, antinutritional factors such as L-DOPA, phytates, oxalates and trypsin inhibitor activity formed a separate cluster, reflecting their common decreasing trend during fermentation.

The clear separation between microbial and antinutritional clusters confirms that detoxification was closely linked to microbial activity. This clustering pattern supports the conclusion that microbial succession during Sonru production represents the primary driver of the observed improvements in nutritional quality and safety of *Mucuna pruriens* seeds

The progressive alkalinization observed during fermentation, together with the dominance of *Bacilluscereus*, indicates that Sonru production follows the biochemical pattern characteristic

of African alkaline-fermented condiments. Similar increases in pH have been attributed to extensive proteolysis and ammonia release during *Bacillus*-mediated fermentation of legume substrates (Ouoba, Rechinger, et al., 2003; Parkouda et al., 2009). The concomitant decline in crude protein and lipid contents further supports the occurrence of intense enzymatic degradation of storage macromolecules. The microbial succession observed in this study closely resembles that previously reported for Sonru, Afitin and Iru, where *Bacillus* species become dominant during fermentation and contribute to texture modification, flavour development and nutrient transformation (Azokpota et al., 2006).

The most significant outcome of the fermentation process was the dramatic reduction of L-DOPA (98.4%). Because L-DOPA represents the principal antinutritional and toxicological constraint limiting the direct consumption of *Mucuna pruriens*, its extensive degradation highlights the potential of Sonru production as a bioprocessing strategy for valorizing this underutilized legume (Sridhar & Bhat, 2007; Vadivel & Janardhanan, 2000).

In addition to L-DOPA, substantial reductions in phytates, oxalates and trypsin inhibitor activity were observed. These changes are nutritionally important because phytates reduce mineral bioavailability through chelation of iron, zinc and calcium, whereas trypsin inhibitors impair protein digestibility (Gupta et al., 2013; Samtiya et al., 2020). The reductions obtained therefore suggest an overall improvement in nutritional quality beyond detoxification alone.

The PCA and HCA analyses confirmed that fermentation time was the principal factor structuring the biochemical and microbiological changes observed during Sonru production. The clear separation between microbial variables and antinutritional compounds suggests that microbial proliferation is closely associated with the detoxification process. Nevertheless, mechanistic studies involving microbial isolation, genomic characterization and enzyme activity measurements are required to identify the microbial populations directly responsible for L-DOPA degradation.

3.7. Limitations of the study

Despite providing novel insights into the detoxification of *Mucuna pruriens* during Sonru production, this study presents several limitations. First, microbial characterization relied primarily on culture-dependent enumeration methods, which do not fully capture the diversity or functional potential of the fermenting microbiota. Molecular approaches such as 16S rRNA gene sequencing, metagenomics or metatranscriptomics would provide a more comprehensive understanding of microbial succession and functionality.

Second, although strong correlations were observed between microbial populations and reductions in L-DOPA and other antinutritional factors, causal relationships could not be established. Direct measurements of enzymatic activities involved in L-DOPA degradation, phytate hydrolysis and proteolysis were not performed.

Third, the study evaluated fermentation under a single traditional processing condition and fermentation duration. The effects of different fermentation temperatures, inoculation strategies and processing variables on detoxification efficiency were not investigated.

Fourth, nutritional improvements were inferred from compositional changes, but mineral bioaccessibility, protein digestibility and bioavailability were not directly assessed. Additional in vitro and in vivo studies are therefore required to confirm the nutritional benefits of the fermented product.

Finally, the safety assessment was based mainly on microbial counts and antinutritional factor reductions. Comprehensive toxicological evaluation, including detection of potential microbial toxins and assessment of residual L-DOPA bioavailability, would strengthen conclusions regarding the safety of Sonru produced from *Mucuna pruriens*.

4. Conclusion

The present study demonstrated that traditional Sonruproduction is an effective bioprocess for the detoxification and nutritional improvement of *Mucuna pruriens* seeds. Fermentation induced significant physicochemical and microbiological changes characterized by progressive alkalinization, reduction in moisture, protein and lipid contents, and a relative increase in ash concentration, reflecting intense microbial metabolism and substrate transformation.

A major outcome of the process was the substantial reduction of antinutritional factors. After 72 h of fermentation, L-DOPA, the principal toxic compound of *Mucuna pruriens*, decreased by approximately 98.4%, from 1905 to 30.3 mg/100 g. Similar reductions were observed for phytates (78.3%), oxalates (73.0%) and trypsin inhibitor activity (46.9%), resulting in final concentrations below recommended safety thresholds for human consumption. These findings confirm the high detoxification potential of Sonruproduction and highlight its suitability as a traditional processing technology for the valorisation of *Mucuna* seeds.

Microbial development, particularly that of *Bacillus cereus* and total viable microorganisms, was strongly associated with antinutrient degradation. Correlation analysis revealed near-perfect negative relationships between microbial growth and L-DOPA concentration (TVC: r = -0.995; *Bacillus cereus*: r = -0.987; $p < 0.001$), indicating that detoxification was largely microbiologically mediated. Principal component analysis and hierarchical cluster analysis further confirmed the close association between microbial proliferation, pH increase and antinutrient reduction throughout fermentation.

Overall, the results provide scientific evidence that traditional Sonru production can transform *Mucuna pruriens* seeds into a safer and nutritionally improved food ingredient. The process offers a promising strategy for promoting the utilization of this underexploited protein-rich

legume in sustainable food systems and may contribute to dietary diversification and food security in sub-Saharan Africa.

Future studies should focus on the identification of the dominant fermentative microorganisms, the elucidation of metabolic pathways involved in L-DOPA degradation, and the evaluation of protein digestibility, amino acid bioavailability and sensory acceptability of the final fermented product. Such investigations would further support the development of standardized and scalable fermentation processes for industrial applications.

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Authors' contribution

DBY: Formal analysis, Investigation, Data curation, Conceptualization, Methodology, Writing, original draft,

JMK: Supervision, Investigation, Visualization, Validation, Software, Writing - review & editing,

KE, GKWT: Methodology, Investigation, Formal analysis, Writing - review & editing,

OHIA, MM, RVCD: Supervision, Investigation, Visualization, Validation, Writing - review & editing,

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests

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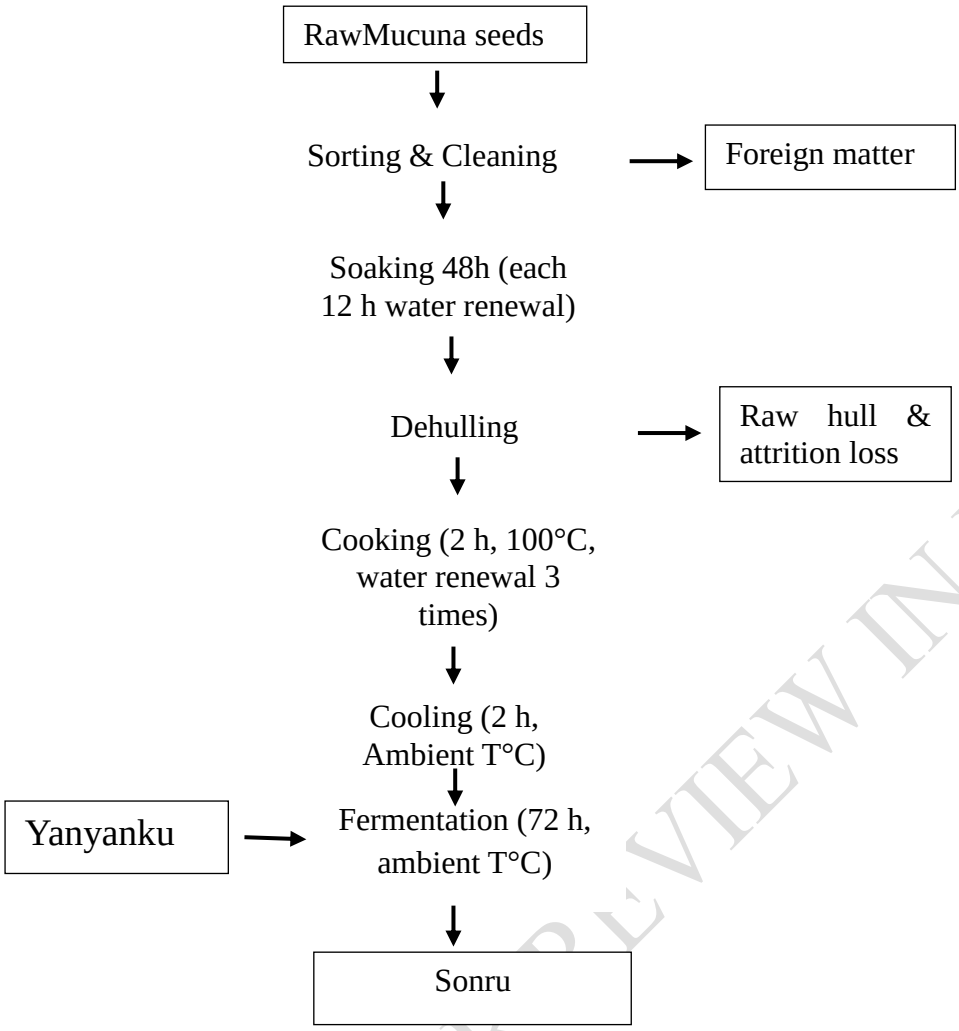


Figure 1 : *Mucuna pruriens* seeds during fermentation in Sonru production process

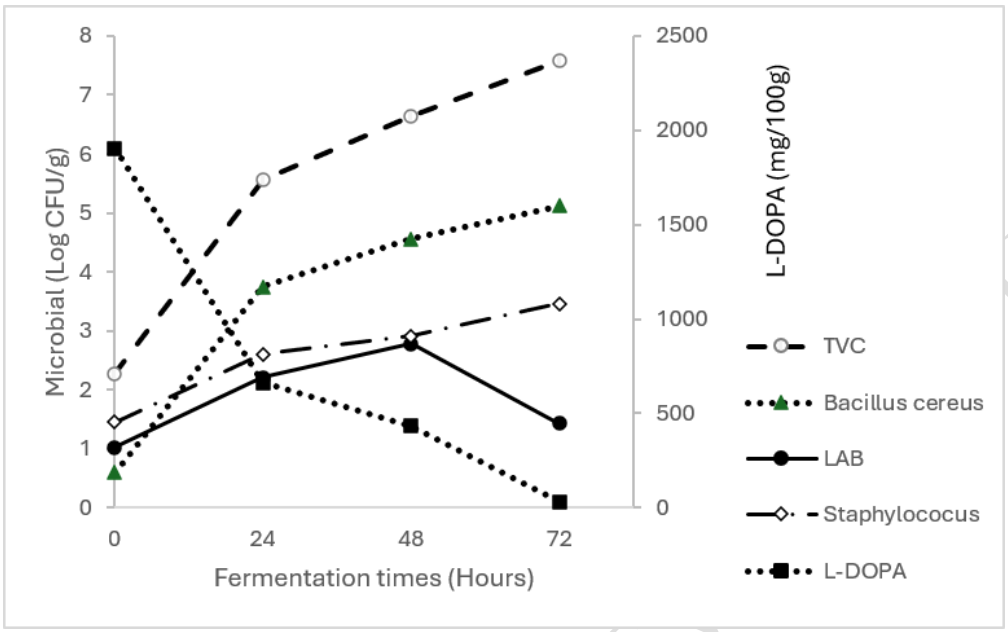
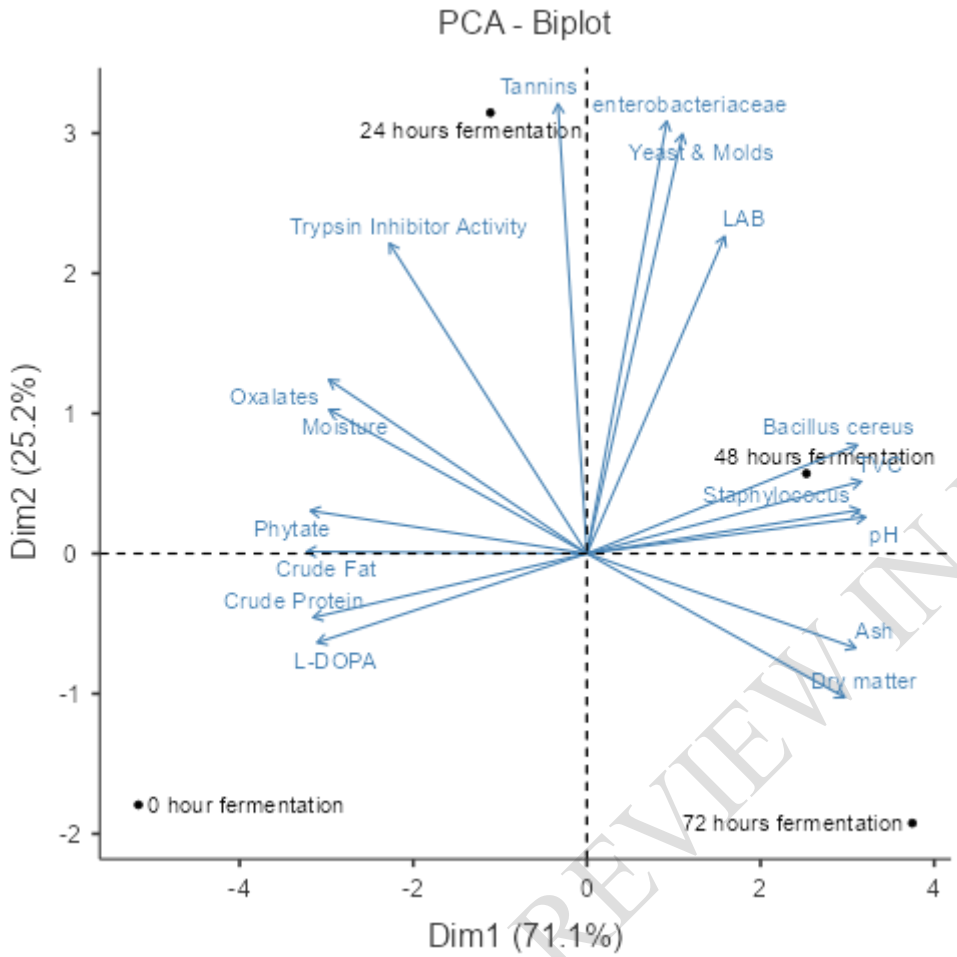


Figure 2changes in microbial and L-DOPA during *Mucuna pruriens* seeds into Sonru at different fermentation times. *TVC=Total viable count; LAB=lactic acid bacteria; L-DOPA= L-3,4-dihydroxyphenylalanine*



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589 Figure 3. Principal Component Analysis (PCA) biplot of physicochemical, microbiological
590 characteristics and antinutrients composition of Sonru based *Mucuna pruriens*

591 *TVC=Total viable count; LAB=lactic acid bacteria; L-DOPA= L-3,4-dihydroxyphenylalanine*

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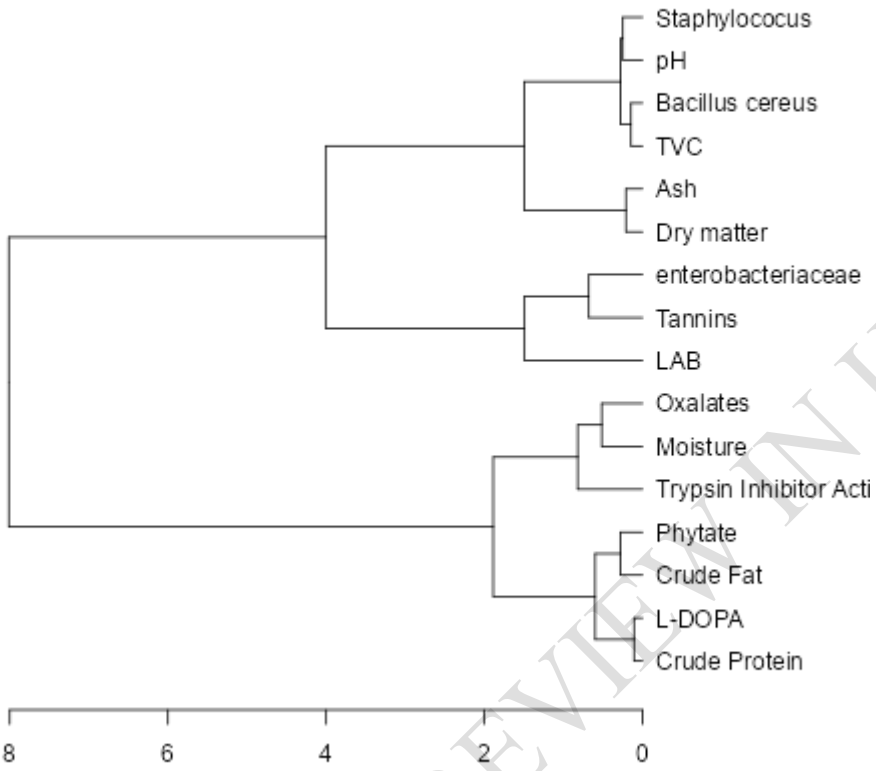

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594 Figure 4Hierarchical Cluster Analysis (HCA)of physicochemical, microbiological and
595 antinutrients variables during fermentation in Sonru production based *Mucuna pruriens*

596 *TVC=Total viable count; LAB=lactic acid bacteria; L-DOPA= L-3,4-dihydroxyphenylalanine*

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600 Table 1. Changes in physicochemical characteristics during processing of Mucuna seeds into
601 Sonru at different fermentation times.
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Parameters	Fermentation times (Hours)			
	0	24	48	72
pH	5.61 ± 0.01a	5.95 ± 0.03b	6.14 ± 0.04c	6.24 ± 0.01c
Moisture (%)	75.2 ± 0.1a	74.7 ± 0.2a	64.4 ± 0.1b	63.7 ± 0.2b
Crude Protein (g/100g)	38.4 ± 0.1a	35.3 ± 0.0b	34.4 ± 0.1c	33.3 ± 0.1d
Crude Fat (g/100g)	4.64 ± 0.3a	3.59 ± 0.0b	2.63 ± 0.0c	2.31 ± 0.1c
Ash (g/100g)	3.87 ± 0.07a	4.24 ± 0.01b	5.69 ± 0.01c	5.78 ± 0.00c

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605 Table 2.Changes in antinutritional factors during processing of *Mucuna seeds* into Sonru at

606 different fermentationtimes.

Parameters	Fermentation times				Recommended
	0	24	48	72	Level
L-DOPA (mg/100 g)	1905 ±34.6a	663 ±6.67b	433 ±4.41c	30.3 ±0.10d	<100
Phytate (mg/100g)	12.3 ±0.03a	8.53 ±0.01b	5.53 ±0.01c	2.67 ±0.27d	<5.0
Tannins (mg TAE/g)	0.03 ±0.01a	0.03 ±0.01a	0.12 ±0.00b	0.00a	<1.0
Oxalates (mg/100 g)	155 ±0.09a	145 ±0.24b	85.4 ±0.05c	41.9 ± 1.67d	<50
Trypsin Inhibitor Activity (TIU/mg protein)	6.26 ±0.26a	8.05 ±0.05b	4.42 ±0.09c	3.32 ±0.07d	<5

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Table 3 : Correlation matrixbetween physicochemical properties, antinutritional factors and microbial populations during Sonru production

	pH	Moisture	Dry matter	Crude Protein	Crude Fat	Ash	L-DOPA	Phytate	Tannins	Oxalates	TIA	TVC	Bacillus cereus	LAB	Staphylococus	Yeast & Molds	enterobacteriaceae
pH	—																
Moisture	-0.870***	—															
Dry matter	0.870***	-1.000***	—														
Crude Protein	-0.974***	0.819**	-0.819**	—													
Crude Fat	-0.961***	0.902***	-0.902***	0.946***	—												
Ash	0.919***	-0.990***	0.990***	-0.871***	-0.947***	—											
L-DOPA	-0.969***	0.781**	-0.781**	0.997***	0.934***	-0.843***	—										
Phytate	-0.969***	0.906***	-0.906***	0.967***	0.965***	-0.938***	0.953***	—									
Tannins	-0.009	0.402	-0.402	-0.040	0.082	-0.298	-0.097	0.188	—								
Oxalates	-0.881***	0.954***	-0.954***	0.854***	0.902***	-0.951***	0.823**	0.954***	0.459	—							
TIA	-0.624*	0.903***	-0.903***	0.560	0.698*	-0.848***	0.505	0.732**	0.732**	0.894***	—						
TVC	0.981***	-0.819**	0.819**	-0.996***	-0.951***	0.877***	-0.995***	-0.965***	0.050	-0.848***	-0.552	—					
Bacillus cereus	0.966***	-0.785**	0.785**	-0.983***	-0.956***	0.852***	-0.987***	-0.937***	0.141	-0.800**	-0.495	0.987***	—				
LAB	0.480	-0.321	0.321	-0.476	-0.378	0.374	-0.490	-0.323	0.535	-0.141	0.054	0.499	0.514	—			
Staphylococus	0.959***	-0.804**	0.804**	-0.984***	-0.918***	0.853***	-0.983***	-0.967***	-0.008	-0.862***	-0.564	0.983***	0.958***	0.451	—		
Yeast & Molds	0.359	-0.051	0.051	-0.401	-0.374	0.163	-0.444	-0.215	0.804**	0.047	0.324	0.429	0.524	0.701*	0.345	—	
enterobacteriaceae	0.359	0.055	-0.055	-0.413	-0.273	0.060	-0.470	-0.199	0.891***	0.095	0.462	0.429	0.500	0.720**	0.383	0.909***	—

* p < .05, ** p < .01, *** p < .001

TVC=Total viable count; LAB=lactic acid bacteria; L-DOPA= L-3,4-dihydroxyphenylalanine, TIA=Trypsin Inhibitor Activity