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Solid-State Fermentation of *Mucuna deeringiana* Seed Flour Using *Lacticaseibacillus rhamnosus*

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Abstract: The genus *Mucuna* is a potential protein source, but it has been underutilized due to the presence of antinutritional factors, especially L-DOPA. Solid-state fermentation with lactic acid bacteria could be an effective and simple method for reducing these antinutritional factors while simultaneously enriching the protein content. In this work, an experimental analysis identified the variables with the greatest influence on the solid-state fermentation of *Mucuna deeringiana*. In general, we observed a decrease in pH due to the production of and increase in lactic acid, resulting in a 91% (6.40 to 0.55 g/100 g sample) reduction in L-DOPA, 51% decrease in phenolic compounds (11.65 to 5.70 g/100 g sample), 97% decrease in tannins (1.26 to 0.04 g/100 g sample), and the antioxidant capacity of the fermented flour was 97%, with an increase in protein content of 12%. Furthermore, it demonstrated greater stability over 24 days compared to the control samples, which remained stable for only 3 days. These results suggest that the bacterium has a positive effect on the production of lactic acid, and the nutritional composition can be enhanced by reducing antinutritional factors, especially L-DOPA, that limit the use of this legume. This process proves to be a cost-effective and sustainable method for developing nutritious feed products derived from *Mucuna* flours.

Keywords: legumes; L-DOPA; lactic acid; antinutritional factors

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1. Introduction

Legume plants of the family *Fabaceae* have the potential to produce protein supplements to improve ruminants' nutrition due to their high protein content, amino acids, minerals, vitamins, and bioactive compounds [1–5]. The high protein levels in these seeds are crucial for adequate nutrition in animal feed. When used in moderate doses or as substitutes for other protein-rich feeds, legume seeds can improve production performance, fattening, and meat quality [6]. Mucuna is an interesting genus of this family because their seed contains between 23 and 30% protein. However, this plant has been underutilized for feed crops largely due to the presence of antinutritional factors (ANFs). The intake of antinutritional compounds can inhibit enzymatic activity in the digestive system and reduce the availability of essential compounds such as proteins, vitamins, and minerals [7]. Low feed intake can decrease feed conversion, growth rate, and animal productivity [8], hormonal changes, and organ damage [9,10]. Although L-DOPA has medical applications in the treatment of Parkinson's disease, in the context of animal nutrition, it has potential neurotoxic effects in rats, especially due to oxidative stress and interaction with

monoamine oxidase B [11,12]. In pigs, L-DOPA in feed results in reduced intake and weight gain. In ruminants, it has a laxative effect if more than 2 kg per day is fed to the cattle [13] (Table 1). The potential as feed for ruminants should be exploited once the adverse effects of antinutritional factors (ANFs) are mitigated [7].

Table 1. Safe consumption levels for certain organisms of antinutritional compounds.

Organisms	Anti-Nutritional Compounds (g/100 g Sample)			Reference
	Tannins	Total Phenol Compound	L-DOPA	
Bovine	1.70–3.50	<0.002	2000	[10,13,14]
Ovine	1.30–1.40	<0.002	NR	[10,14]
Pig	1.00–3.00	18.60–21.43	NR	[15,16]
Rats	NR	NR	0.8	[12]
Chickens	0.07–0.20	4.88–9.56	NR	[15,16]

NR: not reported.

(Table 2) summarizes the content of antinutritional factors such as L-DOPA, phenols, and tannins from some seeds of *Mucuna*, and it shows single and combined processing methods to reduce or eliminate those factors. As can be seen, the L-DOPA and total phenol values are higher than the conventional legumes, such as *Vicia faba* (10.04%) and soya bean (8.75%) [17,18]. Some treatments used to reduce L-DOPA and phenols demand significant energy costs, require specialized equipment, and can produce associated compounds that are not eliminated. Treatments with excessive energy consumption are unviable in terms of sustainability and cost-effectiveness [19].

Hydrothermal treatments, soaking in alkaline solutions and autoclaving, or soaking in citric acid solution and autoclaving are effective for removing L-DOPA, until low quantities (1.5 g per person per day) are achieved that are considered safe for consumption of Mucuna beans [20]. Soaking, cooking, roasting, germination, and fermentation are effective for reducing various ANFs, except phenol, phytate, and tannin, in the seeds of *Mucuna pruriens*. During cooking and autoclaving, the total phenolic content was further reduced to 49%, whereas the tannin content increased to 14%. This increase in tannin content might be attributed to the condensation of free phenols. Vadivel and Pugalenthi [21] suggest that the L-DOPA content in *M. pruriens* is significantly eliminated by dry heat treatment [22], cooking, and autoclaving.

Table 2. Protein content and antinutritional factors such as L-DOPA, phenols, and tannins from some Mucuna seeds submitted to single and combined processing methods.

Legumes	Treatment	Nutritional Modification								Ref.
		Protein (%)		Total Phenolic Contents (g/100 g Sample)		Tannins (g/100 g Sample)		L-DOPA (g/100 g Sample)		
		Raw	Proccesed	Raw	Proccesed	Raw	Proccesed	Raw	Proccesed	
Mucuna cochinchinensis	Fermentation	22.19	36.41	NR	NR	1.07	1.35	NR	NR	[23]
Mucuna pruriens	Soaked	25.34	25.27	1.82	0.73	0.38	0.30	6.83	4.26	[20]
	Cooked	25.34	24.25	1.82	0.69	0.38	0.12	6.83	1.27	
	Roasted	25.34	25.90	1.82	0.69	0.38	0.29	6.83	5.19	
	Germinated	25.34	26.90	1.82	1.82	0.38	0.20	6.83	1.30	
	Fermented	25.34	29.50	1.82	3.02	0.38	0.41	6.83	1.30	
	Soaked + cooked	25.34	24.10	1.82	2.89	0.38	0.14	6.83	2.10	
	Germinated + roasted	25.34	28.78	1.82	3.49	0.38	0.12	6.83	1.23	
	Fermented + roasted	25.34	30.40	1.82	1.71	0.30	0.11	6.83	0.01	
Mucuna pruriens	Fermented (*L. acidophilus*)	29.37	37.36	NR	NR	NR	NR	NR	NR	[22]
	Fermented (*Rhizopus oligosporus*)	30.48	36.85	NR	NR	NR	NR	6.36	1.00	

Table 2. *Cont.*

Legumes	Treatment	Nutritional Modification								Ref.
		Protein (%)		Total Phenolic Contents (g/100 g Sample)		Tannins (g/100 g Sample)		L-DOPA (g/100 g Sample)		
		Raw	Proccesed	Raw	Proccesed	Raw	Proccesed	Raw	Proccesed	
Mucuna deeringiana	Toasted	29.23	29.71	11.80	8.51	2.19	1.26	5.17	2.05	[19]
	Hydratation water	NR	NR	11.80	9.19	2.19	1.06	5.17	3.19	
	Hydratation $NaHCO_3$	NR	NR	11.80	9.48	2.19	0.79	5.17	3.27	
	Hydratation $Ca(OH)_2$	29.23	30.14	11.80	9.67	2.19	0.66	5.17	2.69	
	Hydratation NaCl	29.23	30.02	11.80	8.99	2.19	0.99	5.17	3.12	
	Hydratation + cooked	29.23	32.55	11.80	8.70	2.19	1.33	5.17	2.08	
	Germinated	NR	NR	11.80	11.02	2.19	1.06	5.17	3.80	

NR: not reported.

Among the biological treatments, the utilization of the fungus *Rhizopus oligosporus* in the fermentation of legumes has been investigated as a method to reduce the L-DOPA content [24]. Studies have reported an initial increase in L-DOPA levels during the early stages of fermentation, followed by a subsequent decrease, suggesting a likely synthesis or initial release followed by degradation or utilization by the fungus [18]. It has been proposed that the fungus may have the ability to synthesize L-DOPA from amino acids or release it from more complex molecular structures [18]. However, this increase in L-DOPA content has also been correlated with an increase in antioxidant activity.

Mucuna deeringiana is utilized in Africa, Asia, and Central America. Like other legumes, it is a source of protein (29.23%) [25]. However, the digestibility and utilization of the protein in these seeds are limited by the protein structure and the presence of certain antinutritional factors such as L-DOPA, phenols, and tannins, among others. Given the relevance of *M. deeringiana* flour as a nutritional resource, it is necessary to explore simple strategies that optimize its use in animal feed. Solid-state fermentation (SSF) using lactic acid bacteria (LAB) demonstrates greater efficiency in protein enrichment, reduction in antinutritional factors, and improvement in the digestibility of seed flour [8,23,26–29].

In this context, lactic solid-state fermentation (SSF) emerges as an underexplored methodology in this research field. It stands out as a cost-effective tool compared to conventional methods. The significance of SSF could expand knowledge about *M. deeringiana*, and open new perspectives for its practical and sustainable application in animal feed. Therefore, this research aims to study the effect of solid-state fermentation on the content of L-DOPA, total phenols, antioxidant capacity, and protein of *M. deeringiana* flour with *Lacticaseibacillus rhamnosus* to identify a cost-effective and sustainable method to improve the utilization of this underutilized legume.

2. Materials and Methods

Vitabosa seeds (*M. deeringiana*) were purchased in Agrosemillas "https://agrosemillas.com.co (accessed on 9 January 2024)" in Colombia, which deals in their commercial distribution. These seeds were subjected to pulverization in a hammer mill until a particle size of 450 µm was achieved. The resulting flour from this process was also stored at room temperature.

Lyophilized *Lactobacillus rhamnosus* (CRL1505), which was recently renamed *Lacticaseibacillus rhamnosus* [30], was cultured in Neogen® nutrient agar (catalogue No. NCM0033A) at 37 °C for 3 days. Afterwards, a colony was cultured in Neogen® nutrient broth (catalogue No. NCM0110A) at 37 °C overnight. Each assay required dilution until the optical densities (ODs) were obtained. Absorbance measurements were conducted at 600 nm utilizing a UV-Vis spectrophotometer (Thermo Scientific Evolution 300, Waltham, MA, USA).

Each fermentation sample was prepared in triplicate using sterile sample bags. The substrate composition comprised 8 g of *M. deeringiana* flour and 3 mL of bacterial suspension. The samples were incubated at distinct temperatures, with OD as indicated by the experi-

mental design. The samples were dried at 50 °C for 48 h, cooled, and stored at −10 °C for subsequent analysis.

The SSF was followed by pH measurements and the determination of volatile fatty acids. The extraction and quantification of volatile fatty acids were carried out using the modified method by Fukalova et al. [31]. A total of 0.2 g of each sample was taken, dissolved in 10 mL of distilled water, and stirred for 10 min, followed by a 10 min resting period. Subsequently, 10 µL of the sample was taken and diluted in 190 µL of distilled water. Quantification of volatile fatty acids was performed using High-Performance Liquid Chromatography (HPLC) on a Knauer Azura system equipped with a C-18 column (250 × 4.6 mm, 120 Å) and a UV-Vis detector. The mobile phase consisted of a mixture of dibasic phosphate (567,550, Merk, Boston, MA, USA)/type 1 water/acetonitrile (CAS No. 75-05-8, LiChrosolv®) (pH 3.0) with orthophosphoric acid (CAS 7664-38-2, EMSURE®) flowing at 2.0 mL/min and maintained at 30 °C. Standard solutions (1000 mmol/L) of lactic acid (252,476, Sigma-Aldrich, Burlington, MA, USA), acetic acid (33,209, Sigma-Aldrich), propionic acid (161,810.1611, PanReac AppliChem), isobutyric acid (W222216, Sigma-Aldrich), and butyric acid (CAS No. 107-92-6, Sigma-Aldrich) were prepared with ultrapure water.

Total phenolic content (TPC) was determined using the method by Bhat et al. [32], modified for the analysis of *M. pruriens* phenols. The extraction involved dispersing 0.1 g of material in 5 mL of acidified methanol (9070-03, J.T. Baker). TPC quantification was performed using the Folin–Ciocalteau method [33], with absorbance read at 765 nm on a UV-Vis spectrophotometer.

L-DOPA extraction from samples involved sonication in an 80:20 methanol (9070-03, J.T. Baker)/water solution [34]. Quantification was performed using High-Performance Liquid Chromatography (HPLC) on a Knauer Azura system equipped with a C-18 column (250 × 4.6 mm, 120 Å) and a UV-Vis detector. L-DOPA content (g/100 g sample) was determined using a calibration curve referencing the L-DOPA (D9628, Sigma-Aldrich) standard (1 to 6 mM) [19]. The preparation and extraction of the samples were conducted according to the modified methodology of Banica et al. [35]. In this process, 0.1 g of the sample was weighed and mixed with 4 mL of an 80% methanol–water solution. Subsequently, the mixture was sonicated for 1 h, filtered, and the resulting sample was stored under refrigeration.

The extraction and quantification of tannins (g/100 g sample) were carried out using the methodology of Peng et al. [36]. The extraction involved dispersing 1 g of material in 4 mL of ultrapure water. Subsequently, the mixture was sonicated for 15 min, filtered, and the resulting sample was stored under refrigeration. Tannin quantification was performed using High-Performance Liquid Chromatography (HPLC) on a Knauer Azura system equipped with a C-18 column (250 × 4.6 mm, 120 Å) and a UV-Vis detector. The mobile phase consisted of a mixture of methanol (9070-03, J.T. Baker) and glacial acetic acid (33,209, Sigma-Aldrich) (15–85%) flowing at 1.0 mL/min and maintained at 35 °C, with detection at a wavelength of 210 nm. Standard solutions (0.0625 to 5 mM) of catechin (C1788, Sigma-Aldrich) in methanol (9070-03, J.T. Baker) were prepared.

Antioxidant capacity was determined using the DPPH assay. It was carried out according to the methodology proposed by Gouveia et al. [37]. In this procedure, 100 µL of a methanolic solution was added to 3.5 mL of a DPPH solution (0.06 mol/L) and left in darkness for 30 min. The absorbance was taken at 516 nm using a UV-Vis spectrophotometer (Thermo Scientific Evolution 300, USA). The DPPH radical scavenging activity was calculated as % inhibition:

$$\%\text{Inhibition} = \left(A_{control} - A_{sample}/A_{control}\right) \times 100 \quad (1)$$

where $A_{control}$ is the absorbance of the control solution (DPPH without the sample), and A_{sample} is the absorbance of the solution containing the antioxidant sample.

Dry matter content was determined using method 925.10, while crude protein and nitrogen content were analyzed using method 990.03, according to the guidelines established by the AOAC (2000) [38].

A complete factorial experimental design was implemented as experimental screening, considering three main factors: optical density (OD), temperature, and time, each with three and two levels, respectively.

Once the variables with the greatest influence on antinutritional factors (ANFs), particularly L-DOPA, phenols, lactic acid production, and antioxidant capacity during the fermentation process, were identified, the fermentation time was studied using variance analysis. The data were analyzed using the R program for Windows.

3. Results and Discussion

3.1. Experimental Screening

A factorial design was performed to determine the variables with the greatest influence in the SSF. Lactic acid determination was followed to understand the effectiveness of *M. pruriens* flour in supporting microbial activity during the SSF process. Analyzing the complete factorial experimental design revealed statistically significant differences in lactic acid production. Fermentation time, OD, and the interactions between temperature and time, temperature and OD, and time and OD all affect lactic acid production (p-value < 0.05, R^2 = 99.08%, Table 3).

Table 3. Complete factorial experimental design analyzed considering optical density, temperature, and time as factors influencing the levels of lactic acid, L-DOPA, phenols, and DPPH.

OD	Time (Days)	Temperature (°C)	pH	Lactic Acid (g/100 g Sample)	L-DOPA (g/100 g Sample)	Phenols (g/100 g Sample)	DPPH (%Inhibition)
0.2	1	25	5.92 ± 0.01	47.25 ± 0.015	7.08 ± 0.01	1.82 ± 0.01	87.64 ± 0.70
0.4	1	25	6.02 ± 0.01	16.11 ± 0.06	5.56 ± 0.01	6.77 ± 0.01	86.20 ± 0.00
0.6	1	25	5.96 ± 0.01	59.22 ± 0.010	3.47 ± 0.02	0.74 ± 0.01	86.82 ± 0.02
0.2	3	25	5.60 ± 0.01	18.54 ± 0.035	2.75 ± 0.01	7.08 ± 0.00	97.12 ± 0.12
0.4	3	25	6.96 ± 0.00	31.86± 0.105	3.95 ± 0.10	6.23 ± 0.02	97.11 ± 0.03
0.6	3	25	6.25 ± 0.03	18.18 ± 0.025	3.38 ± 0.04	6.20 ± 0.10	97.08 ± 0.02
0.2	1	30	6.01 ± 0.01	67.68 ± 0.015	5.33 ± 0.05	2.50 ± 0.05	88.36 ± 0.01
0.4	1	30	6.14 ± 0.01	15.16 ± 0.135	4.62 ± 0.01	8.66 ± 0.01	88.46 ± 0.02
0.6	1	30	6.02 ± 0.02	78.21 ± 0.006	4.59 ± 0.02	0.64 ± 0.06	87.84 ± 0.01
0.2	3	30	5.69 ± 0.00	23.40 ± 0.025	2.62 ± 0.01	9.61 ± 0.03	97.06 ± 0.02
0.4	3	30	4.51 ±0.01	11.34 ± 0.015	2.57 ± 0.09	6.79 ± 0.20	97.09 ± 0.01
0.6	3	30	4.99 ± 0.01	22.68 ± 0.025	3.52 ± 0.01	8.32 ±0.00	97.11 ±0.10
0.2	1	35	5.97 ± 0.06	46.89 ± 0.125	4.24 ± 0.02	11.89 ± 0.03	88.47 ±0.02
0.4	1	35	6.23 ± 0.02	12.78 ± 0.025	4.02 ± 0.02	0.43 ±0.05	88.05 ± 0.00
0.6	1	35	6.00 ± 0.00	35.37 ± 0.000	4.67 ± 0.07	7.10 ± 0.10	88.05 ± 0.00
0.2	3	35	6.09 ± 0.01	36.00 ± 0.100	2.65 ± 0.05	5.89 ± 0.01	97.10 ± 0.03
0.4	3	35	5.73 ± 0.01	59.13 ± 0.000	2.97 ± 0.00	2.18 ± 0.00	97.13 ±0.03
0.6	3	35	4.51 ± 0.00	30.33 ± 0.015	3.01 ± 0.02	5.49 ± 0.02	95.30 ± 0.10

Values are given as mean ± SD (n = 3).

On the other hand, L-DOPA and phenols were used as response variables to evaluate the effect of SSF on antinutritional factors. L-DOPA and phenols are influenced by time (p-value < 0.05; R^2 = 77.55%, Table 3) rather than the highest amount of biomass inoculated, temperature, or other unexamined variables. It is important to highlight that the degradation of L-DOPA and phenols may be associated with the acidic medium (formation of lactic acid) and not necessarily the bacterium action.

On the other hand, the antioxidant capacity, measured as DPPH, was also evaluated as a response variable. Antioxidant capacity is affected by time, as with the interaction between temperature and time (p-value < 0.05, R^2 = 99.69%, Table 3).

In general, the results of the experimental screening indicate that lactic acid production is affected by all variables except temperature (Table 3). However, L-DOPA and DPPH are affected solely by time. The interaction between temperature and OD (30° with 0.4 and 25° with 0.2, respectively) showed statistically significant differences (p-value < 0.05) in lactic acid production. This interaction was also considered in the subsequent analysis. Thus, fermentation time was studied using variables such as an OD of 0.2 and 0.4 of *L. rhamosus*, and temperatures of 25 °C and 30 °C.

3.2. The Effect of Fermentation Time on SSF

Figure 1 shows that the production of lactic acid is directly related to the decrease in pH from 5.79 to 3.83 as the fermentation time increased. This decrease in pH is associated with the increase in organic acids produced by the bacteria during SSF [39], as well as the proliferation and dominance of acid-lactic bacteria [40]. Rodríguez de Olmos et al. [41] indicate that robust growth of *L. rhamnosus* should lead to significant acidification within pH ranges of 6.50 to 4.00. Similar results were found in this study. The behavior of *L. rhamnosus* is attributed to its heterofermentative metabolism, which degrades carbohydrates, resulting in the formation of lactic acid [42].

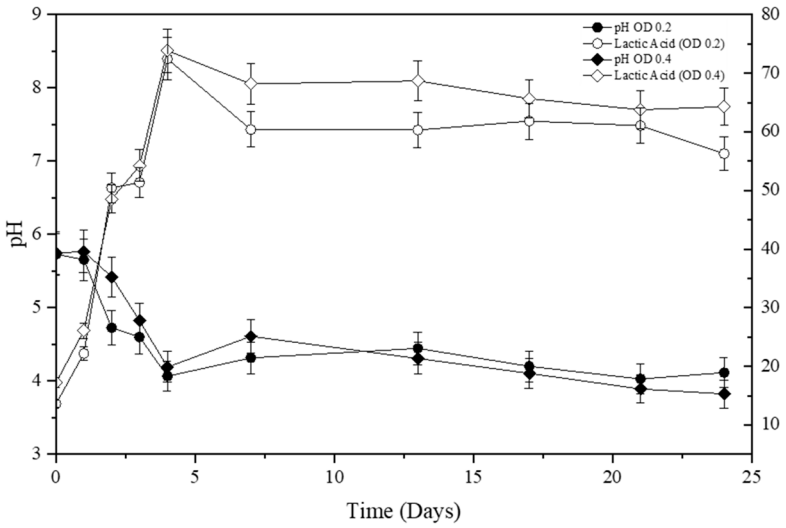

Figure 1. Changes in pH and lactic acid production in *M. deeringiana* flour fermented with *L. rhamnosus* (OD: 0.2, 25 °C, 24 days) and (OD: 0.4, 30 °C, 24 days). Error bars represent means ± SEM (n = 3).

The results indicate that *L. rhamnosus* produces more lactic acid at an OD of 0.4 and a temperature of 30 °C. Akao et al. [43] reported that lactic acid production was optimal at higher temperatures and a low pH of the medium. Lactic acid was not found in the control samples, which mainly evidenced malic acid (3.4 mM); however, during SSF, this acid decreased while lactic acid was generated.

An important effect of adding *L. rhamosus* to the fermentation process is its role in ensuring process viability, as fungal contamination was observed in controls without this bacterium. Lactic acid bacteria (LAB) produce natural antimicrobial compounds such as organic acids that inhibit the growth of pathogenic microorganisms, thus protecting against microbiota, including fungi and spore-forming bacteria [44]. This result can be associated with the stability over 13 days of the obtained product.

The influence of fermentation on *M. deeringiana* seed flour by *L. rhamnosus* on L-DOPA content is reflected in Figure 2A. It was corroborated that the fermentation time influenced the L-DOPA (p-value= 0.004, R^2 = 97.09%), demonstrating a decrease of this compound over time. At the beginning of the fermentation (day 0), the highest quantity of L-DOPA is found, while at 13 days, the quantity decreased (91.40% until 0.55 g/100 g sample). The degradation of L-DOPA content was observed, in agreement with the results reported by Polanowska et al. [24]. They showed L-DOPA degradation from the first day of fermen-

tation, and prolonging the fermentation time coincides with further reduction in this compound. Additionally, Mohd Lazim et al. [45] reported that, using *Lactobacillus acidophilus* co-cultured with *Lactobacillus brevis*, increasing temperature and inoculum size degrades L-DOPA, which is in agreement with our observations. In contrast, Ezegbe et al. [22] found that fermentation with fungi for 3 days significantly reduces the L-DOPA content in *Mucuna pruriens* to levels suitable for consumption.

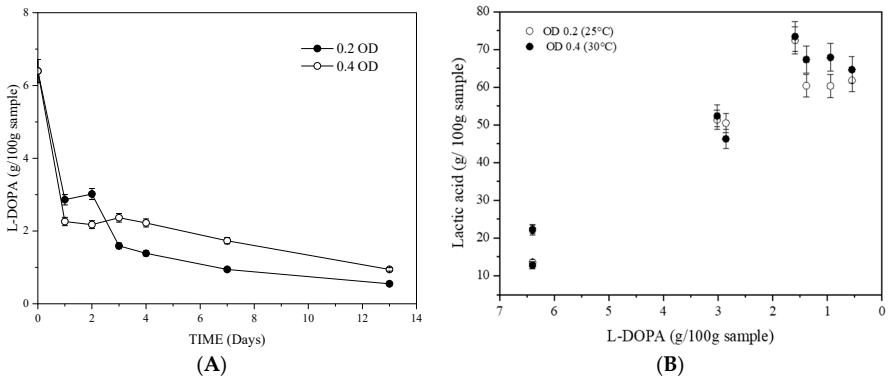

Figure 2. L-DOPA levels during the fermentation of *M. deeringiana* flour fermented with *L. rhamnosus* (OD: 0.2, 25 °C, 13 days) and (OD: 0.4, 30 °C, 13 days). (**A**). Gradual reduction. (**B**). Relationship between lactic acid and L-DOPA concentration. Error bars represent means ± SEM (n = 3).

An inverse proportional relationship was observed between L-DOPA and lactic acid (Figure 2B). As the L-DOPA level decreased, lactic acid increased. The formation of lactic acid can be used as an indicator of the degradation of antinutritional factors, specifically L-DOPA.

This study involves the quantification and assessment of the impact of SSF on another group of ANFs, specifically phenols and tannins. The presence of phenols is also influenced by fermentation time (p-value = 0.014, R^2 = 88.29%). The decrease in phenols over time was quantified from 11.65 to 5.70 g/100 g of sample over 13 days (Figure 3). This result indicates the potential activity of acid lactic bacteria in degrading phenols as fermentation progresses. Similar results were reported by Chen et al. [46], analyzing the effect of lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus acidophilus*) on phenolic component production in strawberry juice. They found a reduction in these components, attributed to prolonged fermentation time and temperature of 37 °C. In contrast, De Pasquale et al. [47] found an increase in phenol content, attributed to the degradation of the cell wall of legumes through the enzymatic activity of *Lactobacillus*; the results indicated that fermented samples had a higher TPC content compared to non-fermented samples. Ezegbe et al. [22] also reported an increase in phenol content in *M. pruriens* seeds fermented with *Rhizopus oligosporus.*

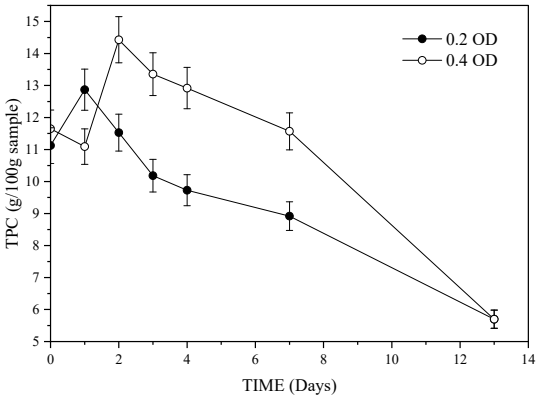

Figure 3. Gradual reduction in TPC levels during the fermentation of *M. deeringiana* flour fermented with *L. rhamnosus* (OD: 0.2, 25 °C, 13 days) and (OD: 0.4, 30 °C, 13 days). Error bars represent means ± SEM (n = 3).

Regarding the decrease in tannins during the SSF process, the results are illustrated in Figure 4. There is a gradual reduction in tannin levels observed during the fermentation time for both OD concentrations (0.2 and 0.4). Although initial levels are relatively high on day 0, they experience a significant decrease after the first day of fermentation. This decrease in tannin levels suggests enzymatic activity induced by the bacteria, leading to the degradation of these phenolic compounds [44]. Additionally, the results reveal that the levels of tannin in controls at 25 °C and 30 °C (without bacteria) decrease slightly compared to the samples containing bacteria. This suggests that *L. rhamnosus* is responsible for the rate of tannin degradation during the fermentation process, as can be seen when compared with the controls.

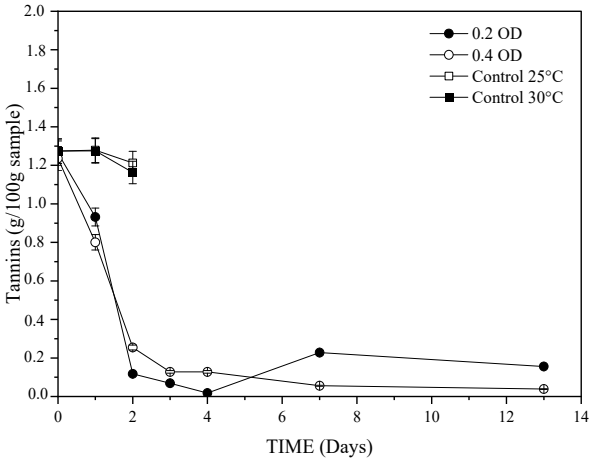

Figure 4. Gradual reduction in tannin levels during the fermentation of *M. deeringiana* flour Fermented with *L. rhamnosus* (OD 0.2, 25 °C, 13 days) and (OD 0.4, 30 °C, 13 days). Error bars represent means ± SEM (n = 3).

Legumes are distinguished by their remarkable content of compounds with antioxidant potential [48]. Figure 5 displays the results, indicating that there are not significant changes in antioxidant capacity. For both samples with OD concentrations of 0.2 and 0.4, as well as for the controls, antioxidant capacity values remained relatively stable throughout the entire assay. Although phenols, tannins, and L-DOPA represent relevant antioxidants, which undergo degradation in the SSF process, it is plausible that other antioxidant compounds are present in the sample, thereby contributing to its overall antioxidant capacity.

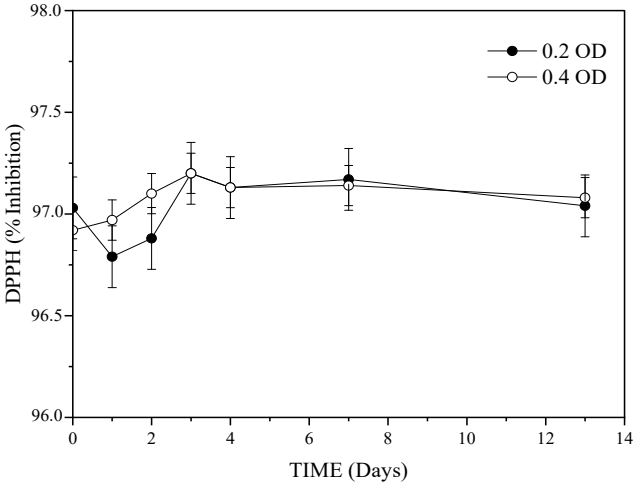

Figure 5. Changes in antioxidant capacity during the fermentation of *M. deeringiana* flour fermented with *L. rhamnosus* (OD 0.2, 25 °C, 13 days) and (OD 0.4, 30 °C, 13 days). Error bars represent means ± SEM (n = 3).

The quantities of L-DOPA (0.55 g/100 g sample), tannins (0.04 g/100 g sample), and phenols (5.7 g/100 g sample) obtained after 13 days of fermentation of *M. deeringiana* flour fall within the ranges reported in Table 1. Consequently, it is possible to indicate that the feed obtained is safe for animal consumption.

Table 4 displays the dry matter and protein percentage of the *M. deeringiana* flour fermented. Although a statistically significant difference is not obtained, a trend of increasing crude protein during fermentation is observed. As can be seen, the protein percentage increases slightly with fermentation time due to the addition, incubation, and growth of *L. rhamosus* in the *M. deeringiana* substrate. Various studies have indicated that bacterial SSF can increase crude protein content due to single-cell protein (protein extracted from cultivated microbial biomass) [49]. In contrast, the fermented samples show a decrease in dry matter. This initial reduction is more pronounced in the early stages of fermentation (1 day). As fermentation progresses, dry matter tends to stabilize, reaching higher values but not returning to the initial levels of the control, reflecting a balance between nutrient consumption and the production of microbial biomass and metabolites. In summary, *M. deeringiana*, being a legume that is easy to harvest and has soil-improving properties, could be used in animal feed after the process of fermentation.

Table 4. Quantification of dry matter and protein content during solid-state fermentation of *M. deeringiana* flour.

Treatment	Crude Protein (%)	Dry Matter (%)
0 Day Without *L. rhamnosus* 25 °C and 30 °C	24.9 ± 0.00	88.8 ± 0.00
1 Day 0.2 OD. 25 °C	27.8 ± 0.01	72.2 ± 0.10
4 Days 0.2 OD. 25 °C	27.2 ± 0.10	71.9 ± 0.02
7 Days 0.2 OD. 25 °C	26.2 ± 0.05	75.7 ± 0.00
1 Day 0.4 OD. 30 °C	23.0 ± 0.02	62.2 ± 0.05
4 Days 0.4 OD. 30 °C	26.9± 0.01	77.5 ± 0.02
7 Days 0.4 OD. 30 °C	26.0 ± 0.00	75.5 ± 0.00

Values are given as mean ± SD (n = 3).

4. Conclusions

Solid-state fermentation with *L. rhamnosus* enhances the nutritional properties of *M. deeringiana* seed flour. By the end of the fermentation process, more than 50% of the phenolic compounds were degraded (total phenolic content reduced by 51%, tannins by 97%, and L-DOPA by 91%), while the antioxidant capacity showed a slight decrease. There was a partial increase in protein content, accompanied by a significant rise in lactic acid content. The formation of lactic acid as the primary product may explain the degradation of antinutritional factors (ANFs). *L. rhamnosus* stabilized and maintained the viability of the fermented feed. This bioprocess demonstrates advantages in enhancing the nutritional value of plant material containing high levels of antinutritional factors and serves as a methodology for both feed production and formulations.

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