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R Pavithra
M.Sc, Clinical Nutrition and Dietetics, Department of Nutrition and Dietetics, Periyar University, Salem, Tamil Nadu, India

L Venipriyadharshini
Assistant Professor, Department of Nutrition and Dietetics, Periyar University, Salem, Tamil Nadu, India

Corresponding Author:
R Pavithra
M.Sc, Clinical Nutrition and Dietetics, Department of Nutrition and Dietetics, Periyar University, Salem, Tamil Nadu, India

Effect of *Lactobacillus* sp. Mediated Fermentation on the Nutritional and Functional Properties of *Vigna aconitifolia*

R Pavithra and L Venipriyadharshini

Abstract
In this experimental study, *Vigna aconitifolia* seeds were soaked for 24 hours at 30°C, followed by fermentation using *Lactobacillus* sp. isolated from curd, incubated at 37°C for another 24 hours. The nutritional composition—including moisture, energy, carbohydrates, protein, fat, fiber, ash, and iron—was assessed. Additionally, total amino acids, polyphenols, and antioxidant levels were evaluated qualitatively. The results demonstrated a significant improvement in nutritional value following fermentation. Protein content increased from 3.6% to 6.19%, while antioxidant activity and total amino acid content were markedly higher in the fermented samples compared to the unfermented ones. Conversely, total polyphenol content was reduced post-fermentation. A gradual rise in pH was observed during fermentation, increasing from 3.5 to 4.2. No microbial contamination was detected throughout the fermentation process.This study concludes that fermentation of *Vigna aconitifolia* using *Lactobacillus* sp. is an effective method to enhance its protein quality, antioxidant potential, and amino acid content, making it a valuable approach for improving the nutritional profile of legume-based foods.

Keywords: *V. aconitifolia*, fermentation, *Lactobacillus* sp, bioavailability

1. Introduction

Processing techniques that involve physical or biochemical modifications can significantly enhance the shelf life and nutritional quality of legumes. Among these, fermentation is a traditional and widely practiced method that involves the biochemical transformation of food components by bacteria or other microorganisms and their enzymes (Cuamatzin-García *et al*., 2022) [1]. Fermentation has been shown to improve the physical, biochemical, and mineral profiles of food, as well as reduce antinutritional factors such as polyphenols. When applied to *Vigna aconitifolia* (commonly known as moth bean), a lesser-known but nutritionally rich legume, fermentation holds the potential to enhance its nutritional value and functional properties. Furthermore, fermentation increases the bioavailability of nutrient**s**, making essential compounds like proteins, amino acids, and minerals more accessible to the body (Siddiqui *et al*., 2023) [2]. Given the importance of traditional and sustainable methods in cereal and legume processing, this study aims to assess the impact of fermentation on the nutritional composition and bioavailability of *Vigna aconitifolia* fermented using *Lactobacillus* sp.

2. Materials and Methods

V. aconitifolia

Vigna aconitifolia seeds were procured from a local market in Salem, Tamil Nadu. The seeds were manually sorted to remove damaged grains and foreign matter, then thoroughly cleaned to eliminate dust and surface impurities in preparation for the fermentation process.

Fermentation

The cleaned *Vigna aconitifolia* seeds were rinsed using potable drinking water and subsequently soaked in the same at a seed-to-water ratio of 1:2. The soaking process was carried out for 24 hours under incubation at 30°C. After soaking, the seeds were ground using a kitchen mixer to obtain a paste-like consistency. To initiate fermentation, *Lactobacillus* sp., isolated from curd, was added to the paste at a concentration of 6.6%.

The mixture was then incubated at 37°C for 20 hours to allow fermentation.

Biochemical Analysis

Proximate analysis was performed to evaluate the fundamental macronutrient composition of *Vigna aconitifolia*, including moisture, fat, protein, carbohydrates, dietary fiber, and energy content, ash and iron. Moisture content was determined by oven-drying the sample at 105°C until a constant weight was achieved. Fat content was estimated using the Soxhlet extraction method with petroleum ether as the solvent. Protein content was quantified using the Kjeldahl method, as per AOAC International (2023) [3], by measuring nitrogen content and applying a standard conversion factor (typically 6.25) to estimate crude protein. Carbohydrates were analyzed using High-Performance Liquid Chromatography (HPLC), which allows precise quantification of both simple and complex sugars. Dietary fiber was measured using the Total Dietary Fiber Method, which involves enzymatic-gravimetric procedures to account for both soluble and insoluble fractions. Energy content was determined through Bomb Calorimetry. Ash content was determined by incinerating the dried sample in a muffle furnace at 550°C until a white or light gray residue was obtained, indicating complete combustion of organic matter. The remaining inorganic residue represents the total mineral content (ash). Iron content was determined using colorimetric or instrumental methods (AOAC, 2023) [3], measuring the heat released during complete combustion of the sample to calculate its calorific value. All analyses were conducted following standard AOAC protocols.

Total polyphenols, Antioxidants, Amino acids and pH

Total polyphenol content was qualitatively estimated using a general spectrophotometric method, as per standard laboratory procedures. The pH of the fermented samples was monitored at regular intervals using the electrometric method and compared to the pH of unfermented *V. aconitifolia* seed flour (AOAC, 2023) [3].

Microbial analysis

Presence of yeast and mold were observed through Potato Dextrose Agar (PDA) and it was kept in observation over 96 hours and examined under Electron Microscope. Other fungal presence was analyzed with Lactophenol Cotton Blue (LCB) - Mounts and the same was examined with electron microscope and both identifications were based of the natural characteristics of yeast, mold and fungi given in International Seed Testing Association (ISTA) Fewer pathogens of fungi can be identified by naked eye or also with lower magnification (AOAC, 2023) [3].

Statistical analysis

Results were examined using independent sample T test to calculate the significance of fermented *V. aconitifolia* from unfermented *V. aconitifolia.*

3. Result and Discussion

Fermentation

Table 1: Microscopic View at 100nm

S. No	Time Duration	Microscopic View at 100nm
1.	8 Hours	
2.	12 Hours	
3.	20 Hours	

Table - 1 outline the microscopic changes observed in *V. aconitifolia* paste during the fermentation process at three different time points: 8 hours, 12 hours, and 20 hours. Microscopic imaging was conducted at 100 nm resolution to monitor the microbial activity and structural changes in the substrate. At 8 hours, initial colonization of *Lactobacillus* sp. was evident, with a sparse distribution of bacterial cells on the surface of the seed matrix. By 12 hours, bacterial proliferation increased significantly, with denser clusters of rod-shaped *Lactobacillus* cells visible, indicating active fermentation. At 20 hours, a mature microbial biofilm structure was observed, along with noticeable degradation of the seed matrix, suggesting advanced enzymatic breakdown and nutrient release. The changes in cell density and seed surface texture throughout the fermentation period reflect the dynamic microbial activity and structural transformation of the substrate (Adebo *et al*., 2022) [4].

Biochemical Analysis

Table 2: Biochemical Analysis

Test Parameters/ 100g	*Unfermented V. *aconitifolia*	Fermented *V. aconitifolia*
Moisture (%)	10.8	75.87
Total Ash (%)	4.8	1.03
Fat (%)	1.1	0.30
Fiber(%)	4.5	1.0
Protein (%)	3.6	6.19
Carbohydrate (%)	56.5	15.12
Energy (k.cal)	330	87.94
Iron (mg)	9.5	2.69

Table 2 presents the comparative nutritional composition of unfermented and fermented *Vigna aconitifolia* per 100 grams of sample. Fermentation resulted in notable changes across several parameters. Moisture content significantly increased from 10.8% to 75.87%, indicating water absorption during the soaking and fermentation processes. Protein content improved from 3.6% to 6.19%, reflecting enhanced proteolytic activity by *Lactobacillus* sp. Similarly, iron content showed a decline from 9.5 mg to 2.69 mg, possibly due to leaching or complexation during fermentation. Conversely, a reduction was observed in carbohydrate (from 56.5% to 15.12%), fat (from 1.1% to 0.30%), fiber (from 4.5% to 1.0%), and ash content (from 4.8% to 1.03%). This suggests microbial utilization and breakdown of complex molecules. Energy content also dropped from 330 kcal to 87.94 kcal, likely as a result of nutrient consumption by fermenting microbes. These changes indicate that fermentation not only alters the nutritional profile but may also enhance protein bioavailability while reducing overall energy density.

Total polyphenols, Antioxidants, Amino acids and pH

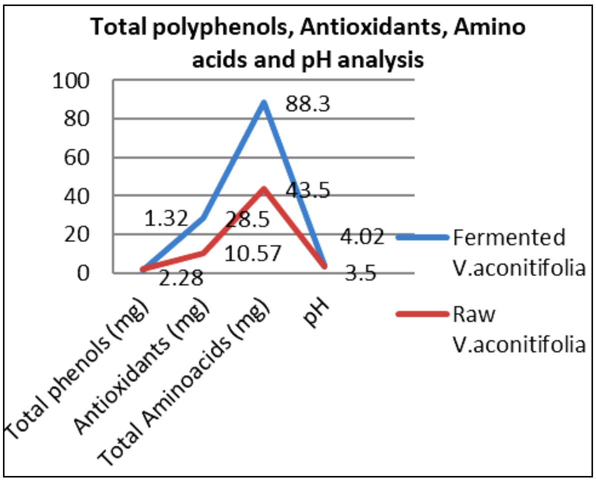

Fig 1: Total polyphenols, Antioxidants, Amino acids and pH analysis

Figure 1 illustrate the comparative analysis of total polyphenols, antioxidant content, total amino acids, and pH levels between raw and fermented *Vigna aconitifolia*. Fermentation resulted in significant biochemical changes. The total amino acid content increased dramatically from 10.57 mg in the raw sample to 88.3 mg in the fermented sample, indicating enhanced protein breakdown and bioavailability due to microbial activity. Antioxidant levels also increased from 2.28 mg to 28.5 mg, suggesting improved functional properties as a result of fermentation (Knez *et al*., 2023) [5]. In contrast, the total polyphenol content slightly decreased from 2.28 mg in the raw sample to 1.32 mg in the fermented one, which is consistent with known reductions in certain antinutritional compounds during fermentation. The pH value increased from 3.5 in the raw sample to 4.02 after fermentation, indicating microbial metabolism and acid production during the process (Frias *et al*., 2019) [9].

Microbial analysis

It is predicted that no significant yeast, mold and fungal growth were observed throughout the time period of 96 hours in either visual or with microscopic examination and hence it shows that the fermentation process was done with no cross contamination (Cichońska P and Ziarno M, 2021) [6].

Statistical analysis

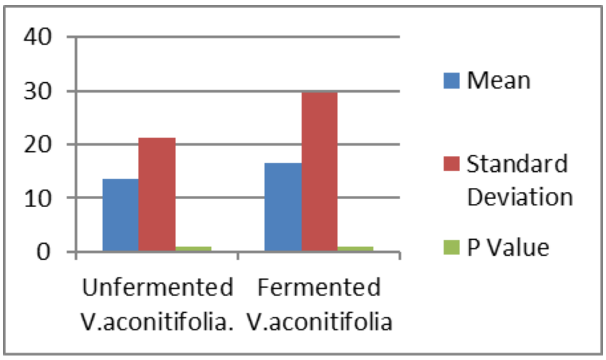

Fig 2: Statistical analysis

Figure 2 presents a comparative statistical analysis of unfermented and fermented *Vigna aconitifolia* samples in terms of mean, standard deviation, and p-value for the measured parameters. The mean values (blue bars) are higher in the fermented sample compared to the unfermented sample, indicating an overall increase in the measured nutritional or functional properties post-fermentation. The standard deviation is also slightly higher in the fermented sample, suggesting greater variability in measurements, possibly due to the dynamic nature of microbial fermentation. The p-values (green bars) for both samples are low, indicating that the observed differences between the unfermented and fermented samples are statistically significant. This supports the conclusion that fermentation has a meaningful impact on the composition of *Vigna aconitifolia*.

4. Conclusion

The present study demonstrates the nutritional and functional enhancement of *Vigna aconitifolia* through fermentation using *Lactobacillus* sp., as supported by proximate analysis, biochemical profiling, and statistical evaluation. Fermentation significantly increased moisture content (from 10.8% to 75.87%), indicating water absorption during the soaking and microbial activity. Most notably, protein content increased from 3.6% to 6.19%, reflecting microbial proteolysis and improved protein bioavailability. Similarly, total amino acid content rose sharply from 10.57 mg to 88.3 mg, as seen in Figure 1, further confirming the protein-enhancing effect of *Lactobacillus*-mediated fermentation.

A substantial rise in antioxidant content (from 2.28 mg to 28.5 mg) was also observed in the fermented sample, suggesting increased functional potential due to microbial release of bioactive compounds. Conversely, the total polyphenol content decreased slightly (from 2.28 mg to 1.32 mg), aligning with known reductions in antinutritional factors during fermentation. The pH value increased from 3.5 to 4.02, indicative of lactic acid production and stable fermentation activity. Despite the enhancements, there was a notable reduction in carbohydrate (from 56.5% to 15.12%), fat (from 1.1% to 0.30%), fiber (from 4.5% to 1.0%), and ash content (from 4.8% to 1.03%). These declines suggest microbial utilization of macronutrients for metabolic processes. Iron content also decreased from 9.5 mg to 2.69 mg, possibly due to mineral complexation or leaching during soaking and fermentation. Statistical analysis (Figure 2) confirms that these differences between unfermented and fermented samples are statistically significant, as indicated by low p-values and observable shifts in mean values and standard deviations across the tested parameters. Overall, this study confirms that fermentation of *Vigna aconitifolia* with *Lactobacillus* sp. enhances protein quality, amino acid availability, and antioxidant activity while reducing antinutrients and caloric density. These improvements position fermented legumes as promising ingredients for developing functional food products, protein supplements, and antioxidant-rich formulations. Future research could explore fermentation of other underutilized legumes using varied strains of *Lactobacillus*, other beneficial bacteria, or yeasts to optimize nutritional outcomes. Such investigations could pave the way for novel food innovations aimed at improving dietary protein intake and functional nutrition.

5. References

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