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RESEARCH PAPER

Fermentation of Rajma Bean by *Bacillus* sp. CAU5 Remove Phytohemagglutinin Completely

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ABSTRACT

Rajma beans (*Phaseolus vulgaris* L.) are a widely consumed legume known for their high protein content and antidiabetic potential. However, their acceptability as a dietary protein source is limited due to the presence of antinutritional and allergenic factors, notably phytohemagglutinin (PHA), a toxic lectin. This study investigates the effectiveness of microbial fermentation using *Bacillus* sp. CAU5 in reducing the antinutritional properties of rajma beans while preserving their sensory and nutritional qualities. Fermentation was carried out under controlled conditions for 20 hours, and the resultant product was analyzed through a phytohemagglutinin assay and sensory evaluation. Results indicated that while boiling reduced PHA activity by approximately 76%, fermentation with *Bacillus* sp. CAU5 completely eliminated detectable PHA activity. Sensory evaluation on a 9-point hedonic scale yielded high acceptability scores for odour (7.4), texture (7.7), taste (7.5), and overall acceptability (7.8). These findings suggest that fermentation is a simple, cost-effective, and scalable method to detoxify rajma beans, making them a promising functional food ingredient for diabetic-friendly diets and vegetarian protein supplementation.

Keywords: Rajma, Fermentation, Antinutritional, Phytohemagglutinin

Rajma beans (*Phaseolus vulgaris* L.), commonly referred to as kidney beans, are a staple legume widely consumed across various cultures due to their rich protein content, affordability, and culinary versatility (Carbas *et al.* 2020). However, despite their nutritional benefits, concerns persist regarding their toxicity and allergenicity, which can pose health risks if not properly managed.

One of the most notable toxic components in kidney beans is phytohemagglutinin (PHA), a type of lectin that can cause severe gastrointestinal distress including nausea, vomiting, and diarrhoea when beans are consumed raw or undercooked (Petroski & Minich, 2020; Wang *et al.* 2021). In addition to lectins, other antinutritional factors such as saponins, phytates, protease inhibitors, and tannins are commonly found in raw beans, which interfere with nutrient absorption and reduce protein digestibility

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(Samtiya *et al.* 2020; Zhang *et al.* 2025). These compounds can hinder the bioavailability of essential minerals like iron and zinc (Mecha *et al.* 2021).

Allergenic proteins present another layer of concern. Kidney beans have been associated with IgE-mediated hypersensitivity reactions, with documented cases of food allergies resulting in conditions such as oral allergy syndrome and allergic rhinitis (Cianferoni, 2024; Wang *et al.* 2021). The allergenic potential is primarily attributed to seed storage proteins and lectins that can act as allergens, particularly in sensitized individuals.

Despite these safety concerns, kidney beans have also been recognized for their health-promoting properties. Several studies have demonstrated their antidiabetic potential, especially due to the presence of α-amylase and α-glucosidase inhibitors, which can help manage postprandial blood glucose levels (Peddio *et al.* 2022; Fukuda *et al.* 2019). In this context, rajma beans are considered functional foods that contribute to glycemic control and metabolic health (Sparvoli *et al.* 2021).

To harness their nutritional advantages while mitigating their antinutritional and allergenic drawbacks, food processing methods such as fermentation, soaking, and thermal treatments have been investigated. Among these, fermentation has emerged as one of the most effective approaches to reduce antinutritional factors like phytates and tannins, improve protein digestibility, and enhance mineral bioavailability (Zhang *et al.* 2025; Samtiya *et al.* 2020). Furthermore, fermentation may modulate the allergenic properties of legumes by degrading or denaturing allergenic proteins (Wang *et al.* 2021).

In our research, we focused on employing fermentation techniques to reduce the levels of antinutritional and allergenic compounds in rajma beans. The goal was to improve their safety and nutritional quality without compromising their beneficial bioactive properties, particularly those relevant to managing type 2 diabetes and providing high-quality plant protein.

MATERIALS AND METHODS

The Rajma beans have been purchased from the local market (*Thangal bazaar, Imphal*). The rabbit blood was obtained from young and healthy farm Rabbits. All the chemicals were procured from authorised agencies and were of the ARS grade.

Fermentation of Rajma bean by *Bacillus* sp. CAU5

The red kidney bean (Rajma) was purchased from the local market. Any unwanted materials and unhealthy seeds were removed, cleaned and soaked in water for 7-8h. Then, it was boiled in a pressure cooker until it was cooked nicely and softened enough to be squeezed in between fingers. After cooling down to room temperature *Bacillus* sp. CAU5 starter (pulverised fermented Rajma with B. sp CAU5 with 10^9 cfu) was inoculated 4g/Kg. It was incubated at 37 °C ± 2°C for 20h. Fermentation was observed by formation of a unique layer of culture on the seed along with the typical aroma. The fermented rajma beans were dried in a mechanical dryer at 60 °C for 12h. The dried product was then pulverised and kept in 4 °C for further use.

20g of the dried and pulverised fermented rajma beans were added with 100 ml of Physiological saline and kept at 4 °C for 20 h in a BOD incubator with intermittent shaking until it was centrifuged at 8000g for 8 min. The supernatant was subjected to rotary evaporation until it was reduced to about 10ml (A_{600} is 0.05). The condensed filtrate thus obtained was subjected to Phytohemagglutinin assay.

Sensory analysis

Sensory evaluation of the fermented rajma bean was carried out by panellists on a 9 point hedonic scale by semi-trained panellists with 1 being dislike extremely and 9 being like extremely. Four parameters viz - 1. Odour 2. Texture 3. Taste 4. Overall Acceptability was tested by the selected forty panellists. The panellists were individually subjected to the test in the sensory evaluation laboratory of the College of agriculture, Central Agricultural University Imphal. The scores were recorded and analysed using statistical methods.

The score of 6 was considered acceptable, lower than 6 in any parameter will be considered unacceptable.

Phytohemagglutinin assay

Lectin-dependent agglutination titers were determined by performing Phytohemagglutinin assays using the washed rabbit RBC. The lectin sample (50 ml) was 2-fold serially diluted with equal volume of 50 mM sodium phosphate buffer pH 7.4 containing 0.9% NaCl in a U-bottom microtiter plate (Tarson, Kolkata, India), followed by the addition of equal volume of rabbit 2% v/v RBC in each well. The contents were mixed gently but thoroughly. Corresponding control wells containing only the mixture of buffer and rabbit RBC were also included as control. After incubation for 1 h at 37 °C, the hemagglutination titer was scored visually (Fig. 3). The lectin activity was expressed as reciprocal of the highest dilution of the lectin sample at which the complete hemagglutination occurred. A Phyto Hemagglutination unit (PHA) was taken as the minimum amount of lectin required for complete hemagglutination under the assay conditions.

RESULTS

Fermentation of Rajma Bean

The process of fermentation of the Rajma bean is described in Fig. 1. The selected *Bacillus* sp. CAU5 was successfully used to produce fermented rajma beans. In the described environment, fermentation of the beans was completed in 20h with desired texture and flavour.

Sensory analysis

The Hedonic scale of 1 to 9 in four parameters was tested for overall acceptability. With average score of 7.4, 7.7, 7.5 and 7.8 for odour, texture, taste and overall acceptability respectively show highly acceptable product (Fig. 2).

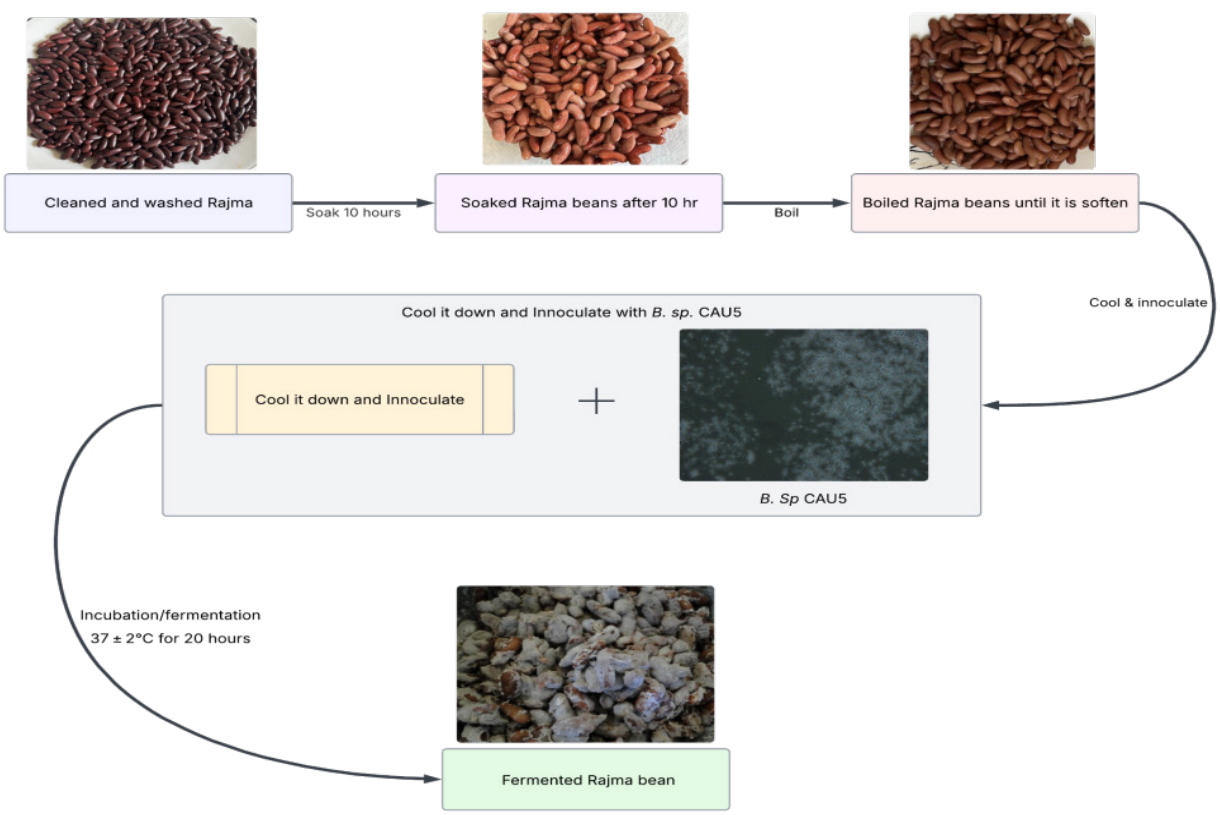

Fig. 1: Fermentation of Rajma beans by *Bacillus* sp. CAU5

Phytohemagglutinin assay

Phytohemagglutinin has been identified as a major hindrance in its acceptability as a protein rich food source in many countries. Although many processing activities removed the toxic level of Rajma beans, only fermentation could remove the PHA completely. Fermentation being very simple and cost effective, it can be easily implemented in rural settings. Moreover, fermentation by *Bacillus* enhanced nutritional as well as medicinal properties. In our study, boiling removed about 76% of Phytohemagglutinin activity of Rajma. But fermentation of rajma by *Bacillus* sp. CAU5 completely removed the phytohemagglutinin activity (Table 1). Also fermentation adds flavour to the product which is otherwise bland in taste. The powder fermented rajma beans could be a good food supplement to protein lacking vegetarian diets of Indians.

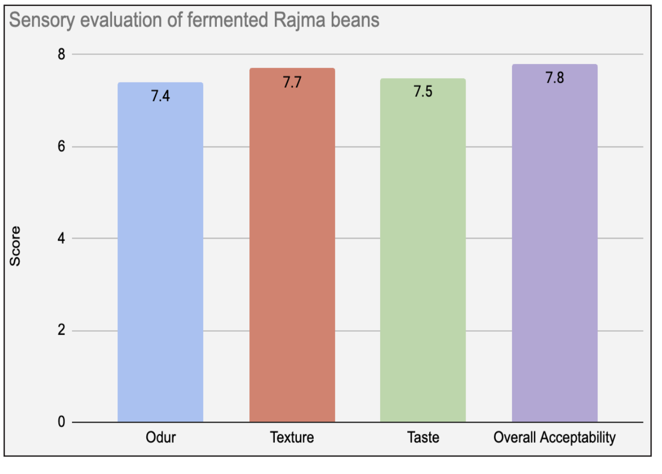

Fig. 2: Sensory evaluation of fermented rajma beans

Table 1: Phytohemagglutinin assay of raw Rajma bean and process rajma bean

Sl. No.	Particular/s	Well No.	Phytohemagglutinin unit (PHA)
1	Raw Rajma bean	10	1024
2	Boiled Rajma bean	8	256
3	Fermented Rajma bean with *bacillus* subtilis CAU5	1	2

Fig. 3: Microtiter plate assay of Phytohemagglutinin

CONCLUSION

Rajma offers a cheaper source of protein in various non vegetarian diets but with its inherent antinutritional factors like trypsin inhibitor, saponins particularly Phytohemagglutinin posses serious health issues. The issue is addressed by various processing methods including soaking in saline water, roasting; cooking but complete removal of PHA was achieved by fermentation of Rajma beans by GRAS grade *Bacillus* sp. CAU5 was previously isolated from Hawaijar, a traditional fermented soybean food of Manipur. The sensory evaluation of the product reaffirms its taste, texture, colour and overall acceptability. With its immense potential the rajma bean could be a novel protein supplement and could be tried as a functional food against type 2 diabetes. This small process could be scaled up for industrial level production and open an avenue for innovative food based on fermented rajma beans.

GENERATIVE AI DECLARATION

The author(s) confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI. The authors developed and verified all scientific content, data analysis, interpretation of results, and conclusions.

The authors take full responsibility for the accuracy, integrity, and originality of the work presented, and no AI tool was listed as an author.

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