The Effect of pH on Steady-State Fermentation of Soya Bean

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Abstract-* Fermentation is also crucial in nutritional and sensory improvements of soybeans thus rendering them to be more food-friendly. To enable the steady fermentation of soybean pods, the research analyses the effects of pH and temperature on three variables, including protein content, moisture level, and growth of microbes. It is carried out with an inoculation of Bacillus subtilis and Lactobacillus plantarum mixed culture and underlying the methodological approach response-surface methodology is used to determine the most favourable operating conditions. The results showed that acidic pH especially at pH of 3 was found to have the greatest protein enrichment and also promoted maximum bacterial activity at moderate temperatures between 25 and 30 o C. The protein content fell at higher pH and high temperatures, the level of moisture also fell with increase in temperature. These results demonstrate that the quality of the products and the performance of the microorganisms depend on the control of the fermentation environment of these microorganisms. This work contributes to a better comprehension of steady processes related to the soybean fermentation, clarifying the influence of pH and temperature on fermentation and improving the practise in the industries. Finally, the investigation demonstrates that nutritional benefits can be enhanced through controlled fermentation in order to raise the chances of sustainable use of soybean.

***Index Terms-* Fermentation, pH, Protein content, Soybean, Temperature**

I. INTRODUCTION

Soybean (*Glycine max* L.) is one of the most nutritionally valuable legumes, widely consumed for its high protein content, unsaturated fatty acids, dietary fiber, and a range of bioactive compounds with demonstrated health benefits [1][2]. Historically cultivated in East Asia, soybeans have become a global commodity, serving as a foundation for foods such as soy milk, tofu, soy flour, and soy oil, as well as fermented products like miso, tempeh, natto, and soy sauce [3][4]. Beyond their nutritional profile, fermented soybean products are valued for improved digestibility, enhanced flavor, and the presence of bioactive metabolites that contribute to food preservation and health promotion [5][6].

Fermentation plays a particularly important role in overcoming some of the challenges of soybean consumption, such as its beany taste, long cooking time, and limited digestibility [7]). Through the action of microbial communities, including *Bacillus subtilis* and lactic acid bacteria, fermentation promotes the breakdown of proteins into peptides and amino acids, enhances sensory quality, and produces metabolites with antimicrobial and antioxidant properties [8][9]. These transformations have made fermented soybean foods an important dietary component in many Asian cultures and a growing interest in Western food industries seeking sustainable plant-based protein sources [10].

Despite their established nutritional and functional benefits, the quality of fermented soybean products is highly dependent on environmental conditions during processing. Parameters such as pH, temperature, and inoculum concentration strongly influence microbial activity, enzymatic reactions, and nutrient availability [11][12]. While temperature has been relatively well studied, less is known about the specific role of pH under steady-state fermentation systems, where microbial growth and metabolite production remain constant over time. This represents a critical gap, as optimizing these parameters is essential for achieving consistent product quality and maximizing protein yield.

Research Gap and Rationale

The interplay amongst pH and temperature during soybean fermentation is yet to be extensively investigated using steady-state controlled conditions despite the fact that the variables exert direct influence on microbial dynamics and the biochemical products. This gap in targeted research restricts the potential of processors to optimise fermentation that can enhance nutritional content, sensory properties, and product shelf life. Addressing this gap is important not only for industrial soybean processing but also for developing affordable, protein-rich foods that can support nutrition security in vulnerable populations [13][14].

Study Objectives

In this paper, the synergetic effects of pH on temperature with respect to the steady-state fermentation of soybean pods with a mixed culture of *Bacillus subtilis* and *Lactobacillus plantarum* will be explored. Specifically, it examines how these parameters have an impact on protein content, moisture retention and microbial growth. The rationale is to identify optimal conditions of fermentation to enrich optimally the microbial performance and proteins, and give practical information regarding enhancement of the production of soybean-based foods.

II. MATERIALS AND METHODS

Materials

The used mature soybean seeds were purchased locally in Katsina, Nigeria. De-hulling and cleaning were carried out by hand to achieve the uniform quality of the seeds and then utilised in the fermentation process. *Bacillus subtilis* and *Lactobacillus plantarum* were the most popular strains used in fermenting soybean and served as inoculum. These were kept in nutrient agar and the MRS agar respectively at a temperature of 4^{o}C until they were required to carry out experiments. The soybean was used to perform fermentation without any nutrient or carbon supplementation, which is in accordance with the earlier studies that provide evidence of the appropriateness of natural sources of carbon and nutrients in the traditional soybean fermentation [5][6].

Experimental Design

The effects of pH, temperature and inoculum concentration in a steady-state fermentation of soybean were to be examined through a Central Composite Design (CCD) with the context of Response Surface Methodology (RSM). The method was chosen due to its effectiveness in the assessment of the interaction effects between many variables and reduced the number of experimental runs [15]. The three independent variables were pH (5.0-7.0), temperature (30- 40^{0}C) and inoculum concentration (1-5 per cent v/v), and the main response variable was the crude protein content at the end of 72 hours fermentation.

Fermentation Process

Soybeans were autoclaved at 121^{o}C and 15 min after being soaked in distilled water (12 h). They were inoculated using the mixed cultures of *B. subtilis* and *L. plantarum* under the experimental design but after cooling. The batch fermenters were used to perform the fermentation process under constant conditions with the temperature kept at 30 - 40^{0}C. The sampling periods were done after 12hrs of each 72hrs of fermentation process. This methodology is in line with methodologies that have been outlined in the past studies regarding the optimization of soybean fermentation parameters [9][16].

Analytical Methods

At every 12-hour interval, a digital pH metre was used to measure the pH of the fermentation broth. The content of crude protein was measured by the Kjeldahl method, in accordance with the AOAC (2016) standards, which are still a classic of protein quantification when analysing food [17]. The content of moisture was determined through drying the samples at a temperature of 105^{0}C until the constant weight was obtained, which followed the conventional procedures of proximate analysis (AOAC, 2016).

Microbial enumeration was carried out using serial dilutions of the fermentation broth plated on selective media: nutrient agar for *B. subtilis* and MRS agar for *L. plantarum*. Plates were incubated at 37°C for 24–48 hours, after which viable colonies were counted and expressed as CFU/mL, consistent with previous studies on fermented soy products [18]. Sensory evaluation was conducted by a panel of 20 trained assessors who rated taste, texture, and aroma using a nine-point hedonic scale, a method widely adopted in food quality research [19].

Statistical Analysis

Experimental data were analyzed using Design-Expert software (Version X, Stat-Ease Inc., Minneapolis, MN, USA). Response Surface Methodology (RSM) was applied to model the effects of pH, temperature, and inoculum concentration on protein content. Model significance was assessed by analysis of variance (ANOVA), with statistical significance set at $p < 0.05$. This combination of RSM and ANOVA has been demonstrated to be effective in optimizing fermentation processes and ensuring reproducibility of results [20][21].

III. RESULTS AND DISCUSSION

Effect of pH on Protein Content

The results revealed that pH exerted a significant influence on protein enrichment during fermentation (Table 1). At room temperature, protein content was highest at pH 3 (0.311 ± 0.002%), while values at neutral and alkaline pH levels were markedly lower. This trend indicates that acidic conditions favor protein hydrolysis and accumulation in fermented soybeans. Similar findings have been reported in studies where acidic environments promoted the activity of proteolytic enzymes, leading to enhanced protein solubilization and peptide release [5]. The preference for acidic conditions is consistent with the metabolic requirements of lactic acid bacteria, which thrive at lower pH values and contribute to improved protein availability [8].

Table 1. Effect of pH and Temperature on Steady-State Fermentation of Soybean

Condition (pH and Temperature)	Protein Content (%)	Moisture Content (%)	Bacterial Colony Count (CFU/mL)
3 (Room temperature)	0.311 ± 0.002	68.99 ± 0.50	Too numerous to count (TNTC)
5 (Room temperature)	0.092 ± 0.001	72.93 ± 0.45	–
7 (Room temperature)	0.053 ± 0.001	58.95 ± 0.35	–
9 (Room temperature)	0.079 ± 0.001	61.36 ± 0.40	–
7 (25°C)	0.096 ± 0.002	64.69 ± 0.30	32
7 (30°C)	0.088 ± 0.001	58.77 ± 0.25	71
7 (35°C)	0.066 ± 0.001	53.34 ± 0.20	41
7 (40°C)	0.061 ± 0.001	36.12 ± 0.15	37

Notes: Values represent mean ± standard error. TNTC = Too numerous to count. Room temperature was approximately 20–25°C.

Effect of Temperature on Protein Content and Microbial Growth

When fermentation was conducted at a fixed pH of 7, temperature exerted a clear impact on both protein content and microbial activity. Protein concentration decreased steadily as temperature increased from 25°C to 40°C, with the highest value (0.096 ± 0.002%) recorded at 25°C. This suggests that moderate temperatures provide optimal conditions for enzymatic activity and protein retention. At higher temperatures, partial denaturation of proteins and reduced enzyme stability may explain the decline in protein yield [9].

Patterns of microbial growth were similar. The highest colony count (71 CFU/mL) was found at 30^{0}C that is consistent with the optimum growth range of *Bacillus subtilis* and *Lactobacillus plantarum* [21]. The activity of bacteria decreased at temperatures exceeding 35^{0}C, which suggests that the presence of high thermal conditions can place stress on the metabolism of microorganisms and decrease the level of fermentation. These findings affirm the previous findings that moderate temperatures of fermentation play a critical role in preserving the balance of microbes and enabling good conversion of substrates [6].

Moisture Content and Its Implications

The moisture content of the soybean substrate also differed with temperature at a significant rate. Moisture was comparatively high at 25degC (64.69 +- 0.30), and the lowest (36.12 +- 0.15) was at 40^{0}C. Reduction in moisture content increasing in temperature can be explained by the evaporation of water and metabolic usage which both influence the dynamics of fermentation. Moisture is also significant in texture and sensory quality and any loss beyond normal can cause firmer products which have less consumer acceptability [9]. Maintaining moderate moisture levels is therefore essential for achieving desirable functional and sensory properties in fermented soybean foods.

Integration with Literature and Practical Implications

Overall, the findings underscore the importance of precise control over pH and temperature in soybean fermentation. Acidic conditions (around pH 3) and moderate temperatures (25–30°C) consistently produced the highest protein content and supported robust microbial activity. These conditions mirror those identified in previous work on soybean and other legume fermentations, where lactic acid bacteria and *Bacillus* species demonstrated optimal functionality under slightly acidic, moderate thermal environments [11][2].

From an industrial perspective, these results provide practical insights for standardizing fermentation processes. By adopting optimal conditions, producers can achieve higher protein enrichment, consistent product quality, and improved microbial efficiency, all of which are essential for scaling soybean fermentation to meet growing consumer demand for nutritious, plant-based foods. Furthermore, the protein enrichment observed in this study highlights the potential of fermented soybeans as an affordable strategy for addressing protein-energy malnutrition, particularly in regions where dietary protein intake is inadequate [13].

CONCLUSION

In this study, pH and temperature factors were significant in the steady fermentation of soybeans. Protein enrichment was best accomplished at acidic (pH 3) conditions, and moderate temperatures (25-30C) were conducive to the microbial activity. Quite on the contrary, high temperatures reduced the level of protein and microbial growth and the level of moisture was also low. These findings show that there is need to strictly control the environmental condition to obtain high-quality fermentation repeatedly.

For practical application, soybean processors should adopt acidic pH and moderate temperature ranges to maximize protein content and microbial efficiency. Maintaining sufficient moisture during fermentation is also important for ensuring desirable texture and sensory properties. These optimized conditions not only improve product quality but also enhance the nutritional potential of soybeans, making them suitable for tackling protein malnutrition in resource-limited settings. Future research should build on these findings by investigating sensory attributes, bioactive compounds, and large-scale feasibility to strengthen both industrial production and consumer acceptance of fermented soybean products.

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