Effects of Single and Mixed Cultures of *Pediococcus pentosaceus* Strain DSM20336 and *Bacillus subtilis* subsp. *subtilis* Strain-168 on the Total Viable Counts and Physicochemical Properties of Fermented Cabbage and Soybeans

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Abstract

The study determined the effects of single and mixed starter cultures of *Pediococcus pentosaceus* strain DSM20336 and *Bacillus subtilis* subsp. *subtilis* strain-168 on the total viable counts (TVCs) of bacteria and physicochemical properties of fermented cabbage and soybeans. A controlled experiment of microbial fermentation was set up in a completely randomized design, using test and standard isolates as starter cultures in single and mixed treatment combinations. Fermentation was allowed to progress at room temperature (28 ± 2°C) from week 1 to week 4. Optimized conditions were established for temperature, pH, and titratable acidity. Results showed that *P. pentosaceus*-based ferments contained the lowest TVCs in both cabbage (19 x 10^6 cfu/g) and soybean (51 x 10^6 cfu/g). The TVC in the mixed culture of *Bacillus* + *Pediococcus* in cabbage ferment was 120.9 x 10^6 cfu/g, while it was 147 x 10^6 cfu/g in soybean ferment. TVC reduced sharply as microbial fermentation progressed over time. In the controlled fermentation of cabbage, it was established that temperature, titratable acidity, and TVCs of fermenters had very high positive relationships with each other. Meanwhile, in the fermentation of soybean product, the TVCs of bacteria were negatively affected by high temperature and low pH (acidity) of the medium. Therefore, lower temperature and pH are recommended for the fermentation of soybean products. The study established some fermentation parameters for cabbage and soybean. This information is important for the commercial production of these products to achieve food security in Nigeria.

Keywords: cabbage; fermentation; fermentation parameters; physicochemical properties; soybeans; TVCs

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1. Introduction

Food fermentation offers enormous nutritional and health benefits, such as improved energy value, amino acid content, and vitamin levels, which play significant roles in the metabolic activities of the human body for healthy living [1,2]. It is a form of food preservation technology that does not require refrigeration or sophisticated methods. This is because metabolites like organic acids, ethanol, and bacteriocins, produced during the fermentation process, exhibit antimicrobial action, thereby reducing the activities of food spoilage microorganisms [1,2]. Microorganisms will continue to grow on the substrate until fermentation products inhibit them. Therefore, growth in a fermenting medium follows the normal phases (lag, log, stationary, and decline phases) expected of bacteria [3].

Fermentation of food can produce diverse types of microorganisms, including unwanted pathogens such as *Salmonella* and *Vibrio*. Although the acidic content and lowered pH of the fermentation medium inhibit the growth of many unwanted microorganisms, some microbial contaminants are able to tolerate extreme conditions and pose potential hazards to humans [4]. Many sources contribute to microbial contamination in fermented food, including post-harvest handling, the presence of vectors, and poor sanitary conditions [5]. Freshly harvested food may contain various vectors and etiological agents, including spore-forming bacteria, viruses, and parasites [5]. Control points are introduced to ensure that microbial loads in food products are kept below their regulatory permissible limits [4].

Many factors have been identified to influence microbial activities during food fermentation. These include the composition

of the substrate (carbohydrate, protein, and fat), the nature of the microbe, and the intrinsic and extrinsic environmental conditions of the medium. It is believed that microbial activities can be controlled by optimizing these influencing factors. These may include moisture, hydrogen ion concentration (pH), salt concentration, temperature, oxygen requirements, nutritional requirements, and the presence of inhibitors [6,7].

2. Methodology

2.1 Sample Collection and Preparation

Cabbage leaves and soybean seeds (5 kg) were purchased from major markets within the State Capital. All materials were packaged in sterile bags and transported to the laboratory for further processing and analysis. Cabbage leaves were cleaned by removing the damaged outer leaf cover. They were shredded after washing with 2 L of sterile distilled water. Hydrated soybean seeds were dehulled manually by rubbing the seeds multiple times with the palms to get them ready for fermentation, while sterile hand gloves were worn to ensure an aseptic condition [8].

2.2 Source of Fermenting Organisms and Preparation of Inocula

Biochemically and molecularly characterized test strains of *Pediococcus pentosaceus* and *Bacillus subtilis* subsp. *subtilis* strain-168 isolates, which were already identified, stored, and duly authenticated, were obtained from a culture collection at the Department of Microbiology, Joseph Sarwuan Tarka University Makurdi. Standard strains of *Lactobacillus fermentum* and *Lactobacillus plantarum* were obtained from the Veterinary Research Institute, Vom, Jos, Plateau State.

2.3 Controlled Fermentation of Cabbage and Soybean using Starter Culture

The fermentation process was set up with plant materials and the inocula. Exactly 0.5% of the test or standard strains were inoculated into 300 g of the shredded cabbage in earth pots lined with sterile aluminum foil [8]. The dehulled germinated soybean seeds were placed in water in a ratio of 1:3 (w/v) g to water in covered, labeled plastic containers of the same sizes. The seeds were allowed to ferment at varied time intervals of 120 h to 1040 h using starter culture (5 to 43 days). The fermented seeds were dried at 50°C in a hot air oven for 50 min [7, 8]. The dried seeds were ground using a Moulinex blender and sieved with a 60-mm mesh size to separate the fine sample flours from coarse particles. The flours were put in labeled plastic containers and stored in the refrigerator.

Fermentation was set up in 24 pots (12 pots for cabbage and 12 pots for soybean experiments). This was a completely randomized design of 8 treatments and 3 replicates per treatment [7, 8].

Treatments for cabbage fermentation were:

C+CT = Cabbage + Control (Spontaneous fermentation)

C+P = Cabbage + *P. pentosaceus*

C+B+P = Cabbage + *B. subtilis* + *P. pentosaceus*

C+B = Cabbage + *B. subtilis*

Treatments for soybean fermentation were:

S+CT = Soybean + Control (Spontaneous fermentation)

S+P = Soybean + *P. pentosaceus*

S+B+P = Soybean + *B. subtilis* + *P. pentosaceus*

S+B = Soybean + *B. subtilis*

Fermentation was allowed to progress at room temperature (28 ± 2°C) from week 1 to week 4 [7].

2.4 Estimation of Total Viable Counts (TVCs) in Fermented Products

Serial dilution, pour plate techniques, and incubation at 37°C for 24 h were employed. Visible colonies on the plates were then counted using a colony counter. The culture media used were Man Rogosa and Sharpe (MRS) Agar and Tryptic Soy Agar. Total viable counts (TVCs) were recorded as colony-forming units per gram (cfu/g) x 10^3. Microbial counts were performed at weekly intervals to monitor the growth of the starter culture throughout the entire fermentation process [9].

2.5 Physicochemical Analysis of Fermented Products

The protocols outlined by the Association of Official Analytical Chemists [10] were adopted for the determination of the physicochemical properties of the fermented products. A 10% suspension of the product was prepared by dissolving 5 g of the sample in 50 mL of distilled water in a 250 mL beaker. The probe of a pH meter (Jenway 3505) was inserted after calibration using buffer solutions of pH 4 and 9. The pH value was then read from the LED display after the electrodes were properly inserted. The temperature (°C) was measured using a mercury-in-glass thermometer inserted into the fermenting sample and read manually. Titratable acidity was determined as follows: approximately 1 g of the sample was weighed and suspended in 10 mL of distilled water. The resulting suspension was titrated against 0.10 N sodium hydroxide using phenolphthalein as the indicator. The volume of 0.10 N sodium hydroxide required to neutralize the sample's acidity was recorded.

2.6 Data Analysis

All phenotypic and biochemical data were analyzed using Minitab 16.0 software for descriptive statistics, analysis of variance (ANOVA), correlation, and regression. The level of significance was set at 0.05 ($P \leq 0.05$).

3. Results

3.1 Total Viable Counts (TVCs) in Fermented Products

The microbial load, expressed as TVCs, in fermented cabbage during the controlled experiment (weeks 1 to 4) is presented in Figure 1. In week 1, the TVC in spontaneous fermentation (C+CT) was less than 100×10^6 cfu/g, whereas it more than doubled in microbially fermented products (>200×10^6 cfu/g). In the spontaneous fermentation group, the TVC increased from 81×10^6 cfu/g in week 1 to 255×10^6 cfu/g by week 4. In contrast, the TVC in microbially inoculated samples decreased significantly from week 2 to week 4. Among these, cabbage fermented with *P. pentosaceus* exhibited the lowest TVC at 19×10^6 cfu/g. The average TVCs of the treated fermented cabbage samples, in descending order, were as follows: Control (139×10^6 cfu/g) > *Bacillus* + *Pediococcus* ferment (120.9×10^6 cfu/g) > *Bacillus*-only ferment (106.9×10^6 cfu/g) > *Pediococcus*-only ferment (92×10^6 cfu/g).

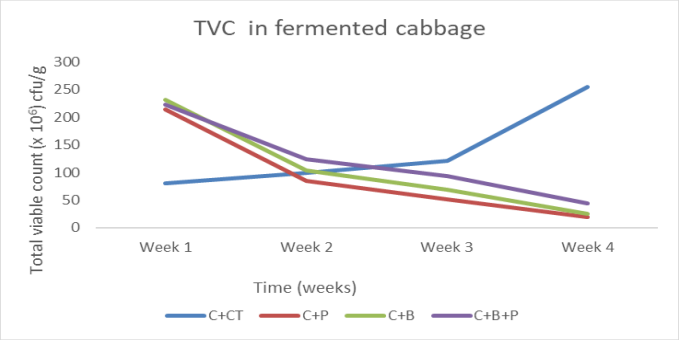

Figure 1: Total viable counts in fermented cabbage.

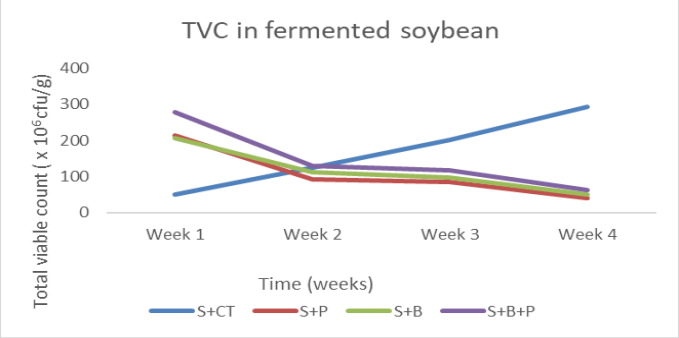

Figure 2: Total viable counts in fermented soybean.

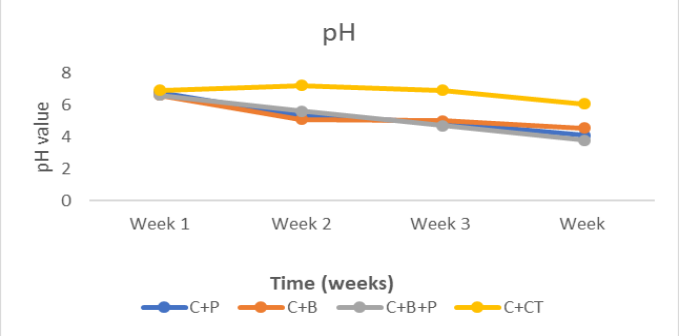

Figure 3: pH of fermented cabbage from weeks 1 to 4.

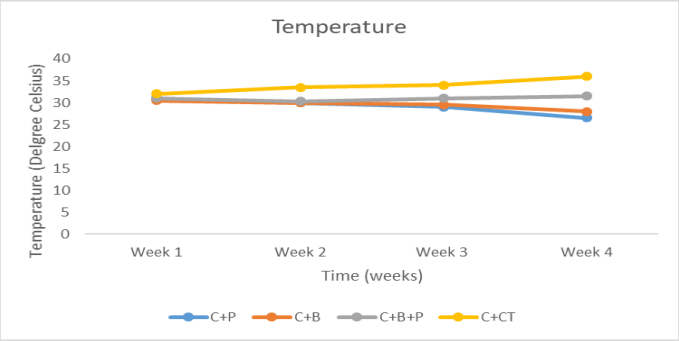

Figure 4: Temperature of fermented cabbage from weeks 1 to 4.

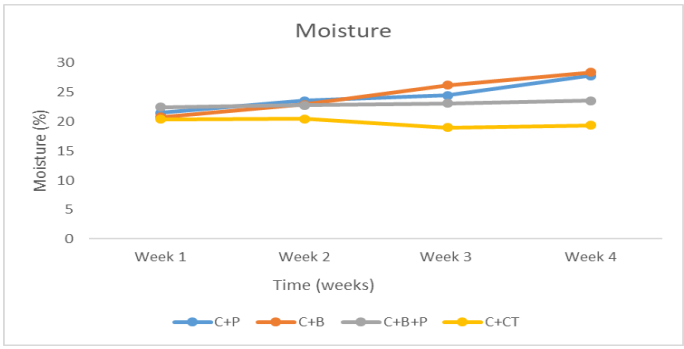

Figure 5: Moisture content of fermented cabbage from weeks 1 to 4.

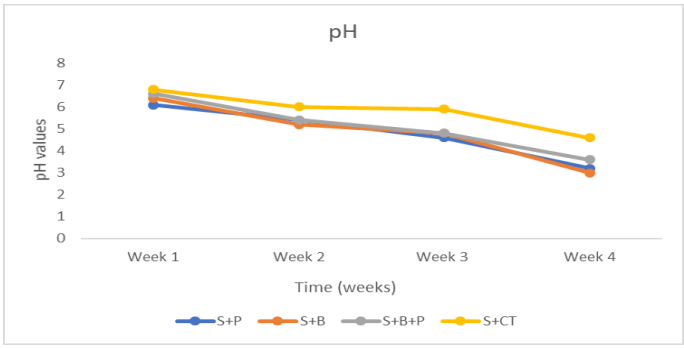

Figure 6: pH of fermented soybean from weeks 1 to 4.

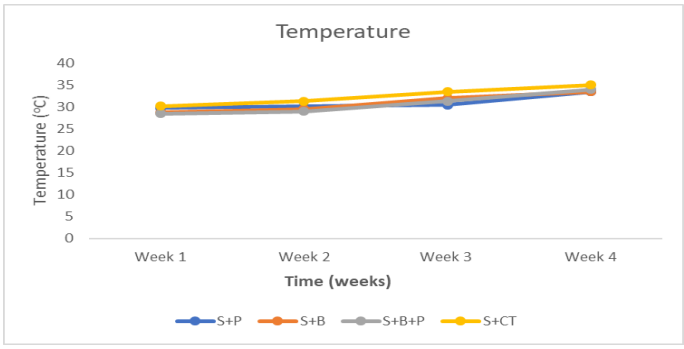

Figure 7: Temperature of fermented soybean from weeks 1 to 4.

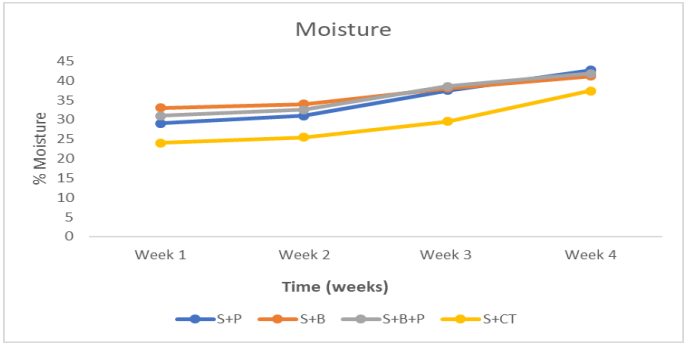

Figure 8: Moisture content of fermented soybean from weeks 1 to 4.

On the other hand, the microbial load in both inoculated and non-inoculated soybean samples from week 1 to week 4 is presented in Figure 2. In week 1, the TVC in spontaneous fermentation (C+CT) was 50×10^6 cfu/g, while it was over four times higher in microbially fermented products (>200×10^6 cfu/g). In the spontaneous fermentation group, the TVC increased from 51×10^6 cfu/g in week 1 to 293×10^6 cfu/g by week 4. In contrast, the TVC in microbially inoculated samples decreased sharply from week 2 to week 4. Among the inoculated samples, soybean fermented with *P. pentosaceus* showed the lowest TVC, at 51×10^6 cfu/g. The average TVCs of the treated fermented soybean samples, in descending order, were as follows: Control (167.5×10^6 cfu/g) > *Bacillus* + *Pediococcus* ferment (147×10^6 cfu/g) > *Bacillus*-only ferment (117.3×10^6 cfu/g) > *Pediococcus*-only ferment (107.3×10^6 cfu/g).

3.2 Physicochemical Properties of Fermented Cabbage

The hydrogen ion concentration (pH) of fermented cabbage in experimental pots from week 1 to week 4 is shown in Figure 3. In spontaneous fermentation (C+CT), the pH was 6.9 at week 1, slightly increased to 7.2 at week 2, and then decreased to a final value of 6.2 at week 4. All pH readings in spontaneous fermentation were higher than those observed in bacterial-induced fermentation. Among the inoculated samples, the pH values ranged from 3.8 in the *Bacillus* + *Pediococcus* ferment (C+B+P) to 4.6 in the *Bacillus*-only ferment (C+B). The results indicate a sharp decline in pH in all starter culture media, shifting from a near-neutral state at week 1 to an acidic state by week 4.

The temperature of the fermented cabbage, as shown in Figure 4, also varied throughout the experimental period. In spontaneous fermentation (C+CT), the temperature was 32°C at week 1 and increased to 36°C by week 4. All temperature readings in spontaneous fermentation were higher than those observed in bacterial-induced fermentation. The temperature in inoculated samples ranged from 26.5°C in the *Pediococcus*-only ferment (C+P) to 31.5°C in the mixed *Bacillus* + *Pediococcus* ferment (C+B+P).

The moisture content of fermented cabbage from week 1 to week 4 is presented in Figure 5. In spontaneous fermentation (C+CT), the moisture content was 20.4% at week 1, decreasing slightly to 19.4% at week 4. Moisture content in spontaneous fermentation was consistently lower than that in bacterial-induced fermentation. Among the inoculated samples, moisture values ranged from 23.6% in the mixed *Bacillus* + *Pediococcus* ferment (C+B+P) to 28.4% in the *Bacillus*-only ferment (C+B). The moisture content of bacterial-induced ferments increased as fermentation progressed from week 1 to week 4, whereas the moisture level in spontaneous fermentation remained relatively stable throughout the experiment.

Table 1 presents the physicochemical properties of fermented cabbage at week 4 (final product) under different inoculum treatments using test and standard microbial isolates. Spontaneous fermentation exhibited the highest pH value, indicating a slightly acidic state (6.05 ± 0.21). In contrast, microbial-induced fermentation significantly reduced the pH to more acidic levels ($p < 0.05$). The combination of *L. fermentum* and *L. plantarum* resulted in the lowest pH value (3.50 ± 0.14). Similarly, a combination of *L. fermentum* and *P. pentosaceus* produced a comparable low pH (3.55 ± 0.07). Among the bacterial-induced fermentations, inoculation with *B. subtilis* alone yielded the highest pH (4.55 ± 0.07), though still within an acidic range.

Spontaneous fermentation also recorded the highest temperature value at 36°C. In microbial-induced fermentation, temperature values were significantly reduced ($p < 0.05$), with the lowest temperature (26.5°C) observed in samples treated with either *L. plantarum*, *P. pentosaceus*, or their combination. Among the bacterial treatments, the mixed inoculation of cabbage with the three lactic acid bacteria (*L. fermentum*, *L. plantarum*, and *P. pentosaceus*) yielded the highest temperature at 34.5°C.

Titratable acidity (TTA) varied significantly across the different inoculum treatments ($p < 0.05$). The highest mean TTA (1.25 ± 0.01) was observed in the mixed treatment with *L. plantarum* and *P. pentosaceus*, while the lowest TTA (0.45 ± 0.07) was recorded in spontaneous fermentation.

Moisture content was lowest in spontaneous fermentation and increased significantly ($p < 0.05$) with microbial inoculation. The highest moisture content was recorded in *B. subtilis*-induced fermentation (28.4%), followed closely by *L. fermentum* (28.3%).

3.3 Physicochemical Properties of Fermented Soybeans

The pH readings of fermented soybeans in experimental pots from week 1 to week 4 are shown in Figure 6. In spontaneous fermentation (S+CT), the pH was 6.8 at week 1 and decreased to 4.6 by week 4. Throughout the experiment, pH readings in spontaneous fermentation were slightly higher than those in bacterial-induced fermentation. In the inoculated samples, pH values ranged from 3.0 in the *Bacillus*-only ferment (S+B) to 3.6 in the mixed *Bacillus* + *Pediococcus* ferment. The results indicate a consistent decline in pH across all fermentation treatments, both spontaneous and inoculated, from week 1 to week 4.

Temperature readings of fermented soybeans from week 1 to week 4 are presented in Figure 7. In spontaneous fermentation (S+CT), the temperature was 30.2°C at week 1 and increased to 35.0°C by week 4. All temperature readings in spontaneous fermentation were slightly higher than those in bacterial-induced fermentation, where values ranged from 33.5°C to 34.0°C. The results show a progressive increase in temperature across all treatments as fermentation advanced.

Moisture content data for fermented soybeans during the same period are shown in Figure 8. In spontaneous fermentation (S+CT), moisture content increased from 24% at week 1 to 37.4% at week 4. Moisture levels were generally higher in the culture-induced fermentations compared to spontaneous fermentation. Across all treatments, moisture content increased as fermentation progressed. By weeks 3 and 4, the moisture content in all cultures appeared to converge, showing similar levels across both spontaneous and inoculated fermentations.

Table 2 presents the physicochemical properties of fermented soybeans at week 4 (final product) under controlled fermentation with different inoculum treatments. Spontaneous fermentation recorded the highest pH value (4.6 ± 0.28), indicating a moderately acidic state. In contrast, microbial-induced fermentation significantly reduced the pH to more acidic levels ($p < 0.05$). Single inoculations with *B. subtilis*, *L. plantarum*, and *P. pentosaceus* resulted in the lowest pH values of 3.00 ± 0.14, 3.05 ± 0.07, and 3.05 ± 0.07, respectively. Among the inoculated treatments, the highest pH value (3.85 ± 0.07) was observed in the mixed inoculation of soybean with both test and standard fermenting strains.

Table 1. Physicochemical properties of cabbage ferments (final product).

Starter Culture	pH	Temperature (°C)	TTA	Moisture (%)
C+L1	4.15 ± 0.07	28.00 ± 0.00	0.89 ± 0.01	28.25 ± 0.21
C+L2	4.25 ± 0.07	26.50 ± 0.71	0.81 ± 0.01	27.55 ± 0.07
C+L3	4.10 ± 0.00	26.50 ± 0.71	0.88 ± 0.01	27.80 ± 0.14
C+B	4.55 ± 0.07	28.00 ± 0.00	0.92 ± 0.01	28.40 ± 0.14
C+L1+L2	3.50 ± 0.14	26.50 ± 0.71	0.90 ± 0.01	25.70 ± 0.42
C+L1+L3	3.55 ± 0.07	28.50 ± 0.71	0.97 ± 0.01	23.70 ± 0.14
C+L1+B	3.80 ± 0.14	30.00 ± 1.41	1.18 ± 0.01	24.75 ± 0.07
C+L2+L3	3.85 ± 0.07	33.00 ± 0.00	1.25 ± 0.01	24.30 ± 0.14
C+L2+B	3.80 ± 0.00	31.50 ± 0.71	1.19 ± 0.01	23.55 ± 0.07
C+L1+L2+L3	3.80 ± 0.14	34.50 ± 0.71	1.22 ± 0.01	23.10 ± 0.00
C+L1+L2+B	4.05 ± 0.07	32.50 ± 0.71	1.12 ± 0.01	22.90 ± 0.14
C+L2+L3+B	3.85 ± 0.07	32.50 ± 0.71	1.20 ± 0.01	23.05 ± 0.07
C+L1+L3+B	3.85 ± 0.07	32.50 ± 0.71	1.14 ± 0.01	23.05 ± 0.07
C+L1+L2+L3+B	3.80 ± 0.14	31.50 ± 2.12	1.21 ± 0.01	21.55 ± 0.21
SP	6.05 ± 0.21	36.00 ± 0.00	0.45 ± 0.07	19.35 ± 1.20
P-value	0.000 (p<0.05)	0.000 (p<0.05)	0.000 (p<0.05)	0.000 (p<0.05)
LSD	0.21	1.82	3.33	0.75

Note: C = Cabbage; L1= *Lactobacillus fermentum*; L2 = *Lactobacillus plantarum*; L3 = *Pediococcus pentosaceus*; B = *Bacillus subtilis*

Table 2. Physicochemical properties of soybean ferments (final product).

Starter Culture	pH	Temperature (°C)	TTA	Moisture
S+L1	3.50 ± 0.00	33.00 ± 0.00	1.81 ± 0.01	44.60 ± 0.14
S+L2	3.05 ± 0.07	33.00 ± 1.41	1.83 ± 0.04	41.95 ± 0.50
S+L3	3.20 ± 0.00	33.50 ± 0.71	1.82 ± 0.02	42.65 ± 0.50
S+B	3.00 ± 0.14	33.50 ± 0.71	1.75 ± 0.08	41.10 ± 0.14
S+L1+L2	3.65 ± 0.07	34.00 ± 0.00	1.73 ± 0.10	41.95 ± 0.35
S+L1+L3	3.45 ± 0.07	32.50 ± 0.71	1.64 ± 0.06	40.65 ± 0.21
S+L1+B	3.65 ± 0.07	33.50 ± 0.71	1.76 ± 0.06	40.95 ± 0.78
S+L2+L3	3.85 ± 0.07	33.00 ± 0.00	1.69 ± 0.01	41.40 ± 0.14
S+L2+B	3.55 ± 0.07	34.00 ± 0.00	1.64 ± 0.06	41.80 ± 0.14
S+L1+L2+L3	3.55 ± 0.07	32.50 ± 0.71	1.63 ± 0.04	41.00 ± 0.28
S+L1+L2+B	3.65 ± 0.07	33.50 ± 0.71	1.65 ± 0.02	42.35 ± 0.07
S+L2+L3+B	3.85 ± 0.07	33.50 ± 0.71	1.66 ± 0.01	41.30 ± 0.85
S+L1+L3+B	3.75 ± 0.07	33.50 ± 0.71	1.65 ± 0.01	41.45 ± 0.21
S+L1+L2+L3+B	3.85 ± 0.07	32.50 ± 0.71	1.66 ± 0.01	41.50 ± 0.99
SP	4.60 ± 0.28	35.00 ± 0.00	0.95 ± 0.07	37.35 ± 1.63
P-value	0.000 (p<0.05)	0.089 (p>0.05)	0.000 (p<0.05)	0.000 (p<0.05)
LSD	0.22		0.10	1.33

Note: S = Soybean; L1 = *Lactobacillus fermentum*; L2 = *Lactobacillus plantarum*; L3 = *Pediococcus pentosaceus*; B = *Bacillus subtilis*

Spontaneous fermentation also recorded the highest temperature (35.0°C). However, there was no significant difference in temperature values between spontaneous and microbial-induced fermentation ($p > 0.05$), with values in the latter ranging from 32.5°C to 34.0°C in some mixed-inoculum treatments. Thus, inoculum treatment had no significant effect on the temperature profile of the soybean ferment.

TTA showed significant differences across the different inoculum applications ($p < 0.05$). The highest mean TTA values were recorded in the single-inoculum treatments with L. fermentum (1.81 ± 0.01), *P. pentosaceus* (1.82 ± 0.02), and *L. plantarum* (1.83 ± 0.04). The lowest TTA value (0.95 ± 0.07) was recorded in spontaneous fermentation. Mixed inoculation significantly lowered the TTA values compared to single-inoculum treatments.

Moisture content was lowest in spontaneous fermentation (37.4%) and increased significantly ($p < 0.05$) in microbial-induced fermentations, reaching the highest value of 44.6% in the L. fermentum-inoculated treatment.

3.4 Relationships among Physicochemical and Bacteriological Properties of Fermented Products

The correlation among the physicochemical properties and TVC of cabbage ferments (Table 3) revealed the following relationships: there was a moderate negative correlation between pH and temperature (r = -0.577), as well as between pH and TTA (r = -0.639). Temperature showed a very strong positive correlation with both TTA (r = +0.977) and TVC (r = +0.945). Additionally, TTA had a very strong positive correlation with TVC (r = +0.916).

Meanwhile, the correlation among the physicochemical properties and TVC of soybean ferments (Table 4) revealed the following relationships: there was a moderate negative correlation between pH and TTA (r = -0.500), and between TVC and TTA (r = -0.662). A very strong negative correlation was observed between pH and temperature (r = -0.933), as well as between TVC and temperature (r = -0.996). Conversely, a very strong positive correlation was found between TVC and pH (r = +0.980).

Table 3. Correlations in physicochemical properties and TVC of cabbage ferments

	pH	Temperature	TTA
Temperature	-0.577		
TTA	-0.639	0.977	
TVC	-0.277	0.945	0.916

Table 4. Correlations in physicochemical properties and TVC of soybean ferments.

	pH	Temperature	TTA
Temperature	-0.933		
TTA	-0.500	0.596	
TVC	0.980	-0.996	-0.662

4. Discussion

Results showed that the single application of *P. pentosaceus* in fermentation yielded the lowest total viable counts (TVCs) in both cabbage and soybean. TVC decreased sharply as microbial fermentation progressed over time. In contrast, mixed application of *P. pentosaceus* and *B. subtilis* increased the bacterial load in both fermented cabbage and soybean. This may be attributed to the synergistic interaction between the two bacteria, as they compete for limited resources in the nutrient medium, thereby supporting the rapid growth of fermenting bacteria while inhibiting non-fermenters. This aligns with previous findings that lactic acid (the main end-product of fermentation) and ammonia (produced during alkaline fermentation) inhibit the growth of other bacteria [3].

The study also observed higher TVC in soybean compared to cabbage. This difference could be due to factors such as the specific characteristics of the microorganisms involved and the environmental conditions, including the protein-rich nature of soybean, which provides nitrogen sources essential for bacterial growth. Akanni et al. [11] similarly reported the diversity and functionality of *Bacillus* species in the alkaline fermentation of Bambara groundnut (*Vigna subterranea* L. *Verdc*) into the traditional African condiment "dawadawa."

The present study established that the physicochemical properties of the fermented products are critical determinants of the final product quality in controlled microbial fermentation of cabbage and soybean. Similar conclusions were reported in the fermentation of malted millet [12], cereals, and legumes [13]. In this work, four key properties were considered: pH, temperature, moisture, and titratable acidity. Acid-tolerant bacteria play a crucial role in food fermentation, as they thrive under acidic conditions [6].

A mixed combination of two lactic acid bacteria (LAB), such as L. *fermentum* + *L. plantarum* or *L. fermentum* + *P. pentosaceus*, resulted in the lowest pH values (3.5–3.6) recorded in cabbage, indicating high acidity. The pH data correlated with titratable acidity, as mixed applications of LAB produced the highest TTA values in cabbage fermentation. These results support existing literature, reaffirming that low pH is a desirable characteristic in acid fermentation mediated by LAB [6].

In contrast, for soybean, single applications of *B. subtilis*, *L. plantarum*, or *P. pentosaceus* produced the lowest pH values (3.00–3.05), while spontaneous fermentation resulted in significantly higher pH. Among bacterial-induced ferments, *Bacillus* + *Pediococcus* in cabbage gave the lowest pH of 3.8, whereas *Bacillus*-induced fermentation in soybean resulted in a pH as low as 3.0.

Fermenting organisms in this study were primarily mesophiles, requiring moderate (optimal) temperature ranges. Temperature changes influence enzyme activity, which is essential for microbial metabolism and the transformation of substrates into fermentation products. Enzymes, being proteins, function best at optimal temperatures and are denatured at higher temperatures, halting all metabolic reactions and leading to cell death [6].

In cabbage fermentation, temperature decreased significantly in bacterial-induced fermentations compared to spontaneous fermentation, reaching as low as 26.5°C in treatments with *L. plantarum*, *P. pentosaceus*, or their combination. While cabbage fermented effectively at lower temperatures, soybean required

higher temperatures for successful fermentation. In cabbage, all temperature readings from spontaneous fermentation were higher than those from bacterial-induced fermentation, with *Pediococcus*-ferment showing the lowest value of 26.5°C. Conversely, in soybean fermentation, temperatures remained consistently high across treatments. Temperature and time are crucial control points in fermentation, influencing acid production and the suppression of unwanted microbes [4]. Typical vegetable fermentations occur at 18–23°C for 3–6 weeks; below this range, LAB activity slows, potentially allowing spoilage or pathogenic bacteria to proliferate. Therefore, optimized temperature and time are essential to selectively favor beneficial fermenters [4].

Moisture content increased in *B. subtilis*- or *L. fermentum*-induced fermentation of cabbage, and in *L. fermentum*-induced fermentation of soybean. This may indicate high water activity, which can promote the growth of undesirable bacteria, leading to spoilage. Notably, minimal moisture was observed in mixed-inoculum treatments. In both cabbage and soybean, moisture content increased over time during bacterial-induced fermentation. Culture-induced ferments showed higher moisture content than spontaneous fermentations. Water is essential for microbial metabolism, and its availability can be manipulated to control microbial activity. Adjustments such as drying, freezing, or modifying solute concentrations (via salt or sugar) are common practices. Growth of desirable fermenters can be promoted by adjusting the water activity while food spoiling microbes can be prevented using this method [14, 15].

The experimental outcomes support the view that controlled fermentation of foods like cabbage and soybeans—mediated by LAB and *B. subtilis*—can be optimized through careful management of critical factors including pH, temperature, oxygen levels, enzymatic activity, inhibitors, and water activity [16, 17]. In this study, these factors were found to be interrelated, affecting each other during the optimization process, consistent with previous research [16, 17].

In cabbage fermentation, temperature, titratable acidity, and TVC were strongly positively correlated, indicating that an acidic environment promoted LAB proliferation and enhanced physiological processes such as enzymatic activity, thereby increasing the fermentation temperature. This pattern has been documented in previous studies [18, 19]. However, in soybean fermentation, high temperatures and low pH negatively affected bacterial TVC, suggesting that lower temperatures and moderate acidity are more suitable for fermenting soybean products. This aligns with prior findings [12, 13], as soybean's high protein content makes it particularly sensitive to temperature variations. Moreover, *B. subtilis* thrives in alkaline conditions typically found in protein-rich fermentations [20].

5. Conclusion

Results showed that *P. pentosaceus*-based ferments had the lowest TVCs in both cabbage and soybean. In contrast, the mixed application of *P. pentosaceus* and *B. subtilis* significantly increased the bacterial load in both products. Therefore, the combined use of these two bacteria is recommended for the fermentation of cabbage and soybean. The study also established that temperature, titratable acidity, and total viable counts of fermenting organisms exhibited interrelated effects that influenced the overall fermentation process, as demonstrated in this report.

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Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions

All authors contributed equally to this work and have agreed to the publication of this paper in this journal.

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