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Preliminary Analysis of Soy Curd by Natural Fermentation Using Green Chilies

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ABSTRACT

Plant-based soy yogurt, as an alternative to traditional dairy yogurt, has gained widespread acceptance owing to its nutritional benefits and versatility. This study investigated soy curd prepared with various starting materials, including chili, cow yogurt starter, and buffalo yogurt starter, and evaluated their bacterial counts in CFU/ml. Significant variations in bacterial counts were observed among different preparations. The Soy curd milk was fermented naturally with the use of green chilies. For this pH was found to be 5.5±0.2, with 1.325g/L TA. The CFU count was found to be 1.1×10^{6}. The antioxidant activity of curd was observed through radical scavenging activity, with a maximum percent RSA of 52.72% at 40 ug/ml by the DPPH assay.. Furthermore, antimicrobial activity by agar well diffusion showed that soy curd inhibited *Staphylococcus aureus* and *Shigella sonnei*. Sensory analysis provided valuable insights into the sensory characteristics of soy yogurt products, which are crucial for consumer acceptance and preference. The one-way ANOVA demonstrate a highly significant difference (p-value < 0.001) in sensory parameters among the three curd samples. This study provides few insights of biochemical and microbiological parameters for prepared Soy curd by natural fermentation using green chilies.

Introduction

In Asian cuisine, soybean curd is an indispensable ingredient with a unique taste and smooth texture. Its colour and flavour can vary owing to distinct fermentation processes, ingredients, and compositions (He and Chung, 2019a). Individuals with lactose intolerance are unable to consume dairy yogurt, which can result in the loss of additional health benefits. However, soybean curd can serve as a suitable alternative (Otolowo *et al.,* 2022). With a carbohydrate ratio of 11.2 to 25.6 g per 100 g of sample, soybean is an oil seed that is an excellent source of dietary protein (Pesic *et al.,* 2005). Numerous scientific investigations have been conducted to explore the incorporation of soybeans and their products, such as soybean curds, into the national diet, owing to their numerous advantages.

Soybean is widely regarded as one of the most important food crops globally because of its high protein content, exceptional nutritional value, exceptional physical and chemical properties, and capacity to absorb fat and water. In addition, it serves as a significant source of isoflavones, phytosterols, phytic acid, and saponins. Some animal studies have suggested that a soy-based diet can lead to increased insulin sensitivity and reduced need for insulin (Colletti *et al.,* 2020).

Curds can be produced using either plant- or animal-based milk. Curd is fermented by specific bacteria, which leads to changes in its health benefits (Otolowo *et al.,* 2022). Owing to its pleasant aroma, taste, unique nutritional value, and fine texture, fermented soybean curd is a popular appetizer.

To enhance preservation and quality and provide a practical and theoretical basis for evaluating the fermentation process, it is crucial to understand the aroma compounds, sensory characteristics, microbiota structure, and their correlation (Wei *et al.,* 2021).

Flavour is a critical factor in determining food acceptability, and the aroma profile is a significant contributor to characterise the taste. The microbiota, aging solution, dressing mixtures, fermentation period, and temperature all affect the amount and composition of volatile flavour components (Wei *et al.,* 2021). The relationship between microbial profiles and food quality is vital for ensuring food safety and quality.

Microorganisms play a significant role in the development of flavour qualities of soybean curds by catalysing various microbial enzymes. Although only a few studies have examined the relationship between flavour and microbiota in soy curd products (He and Chung, 2020), it is essential to understand the microbial community involved, its composition, and its role in the fermentation process to comprehend and improve the taste, quality, and preservation of this traditional food product.

Materials and Methods

Preparation of soymilk

The soybean (*Glycine max*) used in this study was acquired from a local market in Mumbai, India. For the control group, Amul cow and buffalo milk from Amul Dairy, India, was used to produce the yogurt starter. To obtain soymilk, 250 g of soybeans were immersed in water for 12 h, with the water being changed every 2 h. The soybeans were pretreated with 1% sodium bicarbonate solution for 10 mins to reduce the beany flavour of the curd (Rekha and Vijayalakshmi, 2013). Following dehulling of the soybeans by manual rubbing between the palms and rinsing of the okara under running water, the soybeans were ground using a Hamilton Beach blender for 3 min at a high speed (Otolowo *et al.,* 2022). The insoluble residue was removed from the soymilk and gathered in a small container placed in a larger container containing 1.8 litre distilled water on the stove (Wang *et al.,* 2019). The soy slurry was filtered through muslin cloth. After boiling the small pot on the stove for 20 min until the soymilk reached 90 °C, it was cooled to room temperature using an ice bath and utilized for curd production (Cui *et al.,* 2021).

Production of Curd

Green chili, specifically *Capsicum frutescens*, has been utilized as a natural curdling agent for soy curd preparation. In a specific experiment, 500 mL of extracted soymilk was placed in a ceramic container and heated to a warm temperature. For natural fermentation 1, 1½, 2 and 2½ chillies were used. Upon using 1 and 1½ chillies showed no curdling till 12 to 14 hours and 2½ chillies gave a more chilli flavour in the curd.

Thus, two sliced green chilies, complete with their stems, were carefully added to the soymilk to prevent the inclusion of chili seeds. In a separate experiment, cow and buffalo curd from Amul milk were utilized as inoculum for soy milk to produce soy curd as control.

Cow and buffalo curd inoculum was optimized from 1 to 5 % for curdling of Soy milk. Soy milk with chili starter curdled completely at 12 h, while soy milk with 5 % cow and buffalo curd inoculum curdled at 6 h.

Preliminary analysis of soya curd

pH

The pH of the curd sample was determined using a pH paper with a range of 3.5 to 6.0. To accomplish this, the pH paper was immersed in the sample, and a comparison was made with a standard pH colour chart. The pH level of the spontaneously fermented soy curd with chilli inoculum was examined at 0, 2, 4, and 6 h of fermentation along with the other controls, while two replications of the pH measurement were performed to obtain an average value.

Titratable Acidity

The titratable acidity of the soybean curd was evaluated using a titration process that employed standard alkali and phenolphthalein (0.5 g dissolved in 100 mL of 50% ethyl alcohol as an indicator. A 0.1 N standard sodium hydroxide solution was dispensed into a burette, and a conical flask was filled with 10 mL of soy curd. The curd was then titrated until it turned pale pink after adding 3-4 drops of the phenolphthalein indicator. The initial measurements were recorded, and the titration was replicated three times. The titratable acidity of the soy curd and controls was calculated using constant burette readings (CBR).

Bacterial Count and Antibacterial activity of soya curd

To assess microbial count, viable colonies were enumerated at time intervals of 0, 3, 6, 9, and 12 h for the soy curd sample and 0, 2, 4, and 6 h for the control (Fatima and Hekmat, 2020). Three different types of agar media, namely Nutrient Agar (HiMedia-M1884), Sabouraud Dextrose Agar (HiMedia-M1177), and MRS Agar (HiMedia-M1923), were used, all prepared in accordance with the manufacturer's instructions.

The sterilized agar was heated to 121 °C for 15 min and then cooled before use. Aliquots (15 µL) of each yogurt sample were measured and added to 1.5 millilitres of saline solution in an Eppendorf tube, which was then homogenized and dispensed onto agar plates. The plates were incubated at 25 °C for 24 h, after which the colonies were manually counted, and the results were expressed as colony-forming units per mL (CFU/ml) according to the dilution factor used (Otolowo *et al.,* 2022). The average microbial count was determined in triplicates for each sample.

Curd samples were stored under sterile conditions. 0.1 millilitres of fully fermented curd was inoculated into 10 mL MRSA broth and incubated at room temperature for 24 h. The broth was then centrifuged at 10,000 rpm and 44 °C for 20 min. To test the ability of the extracted supernatant to combat three pathogenic bacteria known to significantly impact health, the agar well diffusion method was used (Madhukar, 2021). Three samples of crude bacteriocin were used, including Gram-positive *Staphylococcus aureus* and Gram-negative *Salmonella enterica* and *Shigella sonnei*. Chloramphenicol, a broad-spectrum antibiotic, was used as the positive control (50 µg/mL) and distilled water was used as the blank. Nutrient agar plates were prepared and allowed to solidify. The bacteria were grown in nutrient broth and incubated at 37 °C for 24 h. The optical density (OD) of the culture incubated in nutrient broth was measured at 520 nm using spectrophotometry. 0.1 millilitres of bacterial culture were spreaded onto a solidified nutrient agar plate. Each plate corresponding to the designated bacterium received 40 µl of supernatant from each of the curd samples, antibiotics, and distilled water in the respective wells. The plates were then incubated for 24 h at 37 °C. The diameter of the inhibition zone was measured after incubation. Antibacterial activity was measured in millimetres based on the size of the zone of growth inhibition around the wells (Bang *et al.,* 2022). For each pathogenic bacterium, the experiment was performed three times, and the results were reported as the mean standard deviation (Keawsa-ard *et al.,* 2012).

Antioxidant Activity

The antioxidant activity of soy curd was evaluated using the DPPH radical scavenging method, which assesses the capacity of a sample to neutralize stable free radicals through electron transfer. The decrease in DPPH absorbance at 517 nm following the addition of DPPH solution to the curd sample was measured using a spectrophotometer. The degree of DPPH decolorization is directly proportional to the antioxidant activity of the sample. To evaluate the antioxidant activity of the samples, 10 ml of the curd sample was centrifuged at 3000 rpm for 10 min at 25 °C. Subsequently, 1 ml of the extracted supernatant was combined with a DPPH methanol solution at concentrations of 200, 160, 120, 80, and 40 mg/ml. Ascorbic acid standard concentrations of 200, 160, 120, 80, and 40 mg/ml were prepared in a similar manner.

The mixture was then stored for 24 h at room temperature in the dark (Williams and Chaput, 2010). Methanol served as the blank, whereas the DPPH-methanol solution served as the control (Johny *et al.,* 2019). The formula used to determine the DPPH radical scavenging capacity of soy curd and ascorbic acid was as follows: DPPH Radical Scavenging Activity (%) = [1 - (Absorbance of Sample/Absorbance of Control)] × 100, where At is the absorbance of the sample and Ao is the absorbance of the control (Gondwal *et al.,* 2012). The higher the DPPH radical scavenging efficiency, the greater is the ability of the sample to scavenge DPPH radicals.

Sensory analysis – Taste, Flavour, aroma, thickness, consistency

The sensory properties of the curd samples were assessed 6 hours after their production and storage at 4 °C. The samples and controls were cooled to ambient temperature before analysis. The curd was placed in a 25 mL plastic container and labelled with randomly assigned three-digit numbers (He and Chung, 2019b). Thirty evaluators, were provided with portable water and instructions to rinse their mouths before and after each assessment.

The evaluators were selected based on their familiarity with yogurt products; 18 females and 12 males between 20 and 35 years of age were chosen to avoid bias in the evaluation. Individuals with lactose intolerance were excluded from the study to prevent potential health complications. A 5-point hedonic scale for parameters attributes like Taste, Flavour, Odour, Thickness, Consistency and Appearance was used to evaluate the acceptability of soy curd containing chili, cow, and buffalo inoculum, with 5 indicating extremely satisfactory and 1 indicating extremely unsatisfactory (Otolowo *et al.,* 2022).

Statistical analyses: one way- ANOVA

To determine the significance of differences between the samples, one-way analysis of variance (ANOVA) was performed using SPSS software (version 29) with a significance level set at P=0.05 (Liu *et al.,* 2019). Tukey HSD multiple comparison tests were performed to determine if there were any differences between the curd samples.

Results and Discussion

Preliminary analysis of soya curd

pH

Changes in pH of soybean curd in different fermentation timepoints:

The pH levels of both soybean curd with chili starter and soybean curd with cow and buffalo curd starter were significantly reduced by approximately 0.5-1.0 pH units until the conclusion of their respective fermentation timepoints, as a result of the curd starters. In 1992, Matsuyama *et al.,* (1992) demonstrated that a combination of lactic acid bacterial cultures could achieve reduced pH and increased acidity (Matsuyama *et al.,* 1992).

TA

The level of titratable acidity in soybean curd was determined using phenolphthalein as an indicator by titrating a 10 mL sample against a 0.1 N standard sodium hydroxide solution. The result was expressed as the concentration of lactic acid in g/L, and the CBR reading obtained was used to calculate the titratable acidity of soy curd. The titratable acidity of soybean curd with buffalo curd starter reached 2.8 g/L, which was slightly higher than that of the other curd samples. The titratable capacity of soybean curd with cow starter was slightly lower (1.875 g/L), indicating reduced acidity. Among the three curd samples, the soybean curd with the chili starter had the lowest acidity, measuring 1.325 g/L. Acidity is a crucial aspect in fermented dairy products, as it can obscure the perception of other taste attributes, including sweetness. This can ultimately impact the acceptability of the product, since the likelihood of accepting yogurt is primarily determined by its acidity and sweetness levels (Barnes *et al.,* 1991; de Souza *et al.,* 2021).

Bacterial Count and Antibacterial activity of soya curd

Table 1 illustrates the bacterial count of the curd samples. As depicted in the results, soy curd with cow starter had the lowest total viable bacterial count, amounting to 8.7×10^5 CFU/ml in NA, 17.6 $\times10^5$ CFU/ml in SA, and 20.9 $\times10^5$ CFU/ml in MRSA. In contrast, the soy curd with chili starter had the highest bacterial load, with 34.9 $\times10^5$ CFU/ml in NA, 31.8 $\times10^5$ CFU/ml in SA, and 11.0 $\times10^5$ CFU/ml in MRSA. Good hygiene practices during product production and packaging can be attributed to the presence of bacteria within the permitted range. These findings are consistent with those of Usman and Bolade (2020), and the range of values found for yeasts and moulds falls within the general safe limit (1.0×10^6 CFU/g) for human consumption (Otolowo *et al.,* 2022).

Gram-positive bacteria produce bacteriocins, which are protein-based substances that can be used as food preservatives and potential antibiotic substitutes. Lactic acid bacteria were isolated from curd using MRS broth and their antibacterial activity due to bacteriocins in crude form was assessed using the agar well diffusion method. According to the bar graph in figure 4, the antibacterial activity of the samples varied depending on the soy curd sample tested. The curd sample with buffalo starter showed the highest level of antibacterial activity against *Salmonella sp.* and *Shigella sp.*, with an average zone of inhibition of 11.67±0.2 mm. These findings suggest that soy curd samples have significant antibacterial activity against *Shigella sp*., which may have important therapeutic implications. Further purification of the bacteriocin crude extracts is required. Fermented foods such as Chungkukjang contain probiotics, bioactive peptides, and exopolysaccharides (EPSs) that exhibit anticancer properties. These components counteract oxidative stress, modulate the immune system, and alter the intestinal environment to reduce carcinogen exposure. Studies suggest that fermented foods, such as Chungkukjang, may induce apoptosis in gut cancer cells (Tasdemir and Sanlier, 2020), and fermented soybean tempeh contains bioactive compounds, such as genistein and daidzein, which have been shown to be effective against cancer. These compounds hinder the proliferation of cancer cells by interrupting important signalling pathways and suppressing angiogenicity (Nurkolis *et al.,* 2022).

Antioxidant activity

The antioxidant activity of the soy curd sample was evaluated by comparing its DPPH decolorization at 517 nm to a standard graph of ascorbic acid. Radical Scavenging Activity was employed to calculate the concentration of the sample by comparing its absorbance to a standard at a specific wavelength. From Figure 3, at a concentration of 40 ug/ml, as depicted in the bar graph, it can be inferred that the soy curd sample exhibited the highest Radical Scavenging Activity determined by the DPPH assay. Previous studies on polysaccharides extracted from soybean curd residue have demonstrated dose-dependent increases in DPPH scavenging activity with increasing polysaccharide concentrations owing to their hydrogen-donating capacity (Li *et al.,* 2019).

Similarly, a potent antioxidant effect has been attributed to proteinase activity in the fermentation of soybean okara processed with Bacillus subtilis, which releases bioactive compounds, such as polysaccharides and pectin (Colletti *et al.,* 2020). A study of soy whey-based beverages fermented with water kefir microbiota emphasized the antioxidant properties of soybean curd.

After fermentation, these beverages displayed heightened antioxidant activity owing to the scavenging of reactive oxygen species by lactic acid bacteria. The antioxidant capacity of soybeans is enhanced by the presence of phenols, including isoflavone alkaloids (Azi *et al.,* 2020). Compared to unfermented soy beverages with an enhanced flavour profile, texture, and anti-nutritional constituents such as trypsin inhibitors, phytic acid, bioavailability, minerals, and digestibility, fermented soy exhibits superior antioxidant potential, nutritional value, and health-promoting effects (Fatima and Hekmat, 2020).

Sensory analysis

The characteristics of the control and test curd samples, including taste, flavour, odour, thickness, consistency, and appearance, were evaluated. The results showed that soybean curd with a chili starter yielded the highest flavour, taste, and overall acceptance. The taste, flavour, and thickness of soybean curd with chili starter were not significantly different from those of soybean curd with cow and buffalo curd starters. The thickness of the curd in the test was significantly greater than that of the others. The cohesiveness of a food material, which is a measure of its internal structural resistance, may be indirectly related to curd thickness and volume (Zinia *et al.,* 2022). The results indicated that the taste, flavour, and general acceptability of the control and test curd samples did not differ significantly ($P<0.05$).

The acceptance or rejection of a curd is primarily determined by its flavour and taste, with soy flavour and odour being one of the main factors affecting acceptance (Zinia *et al.,* 2022).

Statistical analyses

A one-way ANOVA revealed statistically significant differences in sensory parameters, including taste, flavour, odour, thickness, consistency, and appearance, among the 3 curd samples with p-values < 0.001 for all categories. The Tukey HSD test showed that odour distinguishes soy curd with chili starter from other samples. These findings highlight the importance of odour in distinguishing between the samples and provide valuable insights for product development.

Statistically significant differences in the sensory analysis results, and different starters for curdling affected the consistency and odour of soybean curd. The analysis showed that soybean curd made with the chili starter had the highest thickness, consistency, and appearance values and was moderately preferred by the panellists.

Table.1 pH of Soy curd with Cow and Buffalo starter in different timepoints

Curd Samples	0hr	3hr	6hr
Cow curd starter	5.5±0.2	4.5±0.2	4.5±0.2
Buffalo curd starter	5.5±0.2	5.0±0.2	4.5±0.2

Table.2 pH of Soy curd with Chilli starter in different timepoints

Curd Samples	0hr	4hr	8hr	12hr
Chilli starter	6.0±0.2	6.0±0.2	6.0±0.2	5.5±0.2

Table.3 Soybean curd with Cow curd starter

Fermentations timepoints (hours)	CFU/ml on NA	CFU/ml on SA	CFU/ml on MRSA
0	0.77×10^6	0.51×10^6	0.28×10^6
3	0.78×10^6	1.53×10^6	0.47×10^6
6	0.87×10^6	1.76×10^6	2.09×10^6

Table.4 Soybean curd with Buffalo curd starter

Fermentations timepoints (hours)	CFU/ml	CFU/ml	CFU/ml
0	1.14×10^6	1.05×10^6	0.63×10^6
3	1.23×10^6	1.32×10^6	0.90×10^6
6	1.32×10^6	2.24×10^6	2.22×10^6

Table.5 Soybean curd with Chilli starter

Fermentations timepoints (hours)	CFU/ml	CFU/ml	CFU/ml
0	1.15×10^6	0.98×10^6	0.59×10^6
4	3.25×10^6	2.77×10^6	0.78×10^6
8	3.49×10^6	3.03×10^6	0.90×10^6
12	3.49×10^6	3.18×10^6	1.11×10^6

Table.6 Antioxidant activity of Soybean curd with different starters in %RSA

Concentration (mg/ml)	200	160	120	80	40
Soybean curd with Cow curd starter	31.9	34.21	35.04	40.99	53.06
Soybean curd with Buffalo curd starter	29.09	30.08	33.55	39.01	45.62
Soybean curd with Chilli starter	21.49	35.87	41.32	44.96	52.72

Table.7 One-way ANOVA of Soybean Curd with Chilli, Cow and Buffalo Starter

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Chilli	Between Groups	36.901	5	7.380	7.469	<.001
	Within Groups	177.867	180	.988		
	Total	214.768	185			
Cow	Between Groups	30.546	5	6.109	4.570	<.001
	Within Groups	240.633	180	1.337		
	Total	271.180	185			
Buffalo	Between Groups	21.040	5	4.208	5.082	<.001
	Within Groups	149.033	180	.828		
	Total	170.073	185			

Figure.1

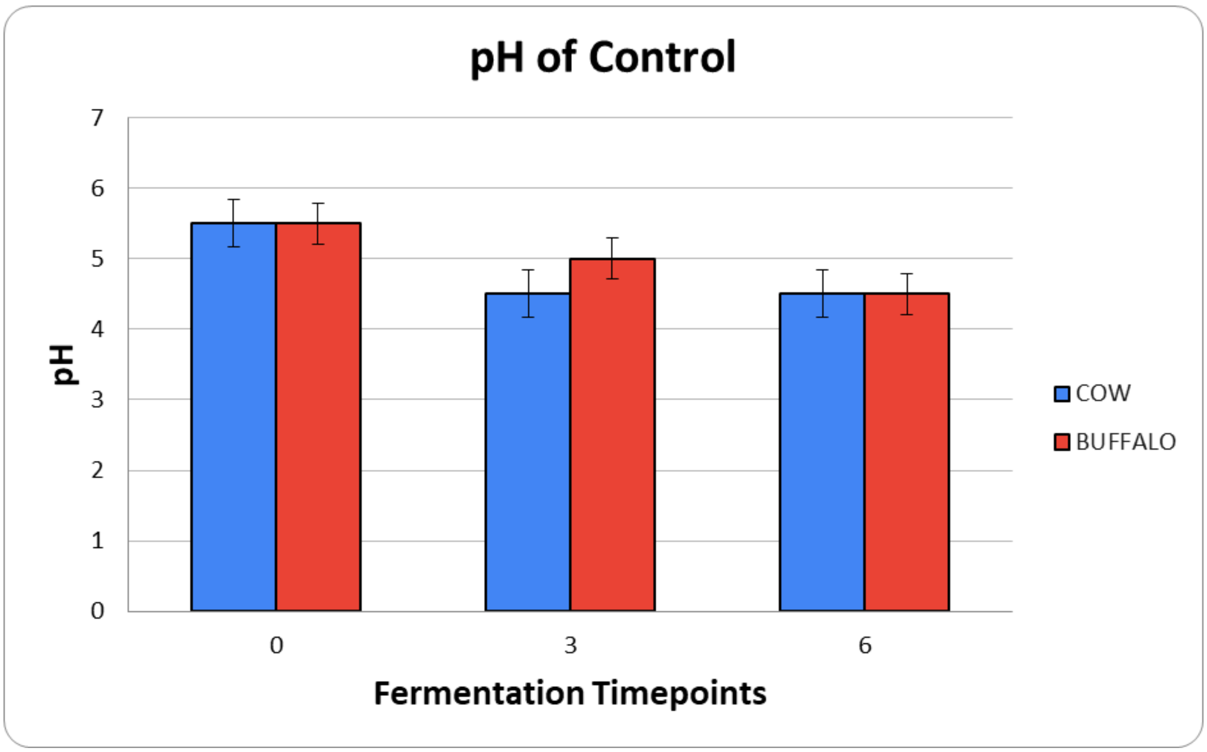


Figure.2

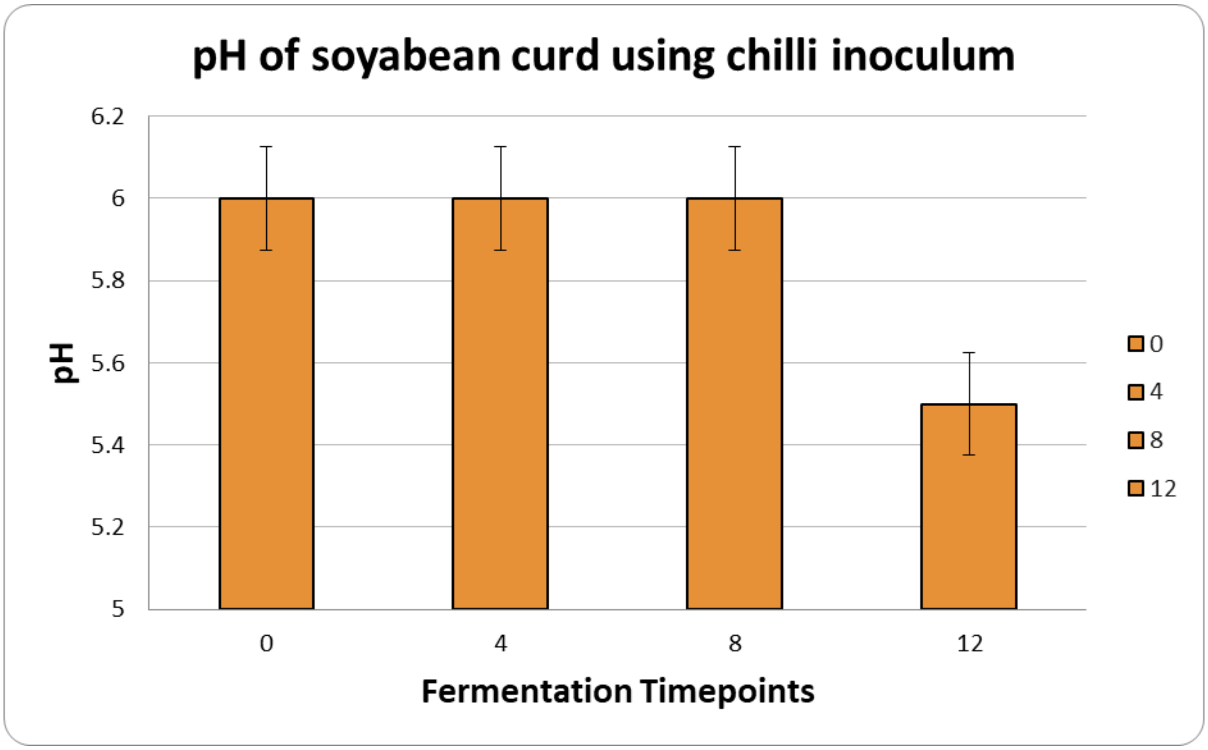


Figure.3 Antibacterial activity of Soy Curd

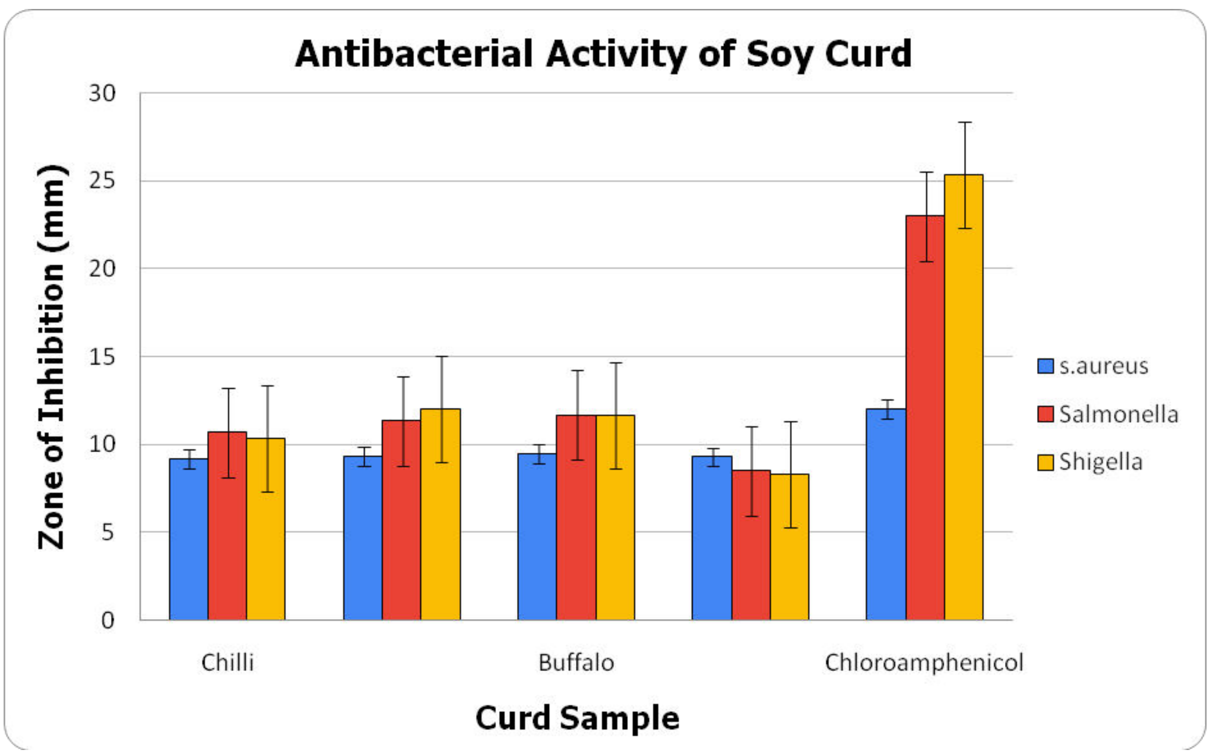
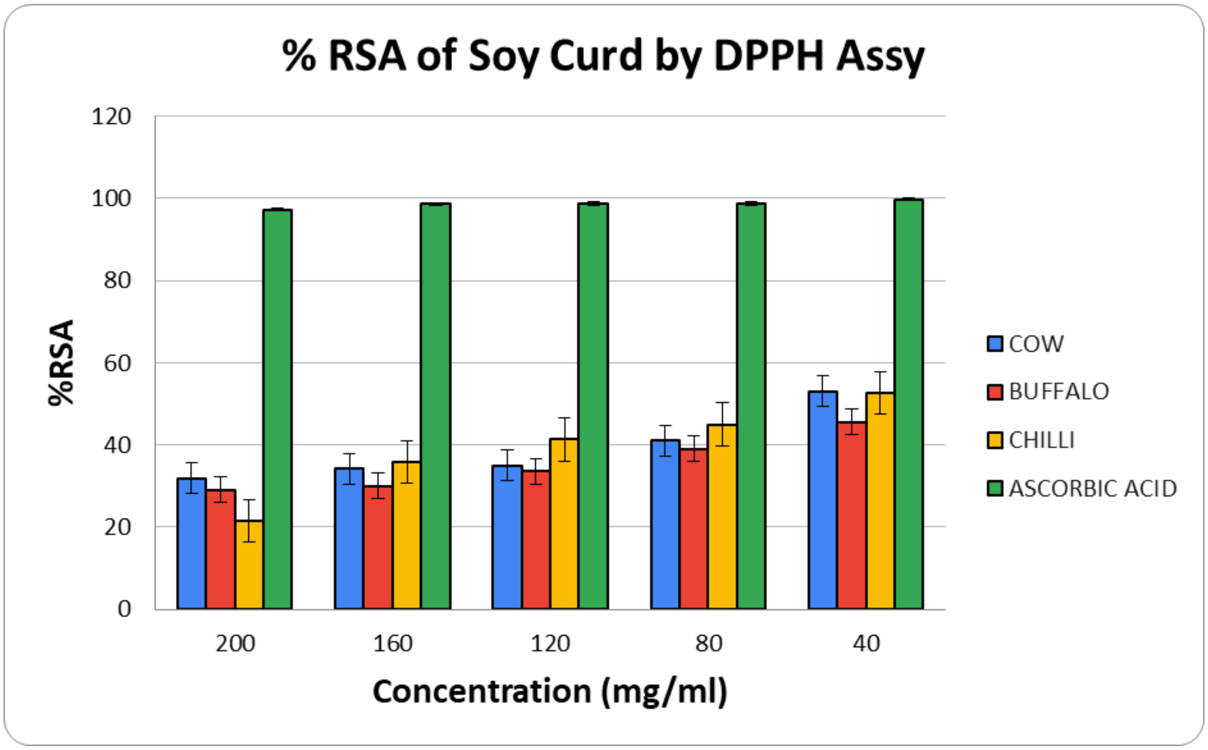


Figure.4 Antioxidant activity of Soy Curd using DPPH Assay



In conclusion the study reports the Preliminary analysis of the three soya curd samples i.e. soy milk with chilli starter, and soy milk with cow and buffalo curd inoculum. The occurrence of bacteria counts within the acceptable level in the entire samples implies that the products are regarded as safe for consumption.

The curd samples exhibited varying levels of titratable acidity, with the soy curd sample treated with buffalo starter displaying the highest acidity and soybean curd treated with chili starter having the lowest acidity among the three samples. Lower acidity levels correspond to reduced risks of microbial growth and contamination, thereby fostering enhanced food safety and potentially requiring fewer acidulants to attain the desired flavour profile, and facilitate the fermentation process.

From antioxidant activity it can be inferred that the prepared soy curd using chilies has maximum Radical Scavenging Activity determined by DPPH assay.

The bacteriocins produced by probiotics, which are protein-based and synthesized by gram-positive bacteria, have potential applications as food preservatives and replacements for antibiotics. The curd sample with buffalo starter exhibited the most significant antibacterial activity against *Salmonella sp*. and *Shigella sp*. among the three samples. These findings suggest that soy curd may have therapeutic potential against *Shigella sp.* and warrant further investigation with purification studies.

Future research can focus on the development of processed soy products, such as cheese, yogurt, and ice cream, to explore regional variations in soy curd production and consumption in relation to cultural and traditional aspects of soy curd.

Additionally, research could be conducted to improve the nutritional profile of soy curd and investigate its potential to mitigate health conditions such as lactose intolerance. Overall, this study highlights the potential of soy curd prepared using natural fermentation with green chilies as a natural alternative to traditional dairy products and could contribute to diversifying the plant-based food market.

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Author Contributions

Akshata Shetty: Investigation, formal analysis, writing—original draft. Manish Bhat: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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