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Optimization of fermentation conditions of fermented rice production through response surface methodology: Effect on nutritional and bio-functional properties

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ABSTRACT

India is considered to be the second-highest rice producer in the world and rice is consumed conventionally as a staple food. However, there is a prevalence of the extensive tradition of making unique fermented rice products with a variety of tastes and textures that are associated with ethnic diversity. This fermented rice product is traditionally prepared by rural women using village art and is popular in Goalpara district, Assam, also, known locally as pachoi in the North Eastern Region of India. In the present investigation, fermentative production of pachoi was carried out by optimizing the fermentation parameters, viz. time, temperature, and initial pH by using response surface methodology (Box-Behnken approach), and nutritional constituents were evaluated under optimized process conditions. Experimental results revealed that carbohydrate, protein, and fat were found 52.671%, 11.89%, and 1.23%, respectively, under optimum conditions of 25°C temperature, 24 h time, and pH 5.49 and higher than the value of carbohydrate, protein, and fat of 32.9%, 12.4%, and 1.1% carried out by conventional natural fermentation FP (N). There is no such difference in phenolic, flavonoid, and DPPH free radical scavenging activity in both FP (L) and FP (N).

1. INTRODUCTION

In Southeast Asian nations, rice (*Oryza sativa*) is a widely utilized cereal crop. It belongs to the Poaceae family and exhibits impressive genetic variation, with numerous cultivars growing throughout Asia. In comparison to its equivalents such as wheat, rye, oats, maize, etc., rice differs from other cereals due to its greater amount of carbohydrates, energy, and a lower percentage of fat [1]. The food-processing process uses a variety of technologies to transform perishable, mostly inedible raw materials-which are also somewhat bulky-into more useful, pleasant foods or consumable liquids with longer shelf lives. Food processing improves the availability and market value of many ethnic foods, reducing waste and loss, and promoting food safety [2]. Since ancient times, rice has been used to make a variety of traditional and ethnic fermented meals and beverages. In addition to regular rice, pigmented rice types possess the ability to be employed as the primary ingredient in rice-based fermented foods due to their phytochemical levels, which are multiplied by many during fermentation. Traditional fermented rice-based foods are becoming less and less popular as a result of urbanization and lifestyle changes [1]. For people with celiac disease, rice flour is preferable since it has qualities including being gluten-free, having a lot of readily digestible carbohydrates, having a bland flavor, being colorless, having low sodium levels, and being hypoallergenic [3].

Rice is the most widely used and easily accessible food item since antiquity, it is employed in the manufacturing of a variety of fermented meals and beverages. The fermented rice matrix is dominated by a variety of probiotics, making it an ideal environment for the development and maintenance of probiotics [4]. In addition, fermentation improves rice's nutritional composition by adding more vitamins, minerals, and amino acids, which raises the product's nutrient value, energy level, and medicinal potential [5]. Fermentation improves the nutrient content and functional qualities of food by transforming substances because of food and microbes' relationship and producing final outcomes that are bioactive or bioavailable, which is good for human health [6]. For a very long time, starter cultures have been used in the production of fermented foods and drinks. When a starting culture is added, the product's taste alters in a consistent, desired, and predictable way, improving its sensory attributes and nutrient content [7]. Fermented items made from cereal have a better nutritional content than nonfermented cereal-based items [8]. Starting cultures for fermented food production are traditionally prepared by backslopping, combining a little quantity of old ferment, utilizing an appropriate

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vessel, and incorporating particular natural items that contain effective bacteria [9]. These age-old techniques, which are still used in small- to mid-scale production units, especially in the creation of household-type products, made it easier to prepare individual or different kinds of fermented meals and beverages [10]. The accessibility of nutritional fibers, indigestible carbohydrates, polyphenolic content, vitamins, and minerals is increased through fermentation. The majority of these substances that are generated during fermentation are known to have prebiotic qualities and to encourage the growth and development of probiotic microbes [10].

According to a recent report [11], rice-fermented food based on *Asparagus racemosus* is an excellent resource for lactic acid bacteria, yeast, *Bifidobacterium* spp., and other microorganisms. It is also rich in minerals, water-soluble vitamins, oligosaccharide (G3-maltotriose), unsaturated fatty acids, and a variety of essential and non-essential amino acids [11]. The external covering of rice grains that breaks down during the milling process is called rice bran. A significant percentage of rice bran 90% is utilized as animal feed, and the remaining portion is removed to make rice bran oil [12]. Rice bran is appropriate to be employed as a substrate in the preparation of high-protein foods since it not only contains macronutrients such as protein, fat, and dietary fiber but also known to include micronutrients such as minerals and vitamin E [12].

Pachoi is a traditional fermented rice product commonly consumed by the Bengali Muslim community in Assam, India. It is primarily consumed during the seasons of autumn and summer and is renowned for its flavor and taste. No literature has been reported on pachoi preparation and on its nutritional effects till now, as per my study, so this study focused on the nutritive values of pachoi and optimizing the fermentation parameters (time, temperature, and initial pH), as well as assessing its nutritional characteristics similar with the pachoi in natural fermentation.

Response surface methodology (RSM), a combination of statistical and mathematical techniques for determining the ideal conditions of factors for desirable responses, is a commonly used optimization technique [13]. RSM's Box-Behnken design is simple to use since it can fully explore an experimental domain, making variables easier to optimize.

2. MATERIALS AND METHODS

2.1. Raw Materials and Sample Preparation

Ranjit raw rice variety and Ahu Kalogoria grain cultivar were considered in this study and purchased from local farmers of Goalpara district, Assam, NER, India. Ahu Kalogoria paddy germinated and sundried according to the method described by Veluppillai *et al*. [14]. The breakdown of germinated part and the husk is done in dheki, and the separation of the husk and germinated part from rice is done. The rice is then powdered into flour and are kept for the further fermentation process. The Ranjit raw rice was cooked for 10 min in induction using a 1:2 ratio of water. After cooking, it is kept to cool down.

2.2. Optimization of Process Conditions for Fermented Rice Product Production

A Box-Behnken central fractional factorial design was used to optimize the process conditions, viz., temperature, time, and initial pH in order to the enrichment of nutrition in pachoiwere observed and the responses chosen were carbohydrate, fats and proteins. To achieve the initial pH for optimization, lactic acid and sodium bicarbonate were used. Finally, for the optimization purpose, the same protocol was followed as indicated in natural fermentation with the only exception where the beaker was covered with aluminum foil. For pachoi preparation 100 g of Ranjit rice (cooked) was taken then 5 g of flour, 0.5 g of salt, and 7 g of sugar were thoroughly combined. Next, 30 mL of water was used to immerse 8 g of husk with germinated roots and shoots for 30 min, after which the mixture was filtered. 3 mL of pasteurized liquid milk Amul Taza (bought from the local market in the Goalpara district of Assam, NER, India) were added to the mixture along with the filtered water. Following complete mixing, the mixture was placed in a 500 mL beaker, covered with aluminum, and allowed to ferment in the incubator in different temperatures, time, and different pH. Then, amount of carbohydrates, fats, and proteins, was measured.

2.3. Protein Analysis

The Kjeldahl method can be used to convert the total nitrogen content into the total protein content. The conversion factor is 6.25 [15].

2.4. Carbohydrate Analysis

Carbohydrate analysis was carried out using the phenol sulphuric acid method by Guo *et al*. [16] with little modification. In this method, D-glucose was used as a standard solution. 500 mg sample was taken in 5 mL of 2.5 N HCL and heated in the water bath for 3 h then make up the volume up to 50 mL with distilled water. Standard was taken in five test tubes in different concentrations and 5% phenol, then 96% sulphuric acid was added. Different test tubes with different samples were taken, followed by the addition of phenol and sulfuric acid. Absorbance was taken in 490 nm using spectrophotometer, and the graph was prepared. From the graph, carbohydrate concentration in the sample was calculated.

2.5. Determination of Fat

Lipid content was estimated by extracting the sample with petroleum ether in Soxhlet apparatus for 8 h, and the amount of lipid was determined after the removal of petroleum ether [17]. The W_1 is the weight of the flask (initial weight). W_2 is the weight of the flask after evaporation of the ether, i.e., the final weight.

$$\text{Fat\%} = W_2 – W_1/\text{sample weight} \times 100$$

2.6. Experimental Design and Statistical Analysis

RSM was used to find the optimum condition for the production of fermented pachoi. A Box-Behnken design with three factors and three levels was chosen to evaluate the combined effect of three independent variables, i.e., fermentation temperature, fermentation Time, and initial pH, coded as A, B, and C, respectively. After preliminary fermentation trials, the upper and lower limits for the independent variables were established. Temperature, time, and pH levels are 25–30°C, 6–24 h, and 4–7, respectively. Three levels of each variable were chosen, and 12 fermenting trials [Table 1] were performed for the evaluation of the optimized condition. A statistical tool (Design-Expert Version 7.0) was used to perform the computations, which included the choice of experimental points, randomization, analysis of variance (ANOVA), fitting of the models, and graphical displays. ANOVA was performed on the data to find variations across formulations that were statistically significant ($P < 0.05$).

2.7. Production of Pachoi by Natural Fermentation

About 100 g of cooked Ranjit rice was mixed well with 5 g of flour, 0.5 g of salt, and 7 g of sugar. Then, 8 g of husk with germinating roots

Table 1: Experimental design for preparation of pachoi.

Standard	Run	Block	A: Temp (°C)	B: Time (h)	C: pH	Response (Fat%)	Response (Carbohydrate%)	Response (Protein%)
2	1	Block 1	30	6	5.5	1.01	69.14	10.94
7	2	Block 1	25	15	7	0.91	74.94	9.32
1	3	Block 1	25	6	5.5	1.1	70.94	10.21
11	4	Block 1	27.5	6	7	1.01	73.29	9.02
14	5	Block 1	27.5	15	5.5	1.15	64.78	11.73
10	6	Block 1	27.5	24	4	1.23	61.54	10.64
15	7	Block 1	27.5	15	5.5	1.19	54.12	10.96
12	8	Block 1	27.5	24	7	1.12	68.76	9.81
5	9	Block 1	25	15	4	1.02	59.34	10.79
9	10	Block 1	27.5	6	4	1.041	67.14	9.19
3	11	Block 1	25	24	5.5	1.41	36.94	13.36
13	12	Block 1	27.5	15	5.5	1.21	45.12	11.12
4	13	Block 1	30	24	5.5	1.43	34.74	13.32
8	14	Block 1	30	15	7	0.94	71.14	9.14
6	15	Block 1	30	15	4	1.14	61.34	10.21

and shoots were soaked in 30 mL of water for 30 min and filtered it. The filtered water was added to the mixture along with 3 mL of pasteurized liquid milk purchased from the local market of Goalpara district, Assam, NER, India. After mixing it thoroughly, the mixture was transferred into a beaker (500 mL), covered by the Petri plate, kept for fermentation, and carbohydrate, fats, proteins, and alcohol were estimated. fat, carbohydrates, and proteins are estimated using the method explained in the subsection of method and materials section.

2.8. Estimation of Alcohol Content

Quantitative estimation of ethanol was performed using the potassium dichromate method [18]. 10–50 µL of absolute alcohol was taken in different test tubes as standard then the volume was made up to 500 µL by adding distilled water. 30 µL of test samples (juice) was taken and the volume makeup to 500 µL by adding distilled water. 1 mL of potassium dichromate reagent was added in each test tube, and then 2 mL of NaOH solution was added in each test tube and incubated at 50°C for 30 min. After incubation absorbance was measured at 600 nm wavelength by spectrophotometer. Moreover, a graph was drawn for the calculation of the ethanol quantity.

2.9. Determination of Total Phenolic Content (TPC)

Folin ciocalteu (FC) was used to estimate the TPC described by Dewanto *et al.* [19]. Each extract 120 µL was taken and followed by 2.5 mL of FC reagent (10%) added, and in between 8 min, 2 mL of sodium carbonate (Na_2CO_3 [7.5%]) was mixed. The samples were vortexed immediately and incubated in the dark for 30 min at 40 °C. Using a Shimadzu ultraviolet (UV)-1800 spectrometer (Shimadzu Inc., Kyoto, Japan), the absorbance of the blue color was measured at 760 nm. The TPC was reported as mg of gallic acid equivalents (GAE)/100 g of sample dry weight, using gallic acid as the standard (DW).

2.10. Determination of Flavonoid content (TFC)

The method of Khatun *et al.* [18] with little modification was used. Crude extract sample preparation in 1 mg/mL methanol stock. A sample of 200 microliters was taken from stock. Make up the volume up to 1 mL in all the test tubes with methanol. Followed by the addition of 0.5 mL of 5% $NaNO_2$ and 0.5 mL of 10% $AlCl_3$. After 5 min of reaction, 2 mL of NaOH (4%) solution was added and incubated at room temperature for 15 min and read the absorbance at 510 nm using a UV-VIS spectrophotometer. Quercetin is used as a standard solution. Blank preparation using methanol (1 mL) and all chemicals except the sample solution. Moreover, the total flavonoid content result is expressed using the graph as quercetin equivalent mg/100 g.

2.11. Determination of Antioxidant Activity by DPPH Method

This method was described by Dewanto *et al.* [19] with some modifications. DPPH gives strong absorbance at 517 nm (deep violet color) due to its unpaired electron. When this radical pairs off in the presence of a free radical scavenger, the absorption vanishes, resulting in decoloration or yellowish color. 200-µL sample extract and make up the volume up to 1 mL with methanol, and control as 1 mL methanol and 3 mL DPPH (0.004%).

$$\text{DPPH scavenged\%} = A_{con} – A_{test}/A_{con} \times 100$$

A_{con}–is the absorbance of the control reaction
A_{test}–is the absorbance of the test sample.

2.12. Determination of Total plate count (TPC)

A widely used technique for assessing microbial levels in food is the TPC method, which measures the population of viable bacteria. In this process, 25 mL of a well-blended food sample is mixed with 225 mL of sterile water in a flask to form a uniform suspension. From this mixture, 1 mL is taken and added to 9 mL of sterile water in a test tube, then thoroughly mixed using a vortex mixer. This dilution is labeled as 10^{-1}. The serial dilution process continues similarly, with each subsequent dilution clearly marked.

From each dilution level, 1 mL of the sample is dispensed into sterile Petri dishes. Then, 15 mL of nutrient agar, previously cooled to 45°C, is poured into each dish. The mixture is gently swirled to ensure uniform dispersion of microorganisms throughout the medium and is allowed to solidify. Once solidified, the plates are incubated upside-down at 37°C for 24–48 h to facilitate bacterial growth. After incubation, only those plates showing 30–300 colonies are selected for enumeration, as this range provides statistically reliable data and avoids errors due to excessive or insufficient colony numbers. Finally, the number of colony-forming units (CFU) per gram or milliliter of the food sample is calculated from the colony counts, providing a quantitative measure of microbial contamination [20].

$$\log_{10} \text{CFU/g} = \log_{10} (\text{Number of colonies/volume plated} \times \text{dilution factor}).$$

3. RESULTS AND DISCUSSION

3.1. Modeling of Experimental Data

Three-dimensional response surface plots were produced to elucidate the relationships between the responses and the experimental levels of each independent variable. The optimum level of each variable for maximum nutritional content was resolved using the response optimizer tool of the software. The following quadratic equation based on linear coefficients of independent variables calculates the optimal point of the given model.

$$X = C_0 + A^* + B^* + C^* + AB^* + AC^* + BC^* + A^{2*} + B^{2*} + C^{2*}$$

Where A is Temperature, B is Time and C is pH.

3.2. Fat Content

The fat content range is between the range of 0.91–1.43%, as shown in [Table 1]. R^2 value of 0.89 indicates 89% of the goodness of fit. [Figure 1] shows that the points are close to the fitted line, indicating good fit [Figure 2] indicates the 3D surface of the fat content. The ANOVA data for the fat content is tabulated in Table 2.

3.3. Carbohydrate Content

Carbohydrates are in the range of 39.3–74.25% as shown in [Table 1]. [Figure 3] shows that the points are not close to the fitted line that indicating there is average fit [Figure 4] indicates the 3D surface of the carbohydrate content. The ANOVA data for the carbohydrate content are tabulated in Table 3.

3.4. Protein Content

The protein content estimated by the Kjeldahl method and the protein range was 9.02–13.32%, as shown in [Table 1]. R^2 value of 0.91 indicates 91% of the goodness of fit. [Figure 5] shows that the points were close to the fitted line indicating a good fit. [Figure 6] indicates the 3D surface of the protein content. The ANOVA data for the protein content are tabulated in Table 4.

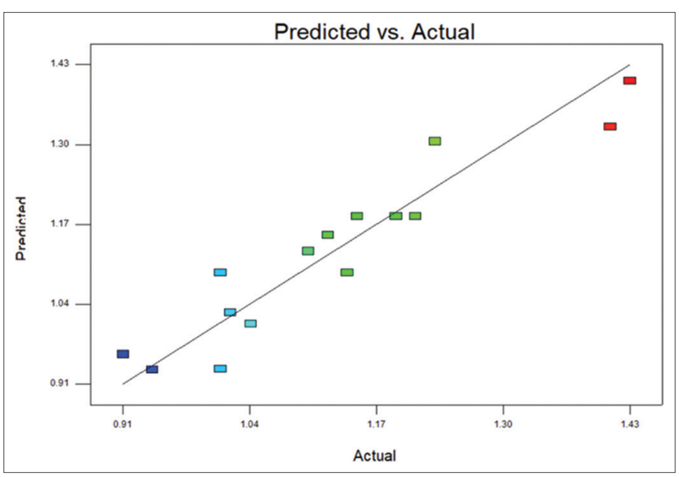

Figure 1: Scatter plot of predicted value versus actual value of fat content from response surface methodology design.

After considering all the 3 responses; the sample with 1.32904% fat, carbohydrate of 51.7548%, and protein of 13.1355% was selected as the best sample with a desirability score of 0.686, as shown in Table 5. With a protein content of 13%, it can be claimed as highly nutritious for human health. Figure 7 shows the 3D surface graphical representation of desirability. According to Prakash Tamang *et al*. [21], during the fermentation procedure, the metabolic activity of bacteria produces a significant quantity of free amino acids. The amount of protein is

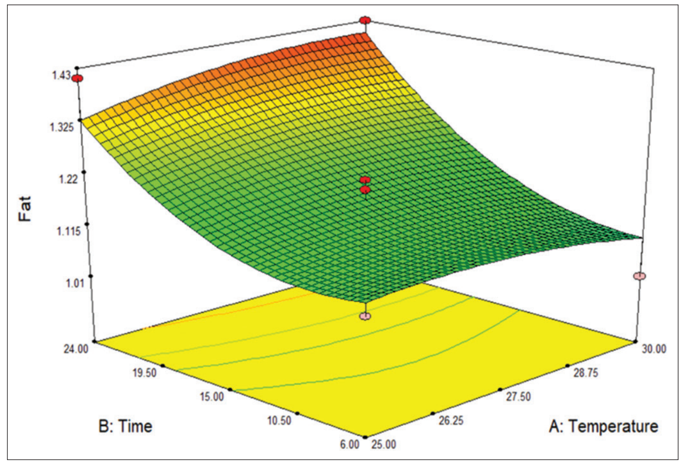

Figure 2: 3D surface graphical representation of fat content.

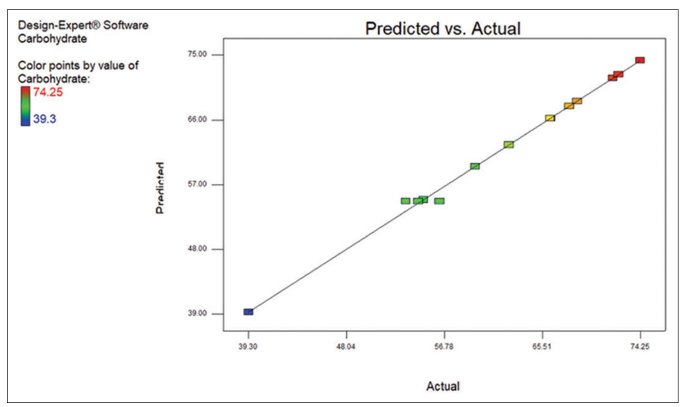

Figure 3: Scatter plot of predicted value versus actual value of carbohydrate content from response surface methodology design.

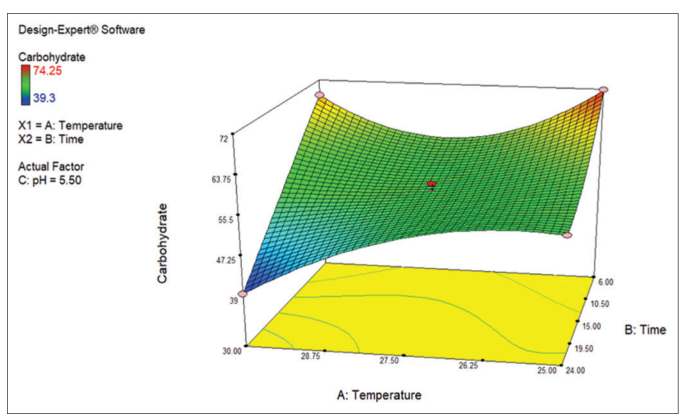

Figure 4: 3D surface graphical representation of carbohydrate content.

Table 2: Analysis of variance table for fat content.

Source	Sum of square	df	Mean square	F value	*P*-value Prob>F	
Model	0.29	9	0.032	4.62	0.0534	Not significant
A-Temperature	8.000E-004	1	8.000E-004	0.12	0.7480	
B-Time	0.13	1	7.55	19.07	0.0072	
C-ph	0.025	1	0.025	3.66	0.1138	
AB	3.025E-003	1	3.025E-003	0.44	0.5384	
AC	2.025E-003	1	2.025E-003	0.29	0.6123	
BC	1.560E-003	1	1.560E-003	0.22	0.6554	
A^2	1.753E-003	1	1.753E-003	0.25	0.6366	
B^2	0.021	1	0.021	3.07	0.1402	
C^2	0.093	1	0.093	13.45	0.0145	
Lack of fit	0.033	3	0.011	11.73	0.0796	Not significant
R-square value	0.896					

Table 3: Analysis of variance table for carbohydrate content.

Source	Sum of square	df	Mean square	F-value	*P*-value Prob>F	
Model	1214.94	12	101.25	43.39	0.0227	Significant
A-Temperature	34.57	1	34.57	14.82	0.0613	
B-Time	52.56	1	52.56	22.53	0.0416	
C-ph	30.80	1	30.80	13.20	0.0681	
R-square value	0.9962					

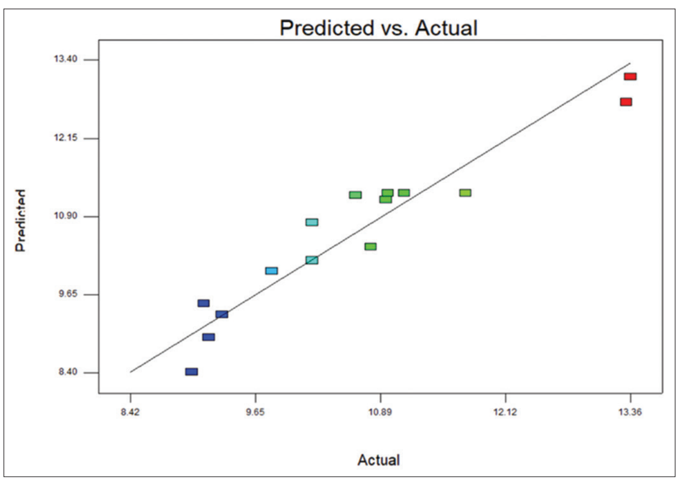

Figure 5: Scatter plot of predicted value versus actual value of protein content from response surface methodology design.

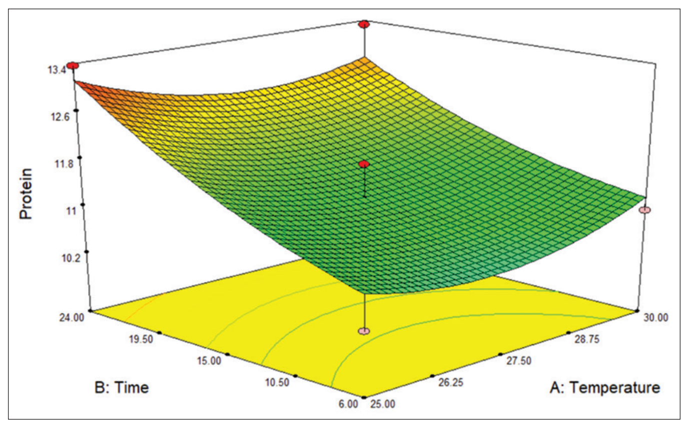

Figure 6: 3D surface graphical representation of protein content.

directly correlated with the density of fermentative bacteria. Increased microbial growth results in higher protein content and nutritional content of the product altered by microbial density.

Table 6 mentioned above indicates that there is little difference between the actual and observed values of fat, protein, and carbohydrates in relation to the values predicted by the software.

3.5. Fat, Carbohydrate, Protein and Alcohol Content of Natural Fermented Rice Product FP (N)

The pH of pachoi was dropped after 24 h of fermentation, from 5.9 to roughly 4.1. It has been documented that various fermented foods experience a pH drop during cereal fermentation. Lactic acid bacteria use the free sugars in cereal fermentation to make lactic acid, which causes the reduction of pH. pH fall increases the activity of microbial enzymes, which gives extra benefits of fermented meals. Furthermore, meals with low pH helps to preserve food by delaying the growth of several other potentially dangerous microbes [21-23]. The total carbohydrate content was analyzed using phenol sulfuric method. The carbohydrates content found in naturally fermented pachoi was 32.9%. Depending on the type of rice and herbs used in various fermented foods, the quantity of carbohydrates varies [24]. The total protein content of pachoi was analyzed by using the Kjeldahl method and found to be 12.4%. Protein content of traditional Indian fermented breakfast idli and dosa was found as 7.2 ± 1.10 g and 6.6 ± 0.25 g, respectively, and significantly less than pachoi [25]. However, according to the study of Krishnamoorthy *et al*. [25], no such change was found in the protein content of fermented rice product idli and non-fermented rice product Khaman.

Table 4: Analysis of variance table for protein content.

Source	Sum of square	df	Mean square	F-value	*P*-value Prob>F	
Model	23.75	9	2.64	5.88	0.0327	Significant
A-Temperature	6.125E-004	1	6.125E-004	1.366E-003	0.9720	
B-Time	7.55	1	7.55	16.83	0.0093	
C-ph	1.57	1	1.57	3.49	0.1206	
AB	0.15	1	0.15	0.33	0.5903	
AC	0.040	1	0.040	0.089	0.7772	
BC	0.11	1	0.11	0.24	0.6431	
A^2	0.73	1	0.73	1.62	0.2589	
B^2	0.22	1	0.22	0.49	0.5155	
C^2	12.62	1	12.62	28.14	0.0032	
Lack of fit	1.91	3	0.64	3.86	0.2125	Not significant
R-square value	0.91					

Table 5: Best sample based on maximum carbohydrate, protein, fat.

No.	Temperature	Time	pH	Fat	Carbohydrate	Protein	Desirability	
1	25	24	5.49	1.32904	51.7548	13.1355	0.686	Selected

Table 6: Predicted versus observed value of carbohydrate, fat and protein in pachoi.

Parameter	Predicted	Observed
Carbohydrate	51.7548	52.671
Protein	13.1355	11.89
Fat	1.32904	1.23

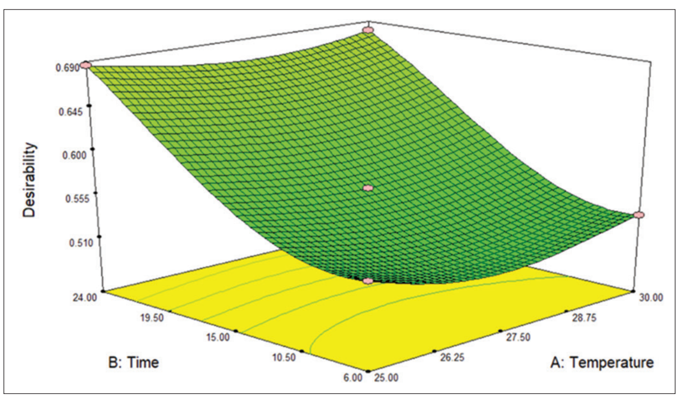

Figure 7: 3D surface graphical representation of desirability.

The fat content of the naturally fermented rice sample was estimated at 1.1%. According to the study of Rajalakshmi and Vanaja [26], traditional Indian breakfast idli and dosa were prepared by using rice and millet mix separately through overnight fermentation. Experimental results revealed that fat content in rice-based idli and millet mix idli were 0.84 ± 0.35 g and 5.2 ± 0.28 g, respectively. However, the fat content in rice-based dosa and millet mix dosa was 1.96 ± 0.26 g and 9.8 ± 0.10g, respectively. According to the study of Hannah and Jyoti [27], the fat content of selroti, an ethnic fermented food, was increased after fermentation.

The total alcohol content estimated in pachoi was found to be 1.3% v/v. Alcohol is produced during the fermentation process due to microbial activity. There is a variation in alcohol concentration during the fermentation. According to the study of Ghosh *et al*. [28], the total alcohol content of the rice beer chuwak in Tripura was found to be 26–35% v/v.

3.6. Phenolic, Flavonoid, and DPPH Radical Scavenging Activity of two samples, FP (L) and FP (N)

The TPC of FP (L) and FP (N) is 563.66 ± 1.15 and 579 ± 1.15 (mg GAE/100 g). The total flavonoid content and DPPH Radical Scavenging content of both FP (L) and FP (N) are 467 ± 1, 478.66 ± 1.15 (mg QE/100 g) and 68.33 ± 1.52, 71.33 ± 0.57% respectively [Table 7]. According to Saharan *et al*. [29], phenolic and antioxidant content increases during fermentation this could be because of the important role that enzymes (including glucosidase, xylanase, and amylase) play in the breakdown of insoluble bound phenolics during fermentation. In rice, 46.98 ± 1.02 µM/g GAE was noted, and on the 5th day of incubation, TPC increased up to eight or nine times more during fermentation. Antioxidant phenol compounds are created throughout the process of fermentation by microbes via a secondary metabolic process or are liberated from the matrix of the substrate by extracellular enzymatic action [30]. According to Ghosh *et al*. [28] 63.42 mg GAE/g total phenol and total Flavonoids 45.36 mg QE/g was observed in Haria a rice fermented food. The fermented rice had a significantly greater number of flavonoids and phenolics. One explanation for this could be that the strain's production of microbial enzymes and acids helped to liberate the flavonoids and phenolics from their complex in dietary fiber and into a form that was easily soluble. On the 4th day of fermentation, the rice-fermented food had a significant level of free radical scavenging activity. It is associated with fermented rice that has greater levels of flavonoids and phenolics [31]. Ghosh *et al*. [28] discovered that Haria had 82.54% antioxidant activity against DPPH free radicals.

3.7. TPC of Two Samples FP (L) and FP (N)

The TPC at the initial stage, i.e., 0 h of fermentation, is 2.07 and 1.02 in FP (N) and FP (L) and then increases after 24 h of fermentation, as shown in Figure 8. The rise in TPC observed during rice fermentation is a typical and predictable outcome, indicating the expansion of

Table 7: Phenolic, flavonoid and DPPH radical scavenging activity of two sample FP (L) and FP (N).

Sample ID	Total phenolic content (TPC; mg GAE/100 g)	Total flavonoid content (TFC; mg QE/100 g)	DPPH radical scavenging activity %
FP (L)	563.66±1.15	467±1	68.33±1.52
FP (N)	579±1.15	478.66±1.15	71.33±0.57

The values are expressed as means±SD of triplicate assays, and the values with different superscripts indicate that they are significantly different (*P*<0.05). TPC: Total phenolic content.

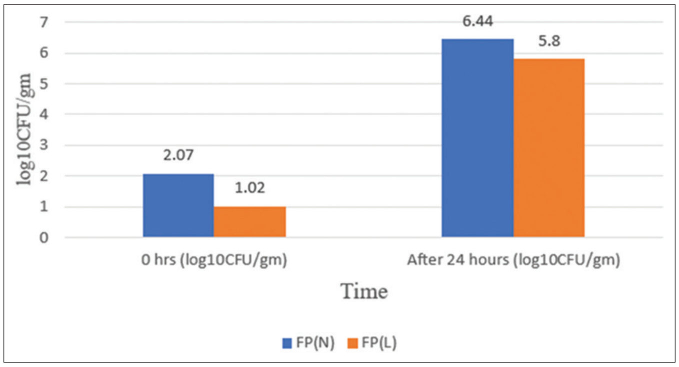

Figure 8: Total plate count of FP (n) and FP (l).

microbial populations due to the presence of abundant nutrients and favorable environmental factors. This microbial activity significantly contributes to altering the sensory qualities-such as the consistency, taste, and shelf life-of the resulting fermented product. According to the study of [32], there is a considerable increase in microbial counts during traditional rice fermentation processes. As microorganisms break down the nutrients found in rice, they alter the surrounding environment-producing factors such as a mildly acidic pH, higher carbon dioxide levels, and various metabolic substances-that support the expansion of a wide range of microbial species, especially in the initial phases of fermentation. The starting ingredients, such as rice and water, naturally contain indigenous microorganisms. When fermentation begins, these native microbes become active, utilizing available resources and reproducing quickly, particularly when there are no chemical preservatives or antimicrobial agents present to restrict their growth [33]. When examining other traditional rice-based fermented drinks, clear variations in the levels of total bacterial populations were evident. For example, Haria, a customary fermented rice drink from West Bengal, showed a markedly elevated count of aerobic bacteria at the initial phase of fermentation, measuring 10.51 $\log_{10}$ CFU/g. These aerobic microorganisms remained predominant throughout the early fermentation period, sustaining their high numbers until the 3rd day [34]. On the other hand, Xaj Pani, a traditional starter used for rice wine preparation in Assam, showed a relatively reduced count of aerobic mesophilic bacteria, with values between 1.2 and 3.1 $\log_{10}$ CFU/g [35].

4. CONCLUSION

Almost every community in the globe has a distinct food tradition that reflects its ethnic background, social structure, and cultural heritage. In this study, the fermented rice-based food product very popular in Goalpara district, Assam, NER of India, has been established as a fermented rice variety of significant nutritional value in comparison to its natural counterpart. Therefore, it can be considered as the first account of the fermented cuisine of the Goalpara district. According to the study's findings, the ideal conditions for preparing this food product are 25°C, 24 h, and 5.49 pH. In comparison to FP (L), FP (N) has somewhat higher levels of phenolic, flavonoid, and DPPH free radical scavenging activities. The enhanced nutritional importance under optimized fermentation conditions and considerably low alcohol content has been established its acceptability as a fermented food in this region of India.

5. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

The Ethical Committee of the Central Institute of Technology Kokrajhar, Assam, India has granted approval for this study on 31May 2025 (Ref. No. CITK/IEC/24).

9. DATA AVAILABILITY

All data underlying the results is available as part of the article.

10. PUBLISHER'S NOTE

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