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# Conceptualization and Evaluation of Fermented Rice Powder

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## Abstract

The convention of fermented rice into fermented rice powder is a natural option with the technology involved. Fermentation is an ancient process that enhances probiotics and maintains gut health. This experimental methodology includes cooking, fermentation, freeze-drying, and analysis of the powder. The fermented rice powder had an acceptability of 8.21 ± 0.32 on a nine-point hedonic scale score. The analyses covered the physical parameters of hand-pound rice, such as length (5.0 mm), width (3.16 mm), thousand-kernel weight (11.22 mg), bulk density, and cooked rice properties like water take-up ratio, and optimum cooking time. Proximate analysis of hand-pound fermented rice powder showed 12.3 ± 0.22% moisture,6.38 ± 0.21% protein, 0.9 ± 0.03% fat, 1.2 ± 0.15% ash, 0.7 ± 0.03% crude fiber, 73.72% carbohydrates, and 328.5 g of energy per 100 grams. The microbial analysis showed no yeast and mold growth. The probiotic count was 238 × $10^4$ CFU/gm, thus interpreting the essential attributes in invitro assays was qualified. The fermented rice powder has bile salt tolerance, 30% stainless steel adherence, tolerated pH 3 acidic conditions, and no evidence of food-borne pathogens. The inclusion of freeze-drying made the fermented rice powder more feasible with a longer shelf life. Fermented rice powder is an organic form of probiotics with traditionally fermented rice characteristics in a unique convenient form.

***Key words*:** Fermentation, Freeze drying, Probiotics, Nutritional properties, Convenience foods

The urbanization process is leading to the discovery of plenty of new convenient probiotic food categories in the market, but are they as healthy as naturally available foods that are debatable. According to USDA, convenience foods are defined as types of foods that save time in food acquisition, preparation, and cleanup. Food fermentation represents one of the oldest known used Biotechnology. This traditional biotechnology has evolved from the natural process in which nutrient availability and the environmental conditions selected particular microorganisms for the fermentation process. One of the cereal-based fermented products is *Pazhaiya soru* or fermented rice, which is a gift from our ancestors for good health. "Health is Wealth" but instead, people think consuming fermented rice led down to their social and wealth status, which is wrong. Health should be our first preference and Nowadays people are willing to spend lots of money on good health and forget old traditions.

Preparation of *Pazhaiya soru* (fermented rice) involves adding water to the cooked rice and later incubating the mixture overnight. In most parts of south India, farmers consume this as an early morning meal before heading to the farm field. Major microbiota isolated from this type of food include *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Pediococcus cerevisiae*, *Enterococcus faecalis*, and *Weissella paramensenteroides* [30]. Probiotics were defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" by the WHO and FAO in 2002. The convention of fermented rice into fermented rice powder is an organic substitute. The fermented rice powder is rich in probiotics, restores healthy intestinal flora, and can heal or prevent gastrointestinal problems like duodenal ulcers, ulcerative colitis, Crohn's disease, irritable bowel syndrome, celiac disease, and infections. Freeze-drying is employed to preserve fermented rice powder ensuring the retention of its qualities and nutrients while maintaining sensory attributes. The fermented rice powder is an organic substitute for the traditional fermented rice. The commercially available probiotic-based powders and supplements in the market are synthetic mediums while this fermented rice powder is a natural option with similar characteristics to the fermented rice. The main objectives of the study are:

- To formulate and evaluate fermented rice powder.
- To evaluate the physical properties of the raw, cooked hand-pound rice and fermented rice powder.
- To estimate the nutritional value, microbial analysis, and probiotic efficacy of the fermented rice powder.

## MATERIALS AND METHODS

*Selection and procurement of raw material*

Hand-pound parboiled rice or ponni rice is called Kaikuthal arisi or Kaikuthal ponni. The scientific name of the rice is *Oryza sativa.* The rice is Hand pounded using Mortar and Pestle/Stone Grinder and the husk is broken down to get the unpolished rice. The selection and procurement of ingredients

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will be from the local market in Chromepet. The selection of the hand-pound rice is by the color of the rice, the texture of the rice, and the aroma of the rice.

The Institutional Human Ethics Committee at the college approved project clearance with the number SDNBVC/IHEC/2023/20. The ingredient is authenticated and coded as O06022406S by Siddha Research Institute. The selection and procurement of raw material, hand-pound rice (*Oryza sativa*) done from the local market.

*Formulation of fermented rice*

The purchased hand-pounded rice will be weighed for 500 grams. The weighed rice will be washed 2 to 3 times with water. In the washed 500 grams of hand-pound rice, water was added until the rice was immersed, for half an hour. The soaked rice was again washed and the rice was pressure cooked in the ratio of 1: 2.

The cooked rice was let to cool down. To the cooled rice 1000ml of water was added. Then they were left for 22 hours for fermentation [31]. It was stated that 22 hours of fermentation leads to 23 phenolic compounds. After 22 hours of fermentation, the fermented rice was added to a mixer along with the rice water and was made into a slurry. The prepared fermented rice slurry was ready for the further process of freeze-drying.

*Freeze drying*

Freeze-drying is a pivotal process for preserving the quality of food products. It involves the sublimation of water from solid (ice) to vapor, bypassing the liquid state, and desorption from the "dry" layer [12]. Freeze drying process was carried out for 52 hours. The desirable temperature used for the freeze drying was -60 to -30 degrees Celsius. This made the fermented rice powder more feasible and convenient to the consumers.

Physical analysis of raw, cooked hand-pound rice and fermented rice powder. The grain dimension, bulk density, 1000 kernel weight, solid density, and porosity of the raw hand-pound rice were analyzed. Assessment of the optimum cooking time and water uptake ratio of the cooked hand-pound rice was carried out. The fermented rice powder was evaluated for total titratable acidity, total soluble solids, and pH.

*Nutrient analysis of fermented rice powder*

The nutrient analysis of fermented rice powder included the assessment of proximate composition, including energy, carbohydrates, protein, fat, crude fiber, ash, and moisture content [1].

*Microbial analysis of fermented rice powder*

The total Bacterial count, yeast, Mold, and probiotic count were assessed.

*Morphological tests and probiotic efficacy test for fermented rice powder*

The fermented rice powder was tested for morphological tests-KOH test, Catalase test, and Spore staining [21]. The probiotic efficacy was also tested for the fermented rice powder [19].

*Sensory analysis of fermented rice powder*

For assessing the acceptability of the rehydrated fermented rice powder, a nine-point hedonic scale was employed [11].

*Shelf-life analysis*

The shelf life of the fermented rice powder will be evaluated over 20 days ($0^{th}$, $5^{th}$, $10^{th}$, $15^{th}$, and $20^{th}$ days) at room temperature and refrigerated sample. The sensory analysis was also performed for the shelf-life analysis, on the $0^{th}$, $5^{th}$, $10^{th}$, $15^{th}$, and $20^{th}$ days.

## RESULTS AND DISCUSSION

*Formulation of fermented rice powder*

In the formulation of fermented rice powder, a systematic and comprehensive approach was undertaken. The methodologies included were pressure cooking, fermentation, and freeze-drying. Subsequently, the sensory quality of the sample was judiciously assessed utilizing a rigorous 9-point hedonic scale. This detailed evaluation aimed to discern the overall acceptability of the fermented rice powder.

Table 1 Physical properties of raw hand-pound rice

| Physical properties | Values |
|---|---|
| Length | 5.0 ± 0.1 mm |
| Width | 3.16 ± 0.1mm |
| Bulk density | 0.83 ± 0.3 g/cm |
| 1000 kernel weight | 11.22 ± 0.2 mg |
| Solid density | 1.23 ± 0.3 g/cm |
| Porosity | 38.67% |

*Evaluation of physical properties*

The physical properties of raw hand-pound rice, cooked hand-pound rice, and the final product which is fermented rice powder were analyzed.

Table 2 Physical properties of cooked hand-pound rice

| Physical properties | Values |
|---|---|
| Water uptake ratio | 2.59% |
| Optimum cooking time | 35 Minutes |

*Physical properties of raw hand-pound rice*

*Grain dimensions*

The type of paddy is classified by the length of the whole brown rice grain. By the standard, the results show that, selected hand-pound rice is a short type of paddy which is of a length of 5.0mm. The width of the rice was found to be 3.15mm.

*Bulk density*

In this study, the bulk density was found to be 0.83±0.3 g/cm which is similar to the study by [20] and the recorded values were from 0.77g/cm to 0.87g/cm.

*1000 kernel weight*

The thousand kernel weight was found to be 11.22 g, while the [20] study shows 24.37. The difference between these might be because of the growth pattern, and environmental factors.

*Solid density*

The study recorded that the solid density of the rice grains with values between 1.19 and 1.27 g/cm$^3$ [35] and this study has similar values of the solid density of the hand-pound rice which was found to be 1.23g/cm$^3$.

Table 3 Physical properties of fermented rice powder

| Physical properties | Values |
|---|---|
| Total titratable acidity | 0.65% |
| Total soluble solids | 32.60 |
| pH | 5.8 |

*Porosity*

The porosity of the hand-pound rice was 38.67%, the Closest value to this study 45.30%, was measured by the study [29].

*Physical properties of cooked hand-pound rice*

*Water uptake ratio*

Water uptake measurement was involved in the set of analyses, as it pictures another textural characteristic according to rice rather than simply cooking. The range values for the water uptake ratio varied from 1.13 to 3.35 in the study [24]. The present study value of water uptake value comes under this range, 2.59%.

Table 4 Tolerance to low pH

| pH 2 OD value | | pH 3 OD value | |
|---|---|---|---|
| 0.187 | 0.198 | 0.239 | 0.378 |

Table 5 Antibiotic susceptibility

| Antibiotics | Activity |
|---|---|
| Oxacillin | Resistance |
| Chloramphenicol | Resistance |
| Cefoxitin | Resistance |
| Tetracycline | Sensitive |
| Vancomycin | Sensitive |
| Amoxycillin | Sensitive |
| Penicillin | Sensitive |

*Optimum cooking time*

Cooking time is very important information concerning consumer preferences and also certain types of rice cultivars and their utilization. According to the study the cooking process is an important operation [33]. The cooking length and final texture of the rice kernels are crucial for customers to select the optimal variety for their consumption. This study shows 35 minutes as an optimum cooking time.


Fig 1 Optimum cooking time

*Physical properties of fermented rice powder*

*Total titratable acidity*

It was reported acidity of peanut milk fermented with *Lactobacillus acidophilus* ranged from 0.38 to 0.53% [3]. The findings show that the fermented rice powder has 0.65% of total titratable acidity which is slightly higher.

*Total soluble solids*

Yakult is a commercial fermented product. they contained 18.9 of TSS while results show that fermented rice powder has 32.60 of total soluble solids.

*pH*

The measurement of pH serves as an indicator of free acidity in the food [18]. The pH of the fermented rice powder was found to be 5.8, and the study, [3] shows a pH range of 4.76-4.43 for the fermented peanut milk.

*Proximate analysis of fermented rice powder*

The proximate analysis of the fermented rice powder was analyzed. An essential consideration in nutrition is the quantity of energy released by specific foods. Excessive calorie consumption has been associated with various chronic ailments such as obesity, diabetes, and cardiovascular disease [13]. The fermented rice powder has an energy of 328.5 kcal. Many have reported increased protein quality due to fermentation [7]. The present shows the protein value is 6.38 g, which is similar to the value 6.93 in [9]. No significant difference in protein content is observed between the two states. The finding aligns with the known benefits of freeze-drying, as highlighted by Tolera and Abera in 2017, where the process enhances protein retention.

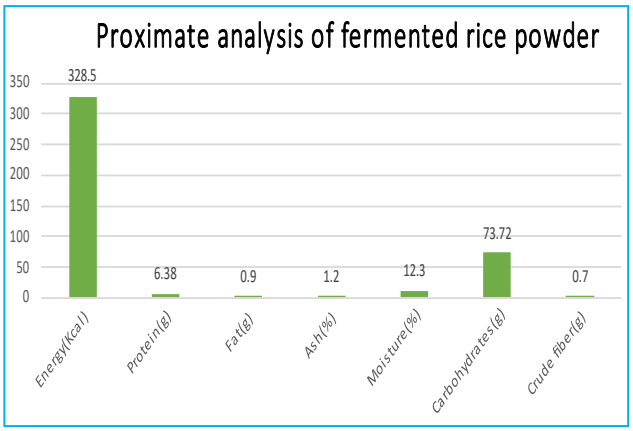


Fig 2 Proximate analysis of fermented rice powder

According to [16], meeting a minimum of 20% is essential to ensure adequate intake of total energy, essential fatty acids, and fat-soluble vitamins. The fermented rice powder has 0.9 g of fat which is a slight difference from the reported value of 12 hours of fermented rice is 1.66g. There is a significant decrease in the fat of the fermented rice powder. Ash refers to the inorganic residue remaining after either ignition or oxidation of organic matter in food. Ash analysis is employed to determine the total mineral content available in the food. The fermentation decreased the ash content probably because of the loss of dry matter, which normally occurs during fermentation [22]. The ash content of the fermented rice powder is 1.2%. Ash analysis is employed to determine the total mineral content available in the food. The ash content of the fermented rice powder is 1.2%. The slight increase in ash content is due to moisture reduction in fermented rice, which increases the dry matter concentration. The moisture content of fermented rice powder is 12.3%. A significant reduction in moisture content is observed, likely due to the evaporation of moisture during freeze-drying.

Within food processing, carbohydrate analysis stands as a vital technique for quality control, ensuring that the ingredient proportions in foods adhere to established standards. The carbohydrate value of the fermented rice powder is 73.72 g which is lower than the traditionally fermented rice 88.88g. The lower value of the carbohydrate may be due to the freeze-drying process. The crude fiber of the fermented rice powder is 0.7g. Loss of moisture increases dry matter of which fiber is one and the high fiber content of foods is good for quick bowl evacuation [17].

Table 6 Summary of shelf-life analysis of fermented rice powder

| Day | Room temperature | Refrigerator |
|---|---|---|
| 0th | No visible changes in the flavor, texture, or appearance of the fermented rice powder | No visible changes in the flavor, texture, or appearance of the fermented rice powder |
| 5th | No visible changes in the flavor, texture, or appearance of the fermented rice powder | No visible changes in the flavor, texture, or appearance of the fermented rice powder |
| 10th | No visible changes in the flavor, texture, or appearance of the fermented rice powder | No visible changes in the flavor, texture, or appearance of the fermented rice powder |
| 15th | Changes in the texture, flavor, and appearance of the fermented rice powder | No visible changes in the flavor, texture, or appearance of the fermented rice powder |
| 20th | Changes in the texture, flavor, and appearance of the fermented rice powder | No visible changes in the flavor, texture, or appearance of the fermented rice powder |

Table 7 Sensory evaluation for shelf life

| Treatment (1:6) | Appearance | Taste | Flavor | Texture | Color | Overall acceptability |
|---|---|---|---|---|---|---|
| 0th day | 8.2±0.45 | 8±0.5 | 8.25±0.43 | 7.5±0.5 | 8.5±0.9 | 8.21±0.25 |
| 5th day | 8.05±0.34 | 7.42±0.78 | 7.56±0.77 | 7.65±0.55 | 8.35±0.65 | 8.32±0.20 |
| 10th day | 8±0.23 | 7.54±0.88 | 7.43±0.45 | 7.34±0.65 | 8.32±0.77 | 8.05±0.12 |
| 15th day | 8.15±0.31 | 7.3±0.67 | 7.89±0.33 | 7.23±0.54 | 8.56±0.42 | 7.95±0.15 |
| 20th day | 8.05±0.44 | 7.04±0.55 | 7.45±0.62 | 7.22±0.63 | 8.54±0.28 | 8.05±0.10 |

Table 8 Significance of the sensory evaluation of the shelf life

| Day | 0th day | 20th day | P Value | Significance |
|---|---|---|---|---|
| Appearance | 8.2±0.45 | 8.05±0.44 | 0.421648255 | Not Significant |
| Taste | 8±0.5 | 7.04±0.55 | 0.109770083 | Not Significant |
| Flavor | 8.25±0.43 | 7.45±0.62 | 0.25081536 | Not Significant |
| Texture | 7.5±0.5 | 7.22±0.63 | 0.870901746 | Not Significant |
| Color | 8.5±0.9 | 8.54±0.28 | 0.61413955 | Not Significant |
| Overall acceptability | 8.21±0.25 | 8.05±0.10 | 0.946651164 | Not Significant |

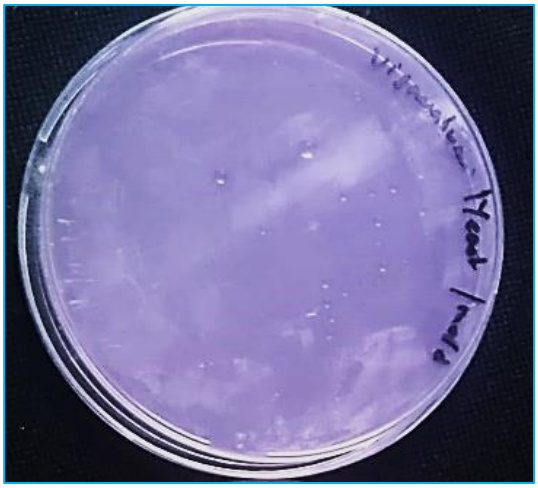

Fig 3 Yeast and mold count

*Microbial characteristics*

Food-borne pathogens play a major role in food-borne illnesses. Therefore, food handling should be done with caution as food-borne pathogens can spread rapidly through food, contaminate food, and infect humans. Food pathogen growth can be accelerated if equipment and production surfaces used in the production of food products are inadequately sanitized [34]. In this study the bacterial count was NIL. The Microbial examination of fermented rice powder demonstrated a complete absence of yeast and mold growth. This absence is directly linked to the reduced water activity achieved through the freeze-drying process, establishing it as a potent preservation method. The efficacy of freeze-drying is further highlighted as it prevents microbial proliferation and contributes significantly to the product's extended shelf life and overall quality. The probiotics need to stabilize themselves in GIT at least of cell counts become 106 CFU g was reported from the study by [25]. While in this study the probiotic count was found to be $238\times10^4$ cfu/gm.

*Morphological tests and probiotic efficacy test for fermented rice powder*

*Morphological test*

The isolate was found to be KOH-negative, gram-positive, and catalase-negative. The KOH test was negative, which means the isolates are gram-positive. The catalase test showed a positive reaction characterized by the formation of oxygen bubbles that indicate the production of catalase enzyme by the test bacterium. Therefore, the isolates, that did not give gas bubbles, so they were catalase test was negative. During the spore staining test, the isolates did not form endospores the isolate is non-spore forming [8].

*Probiotic efficacy test*

*Tolerance to low pH*

The percentage survival of probiotic LAB at different pH levels was evaluated in the fermented rice powder. The selected isolate was tested for acid tolerance test at pH 2 and pH 3 for 3 to 6 hours. The study conducted states Out of the total 56 LAB isolates, 4 (7.14%) isolates survived pH 2.0, 2.5, and 3.0 upon exposure for 3 and 6 hours [19]. In this study, the increase in OD value in pH 3, interprets the culture was able to grow in the pH 3 acidic condition.

*Tolerance to bile salts*

A probiotic strain can be chosen and evaluated based on the physiological concentration of human bile in the duodenum, which is around 0.3 percent [28], and thus 0.3 percent bile was used in this study. In this study, the OD value without bile salt was noted to be 0.268 and with bile salt 0.262, the results

interpret no change in the OD value after 24 hours of incubation which states that survival of the probiotic.

*Bacterial adhesion to stainless steel plate*

The adherence ability of the potential probiotic LAB isolates was found to range between 32.75 and 36.30% [19]. In this study, the adherence ability was found to be 30% which is 2 % different from that study, and it was reported that tolerance to high bile salt conditions is strain-specific [23].

*Antimicrobial activity*

The selected strains' antimicrobial capability was evaluated using Staphylococcus aureus, and *E. coli*. *Staphylococcus aureus* has an inhibition zone of 11 while *E. coli* has no zone. The occurrence of any food-borne illness is NIL.

*Antibiotic susceptibility*

In the antibiotic susceptibility testing, certain antibiotics were identified as ineffective against the target bacteria, indicating resistance. This resistance could be attributed to various factors, including genetic mutations or the acquisition of resistance genes by the bacteria. All of the tested four Lactobacillus strains were found to be resistant to streptomycin and kanamycin but sensitive towards tetracycline, ampicillin, and erythromycin [19]. In this study Oxacillin, Chloramphenicol, and cefoxitin are resistant and Tetracycline, vancomycin, Amoxycillin, and Penocillin are sensitive.

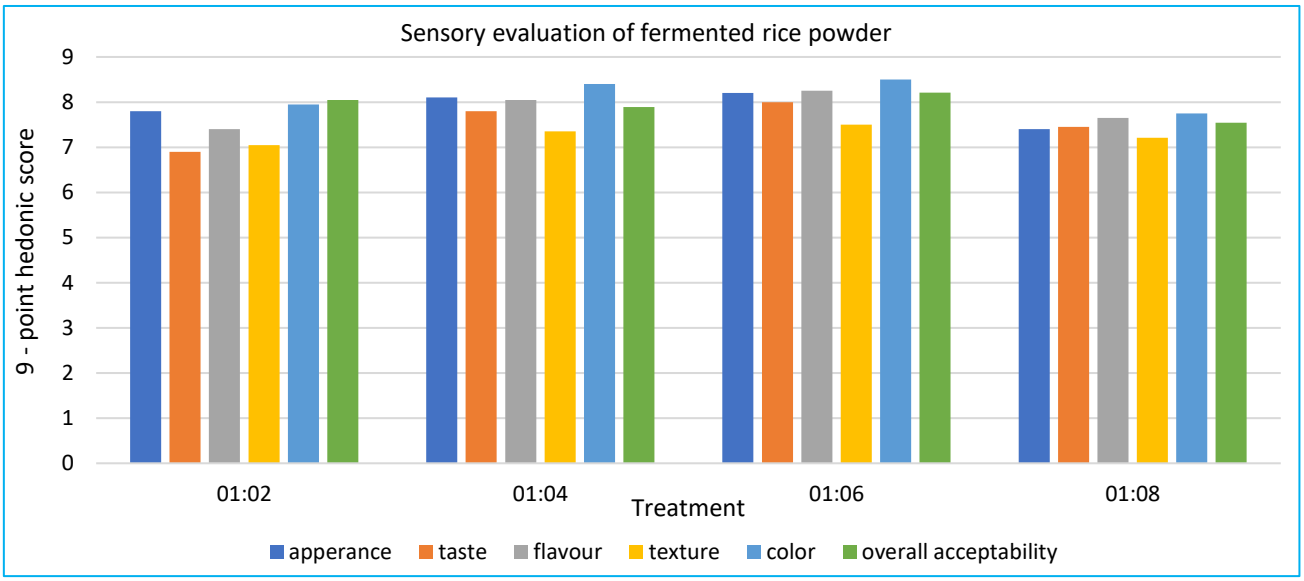


Fig 4 Sensory evaluation of reconstituted fermented rice powder

Fig 4 Fermented rice powder

*Sensory analysis of reconstituted fermented rice powder*

The Reconstitution ratio of fermented rice powder is done by mixing it with lukewarm water (36 °C) in a ratio of 1:2, 1:4, 1:6, and 1:8 (Fermented rice powder: water) [14]. The sensory evaluation was done with 15 untrained panelists using a nine-point hedonic scale for all the ratios. Among all the ratios 1:6 was best with desirable characteristics of appearance, taste, flavor, texture, color, and overall acceptability. (8.2±0.3, 8±0.4, 8.25±0.1, 7.5±0.54, 8.5±0.76, 8.21±0.54 respectively). The graph shows the various sensory attributes and the average score of the fermented rice powder.

*Shelf-life analysis*

The shelf life of the sample was analyzed using a nine-point hedonic scale and visual examination for 20 days. The fermented rice powder was mixed with lukewarm water for activation and tasted daily on the $0^{th}$,$5^{th}$,$10^{th}$,$15^{th,}$ and $20^{th}$ days and visually examined for 20 days. The fermented rice powder was stored at room temperature as well as in a refrigerator in a zip lock. There was no characteristic change in the sample which was stored in the refrigerator while the sample at room temperature had changes in the flavor and texture.

The sensory attributes were tested by rehydrating the fermented rice powder with a ratio of 1:6 for 0 $^{th}$,$5^{th}$,$10^{th}$, $15^{th,}$ and $20^{th}$ day. The sensory evaluation for shelf-life analysis was done. The significance of the $0^{th}$ and $20^{th}$ days was calculated and the p-value showed there was no significant change in the $0^{th}$ and $20^{th}$ days.

## CONCLUSION

The conceptualization and evaluation of the fermented rice powder resulted in a convenient product. The characteristics of the formulated fermented rice powder were similar to that of fermented rice. The freeze-drying process helped to retain the quality of the product and to extend shelf life. The fermented rice powder is a modern innovation and a value-added product to the market for workaholic people with all the desirable qualities. The utilization of fermented rice powder as an innovative approach presents a promising solution to retain food security. This method not only adds value to surplus rice but also contributes to sustainable food practices.

By harnessing the benefits of fermentation, we enhance the nutritional profile and shelf life of rice, addressing both food waste concerns and the need for long-term food stability. It is a convenient food with probiotic organisms similar to that of traditional fermented rice.

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