Spring 2024

Overnight Rice Fermentation: An Analysis of the Physicochemical Changes in White, Brown, and Enriched Rice Varieties

Kavin Sivakumar
San Jose State University

Recommended Citation

Sivakumar, Kavin, "Overnight Rice Fermentation: An Analysis of the Physicochemical Changes in White, Brown, and Enriched Rice Varieties" (2024). *Master's Theses*. 5525.
DOI: https://doi.org/10.31979/etd.ejxf-3a5h
https://scholarworks.sjsu.edu/etd_theses/5525

OVERNIGHT RICE FERMENTATION: AN ANALYSIS OF THE PHYSICOCHEMICAL CHANGES IN WHITE, BROWN, AND ENRICHED RICE VARIETIES

A Thesis

Presented to

The Faculty of the Department of Nutrition, Food Science and Packaging

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Kavin Sivakumar

May 2024

The Designated Thesis Committee Approves the Thesis Titled

OVERNIGHT RICE FERMENTATION: AN ANALYSIS OF THE PHYSICOCHEMICAL CHANGES IN WHITE, BROWN, AND ENRICHED RICE VARIETIES

by

Kavin Sivakumar

APPROVED FOR THE DEPARTMENT OF NUTRITION, FOOD SCIENCE AND PACKAGING

SAN JOSÉ STATE UNIVERSITY

May 2024

Xi Feng, Ph.D.	Department of Nutrition, Food Science, and Packaging, San Jose State University, United States
John Gieng, Ph.D.	Department of Nutrition, Food Science, and Packaging, San Jose State University, United States
Oluwafemi Adebo, Ph.D.	Centre for Innovative Food Research, Department of Biotechnology & Food Technology, University of Johannesburg, South Africa

ABSTRACT

OVERNIGHT RICE FERMENTATION: AN ANALYSIS OF THE PHYSICOCHEMICAL CHANGES IN WHITE, BROWN, AND ENRICHED RICE VARIETIES

by Kavin Sivakumar

Fermentation refers to the activity of microorganisms breaking down substrates, resulting in changes to a food product. One fermented food practice from the Indian subcontinent is overnight fermented rice. The traditional preparation consists of covering cooled cooked rice in water and leaving it at room temperature to ferment overnight. The objective of this project was to investigate overnight rice fermentation through quantifying its physicochemical changes. The study utilized three types of rice: white, enriched white, and brown. Mixtures of cooked rice and water at a 1 gram: 2 milliliter ratio were fermented at 29 degrees Celsius and 60% humidity for a duration of 24 hours. Samples were collected every 6 hours. The samples were measured for pH, titratable acidity, mineral contents, free amino acids, reducing sugars, soluble protein, and antioxidant content. The fermentation resulted in decreases in pH and increases in titratable acidity. Mineral content was higher in enriched and brown rice compared to white rice. Varied trends were exhibited for soluble protein and amino acid content, similar trends were observed in reducing sugars and antioxidant activity across fermentation time. This study revealed the chemical changes that occurred during the overnight fermentation of three rice varieties. The results of this study offer insights to the potential health benefits of fermentation, through changes in nutritional composition. Future research can evaluate further fermentation conditions, identify the specific microbes involved, and conduct clinical studies on the perceived health benefits of overnight fermented rice.

ACKNOWLEDGMENTS

I would first like to thank my thesis advisor Dr. Xi Feng. I am deeply grateful for your unwavering support and guidance through this process. I am truly inspired by your dedication to student success in and out of the classroom. Thank you to my thesis committee advisors, Dr. Oluwafemi Adebo and Dr. John Gieng, for your insightful feedback and thoughtful comments. Your input greatly enhanced my work. I would like to extend my gratitude to the faculty at the NUFS department. Thank you to Dr. Adrianne Widaman for assuring me I could pursue my interests in food science in the graduate program. Thank you to Dr. Giselle Pignotti for refining my writing skills in NuFS 217. Thank you to Dr. Susan Chen for the guidance in statistical analysis. Thank you to Leah Olaivar and Christina De Leon for unlocking the laboratory rooms whenever I needed them. Thank you to Wendee Augustiro for your support in ensuring my success in my lab experiments. I would like to thank the Student Research Assistance Committee and for the Circle of Friends Student Research Assistance Award. Thank you to Mrs. and Mr. Rauen whose donation was instrumental in completing my research.

Thank you to my family, who supported me through this journey. Amma, your endless flow of ideas, inspired me to select this topic and represent our food culture. Appa, your love of cooking was my first exposure to food science. Kavya Sivakumar you are the sweetest sister a brother could have. To my dear best friends, thank you for your endless support. Danette Loo & Sofia Paborji you two passed the Spartan torch to me, and stayed right by my side. Nathan Ma your calm & collected encouragement kept me going. To Wei Deng, thank you. Your guidance got me into grad school and took me all the way through the end. Here's to our continued friendship and success in academia.

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Introduction

Fermentation is a food practice that has been used for thousands of years to preserve and enhance the flavor of food and, in some instances, its nutritional value. The process of fermentation is characterized by the activity of microorganisms breaking down substrates, such as carbohydrates, resulting in changes to a food product through the production of organic acids and alcohols. As the fermentative process results in significant physicochemical changes to the food, many parameters can be studied to determine the effectiveness and extent of fermentation.

This fermentative process has been applied to various foods, including grains. Grain-based fermentation extends to fermented rice products, which are staple foods in many parts of the world, particularly in South Asia. Extensive research has shed light on the nutritional value, health benefits, microbial diversity, and sensory properties of rice fermentation. Considering the vast array of diverse fermented rice-based dishes, some have not been explored, such as overnight fermented rice, originating in South Asia, where rice covered in water is fermented over 12 hours. This review aims to provide a comprehensive understanding of fermented rice-based foods, their potential health benefits, and their ability to address global nutritional concerns.

Global Rice Consumption and Nutrient Concerns

Rice is a staple food for over half of the global population, providing more than 20% of the world's kilocalories (Fukagawa & Ziska, 2019). According to a recent statistical report 520.4 million metric tons of rice was consumed in the 2022/2023 crop year (Shahbandeh, 2024). It grows in over 100 countries, with ten of the highest rice-producing countries in Asia

(Childs, 2023). After harvest and processing, rice is typically categorized as either white or brown, with white rice having the bran removed, resulting in the loss of certain nutrients and phytochemicals (Fukagawa & Ziska, 2019). During the bran removal or polishing process, 85% of the fat, 15% of protein, 75% of phosphorus, 90% of calcium, and 70% of B vitamins (B1, B2, and B3) are lost (Ravichanthiran et al., 2018). While white rice remains a significant source of carbohydrates and trace micronutrients, the nutrient losses during polishing are significant. Furthermore, with white rice being a significant source of energy for billions of people, a lack of diet diversity contributes to micronutrient deficiencies, including iron, vitamin A, and folate, which can lead to iron deficiency anemia (Peña-Rosas et al., 2019). This is further exacerbated in developing regions, like South Asia. Despite economic growth, and health care improvement, micronutrient deficiencies of iodine, iron, vitamin A, and zinc are still high in South Asia (Harding et al., 2018).

One method to address these deficiencies would be to increase the consumption of unpolished rice in micronutrient-deficient populations. However, cultural factors and the influence of regional preferences pose a challenge to the acceptability of nutritionally beneficial rice products. Some studies on consumer preferences shed light on methods to overcome these obstacles in broader populations. A study conducted in the city of Chennai in Tamil Nadu, India compared polished rice varieties and consumer acceptance. It found that participants strongly preferred the polished rice samples in terms of color, appearance, texture, taste, and overall quality. In contrast, unmilled brown rice received lower preference ratings. However, upon learning of the nutritional benefits of unmilled rice, 93% of the participants expressed a willingness to switch to unmilled rice varieties (Sudha et al., 2013).

In another study conducted in Nepal, participants also expressed interest in incorporating brown rice into their diets upon learning of the health benefits (Gyawali et al., 2022). These studies showed that nutrition education and knowledge played a key role. This illustrates that nutrition education could be a potential solution to address the hesitation in the adoption of healthier rice products.

In response to global nutritional concerns, fortification, and enrichment have emerged as strategies to enhance the nutrient content of food products. Fortification and enrichment are food processing methods in which micronutrients, such as iron and B vitamins, are added to foods to increase their nutrient value (Newman et al., 2019). Enrichment involves the replacement of the specific micronutrients lost in the milling process, and fortification includes the addition of any beneficial micronutrient. Globally, 140 countries have regulations for fortification programs, with iodized salt and fortified grain among the most common (Olson et al., 2021). Rice fortification could be one way to potentially address micronutrient deficiencies. As a major global food source, rice can serve as an optimal vessel for fortification to address micronutrient deficiencies at a population level (Peña-Rosas et al., 2019). A study was conducted in Bangladesh to address anemia and zinc deficiency among women of reproductive age. The researchers provided participants with fortified rice containing added Vitamin A, Vitamin B1, Vitamin B12, folic acid, iron, and zinc. This resulted in a decrease in anemia prevalence by 4.8% (Ara et al., 2019). Furthermore, voluntary fortification also emerges. Some private companies, such as Olam in Ghana, have elected to add iron, zinc, and B-complex vitamins to their long-grain rice, providing consumers with 15% of their recommended dietary allowance (Olson et al., 2021).

Benefits of Rice Fermentation

Fermentation as a food practice has been adopted by various food cultures across the world. During fermentation, the fermentative microorganisms impart several changes to the food products, including extending preservation time, enhancing flavor profiles, and increasing nutritional values. Rice-based fermented foods are often the result of endogenous microorganisms. The primary fermentative organisms in rice-based fermented foods are lactic acid bacteria (LAB), lactobacilli, bifidobacteria, yeasts, and molds (Ray et al., 2016). LAB are known to produce antioxidants in fermented foods, including active phenolic metabolites, chlorogenic acid, and sulforaphane (Wang et al., 2021). Previous studies have also identified several LAB strains as probiotic candidates, such as *Lactobacillus fermentum* KKL1 (Ghosh, Ray, Adak, Halder, et al., 2015; Thakkar et al., 2015). This combination of antioxidants and probiotics in rice-based LAB fermented foods showcase its health benefits.

Another benefit of fermentation is its ability to enhance and improve the nutritional value of foods. This is achieved through the elimination of anti-nutrient factors. Some cereals and legumes contain trypsin inhibitors and phytates, which impede protein digestion and mineral release, respectively (Nkhata et al., 2018). Fermentation of these cereals and legumes results in the activation of endogenous enzymes, which disrupt the function of these inhibitors and phytates (Nkhata et al., 2018). The removal of these anti-nutrient factors makes macronutrients more accessible. LAB fermented foods have also been shown to increase the bioavailability of certain minerals from plant sources, including calcium, zinc, and iron (Gan et al., 2023; Knez et al., 2023). Fermentation of germinated brown rice has also been shown to increase its bioactive compound profiles and anti-inflammatory properties (Pino et al., 2022). One specific rice-based fermented food, originating from Tamil Nadu, India is fermented cooked rice water or “pazhaya sadham” in Tamil (Thilagavathi et al., 2019). This fermented rice dish offers not only nutritional benefits but also potential health applications. A study has shown that fermented cooked rice water effectively inhibits the proliferation of hepatocellular carcinoma cells in an *in vitro* assay, making it a potential source of natural therapeutic agents against cancer (Thilagavathi et al., 2019).

Evaluation of Fermented Rice Foods

The process of fermentation is characterized by several biochemical and molecular changes through microorganism activities. Various approaches are employed to explore the microbial, biochemical, and sensory profiles of these foods.

Microbial analysis is a fundamental aspect of fermentation research as the fermentative microorganisms, including LAB, yeasts, and molds are known to fluctuate during the process (Ray et al., 2016). The microbial succession influences the development and outcome of the fermentation process over time. In the study of the fermentation of Haria, an east Indian rice beer, molds and yeasts peaked on the 2nd day of fermentation while LAB and Bifidobacterium increased simultaneously (Ghosh, Ray, Adak, Dey, et al., 2015). The enumeration of these microorganisms was conducted on specialized media with figures outlining peaks of their activity (Ghosh et al., 2015). A study on idli fermentation, an Indian fermented food made from rice and black gram, used advanced techniques, such as 16S rRNA amplicon sequencing, denaturing gradient gel electrophoresis, and quantitative PCR to explore the microbial dynamics of the process (Mandhania et al., 2019). These analyses provided insights into the diversity and succession of microbial populations throughout the fermentation process.

Biochemical analysis is another important component to understanding rice fermentation. During the process, fermentative microbes enzymatically degrade macromolecules in food substrates, producing amino acids, sugars, and fatty acids which participate in further biological and chemical reactions (Park & Kim, 2019). The fermentative process in rice yields various compounds, including, organic acids, phenolics, saccharides, antioxidants, and amino acids. Quantification methods such as Gas chromatography–mass spectrometry (GC-MS) allow for the assessment of saccharides and their derivatives (Ghosh et al., 2015). Organic acid extracts from fermented rice have been analyzed by High-Performance Liquid Chromatography (HPLC) (Hor et al., 2022). These specialized methods and tools enable the identification and quantification of minute biochemical changes in fermented rice foods.

Sensory evaluation plays a key role in the development and improvement of rice-based fermented foods. By systematically assessing taste, aroma, and texture, it is possible to measure product quality and consumer acceptance (Pandey et al., 2021). A study by Zhang et al., (2022) explored the effects of solid-state fermentation on the sensory characteristics of brown rice. The study found that fermentation significantly improved the overall sensory score of brown rice, raising it from 59.82 (control group) to 74.22 (highest score) ($p < 0.05$) (Zhang et al., 2022). This improvement was attributed to the production of unique flavors through the release of volatile compounds during fermentation, as well as the increase in free amino acids, such as glutamic and aspartic acids. Additionally, the hydrolysis of the rice softened the texture and enhanced the appearance, taste, and overall sensory quality. A study by Chelliah et al., (2016) examined the impact of adding curry leaves to idli batter and how this affected sensory qualities including appearance, texture, mouthfeel, flavor, aftertaste, and overall acceptability. The results showed a clear preference for idlis made with curry leaves. demonstrating higher sensory scores across all evaluated attributes compared to control idlis. This study not only confirms the acceptance of fermented rice-based products, but specifically shows an increased interest in the aspect of enrichment.

Conclusion

The prevalence of global rice consumption can often lead to micronutrient deficiencies in vulnerable populations, particularly in Asia. While whole grain and fortified rice products can provide a method to address these deficiencies, cultural and regional factors pose a barrier. Rice fermentation also showcases the ability of microorganisms, including (LAB) to alter the nutrient composition of rice. Through the elimination of antinutrient factors and the increase of antioxidants, the fermentative process can yield nutritional benefits. Through nutrition education and the development of novel fortified fermented rice foods, micronutrient deficiencies may be addressed. Rice fermentation has the potential to bridge nutritional gaps and promote health.

Chapter 2: Journal Article

OVERNIGHT RICE FERMENTATION: AN ANALYSIS OF THE PHYSICOCHEMICAL CHANGES IN WHITE, BROWN, AND ENRICHED RICE VARIETIES

ABSTRACT

Fermentation refers to the activity of microorganisms breaking down substrates, resulting in changes to a food product. One fermented food practice from the Indian subcontinent is overnight fermented rice. The traditional preparation consists of covering cooled cooked rice in water and leaving it at room temperature to ferment overnight. The objective of this project was to investigate overnight rice fermentation through quantifying its physicochemical changes. The study utilized three types of rice: white, enriched white, and brown. Mixtures of cooked rice and water at a 1 gram: 2 milliliter ratio were fermented at 29 degrees Celsius and 60% humidity for a duration of 24 hours. Samples were collected every 6 hours. The samples were measured for pH, titratable acidity, mineral contents, free amino acids, reducing sugars, soluble protein, and antioxidant content. The fermentation resulted in decreases in pH and increases in titratable acidity. Mineral content was higher in enriched and brown rice compared to white rice. Varied trends were exhibited for soluble protein and amino acid content, similar trends were observed in reducing sugars and antioxidant activity across fermentation time. This study revealed the chemical changes that occurred during the overnight fermentation of three rice varieties. The results of this study offer insights to the potential health benefits of fermentation, through changes in nutritional composition. Future research can evaluate further fermentation conditions, identify the specific microbes involved, and conduct clinical studies on the perceived health benefits of overnight fermented rice.

Keywords: Overnight fermented rice, titratable acidity, mineral contents, antioxidant properties

Introduction

Fermentation is the biological process in which microorganisms convert substrates, such as carbohydrates into organic acids and alcohol. The fermentative process has been applied to many foods, notably to grains. One such fermented food practice from the Indian subcontinent is overnight fermented rice, commonly referred to as ‘pazhaya sadham’ in Tamil, ‘poita bhat’ in Hindi, or just as ‘sour rice’. The traditional and most common preparation consists of covering cooled cooked rice in water and leaving it out at room temperature overnight (Ray et al., 2016). The rice and water are then consumed the next day. The endogenous microorganisms responsible for the overnight rice fermentation have been identified as lactic acid bacteria (LAB) (Ray et al., 2016).

The traditional overnight fermentation process has commonly been with white rice (Ray et al., 2016). Rice is a staple food for over half of the global population, providing more than 20% of the world's kilocalories (Fukagawa & Ziska, 2019). Despite its importance, rice undergoes a polishing process that removes the bran, resulting in the loss of nutrients such as phosphorus, calcium, and B vitamins (Ravichanthiran et al., 2018). These nutrient losses, coupled with a lack of diet diversity, can contribute to micronutrient deficiencies, such as iron, vitamin A, and folate, which are particularly prevalent in developing regions like South Asia (Peña-Rosas et al., 2019).

There is limited research comparing the fermentation of other types of rice, such as brown rice and enriched white rice. This research gap presents an opportunity to explore the potential benefits in using these nutrient-rich rice varieties in traditional fermentation practices. Studying the fermentation of different rice types is important because of their distinct nutritional profiles. Comparing the fermentation of white, brown, and enriched white rice can reveal how nutritional differences impact the fermentation process and outcomes. Furthermore, this comparison provides an opportunity to implement nutrient rich rice varieties in traditional culinary contexts.

Fermentation has been shown to enhance rice nutrition by improving protein digestibility, mineral availability, antioxidant activity, and through the releasing of peptides and amino acids (Lim et al., 2023). Exploring the fermentation of brown rice is especially promising due to its higher fiber and reduced carbohydrate content compared to white rice, while enriched white rice offers added minerals. This comparison has the potential to address micronutrient deficiencies and optimize traditional fermented food practices, shedding light on the use of nutrient-rich rice varieties in traditional practices and their potential health benefits.

The objective of this project was to investigate the process of overnight fermentation of white, enriched, and brown rice varieties through quantifying the physical and chemical changes that occur during the fermentation process. As fermentation is characterized by several chemical and physical changes, there are several measures of interest. This project focused on changes in pH, titratable acidity, reducing sugar content, antioxidant properties, free amino acids, soluble protein, and mineral contents. Our hypothesis was that the titratable acidity, reducing sugars, free amino acids and soluble protein contents increased with fermentation. Additionally, the brown rice and enriched white rice exhibited higher mineral content than plain white rice across fermentation.

Materials and Methods

Rice Preparation

Three types of rice, white long grain, enriched white long grain, and brown long grain rice, were prepared. The study utilized California White Basmati Gourmet Rice (Lundberg Family Farms, Richvale, CA, USA), Long Grain Enriched Rice (Great Value, Walmart, Bentonville, AR, USA), and Brown Whole Grain Rice (Mahatma, Riviana Foods, Houston, TX, USA). The nutritional information obtained from the packaging of the rice is presented in Table 1.

The rice varieties were prepared on a stove top according to the ratios specified on the package instructions, with the omission of any specified rinsing step. White rice was prepared by combining 1 cup of rice with 1.5 cups of distilled water in a 3-quart glass pot. The rice and water were heated until it reached boiling point. Once boiling, the pot was covered with a tight-fitting lid, and the heat was reduced to maintain a low simmer. The rice was cooked for a duration of 15 minutes. The pot was then removed from the heat source while still covered, and the rice was steamed for an additional 10 minutes.

The enriched rice was prepared by combining 1 cup of enriched rice with 2 cups of distilled water in a 3-quart glass pot. The enriched rice and distilled water were heated until it reached boiling point. Once boiling, the pot was covered with a tight-fitting lid, and the heat was reduced to maintain a low simmer. The rice was cooked for a duration of 20 minutes, until all the water had been absorbed.

The brown rice was prepared by combining 1 cup of brown rice with 2.5 cups of boiling distilled water in a 3-quart glass pot. The pot was then covered with a tight-fitting lid, and the heat was reduced to maintain a low simmer. The rice was cooked for 50 minutes, until all the water had been absorbed.

After cooking, all three rices were cooled to 20 ℃ immediately and left for 12 h. Three replications were utilized in this study.

Fermentation

The fermentation process began after 12 h post preparation. The cooked rice and distilled water were combined in a 1:2 ratio (g/ml). The rice and water were placed in airtight plastic deli containers. The rice and water samples were then fermented in an incubator at 29 ℃ (60 % humidity) for 24 h. Samples were collected every six hours (0, 6, 12, 18, and 24h).

Sample Preparation

The fermented rice water samples were homogenized at 12,000 rpm for 90 seconds with a homogenizer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The homogenized rice and water samples were utilized to measure pH, titratable acidity, and mineral content. The homogenized samples were centrifuged at 10,000 rpm for 2 minutes to obtain a clear liquid supernatant. The supernatant was utilized to test free amino acids, reducing sugars, soluble protein, and antioxidant properties measurements.

pH and titratable acidity measurement

The pH of the samples was measured with a Mettler Toledo FG2/EL2 pH meter (Columbus, OH, USA). The titratable acidity was conducted with 25 mL homogenized samples diluted with 50 mL of distilled water. The diluted samples were titrated with 0.05 N

NaOH. A phenolphthalein solution was utilized as an indicator and the titration stopped when the solution reached a light pink color. Results were expressed in grams of lactic acid per 100 mL of sample.

Reducing sugars measurement

Reducing sugars were measured based on the Somogyi-Nelson Methodology (Somogyi, 1952). The reducing sugars were analyzed with 2 ml of supernatant obtained from each sample in duplicate. A spectrophotometer UV-2600 (Shimadzu Scientific Instruments (SSI), Columbia, MD, USA) was used to read the absorbance at 520 nm. Polymethyl methacrylate cuvettes were used (BrandTech Scientific Inc., Essex, CT, USA). A standard curve was prepared with glucose (Sigma-Aldrich, St. Louis, MO, USA) to calculate the reducing sugar content in the samples.

Free amino acids and soluble protein

The free amino acids measuring method was adapted from Free Amino Nitrogen (FAN) Measurement in Beer using the Eppendorf BioSpectrometer® protocol (Eppendorf, Hamburg, Germany). The total free amino acid content samples were determined with a standard curve of glycine. The absorbance of samples was measured at 570 nm with a spectrophotometer UV-2600 (Shimadzu Scientific Instruments (SSI), Columbia, MD, USA).

Soluble protein measurements were determined utilizing the Bio-Rad DC Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA). A standard curve of albumin was used to determine the soluble protein content of the rice samples.

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) was measured using an Invitrogen Ferric Antioxidant Status Detection Kit from Thermo Fisher (Thermo Fisher Scientific Inc., Waltham, Massachusetts, U.S).

Mineral analysis

Mineral content was measured by the University of San Francisco, MLK Core Facility in Oakland, California. Analysis included calcium, iron, zinc, potassium, magnesium, and phosphorus. The rice samples were measured for elemental analysis through Inductively Coupled Plasma-Optical Emission Spectrometry. 50 µL of each samples was digested with 70% OmniTrace nitric acid (VWR) for 12-16 hours, diluted to 5% nitric acid with OmniTrace water (VWR), vortexed 10-30 sec, centrifuged at 4,000 g for 10 minutes, and analyzed on a 5100 Synchronous Vertical Dual View ICP-OES (Agilent Technologies, USA).

Statistical analysis

Statistical data was calculated by IBM® SPSS® software 29.0. Average values were calculated. Post-hoc comparison was used to determine statistical significance across time point and rice type. Two-way Analysis of Variance (ANOVA) testing was conducted to determine the effects of time point and rice type on the mean pH, titratable acidity, free amino acids, soluble protein, free reducing sugars, antioxidants, and mineral content (SPSS 29, Chicago, IL, USA) ($p < 0.05$).

Results and Discussion:

pH and titratable acidity

A key characteristic of LAB fermentation is the production of organic acids, including acetic acid and lactic acid (Park & Kim, 2019). The pH showed significant changes during the 24-hour fermentation period across all rice types ($p < 0.05$) as shown in Table 2.

All three rice types experienced a significant decrease in pH from the initial mean at 0 hours to the final mean at 24 hours. The starting pH of brown rice at 0 h was significantly lower than the white and enriched rice varieties at 0 h. However, the final pH values across all three rice types at 24 h were similar and not statistically different. This implies that while at the start the brown rice was inherently more acidic, the fermentation process resulted in a similar pH end point for all three varieties.

The white and enriched rice showed greater decreases in pH from 0 h to 24 h compared to the brown rice. The decrease in pH for white rice was 1.30, and for enriched rice, it was 1.44. Comparatively the decrease in pH for brown rice was 1.07. This is likely due to differences in substrates available for LAB between the rice varieties. The white and enriched rice have slightly higher carbohydrate content than brown rice. Rices that have higher starch content demonstrate greater decreases in pH due to increased lactic acid production by LAB (Wang et al., 2021).

All three rice varieties experienced significant increases in titratable acidity (TA) across the 24 h fermentation period ($p < 0.05$) as shown in Table 2. The inherent acidity of the brown rice was also evident in the measures of TA. Brown rice had the highest TA content both at the start (0 h) and end (24 h) of the fermentation.

Soluble protein and amino acids

The results indicated that there were significant increases in the mean soluble protein content during the fermentation between specific time points across the different rice types as shown in Table 2. The two-way ANOVA indicated a very significant interaction between rice type and time point for soluble protein content ($p < 0.05$). The most significant increases of soluble protein were observed in white and enriched rice which resulted in a 163.1% and 178.7% increase from 0 h to 24 h respectively. Comparatively, brown rice did not experience a significant difference in soluble protein from 0 h to 24 h. Increases in protein content have been observed in grain fermentations. Previous studies on maize fermentation have noted substantial increases in protein content across different maize varieties following fermentation (Cui et al., 2012). However, it is also noted that the impact of fermentation on protein content can vary significantly. Nkhata et al., (2018) emphasized the inconsistencies in previous research, suggesting that factors like experimental design and initial protein profiles contribute to variations in protein measurements across fermentation. The observed increases in protein content may be attributed to the loss of dry matter during fermentation, as microorganisms metabolize carbohydrates and fats. This process results in a higher concentration of protein in fermented foods (Nkhata et al., 2018).

There were no significant changes in free amino acids ($p = 0.501$) during the 24-hour fermentation period across all rice types as shown in Table 2. Across the fermentation the free amino acid contents were mostly stable, with some slight variations. The variation included a 50.1% increase in free amino acids from 0 h to 12 h in white rice. However, similar increases were not observed in either brown or enriched rice. This increase of free amino acids could be attributed to proteolysis caused by the fermentative microorganisms. LAB fermentation of rice often results in the concentration of essential amino acids (Ray et al., 2016). The increases of free amino acids aligned with findings of previous studies (Heo et al., 2020). In studies of mixed grain fermentations, it has been found amino acids including arginine, citrulline, ornithine, and agmatine increased across fermentation. This increase in amino acids was attributed to the altering of the metabolites involved in the urea cycle and polyamine pathway (Heo et al., 2020).

Ferric reducing antioxidant power (FRAP)

Antioxidant activities for the three varieties of rice were measured through the reduction of ferric ions ($FeCl_3$) as shown in Table 2. The two way ANOVA revealed a highly significant interaction between rice type and time point for FRAP ($p < 0.001$). In white rice, the initial FRAP values were highest at 0 h, followed by a significant decrease of 36.6% at 12 h. Enriched rice also exhibited slight variations in FRAP values throughout the fermentation. The only significant change in FRAP in enriched rice was a 53.5% increase from 0 h to 18 h.

Brown rice began with the highest initial antioxidant activity, 11.8% higher than white rice and 47.4% higher than enriched. For the first 18 h of fermentation brown rice had significantly higher antioxidant power compared to the enriched and white rice. This is because whole grains, like brown rice, are higher in phenolics which are potent antioxidants (Ghasemzadeh et al., 2018). The phenolic content is especially high in the rice bran, therefore the removal of the bran during the production of polished rice (white and enriched) leads to lower antioxidant activity (Ghasemzadeh et al., 2018). It is also worth noting that some

LABs are capable of metabolizing phenolic compounds (Wang et al., 2021). This could explain the fluctuations in antioxidant activity in brown rice as the fermentation progresses.

Reducing sugar

There was no significant interaction between rice type and time point on reducing sugars (p = 0.189) as shown in Table 2. While all three rice types experienced decreases from 0 h to 24 h, only white rice experienced a statistically significant decrease in reducing sugar content from 0 h to 24 h (39.63% decrease). Brown rice, although having a lower carbohydrate content compared to white and enriched rice, had the highest starting reducing sugar content at 0 h. The reducing sugar content of brown rice was 50.7% higher than white rice and 172.2% higher than enriched rice. The slight variations in reducing sugars across fermentation could be attributed to the enzymatic hydrolysis of starch in the rice by LAB (Nkhata et al., 2018). However, these changes in reducing sugar were not significant.

Mineral contents:

In the context of fermented grains, it is expected that the mineral contents do not change. This is mostly reflected in the results of this study displayed in Table 3. The fermentation process has been shown to improve the bioavailability of certain minerals from plant sources, including calcium, zinc, and iron. (Knez et al., 2023). The improvement of mineral bioavailability is through the elimination of the anti-nutrient phytic acid by the fermentative microorganisms (Gupta et al., 2015). Furthermore, the fermentation process has also been known to liberate minerals bound in the matrices of foods, leading to greater bioavailability (Nkhata et al., 2018). Therefore, while fermentation may impact the bioavailability of the minerals present in the food, the mineral content itself is expected to be unaffected. However, across the fermentation certain rice varieties did experience some fluctuations.

Calcium

There was no significant interaction between rice type and time point on calcium content ($p = 0.221$) as seen in Table 3. All three rice types show relatively stable calcium levels during fermentation, with minor fluctuations, none of which were significant. At 0 h Brown rice had the highest calcium content 19.23% higher than enriched and 2.86% higher than white rice.

Iron

There was a significant interaction between rice type and time point on iron content ($p < 0.001$) as described in Table 3. This significant interaction was attributed to the differences in iron content across the three rice types. The iron content of enriched and brown rice was significantly higher than white rice across all time points. Enriched rice had the highest iron content at 0 h 64.96% higher than white and 19.62% higher than brown. The three rice types did show some variation in iron content across fermentation. White rice had a notable decrease of 56.8% from 0 h to 12 h followed by an increase of 171.4% at 24 h. Enriched rice shows consistent iron levels throughout fermentation with a significant overall increase of 26.5%. These large variations across the fermentation are likely due to inconsistent sampling and potential iron contamination.

Zinc

There was a significant interaction between rice type and time point on zinc content ($p < 0.001$) as shown in Table 3. White rice experiences a significant increase in zinc content of approximately 115.2% from 2.33 µg/g at 0 hours to 5.02 µg/g at 12 hours, followed by a decrease in zinc content at 18 hours and 24 hours. Enriched rice remains relatively stable with a minor overall increase of 50.8% at 24 hours compared to 0 hours. Brown rice shows some fluctuations, including a significant decrease at 12 hours and slight recovery at 18 and 24 hours. Of the three rice types at 0 h, white rice had the highest zinc content, 41.74% higher than enriched rice and 15.38% higher than brown rice.

Potassium

There was a significant interaction between rice type and time point on potassium content ($p < 0.001$) as shown in Table 3. These differences in potassium content during the fermentation process were mainly attributed to the differences in types of rice. Brown rice had the highest potassium content, which was 68.75% higher than white rice and 192.90% higher than enriched rice.

Magnesium

There was no significant interaction between rice type and time point on magnesium content ($p = 0.593$) as shown in Table 3. This indicated that the changes in magnesium content during the fermentation process were not significantly affected by the type of rice used. The rice type with the highest magnesium content was brown rice, 164.01% higher than white rice and 339.61% higher than enriched rice.

Phosphorus

There was no significant interaction between rice type and time point on phosphorus content ($p = 0.117$) as shown in Table 3. The changes in phosphorus content were not significantly different across the fermentation period. Brown rice had the highest phosphorus content, 164.01% higher than white rice and 339.61% higher than enriched rice.

Mineral content should generally remain stable during fermentation since these compounds are not typically altered by microbial activity. Perceived increases in mineral content can be attributed to the loss of dry matter during fermentation through microbial degradation, in turn resulting in an increase of the concentration of minerals (Nkhata et al., 2018). Across all three rice types, brown rice had the highest calcium, potassium, phosphorus, and magnesium content at hour 0. With potassium, magnesium, and phosphorus being significantly higher in brown rice than in enriched and white rice. While not statistically significant, enriched rice had the highest iron content of the three rice types at 0 h. White rice had significantly higher zinc content of the three rice types at 0 h. The differences in mineral content across rice types can be attributed to their inherent nutrient compositions. Brown rice, which retains its bran layer, contains higher levels of minerals compared to white rice and enriched rice. Enriched rice resulted in a higher iron content through the fortification process.

Conclusion

This study provides insights into the physicochemical changes that occur during the fermentation of white, enriched white, and brown rice. The results revealed variations across the three rice types in terms of pH, titratable acidity, soluble protein, free amino acids, reducing sugar content, FRAP and mineral contents. Of the three rice types investigated, brown rice is the most nutrient rich. Brown rice showed the best overall nutrient profile, including higher soluble protein, antioxidant levels and mineral contents.

The findings of this study reveal potential applications of fermented foods informed by traditional culinary practices. The variations in mineral content, particularly in brown and enriched rice, underscore the importance of choosing rice varieties based on their nutritional value. Future research can further study the mechanisms of fermented rice through microbial analysis. Additionally, the expansion of the scope of research to include other rice varieties and fermentation parameters can offer a broader perspective on the rice fermentation. Future studies can also investigate the sensory attributes of fermented rice to determine novel fermented food products.

Table 1
Nutritional Facts of Dry White, Enriched, and Brown rice in 45 g

	Total Carbohydrates (g)	**Protein (g)**	**Calcium (mg)**	**Iron (mg)**	**Potassium (mg)**
White	36	3	13	0	50
Enriched	36	3	10	1.9	50
Brown	34	3	0	1	115

Table 2

Mean pH, TA, Soluble Protein, Amino Acids, FRAP, & Reducing Sugars of White, Enriched, & Brown Rice Across Fermentation

pH

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours
white	6.75 ^{x,a}	6.51 ^{x,a}	5.95 ^{x,b}	5.64 ^{x,b,c}	5.45 ^{x,c}
enriched	6.91 ^{x,a}	6.82 ^{x,a}	6.32 ^{y,b}	5.69 ^{x,c}	5.47 ^{x,c}
brown	6.53 ^{y,a}	6.40 ^{x,ab}	6.17 ^{y,bc}	5.90 ^{x,c}	5.46 ^{x,d}

Titratable Acidity (g/100mL)

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours
white	0.0087 ^{x,a}	0.0135 ^{x,b}	0.0185 ^{x,c}	0.0233 ^{x,d}	0.0249 ^{x,d}
enriched	0.0042 ^{y,a}	0.0062 ^{y,a}	0.0101 ^{y,b}	0.0155 ^{y,c}	0.0162 ^{y,c}
brown	0.0101 ^{z,a}	0.0139 ^{x,ab}	0.0190 ^{x,b}	0.0262 ^{x,c}	0.0334 ^{z,d}

Soluble Protein (mg/mL)

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours
white	0.252 ^{x,a}	0.249 ^{x,a}	0.545 ^{x,b}	0.635 ^{x,b}	0.663 ^{x,b}
enriched	0.136 ^{y,a}	0.170 ^{x,a}	0.370 ^{y,b}	0.373 ^{y,b}	0.379 ^{y,b}
brown	0.500 ^{z,ab}	0.621 ^{y,a}	0.564 ^{x,a}	0.405 ^{y,b}	0.492 ^{y,ab}

Free Amino Acids (mg/L)

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours
white	7.21 ^{x,a}	8.77 ^{x,ab}	10.82 ^{x,b}	9.12 ^{x,ab}	9.18 ^{x,ab}
enriched	8.18 ^{x,a}	8.13 ^{x,a}	7.70 ^{x,a}	8.41 ^{x,a}	8.93 ^{x,a}
brown	8.53 ^{x,a}	8.90 ^{x,a}	8.66 ^{x,a}	8.85 ^{x,a}	8.70 ^{x,a}

FRAP (µM FeCl$_3$)

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours
white	299.94 ^{x,a}	245.48 ^{x,ab}	190.25 ^{x,b}	238.91 ^{x,ab}	239.47 ^{x,ab}
enriched	227.54 ^{x,a}	246.09 ^{x,a}	213.83 ^{x,a}	349.35 ^{y,b}	228.64 ^{x,a}
brown	335.30 ^{x,a}	443.89 ^{y,a}	363.16 ^{y,a}	322.43 ^{y,a}	162.78 ^{y,b}

Reducing Sugars (µg glucose /mL)

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours
white	115.33 ^{x,a}	88.61 ^{x,acd}	48.79 ^{x,b}	61.37 ^{x,bc}	69.62 ^{x,bd}
enriched	63.86 ^{y,a}	60.25 ^{x,a}	47.18 ^{x,a}	44.29 ^{x,a}	36.59 ^{x,a}
brown	173.85 ^{z,a}	143.58 ^{y,a}	140.56 ^{y,a}	124.38 ^{y,a}	106.91 ^{y,a}

Note: Uncommon letters (a, b, c, d) in the same row indicate significant differences between time points at $p < 0.05$ Uncommon letters (x,y,z) in the same column within each subtable indicate significant differences between rice type at $p < 0.05$

Table 3

Mean Mineral Content of White, Enriched, & Brown Rice Across Fermentation

Calcium (µg /g)

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours
white	8.862 $^{x, a}$	8.367 $^{x, a}$	8.320 $^{x, a}$	8.553 $^{x, a}$	9.871 $^{x, a}$
enriched	10.272 $^{y, a}$	10.581 $^{y, a}$	10.756 $^{y, a}$	10.585 $^{y, a}$	11.446 $^{y, a}$
brown	10.566 $^{y, a}$	10.429 $^{y, a}$	9.717 $^{y, a}$	9.688 $^{y, a}$	10.100 $^{y, a}$

Iron (µg /g)

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours
white	0.839 $^{x, ab}$	0.528 $^{x, ab}$	0.363 $^{x, a}$	0.537 $^{x, ab}$	0.976 $^{x, b}$
enriched	1.384 $^{y, a}$	1.461 $^{y, a}$	1.578 $^{y, a}$	1.453 $^{y, a}$	1.752 $^{y, a}$
brown	1.157 $^{y, a}$	1.289 $^{y, ab}$	1.660 $^{y, b}$	1.031 $^{z, a}$	1.063 $^{z, a}$

Zinc (µg /g)

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours
white	2.333 $^{x, a}$	2.424 $^{x, a}$	5.019 $^{x, b}$	1.891 $^{x, a}$	1.818 $^{x, a}$
enriched	1.646 $^{y, a}$	1.781 $^{y, a}$	1.852 $^{y, ab}$	2.100 $^{y, ab}$	2.483 $^{y, b}$
brown	2.022 $^{y, ab}$	2.102 $^{y, a}$	1.973 $^{y, ab}$	1.828 $^{y, b}$	1.852 $^{y, ab}$

Potassium (µg/g)

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours
white	163.675 $^{x, ab}$	163.807 $^{x, ab}$	158.040 $^{x, a}$	162.125 $^{x, ab}$	165.869 $^{x, b}$
enriched	94.300 $^{y, a}$	94.695 $^{y, a}$	95.123 $^{y, a}$	91.764 $^{y, a}$	96.472 $^{y, a}$
brown	276.203 $^{z, a}$	254.245 $^{z, b}$	262.372 $^{z, bc}$	259.627 $^{z, bc}$	267.846 $^{z, ac}$

Magnesium (µg/g)

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours
white	48.686 $^{x, a}$	48.498 $^{x, a}$	45.744 $^{x, a}$	45.899 $^{x, a}$	47.254 $^{x, a}$
enriched	29.238 $^{y, a}$	29.146 $^{y, a}$	28.568 $^{y, a}$	28.519 $^{y, a}$	30.135 $^{y, a}$
brown	128.534 $^{z, a}$	126.613 $^{z, a}$	119.254 $^{z, a}$	115.810 $^{z, a}$	121.475 $^{z, a}$

Phosphorus (µg/g)

Rice Type	0 hours	6 hours	12 hours	18 hours	24 hours

white	176.675 $^{x, a}$	181.587 $^{x, a}$	162.755 $^{x, a}$	176.091 $^{x, a}$	177.048 $^{x, a}$
enriched	118.517 $^{y, a}$	119.132 $^{y, a}$	114.379 $^{y, a}$	114.668 $^{y, a}$	115.903 $^{y, a}$
brown	346.815 $^{z, a}$	332.044 $^{z, ac}$	332.504 $^{z, ac}$	315.835 $^{z, b}$	329.725 $^{z, bc}$

Note: Uncommon letters (a, b, c, d) in the same row indicate significant differences between time points at $p < 0.05$ Uncommon letters (x,y,z) in the same column within each subtable indicate significant differences between rice type at $p < 0.05$

Chapter 3: Summary and Recommendations

Summary

Fermentation is the breakdown of a substrate in food through the activities of microorganisms. In the context of food processing, fermentation has long been used to add value to foods. Various cultures around the world have utilized fermentation as a means to preserve and enhance food flavor. This study delved further into a specific food practice, overnight fermented rice, commonly referred to in Tamil as Pazhaya Sadham, or “old rice.” The centuries old practice consisted of covering cooked rice with water and leaving it to ferment overnight, for consumption the next day (Ray et al., 2016). This process has not been extensively studied and investigated scientifically. The study measured changes in titratable acidity, pH, reducing sugars, soluble protein, free amino acids, antioxidants, and mineral content in white, brown and enriched rice. White rice represented the standard preparation while enriched and brown rice represented nutrient dense treatments. These measurements were used to understand the physicochemical changes occurring during the process of fermentation.

The key findings of the study included significant changes in pH, titratable acidity, soluble protein content, reducing sugar content, antioxidant capacities throughout the fermentation. There was no significant change in the free amino acid content. While all rice types experienced similar physicochemical changes, enriched and brown rice exhibit higher mineral content compared to white rice. However, mineral content remains relatively stable throughout fermentation. The finding of this study aligned with prior research of rice based fermented foods (Nkhata et al., 2018; Pino et al., 2022; Thilagavathi et al., 2019).

The findings of this study have several implications. Firstly, it adds more information to the limited existing literature on overnight fermentation. The insights gained from the measured physicochemical parameters contribute a better understanding of fermented food. The findings can also inform public health through addressing micronutrient deficiencies. The enriched and brown rice varieties were higher in micronutrient content but had similar fermentative outcomes to the white rice. This shows that broadly, fermented foods that involve white rice can also be replicated with enriched or brown rice. Some of the limitations of this study include the lack of microbial analysis and small sample size. As only three specific types of rice were measured, the generalizability of this study is decreased. Future research can focus on investigating the impact specific microbes have on the fermentation.

Recommendations

This study did not conduct any form of microbial analysis to identify the microorganisms responsible for the fermentation. It is recommended that future studies explore which lactic acid bacteria (LAB) and/or yeasts specifically contribute to the fermentation of rice in this process. Furthermore, microbial analysis can be used to evaluate the safety of this fermented food. In addition, as the overnight rice fermentation is spontaneous, the region and local microbiota may have an impact on the fermentation. The fermentation conditions explored in this study controlled for the same temperature, humidity, and time for all three rice varieties. An avenue of exploration could be altering these conditions to find which combination of temperature, humidity, and time contribute to the optimal fermented rice product. It is recommended to conduct sensory and clinical study. Community interventions regarding the selection of rice type is also worthy to be further explored. This will further the understanding of food selection and identify methods in which education can be used to inform people about nutritionally beneficial foods.

References:

Ara, G., Khanam, M., Rahman, A. S., Islam, Z., Farhad, S., Sanin, K. I., Khan, S. S., Rahman, M. M., Majoor, H., & Ahmed, T. (2019). Effectiveness of micronutrient-fortified rice consumption on anaemia and zinc status among vulnerable women in Bangladesh. *PloS One*, *14*(1), e0210501. https://doi.org/10.1371/journal.pone.0210501

Chelliah, R., Ramakrishnan, S. R., Premkumar, D., & Antony, U. (2016). Bio-fortification and shelf-life extension of idli batter using curry leaves (Murraya koenigii). *Journal of Food Science and Technology*, *53*(6), 2851–2862. https://doi.org/10.1007/s13197-016-2264-2

Childs, N. (2023, September 27). Rice sector at a glance. USDA ERS - Rice Sector at a Glance. https://www.ers.usda.gov/topics/crops/rice/rice-sector-at-a-glance/

Cui, L., Li, D.-J., & Liu, C.-Q. (2012). Effect of fermentation on the nutritive value of maize. *International Journal of Food Science & Technology*, *47*(4), 755–760. https://doi.org/10.1111/j.1365-2621.2011.02904.x

Fukagawa, N. K., & Ziska, L. H. (2019). Rice: Importance for global nutrition. *Journal of Nutritional Science and Vitaminology*, *65*(Supplement), S2–S3. https://doi.org/10.3177/jnsv.65.S2

Gan, J., Kong, X., Wang, K., Chen, Y., Du, M., Xu, B., Xu, J., Wang, Z., Cheng, Y., & Yu, T. (2023). Effect of fermentation using different lactic acid bacteria strains on the nutrient components and mineral bioavailability of soybean yogurt alternative. *Frontiers in Nutrition*, *10*, 1198456. https://doi.org/10.3389/fnut.2023.1198456

Ghasemzadeh, A., Karbalaii, M. T., Jaafar, H. Z. E., & Rahmat, A. (2018). Phytochemical constituents, antioxidant activity, and antiproliferative properties of black, red, and brown rice bran. *Chemistry Central Journal*, *12*(1), 17. https://doi.org/10.1186/s13065-018-0382-9

Ghosh, K., Ray, M., Adak, A., Dey, P., Halder, S. K., Das, A., Jana, A., Parua Mondal, S., Das Mohapatra, P. K., Pati, B. R., & Mondal, K. C. (2015). Microbial, saccharifying and antioxidant properties of an Indian rice based fermented beverage. *Food Chemistry*, *168*, 196–202. https://doi.org/10.1016/j.foodchem.2014.07.042

Ghosh, K., Ray, M., Adak, A., Halder, S. K., Das, A., Jana, A., Parua (Mondal), S., Vágvölgyi, C., Das Mohapatra, P. K., Pati, B. R., & Mondal, K. C. (2015). Role of probiotic Lactobacillus fermentum KKL1 in the preparation of a rice based fermented beverage. *Bioresource Technology*, *188*, 161–168. https://doi.org/10.1016/j.biortech.2015.01.130

Gupta, R. K., Gangoliya, S. S., & Singh, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology*, *52*(2), 676–684. https://doi.org/10.1007/s13197-013-0978-y

Gyawali, P., Tamrakar, D., Shrestha, A., Shrestha, H., Karmacharya, S., Bhattarai, S., Bhandari, N., Malik, V., Mattei, J., Spiegelman, D., & Shrestha, A. (2022). Consumer acceptance and preference for brown rice-A mixed-method qualitative study from Nepal. *Food Science & Nutrition*, *10*(6), 1864–1874. https://doi.org/10.1002/fsn3.2803

Harding, K. L., Aguayo, V. M., & Webb, P. (2018). Hidden hunger in South Asia: A review of recent trends and persistent challenges. *Public Health Nutrition*, *21*(4), 785–795. https://doi.org/10.1017/S1368980017003202

Heo, S. J., Kim, A.-J., Park, M.-J., Kang, K., & Soung, D. Y. (2020). Nutritional and functional properties of fermented mixed grains by solid-state fermentation with Bacillus amyloliquefaciens 245. *Foods (Basel, Switzerland)*, *9*(11). https://doi.org/10.3390/foods9111693

Hor, P. K., Goswami, D., Ghosh, K., Takó, M., Halder, S. K., & Mondal, K. C. (2022). Preparation of rice fermented food using root of Asparagus racemosus as herbal starter and assessment of its nutrient profile. *Systems Microbiology and Biomanufacturing*, *2*(1), 147–156. https://doi.org/10.1007/s43393-021-00046-8

Knez, E., Kadac-Czapska, K., & Grembecka, M. (2023). Effect of fermentation on the nutritional quality of the selected vegetables and legumes and their health effects. *Life*, *13*(3). https://doi.org/10.3390/life13030655

Lim, M.-J., Barathikannan, K., Jeong, Y.-J., Chelliah, R., Vijayalakshmi, S., Park, S.-J., & Oh, D.-H. (2023). Exploring the impact of fermentation on brown rice: Health benefits and value-added foods—a comprehensive meta-analysis. *Fermentation*, *10*(1), 3. https://doi.org/10.3390/fermentation10010003

Mandhania, M. H., Paul, D., Suryavanshi, M. V., Sharma, L., Chowdhury, S., Diwanay, S. S., Diwanay, S. S., Shouche, Y. S., & Patole, M. S. (2019). Diversity and succession of microbiota during fermentation of the traditional Indian food idli. *Applied and Environmental Microbiology*, *85*(13). https://doi.org/10.1128/AEM.00368-19

Newman, J. C., Malek, A. M., Hunt, K. J., & Marriott, B. P. (2019). Nutrients in the US diet: Naturally occurring or enriched/fortified food and beverage sources, plus dietary supplements: NHANES 2009–2012. *The Journal of Nutrition*, *149*(8), 1404–1412. https://doi.org/10.1093/jn/nxz066

Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J.-B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science & Nutrition*, *6*(8), 2446–2458. https://doi.org/10.1002/fsn3.846

Olson, R., Gavin-Smith, B., Ferraboschi, C., & Kraemer, K. (2021). Food fortification: The advantages, disadvantages and lessons from sight and life programs. *Nutrients*, *13*(4). https://doi.org/10.3390/nu13041118

Pandey, S. (2021). Quality aspects of rice-based fermented beverage. *Biomedical Journal of Scientific & Technical Research*, *34*(5). https://doi.org/10.26717/bjstr.2021.34.005609

Park, M. K., & Kim, Y.-S. (2019). Distinctive formation of volatile compounds in fermented rice inoculated by different molds, yeasts, and lactic acid bacteria. *Molecules*, *24*(11). https://doi.org/10.3390/molecules24112123

Peña-Rosas, J. P., Mithra, P., Unnikrishnan, B., Kumar, N., De-Regil, L. M., Nair, N. S., Garcia-Casal, M. N., & Solon, J. A. (2019). Fortification of rice with vitamins and minerals for addressing micronutrient malnutrition. *Cochrane Database of Systematic Reviews*, *2019*(10). https://doi.org/10.1002/14651858.CD009902.pub2

Pino, A., Nicosia, F. D., Agolino, G., Timpanaro, N., Barbagallo, I., Ronsisvalle, S., Caggia, C., & Randazzo, C. L. (2022). Formulation of germinated brown rice fermented products functionalized by probiotics. *Innovative Food Science & Emerging Technologies*, *80*, 103076. https://doi.org/10.1016/j.ifset.2022.103076

Ravichanthiran, K., Ma, Z. F., Zhang, H., Cao, Y., Wang, C. W., Muhammad, S., Aglago, E. K., Zhang, Y., Jin, Y., & Pan, B. (2018). Phytochemical profile of brown rice and its nutrigenomic implications. *Antioxidants*, *7*(6). https://doi.org/10.3390/antiox7060071

Ray, M., Ghosh, K., Singh, S., & Chandra Mondal, K. (2016). Folk to functional: An explorative overview of rice-based fermented foods and beverages in India. *Journal of Ethnic Foods*, *3*(1), 5–18. https://doi.org/10.1016/j.jef.2016.02.002

Shahbandeh, M. (2024, January 31). *Total global rice consumption 2023/24*. Statista. https://www.statista.com/statistics/255977/total-global-rice-consumption/

Sudha, V., Spiegelman, D., Hong, B., Malik, V., Jones, C., Wedick, N. M., Hu, F. B., Willett, W., Bai, M. R., Ponnalagu, M. M., Arumugam, K., & Mohan, V. (2013). Consumer Acceptance and Preference Study (CAPS) on brown and undermilled Indian rice varieties in Chennai, India. *Journal of the American College of Nutrition*, *32*(1), 50–57. https://doi.org/10.1080/07315724.2013.767672

Somogyi, M. (1952). Notes on sugar determination. *Journal of Biological Chemistry, 195*(1), 19-23. https://doi.org/10.1016/S0021-9258(19)50870-5

Thakkar, P., Modi, H. A., & Prajapati, J. B. (2015). Isolation, characterization and safety assessment of lactic acid bacterial isolates from fermented food products. *International Journal of Current Microbiology and Applied Sciences*, *4*(4), 713-725.

Thilagavathi, P., Rekha, A., & Soundhari, C. (2019). Probiotic and anticancer activity of fermented rice water. *The Pharma Innovation Journal*, *8*(7), 290–295. https://www.thepharmajournal.com/archives/2019/vol8issue7/PartE/8-7-37-429.pdf

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., & Geng, W. (2021). Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Frontiers in Bioengineering and Biotechnology*, *9*, 612285. https://doi.org/10.3389/fbioe.2021.612285

Zhang, D., Ye, Y., Wang, L., & Tan, B. (2022). Nutrition and sensory evaluation of solid-state fermented brown rice based on cluster and principal component analysis. *Foods*, *11*(11). https://doi.org/10.3390/foods11111560