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Microbiome profile and nutritional benefits of traditional overnight soaked cooked rice

Sadia Afrin [a], Caroline Hayley Orr [a,b], Shweta Kuba [a,b], Showti Raheel Naser [c], Rakeem Farooq [a], Salim Khan [c], Manoj Menon [d], Laura Zanetti Domingues [e], Mosharraf Hossain Sarker [f,*]

[a] Science Department, School of Health & Life Sciences, Teesside University, UK
[b] National Horizons Centre, Teesside University, UK
[c] Genomic Research Laboratory, Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh
[d] Department of Geography, School of Geography and Planning, University of Sheffield, UK
[e] Octopus Facility, STFC, UK
[f] School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, UK

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ABSTRACT

This study explores the microbial composition, nutrient content, and health benefits of "Panta Bhat," a traditional dish made from cooked rice soaked overnight, commonly consumed in Southeast Asia, particularly in Bangladesh and India. The research aims to evaluate its potential as an affordable food to enhance nutrition and combat malnutrition in population where rice is a staple. The findings reveal that soaking cooked rice overnight initiates mild fermentation, leading to the consistent growth of beneficial bacteria from the *Bacillota* phylum. 16S rRNA sequencing identified two Gram-positive bacteria, *Leuconostoc lactis* and *Weissella confusa*, known for their probiotic and lactic acid-producing capabilities. Furthermore, mineral analysis via ICP-MS showed significant increases in free iron, zinc, manganese, boron, calcium, magnesium, and potassium in soaked rice compared to unsoaked rice. Notably, there was a significant reduction in glycaemic response after consuming soaked rice ($P < 0.01$). This study suggests that overnight-soaked cooked rice is a promising source of probiotics and enhanced micronutrients, making it a valuable and cost-effective, low-glycaemic food option.

1. Introduction

Traditional soaking of leftover cooked rice, known as 'panta bhat,' is an ancestral method of overnight food preservation in Southeast Asia, particularly in Bengal delta region. This culinary tradition involves soaking cooked rice in water for a minimum of 12 h or overnight (Goswami, Boro, & Barooah, 2016). Panta Bhat serves as a staple, low-cost breakfast option, known for its energy-boosting properties, balancing micronutrient and effective hydration benefits (Ray, Ghosh, Singh, & Mondal, 2016). It is typically served accompanied by the flavours of green chillies, salt, and onions, enhancing its taste profile (Nath et al., 2021). It holds particular significance for impoverished farmers who endure the intense morning heat while working in the fields. Panta Bhat not only provides essential nourishment but also reflects cultural heritage and sustainable food practices rooted in the region's agricultural lifestyle. This practice is deeply interwoven with the region's rich culinary traditions and holds a significant place in its cultural fabric. Panta Bhat, despite its simple preparation and nutrient-richness, embodies the agricultural heritage, economic wisdom, and societal norms of Bengal (Bhattacharjee, 2016; Ray et al., 2016; Sireesha, 2024). It serves as a symbol of Bengali identity and communal unity, bridging different social and economic strata. The dish's simplicity reflects the essential Bengali ethos of finding joy and satisfaction in the basic pleasures of life. However, technological advancements have steadily transformed human nutritional habits, leading to the decline of this traditional practice. As a result, Panta Bhat has become a celebratory custom in the Bengali New Year to symbolize rejuvenation and cleanliness.

Fermentation, an ancient culinary technique embraced for food preservation, elevates the intrinsic worth of foods by unlocking essential micronutrients (Hwang, Kim, Moon, Yang, & Kim, 2017). In traditional biotechnological practices, fermented food preparation takes centre

* Correspondence to: School of Pharmacy and Biomolecular Sciences; Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, UK.
E-mail address: M.H.Sarker@ljmu.ac.uk (M.H. Sarker).

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stage. This microbially mediated process converts carbohydrates into essential nutrients, including organic acids, alcohol and vitamins and makes foods easily digestible. Concurrently, it also gives rise to antimicrobial compounds like bacteriocins, contributing to the growth suppression of foodborne pathogens (Kim et al., 2016; Nout, 2014).

Recent evidence shows that overnight soaking of cooked rice promotes the growth of bacteria that may cause mild fermentation of the rice (Rahman et al., 2021). The fermentative bacteria release a raft of micronutrients from the rice during the fermentation process potentially increasing its nutritional value. A study specially spotlighted the findings about the nutritional value of Panta Bhat, due to the uncovering the prominence of a probiotic bacterium such as *Weisella confusa* strain in overnight soaked rice. This bacterium was found to curb the growth of other bacteria and fortifies the defence against invading pathogens (Nath, Roy, Sikidar, & Deb, 2020).

Malnutrition, including micronutrient deficiencies and diabetes, is a prevalent cause of metabolic disorders in low- and middle-income countries like Bangladesh. Dietary imbalance is a significant contributing factor to the persistent and unevenly distributed malnutrition observed across Bangladeshi society. Projections indicate that the incidence of diabetes in Bangladesh could surge by over 50 % in the next 15 years (Biswas, Islam, Rawal, & Islam, 2016). This increase is closely linked to dietary habits, as high glycaemic index foods such as rice, and sugary treats are staples for many, exacerbating the diabetic landscape (Choudhury, Furbish, & Chowdhury, 2016). The ubiquity of rice as a dietary cornerstone in Asian cultures, particularly in Bangladesh, further intensifies the burden of dietary glycaemic load (Boers, Seijen ten Hoorn, & Mela, 2015). Given that Bangladesh holds the title of the world's highest per capita rice consumer, with an average intake of approximately 500 g of uncooked rice per day, the significance of traditional rice preservation techniques becomes profoundly important. Beyond its role in staving off spoilage, enhancement of rice's nutritional profile and digestibility through fermentation (Hwang et al., 2017), this research investigates whether Panta Bhat could play a role in combating malnutrition, particularly in regions where rice is a staple, but dietary deficiencies are prevalent. This study scientifically examines the practice of soaking rice to understand its microbial load, nutritional content, and potential role as a low glycaemic food. Using general microbiology and 16S rRNA gene sequencing, the beneficial microbes in soaked rice were identified. Additionally, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and flame photometry will determine the micronutrient profile of fermented cooked rice. The investigation also explored Panta Bhat's impact on blood glucose levels in healthy, non-diabetic individuals.

This research aims to reveal the hidden benefits of a traditional cherished dish, exploring its potential as a practical, low-cost solution for enhancing nutrition and health to population where rice is a staple.

2. Materials and methods

The study comprised two phases. Initially, the focus was on assessing the alterations in micronutrient levels and the glycaemic impact post-consumption. Subsequently, the investigation shifted towards evaluating the microbiome load.

2.1. Preparation of socked rice

Cooked and overnight soaked rice was prepared following traditional Bangladeshi methods. In brief, white Basmati rice grains collected from a local supermarket were washed and cooked with tap water for 20–30 min in an aluminium pot and then cooled to room temperature. Drinking water was added into the pot until all rice were submerged. The pot was covered with a lid and kept overnight at 30 °C and room temperature for 12 h for further analysis. Control samples were cooked rice samples without overnight soaking (unsoaked).

2.2. Determination of Blood Sugar

16 volunteers from the Bangladeshi community living at Tees Valley area, in UK were selected. Participants were selected based on their experiences making and eating soaked rice or their knowledge of this traditional food (as per the recommendation by the TU Ethics committee; Ref: 2023 Mar 15182 FAROOQ_TU). Out of 16 participants, three of them were previously diagnosed as type 2 diabetic and hence their data were excluded from the analysis.

The experiment was conducted at one of the participant's homes. The homeowner had prepared the food as per the instruction provided. The experiments were conducted in two separate days. Each morning at 9 am, the participants blood was sampled with a handheld glucometer to measure their blood sugar level reading at a fasted state. Once the first glucose level was collected, the participants were then allowed to consume around 5 gm/kg body weight of soaked rice. Following consumption, the participants' blood glucose level was tested every 20 min over the next two hours to obtain a further 6 blood glucose readings. On the second day, control experiment was performed using the same procedure with unsoaked rice.

2.3. Measurement of mineral nutrients

Mineral nutrients such as Zinc (Zn), Iron (Fe), Calcium (Ca), Magnesium (Mg), Manganese (Mn), Copper (Cu), Boron (B), Potassium (K), and Sodium (Na) were analysed in both unsoaked and soaked rice samples that had been oven dried for 48 h. The rice was cooked with deionized water and then soaked in deionized water for 12 h. After soaking, the rice was blended, oven-dried at 60 °C for 48 h to remove moisture and grounded into a fine powder using a pestle and mortar. Ten grams of the powdered sample were placed in polypropylene tubes and treated with 10 ml of nitric acid. The samples were heated in a block system at 95 °C for 2 h. Following filtration, the total content of Ca, Fe, Mn, Mg, B and Zn were determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES 5800 Agilent), while the Na and K contents were analysed using flame photometry technique.

Following experiment were undertaken to determine pattern of bacterial growth and their identification in soaked rice.

2.4. Quantification of colony-forming units per millilitre (cfu/ml)

Culturable bacteria were quantified through serial dilution and spread plate techniques. Two distinct agar media were employed: 1) de Man, Rogosa & Sharpe (MRS), a selective culture medium fostering Lactobacilli growth, and 2) Luria-Bertani (LB), a non-selective medium encouraging general bacterial growth. Unsoaked rice, as well as rice soaked at 20 °C and 30 °C, cultivated for 24 h in MRS and LB media, underwent serial dilution in a 1:10 dilution series using sterile phosphate-buffered saline (PBS). Subsequently, 100 μl from each dilution was spread onto MRS and LB plates using a glass plate spreader. The plates were then incubated overnight at 37 °C, and the colonies were enumerated to calculate colony-forming units per milliliter (cfu/ml). Each sample was triplicated on the agar plates. The dilution plate displaying 20–300 colonies was utilized for cfu/ml calculation. If fewer than 20 colonies were observed, it was recorded as "too few to count (TFTC)," while if more than 300 colonies were present, it was noted as "too many to count (TMTC).".

2.5. Determination of growth curve

The bacterial growth curve was constructed by assessing the optical density (OD) of the bacterial culture. A spectrophotometer was employed to measure OD at a wavelength of 600 nm. Initially, glycerol stocks of *Lactobacillus buchneri (L. buchneri)* and *Escherichia coli* K-12 W3110 (*E. coli*) were inoculated into separate 50 ml conical flasks containing 25 ml of autoclaved MRS and LB broths, respectively. These

flasks were then incubated overnight at 37 °C. The following day, OD was measured at 600 nm, and the quantities of negative control (media only), positive control (media plus *L. buchneri* or *E. coli*), and test samples (media plus rice samples) required for the 25 ml culture media were calculated to establish the growth curve.

Unsoaked rice, as well as rice soaked at 20 °C and 30 °C, were prepared the day before. Ten grains of rice from each test sample, along with the calculated liquid broth from soaked rice, were added to separate 50 ml conical flasks containing MRS and LB broths. Negative and positive controls (*L. buchneri* for MRS media and Wild type *E. coli* for LB media) were also prepared in separate conical flasks. Each test sample was prepared in triplicates for both media, and the initial OD was recorded. The flasks were then placed in two shaking incubators set at 20 °C and 30 °C, rotating at a speed of 150 rpm, for 24 h. OD measurements were taken every 4 h during the incubation period. The pH of soaked rice sample was determined using a Jenway 3510 pH meter at 2 h interval for first 12 h and then after 24 h at the end of the experiment.

2.6. Whole DNA extraction and sequencing

DNA from rice samples soaked at 20 °C and 30 °C was extracted using the DNeasy® PowerFood® Microbial Kit, following a modified protocol designed for optimal DNA yield as per the manufacturer's guidelines. Initially, 0.25 g of soaked rice sample was combined with 1.8 ml of rice broth in a 2 ml microcentrifuge tube. The samples were homogenized using a FastPrep-24™ 5 G Homogenizer at a speed of 8 m/s for 6 mins.

The resulting microbial food culture was centrifuged, and the supernatant was removed. This process was repeated until 10 ml of soaked rice broth was centrifuged to obtain a concentrated rice pellet.

The cell pellet was resuspended in 450 µl of pre-warmed (55 °C) MBL solution and transferred to a PowerBead Tube. Instead of using a vortex adapter, the PowerBead Tubes were placed in the FastPrep-24™ 5 G Homogenizer, vortexed horizontally for 10 min at maximum speed. After centrifugation, the supernatant was transferred to a clean tube, and IRS solution was added and incubated before another centrifugation step. The resulting supernatant was mixed with MR solution and loaded onto an MB Spin Column. After several wash steps, the purified DNA was eluted using 50 µl of elution buffer (EB).

The purified DNA samples were quantified using a NanoDrop™ (One Microvolume UV-Vis Spectrophotometer, ThermoScientific). Samples with a concentration of at least 10 ng/µl were selected and sent to Novogene (Genomic services, Cambridge, UK) for sequencing. The V4 region of the 16S rRNA gene was amplified using primers 515 F (5′ GTGCCAGCMGCCGCGGTAA 3′) and 806 R (5′ GGACTACHVGGGTWTCTAAT 3′). Resulting PCR products were subjected to pair-end 250 bp sequencing using Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) at Novogene (Beijing, PR China) at read depth of 30,000 tags per sample.

The raw sequencing reads were processed in FASTQ.

2.7. Functional Pathway Analysis

The 16S rRNA gene sequences were further processed by different bioinformatic tools for analysing taxonomic classification of the microbial community, extraction of the open reading frame, functional profiling, and visualization of microbiota present in soaked rice. DADA2 (Callahan et al., 2016), was used to dereplicate the reads and to get feature tables and feature sequences. The relative abundance was estimated to identify the bacterial taxonomy. The QIIME2 v2020.2 analysed alpha and beta diversity (Bolyen et al., 2019). PICRUSt software (Langille et al., 2013) provided predictive metagenome functions from bacterial 16S rRNA gene data.

2.8. Species identification through 16S rRNA gene amplification and sequence

An attempt was made for bacterial identification from the colonies grown in MRS broth by inoculation of soaked rice. Soaked rice was cultivated at 20 °C and 30 °C for 24 h in MRS plates and eight colonies were randomly selected (four from 20 °C and four from 30 °C) for species identification. Colonies were resuspended in 200 µl of nuclease free water and heated at 100 °C for 10 min using a heat block (Thermo Scientific_TM digital dry bath) before centrifuging at 13000 rpm for 3 min (Head, Hiorns, Embley, McCarthy, & Saunders, 1993). The 16S rRNA gene was amplified from extracted DNA by PCR. The universal primers for 16S rRNA were used: 27 F, 5'-GTGCTGCAGAGAGTTTGATCCTGGCTCAG-3' and 1492 R, 5'-CACGGATCCTACGGGTACCTTGTTACGACTT-3'. Bio-Rad C1000 Touch thermal cycler was used for amplification: Initial denaturing at 94 °C for 2 min, denaturing at 95 °C for 1 min, annealing at 49 °C, extension at 72 °C for 90 s, for 35 cycles before final extension at 72 °C for 5 min. QI Aquick PCR purification kit (Qiagen) was used to purify the amplified products. The purified PCR products were sent to Eurofins, Ebersberg, Germany for 16S rRNA gene sequencing. The sequencing data were analysed by nucleotide BLAST tool from NCBI (https://blast.ncbi.nlm.nih.gov/Blast) for bacterial identification. The isolates were identified at species level and these findings were confirmed by comparative BLASN database queries based on sequence similarity. A phylogenetic analysis of isolated bacteria was created using distance-based approach, neighbor-joining method based on a pairwise distance matrix computed for all sequences under consideration.

2.9. Statistical analysis of blood sugar levels

A t-test with a Mann-Whitney U post hoc test was conducted to compare the differences in blood sugar analysis upon consumption of unsoaked cooked basmati rice and Panta Bhat. A p-value of $P < 0.01$ at the 95 % confidence interval was considered as statistically significant.

3. Results

3.1. Glycaemic effect of soaked rice on participants without diabetes

Significant differences in glycaemic level were observed of consuming soaked rice as compared to traditionally cooked rice in healthy individuals. The consumption of unsoaked rice led to a marked increase in blood glucose levels, whereas soaked rice resulted in a more stable blood glucose profile, as illustrated in Fig. 1. Although both types of rice initially caused a rise in blood glucose levels 20 min after consumption, soaked rice exhibited much slower rise with blood glucose levels stable after reaching their peak at 25 min post-consumption. The statistical analysis revealed a significant difference ($p < 0.01$) in blood glucose levels between those the participants who consumed unsoaked

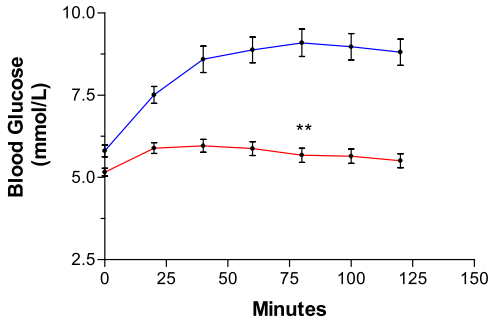

Fig. 1. The impact of overnight soaked rice (red line) and unsoaked rice (blue line) composition on blood glucose levels in healthy, non-diabetic participants (n = 13). Error bars show standard error of means (s.e.m).

rice and those who consumed soaked rice.

3.2. Micronutrient analysis in fermented soaked rice

A comparative analysis of mineral content was conducted between soaked and unsoaked cooked rice (used as the control). Soaked exhibited a substantial increase in several metal nutrients (Fig. 2), with iron (Fe), zinc (Zn) copper (Cu), calcium (Ca), and magnesium (Mg) being the most abundant among the quantified metals. Their levels were recorded at 2.24 μg/g, 0.51 μg/g, 0.46 μg/g, 0.41 μg/g and 0.40 μg/g of material, respectively. Additionally, soaked rice contained other trace elements such as magnesium (Mg), manganese (Mn), boron and potassium (K), with levels measured at 0.0.16 μg/g, 0.012 μg/g, 0.49 μg/g and 0.011 μg/g, respectively, in comparison to the control group.

3.3. Fermentation of soaked rice as monitored by pH changes

The pH of soaked rice which was kept at 20 °C and 30 °C were measured every 2 h for 24 h. The pH gradually decreased from neutral towards acidic pH (Table 1). The pH reduced more in soaked rice which was kept at 30 °C compared to soaked rice at 20 °C.

3.4. Bacterial population counts

The microbial population was counted on MRS and LB culture media from unsoaked rice, soaked rice fermented at room temperature and soaked rice fermented at 30 °C after 12 h. The total count varied between the two different media. The bacterial count was higher on LB media than MRS media from all three sample types. There was no significant difference in bacterial counts between ($P = 0.0585$ on MRS and $P = 0.53928$ on LB media).

3.5. Bacterial Growth curve

The density of bacteria was measured by determining the optical desity of the broth culture and the results were plotted as a function of time (Fig. 3). The growth patterns of bacteria present in unsoaked rice, soaked rice fermented at 20 °C and soaked rice fermented at 30 °C in MRS and LB media were observed. From the MRS cultures (Fig. 3a), the growth of bacteria present in soaked rice at 20 °C were different from the bacteria of soaked rice at 30 °C with higher optical density in rice soaked at 30 °C at all time points (4 h $P = 1.29 \times 10^{-4}$, 8 h $P = 9.35 \times 10^{-8}$, 12 h $P = 4.59 \times 10^{-6}$, 24 h $P = 2.26 \times 10^{-4}$). Rice soaked at 20 °C also showed higher optical density than unsoaked rice at 8 and 12 h ($P = 8.11 \times 10^{-8}$ and $P = 6.32 \times 10^{-4}$ respectively).

In the LB cultures (Fig. 3b), the OD for all rice types was similar after 24 h. However, at 4, 8 and 12 h OD was higher for soaked rice at 20 °C compared to unsoaked rice ($P = 3.05 \times 10^{-4}$, 4.827×10^{-4} and 2.98×10^{-4} respectively) and was higher for soaked rice at 30 °C compared to unsoaked rice at 4 ($P = 2.54 \times 10^{-5}$) and 12 ($P = 2.67 \times 10^{-4}$) hours.

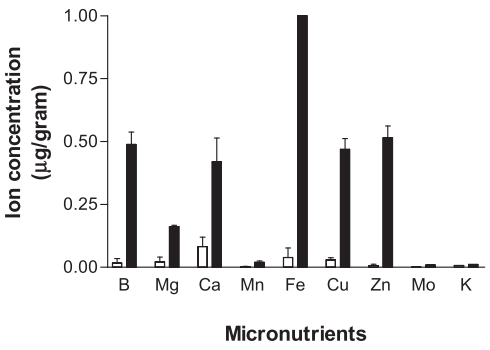

Fig. 2. Comparison of micronutrient composition within unsoaked rice and soaked rice. The open bars are unsoaked rice and the filled bars are test soaked rice samples (n = 3).

3.6. Determination of the microbial communities in soaked rice

Changes of relative abundance of phyla were measured between unsoaked rice and soaked rice each at two different temperatures (20 °C and 30 °C). It was observed that, Bacillota increased significantly ($P = 0.0467$) in both soaked rice samples at two temperatures compared to the unsoaked rice.

3.7. Functional pathway analysis

DNA sequence data was used to make assumptions around metabolic pathways involved in the soaked rice fermentation process. The pathways upregulated among samples were compared in the heatmap shown in Fig. 5(a). A Venn diagram was generated showing the similarities and unique pathways among the samples and compared in Fig. 5(b). The two soaked rice samples at 20 °C and 30 °C shared a common pathway for biosynthesis of CMP-pseudaminc acid which was upregulated in both samples and uniquely found in soaked rice samples.

3.8. Analysis of 16S rRNA gene sequencing

All 8 colonies were successfully sequenced by Sanger sequencing with 1500 bp sequence which is identified the isolates at species level. Neighbor-joining tree clearly clustered in separate groups according to the species' sequences. High bootstrap values were identified in all groups which supports the values that means every time the same branch was observed when the phylogenetic tree was generated on a resampled set of data. Isolated from 20 °C-soaked rice exhibited 99.62 % resemblance with *Leuconostoc lactis* and other closely related species were *Leuconostoc citreum* and *Leuconostoc holzapfelii*. On the other hand, isolates from 30 °C-soaked rice showed 99.09 % similarity with *Weissella confusa* and other related species such as *Weissella cibaria*.

4. Discussion

4.1. Blood glucose levels do not rise following consumption of soaked rice

To explore the anecdotal evidence that soaking rice overnight can initiate a mild fermentation process and potentially enhance its nutrient profile, this study investigated the effects of the traditional rice soaking method on microbial growth, micronutrient changes, and post-consumption blood sugar levels.

Recognizing the critical role of glycaemic index in understanding the impact of soaked rice consumption, the study conducted blood sugar analyses in healthy individuals. White basmati rice is recognized for its high glycaemic index (GI) (Nayar and Madhu, 2020; Miller, Pang, & Bramall, 1992). The long-term consumption of high GI food has been linked to chronic diseases such as obesity, cardiovascular diseases, and type II diabetes (Ding and Malik, 2008). The findings from this research indicate no significant changes in blood glucose levels among non-diabetic individuals who consumed soaked rice compared to pre-consumption level. However, consumption of unsoaked rice resulted in significantly elevated blood glucose levels. There is evidence that fermentation of rice can influence its absorption and glycaemic index (GI) in several ways, primarily due to changes in its chemical composition and structure. Research conducted by (Tu et al., 2021) provides compelling evidence supporting the notion that the fermentation process applied to rice during food preparation induces significant structural and chemical transformations including conversion into resistance starch. Microbial activity during fermentation can also increase fibre content of the rice. These alterations, in turn, significantly impact the digestion of rice starch, making it less accessible to digestive enzymes. The modified structure resulting from fermentation has been identified

Table 1
pH readings of soaked rice at different temperatures within 24 h.

Samples	Time (hrs)	0	2	4	6	8	10	12	24
Soaked rice at 20 °C	pH	7.2	7.06	6.77	6.31	6.14	6.04	5.81	5.12
Soaked rice at 30 °C	pH	7.25	7.16	6.88	5.93	5.12	4.93	4.86	4.33

Table 2
Colony forming units of microorganisms grown from unsoaked and soaked rice on MRS and LB media.

Media	Samples	CFU/ml ± SD
MRS	Unsoaked rice	$4.6 \times 10^8 \pm 2.91 \times 10^8$
	Soaked rice at 20 °C	$2.45 \times 10^7 \pm 1.04 \times 10^7$
	Soaked rice at 30 °C	$3.95 \times 10^8 \pm 1.41 \times 10^8$
LB	Unsoaked rice	$9.65 \times 10^{10} \pm 1.26 \times 10^{10}$
	Soaked rice at 20 °C	$1.1 \times 10^{11} \pm 3.41 \times 10^{10}$
	Soaked rice at 30 °C	$1.22 \times 10^{11} \pm 1.20 \times 1010$

Table 3
Unique and shared functional alteration caused by the microbial change in the samples through PICRUSt analysis.

Sample	Pathway ID: Pathway Description
Unique pathways in sample A – unsoaked rice	PWY−622: starch biosynthesis PWY−5392: reductive TCA cycle II PWY−7347: sucrose biosynthesis III SUCSYN-PWY: sucrose biosynthesis I PWY−7084: nitrifier denitrification PWY−5744: glyoxylate assimilation CENTBENZCOA-PWY: benzoyl-CoA degradation II (anaerobic) PWY−5743: 3-hydroxypropanoate cycle PWY−5656: mannosyl glycerate biosynthesis I PWY−7024: superpathway of the 3-hydroxy-propanoate cycle
Unique pathways in sample B soaked rice	PWY−4722: creatinine degradation II PWY−6830: superpathway of methanogenesis LIPASYN-PWY: Phospholipase PWY−5823: superpathway of CDP-glucose-derived O-antigen building blocks biosynthesis
Unique pathways in sample C unsoaked rice at 30 °C	PWY−1422: vitamin E biosynthesis (tocopherols) PWY−3661: glycine Betaine Degradation
Unique pathways in sample D soaked rice at 30 °C	PWY−6167: flavin biosynthesis PWY−7209: superpathway of pyrimidine ribonucleosides degradation PWY−7031: protein N-glycosylation (bacterial) PWY−6486: D-galacturonate degradation PWY−6760: D-xylose degradation
Common pathways among unsoaked rice at both temperatures	P621-PWY: nylon−6 oligomer destruction PWY−1501: mandelate degradation I PWY−6957: mandelate degradation to acetyl-CoA PWY−7046: 4-coumarate degradation (anaerobic)
Common pathways among soaked rice at both temperatures	PWY−6143: CMP-pseudaminic acid biosynthesis / CMP-pseudaminate biosynthesis

as a key factor that can impact the postprandial absorption of the rice.

This finding underscores the intricate interplay between food processing methods, structural and chemical modifications, and subsequent physiological effects, shedding light on the potential implications for digestive processes and nutrient absorption in the context of rice consumption. Apart from this the fermentation has been shown to increase acetic acid and lactic acid and other health beneficial bioactive components such as phenolic compounds, antioxidants, Gamma-aminobutyric acid (GABA), as well as enhanced nutritional values including vitamins, short-chain fatty acids, bacteriocins, and exopolysaccharides. These compounds have been associated with demonstrating antidiabetic properties and the capacity to lower blood glucose levels

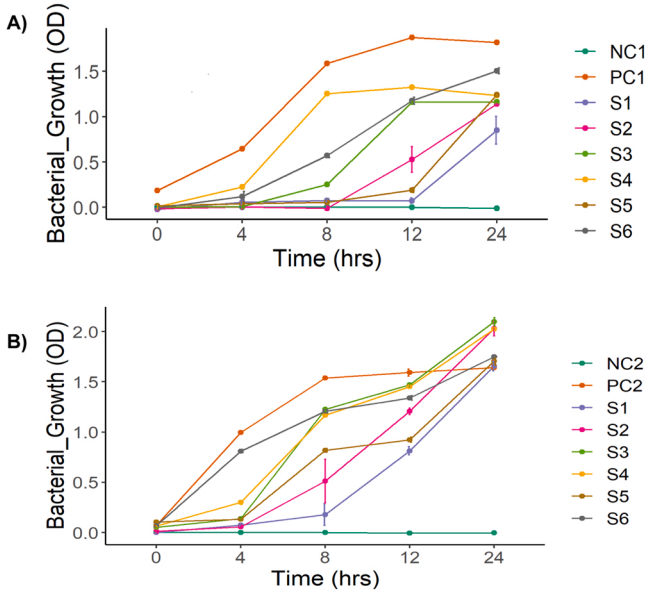

Fig. 3. The bacterial growth curve of soaked and unsoaked rice cultures under different temperatures. A) MRS broth. B) LB broth (NC- Control media, PC1-*L. buchneri*, PC2-*E. coli,* S1 Uncooked rice, S2- Cooked unsoaked rice, S3-soaked at 20 °C; S4 -soaked at 30 °C, S5-Soaked uncooked rice at 20 °C and S6-Soaked rice at 30 °C). Error bars show standard deviation.

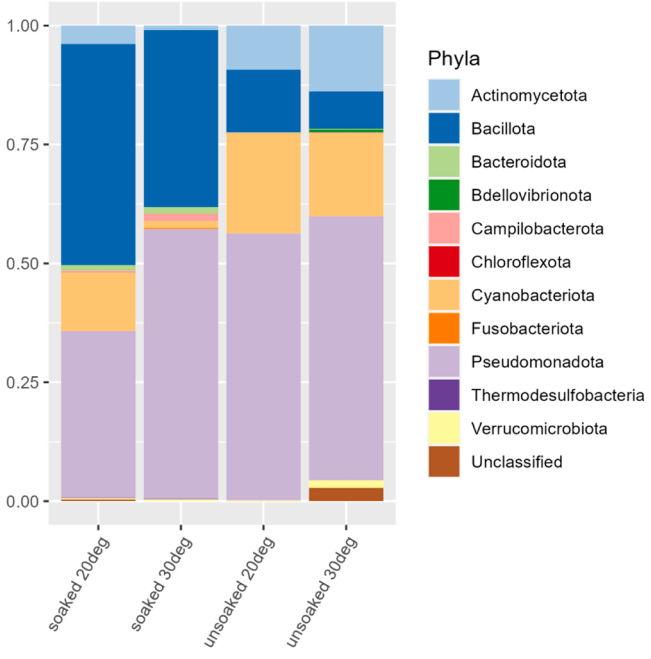

Fig. 4. Relative abundance changes of Phyla in soaked and unsoaked rice at different temperatures.

(Hwang et al., 2017; Hyun, Kim, Jung, & Kim, 2021; Sivamaruthi, Kesika, Prashanth, & Chaiyacut, 2018; Teo, See, Ramazanu, Chan, & Wu, 2022). Organic acids has been shown to slow gastric emptying, the

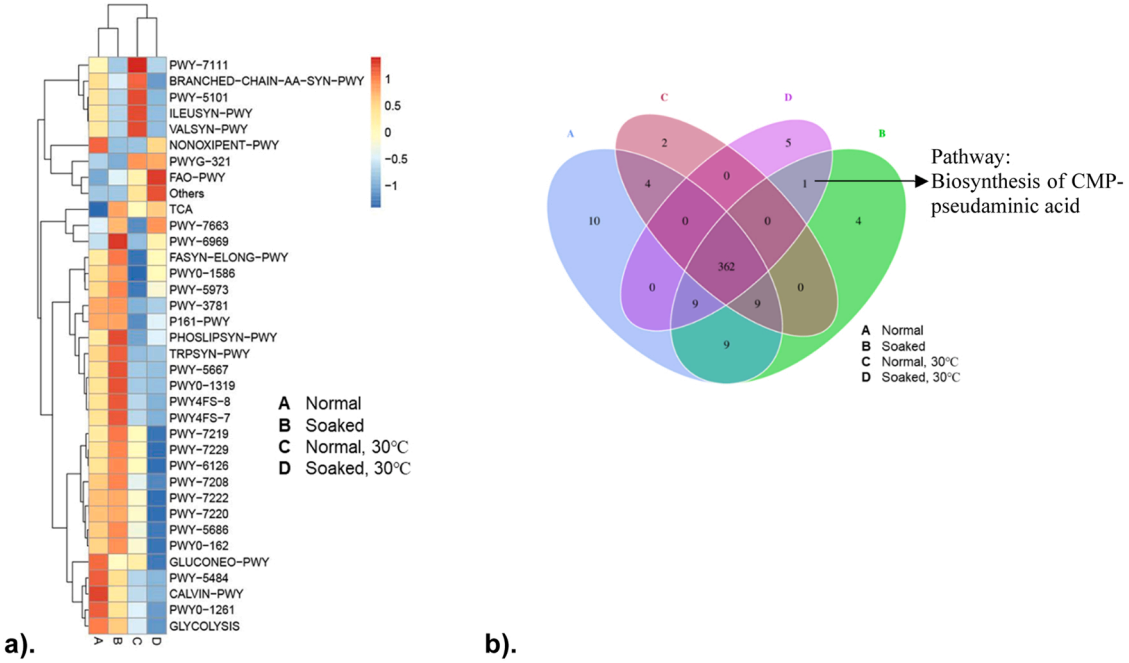

Fig. 5. (a) Heatmap showing top 35 pathways identified in the samples showing upregulated pathways in comparison to other samples. (b) Venn diagram showing numbers of unique and shared pathways among samples.

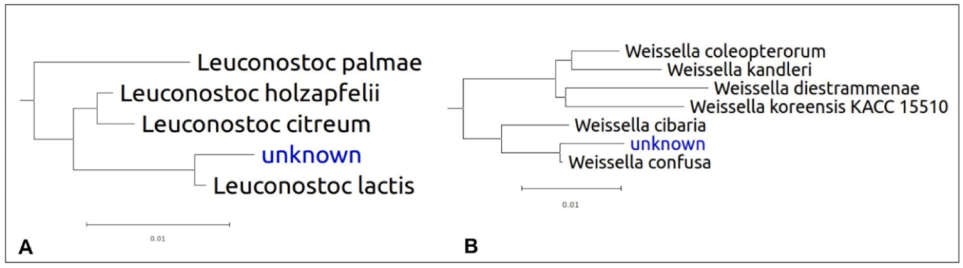

Fig. 6. Phylogenetic Tree. Identification of bacterial species from soaked rice by 16S rRNA sequence data. A phylogenetic tree created based on the neighbor-joining of 16S rRNA gene sequences. The sequences identified in this research are shown in blue. (A) Dendrogram of isolated bacterial sequences of soaked rice prepared at 20 °C and (B) bacterial sequences of 30 °C-soaked rice. 0.01 mentions to nucleotides per site in the alignment, a measure of the scale of the genetic distance between each of the bacterial groups.

rate at which food leaves the stomach and enters the small intestine, thus slowing down the absorption of sugars and leading to improved blood sugar control (Noh, Lee, Lee, & Pyo, 2020).

Our study offers preliminary insights into the potential beneficial impact of soaked rice on reducing the glycaemic response of rice. However, it is essential to underline the need for further investigations involving a larger population, encompassing both diabetic and non-diabetic individuals, to comprehensively understand the implications of soaked rice consumption on blood sugar levels. The findings of this research hold promise for unravelling novel dietary strategies that may contribute to glycaemic control and overall health in populations where rice is a staple diet.

4.2. *Micronutrients are elevated in soaked rice*

Micronutrient malnutrition poses a significant challenge in developing countries, including Bangladesh, with elevated rates of iron and zinc deficiencies, particularly among children and pregnant women (Gupta, Brazier, A. K., & Lowe, 2020). In this study there were significant enhancement of micronutrients such as iron, zinc, and calcium was found when cooked rice was soaked overnight. There were also considerable enhancement of other micronutrients like, magnesium, manganese, barium, copper, cobalt, bismuth and potassium. Iron concentration have previously been reported as higher as 73.91 mg/100 g sample in fermented sour rice (Bhattacharyya, 2014) which strongly support the present study. The newspaper report also claimed that there are significant increase of potassium (839 mg/100 g sample), sodium (303 mg/100 g sample) and calcium (850 mg/100 g sample) after 12 h fermentation due to soaking process.

Evidence suggests that rice fermentation could result in dephytinization facilitated by microbial enzyme phytase. Studies indicate an increase in this enzyme's activity in rice fermented with the probiotic yeast strain, *Saccharomyces cerevisiae*. This dephytinization may play a potential role in increasing release of minerals (Banik, Ghosh, & Mondal, 2020). Additionally, evidence suggests that the fermentation process reduces anti-nutritional factors like trypsin inhibitors, a factor which may also play a role in increasing micronutrient contents in the grains (Badau, Nkama, & Jideani, 2005) and (Nadeem et al., 2010). By mitigating anti-nutritional factors and enhancing nutrient bioavailability, the rice soaking process has the potential to improve the dietary quality of fermented food, thereby contributing to addressing micronutrient deficiencies in impoverished communities.

4.3. The microbial community within fermented rice may aid health benefit

Analysing the number of viable cells of bacteria in samples by colony forming unit measurement using two different medias showed more colonies on LB media than the MRS media. In MRS broth OD was higher at all time points across 24 h for rice soaked at 30 °C compared to unsoaked rice. Rice soaked at 20 °C showed higher OD compared to unsoaked rice at 8 and 12 h. In LB broth OD was higher at 4, 8 and 12 h for rice soaked at 20 °C and at 4 and 12 h for rice soaked at 30 °C compared with unsoaked rice. This suggests that overnight soaking, particularly within a warm climate, will increase the overall microbial community of rice and that potentially probiotic microorganisms which are selected for within MRS media may be increased during fermentation.

The pH of soaked rice was measured every two hours for 12 h and showed that the pH gradually reduced from neutral to acidic pH (between 5–4). Studies state that bacteria belonging to the genus *Lactobacillus* and *Leuconostoc* can grow in temperature ranging from 2 to 53 °C and pH varying between 4.5 and 6.5 (Śliżewska and Chlebicz-Wójcik, 2020). This result gives an indication that soaked rice could undergo fermentation due presence of fermenting lactic acid bacteria species.

16S rRNA gene amplicon sequencing was used to analyse the microbial communities present within the rice. By using different bioinformatics tools, the sequence data was taken to identify the relative abundance of bacteria at the phylum level, and it was found that bacteria belonging to the Bacillota phylum increased in soaked rice. Bacillota (previously known as Firmicutes) are dominant phyla present in the human gut and known to maintain intestinal homeostasis, development, and protection against pathogens (Stojanov, Berlec, & Štrukelj, 2020; Rinninella et al., 2019). Probiotic microbes belong to the Bacillota phylum play an important probiotic role within the gut microbiome (Rastogi and Singh, 2022).

Identification of microbes growing in culture using whole 16S gene sequencing, and supported by 16S rRNA amplicon sequencing, showed presence of *Lactococcus and Leuconostoc* was increased in rice soaked at 20 °C and *W. confusa* was increased in rice soaked at 30 °C. There could be understandable concern about the potential for an increased microbial community within a fermented product to lead to an increased likelihood of pathogens present within the rice. However, both *Leuconostoc* and *Weissella* have been previously identified within fermented foods and have been recognised for their probiotic potential (Dou and Teixeira et al., 2023, 2021). *W. confusa* are known to produce non-digestible oligosaccharides and extracellular polysaccharides and this may explain the conversion of soaked rice to become low glycaemic food (Fusco et al., 2015). Both *Leuconostoc* and *Weisella* have also been shown to release bacteriocins and organic acids which can reduce pathogenic bacteria as well as production of antioxidants and release of cholesterol reducing compounds, resulting in health benefits (Anandharaj, Rani, & Swain, 2021; Dey, Khan, & Kang, 2019).

Functional pathway analysis showed the upregulation and downregulation of different pathways regulated by the bacterial species in soaked rice at two different temperatures and was found that both temperatures of soaked rice shared a common pathway which was upregulated and uniquely found only in soaked rice. This pathway was the biosynthesis of CMP-pseudaminic acid. Studies suggests that CMP-pseudaminic acid is a component which is responsible for inhibiting an enzyme that allows the assembly of cell surface virulence factors of pathogenic bacteria (McNally et al., 2008). Potentially the beneficial lactic acid bacteria present in soaked rice upregulate pathways involved in the suppression of growth of pathogenic bacteria.

4.3.1. Study limitations

This preliminary study was primarily conducted by MSc and undergraduate students, with volunteer recruitment being limited due to ethical regulations and issues. All experiments were performed on simulated rice, and only one type of rice was used, without replication across other varieties. Therefore, the results should be interpreted with caution.

5. Conclusion

This interdisciplinary research underscores the traditional practice of consuming "Panta Bhat," and validates its nutritional value. By analysing this traditional food practice, we believe this study can significantly highlight the nutritional importance of this food to people like Bangladeshi population, where rice is a staple. Our research aligns with contemporary trends that advocate for low-cost, healthier dietary choices and solidify and validate the importance of such practices in health and wellbeing and reducing global food waste. The awareness of indigenous knowledge embedded in panta Bhat and role of the beneficial microbes and nutrients they contain could facilitate its importance in modern lifestyle. The region's climate, with temperatures ranging from 26–35 °C, facilitates the fermentation process, making it a low-energy, cost-effective culinary practice for nutritional well-being.

Ethical Clearance

This study has received research ethics Clearance from Teesside University ethical Committee (Reference: 2023 Mar 15182 FAROOQ).

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CRediT authorship contribution statement

Caroline Orr: Formal analysis, Supervision, Writing – review & editing. **Sadia Afrin:** Investigation, Writing – review & editing. **Showti Naser:** Investigation, Writing – review & editing. **Shweta Kuba:** Conceptualization, Supervision, Writing – review & editing. **Salim Khan:** Conceptualization, Writing – review & editing. **Rakeem Farook:** Investigation. **Laura Domingues:** Conceptualization. **Manoj Menon:** Conceptualization, Writing – review & editing. **Mosharraf Sarker:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

I declare that the manuscript, or part of it, has neither been published (except in form of abstract or thesis) nor is currently under consideration for publication by any other journal; and that my co-authors have read the manuscript and approved its submission to Journal of Food and Humanity. On behave of all authors I also confirm that we do not have any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations in relation to this publication.

Data availability

Raw FastQ files for 16 s rRNA sequencing have been deposited in ArrayExpress (https://www.ebi.ac.uk/arrayexpress/) on 9 February 2024 with the accession number E-MTAB-13807 (https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-13807).

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