



Quality Profiling of Gluten and Dairy Free Probiotic Beverages from Finger Millet

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10.18805/ajdfr.DR-2306

ABSTRACT

Background: Fermented millet-based beverages are a nutrient-dense non-dairy beverage choice that has been a part of our diets since ancient times. Finger millet is a nutritious cereal rich in vitamins, minerals, dietary fiber and other phytochemicals. The present study aimed to ascertain the use of finger millets in the formulation of non-dairy probiotic beverages and examine the effect of different treatments on the sensory acceptance of the beverages.

Methods: Finger millet-based beverages were developed using four distinct pretreatment methods, including soaking and germination of grains, while the control beverage was prepared with grains that were neither soaked nor germinated. pH, total acidity, specific gravity and TSS of the beverages were investigated along with microbial quality.

Result: Results revealed that finger millet stands out as an excellent substrate for the development of fermented beverages, particularly when combined with probiotic strains such as *Lactobacillus acidophilus* and *Streptococcus thermophilus*. Treatment S₃ was the most acceptable beverage among the developed formulations based on sensory evaluation results.

Key words: Beverage, Fermentation, Finger millet, Probiotic, Sensory.

INTRODUCTION

Millets are small seeds belonging to the grass family that can be processed in a variety of ways to provide a range of valuable foods. Their high nutritional content, gluten-free status and ability to withstand challenging growth conditions make them a promising crop for the future (Panigrahi *et al.*, 2024). Finger millet was referred to as “nrittakondaka” meaning the Dancing grain in ancient Indian sanskrit literature which is a super food with various health benefits that can fight against malnutrition and other illness including osteoporosis, diabetes, rickets and obesity (Maharajan and Ceasar, 2022). Food products made from finger millets have been shown to have anti-cancer and anti-aging properties and are thought to lower blood pressure and cholesterol (Gebreyohannes *et al.*, 2024). The fast rising prevalence of coeliac disease and lactose intolerance has raised demand for gluten-free and dairy-free products. Finger millet therefore has the potential to serve as a primary ingredient for producing a functional beverage that is free of gluten and dairy.

World Health Organization defined probiotics as “live microbial supplements when administered in adequate amounts, confer health benefits on the host” (Sugandhi, 2018). The tradition of consuming foods containing beneficial microbes or fermented foods has been in the long run culture of mankind (Narayanan and Subramonian, 2023). Probiotics are regarded as microbiological sources of viable nutraceuticals that, provides a number of health benefits (Adriani *et al.*, 2024). Furthermore, probiotics support intestinal health and significantly boost immunity (Bhavya and Lakhvinder, 2023). The most often utilized probiotics in food items are microbial species from the genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus*

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How to cite this article: Shruthi, P., Divakar, S., Anith, K.N. and Beela, G.K. (2025). Quality Profiling of Gluten and Dairy Free Probiotic Beverages from Finger Millet. Asian Journal of Dairy and Food Research. 1-7. doi: 10.18805/ajdfr.DR-2306.

Submitted: 20-12-2024 **Accepted:** 14-04-2025 **Online:** 14-05-2025

(Vijaya Kumar *et al.*, 2015). The incorporation of microbial cultures into food not only enhances its nutritional qualities but also augments its flavor and extends its shelf life, a practice that has been recognized for generations.

The rise in consumer preference favoring vegan, healthy, nutrient dense food and beverage choices increased the demand of novel ingredients as substrates for probiotic supplementation. This shift has fuelled the formulation of various functional foods and beverages and its market growth. Hence, the present study focused on developing millet based probiotic beverages using the bioprocessed finger millet and their sensory evaluation. Finger millet beverages were prepared by inoculating probiotic strains of *Lactobacillus acidophilus* and *Streptococcus thermophilus* and incubating at 37°C for 24 hours.

MATERIALS AND METHODS

This study was carried out in the Food and Nutrition Laboratory, Department of Community Science, College of Agriculture, Vellayani and the Laboratory of Microbiology,

Department of Microbiology at the College of Agriculture Vellayani, Kerala Agricultural University, Trivandrum for a research period of 12 months.

Procurement of materials

Finger millet (*Eleusinecoracana*) commonly known as ragi, was procured from the nearby supermarket store, at Trivandrum. The probiotic starter culture strains *Lactobacillus acidophilus* NCDC 13 and *Streptococcus thermophilus* NCDC 75 was procured from NCDC NDRI Karnal for developing probiotic beverages. Glass bottles and Sugar was purchased from local market of Trivandrum.

Starter culture preparation

The probiotic strains *Lactobacillus acidophilus* NCDC 13 and *Streptococcus thermophilus* NCDC 75 were activated by pure culturing in *Lactobacillus acidophilus* media and *Streptococcus thermophilus* media at 37°C for 24 hours and then on broth for required growth. The microbial culture was centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded and the pellets were washed with double distilled water. An initial concentration of 108cfu/ml was constituted by diluting the microbial culture with sterile distilled water with aid of a turbidity meter and the starter culture was stored under 4°C.

Production of the beverage samples

The finger millet grains were first cleaned to remove any dirt and impurities, then washed thoroughly with tap water and dried properly. For soaking, the grains were immersed in water for either 12 hours or 24 hours. For sprouting, they were initially soaked for 8 hours and then left to germinate for two different time periods-12 hours and 24 hours. The control sample was prepared by using the ragi grains without soaking or germinating. In order to extract the milk, 250 millilitres of water was added to grind the treatment variants of finger millet grains. The extracted finger millet milk was strained and a known quantity of sugar was added

to the extracted milk treatments after they had been heated to 100°C for 10 minutes. After cooling to room temperature, 8% probiotic culture mix was added and the beverage mixes were incubated for 24 hours at 37°C before being stored below 4°C. Fig 1 depicts the flowchart of beverage formulation. Table 1 represents the treatment details of beverage samples.

Physico-chemical parameters of the probiotic millet beverage samples

The quality parameters including pH, total acidity, specific gravity and total soluble solids of the developed beverage samples were analyzed using standard AOAC procedures (AOAC 2000). The pH of the beverage samples were measured using a pH meter. The total acidity content of the samples was measured by titrating the samples with standardized Sodium Hydroxide solution to the phenolphthalein end point. The total soluble solids were measured by standard AOAC method using a refractometer (AOAC 2000). Specific gravity of beverage samples were measured using pycnometer according to Hossin *et al.* (2021); Memon *et al.* (2009).

Microbial monitoring of the beverage samples

Microbial enumeration was conducted to determine the total viable count, total bacterial count, total fungal count and total coliform count in the beverage samples. The total viable count of *Lactobacillus acidophilus* and *Streptococcus thermophilus* in the beverage samples was enumerated by spread plate count method. Plates were incubated in inverted position in an incubator at 37°C for 24 hrs. Spread plate count on Nutrient agar media, Rose Bengal Agar Media and EMB Agar media was used to measure total bacterial count, total fungal count and total coliform Count (Saeid and Rana, 2020).

Total plate count (CFU/ml) =

$$\frac{(\text{No. of colonies} \times \text{Dilution factor})}{\text{Volume of sample taken}}$$

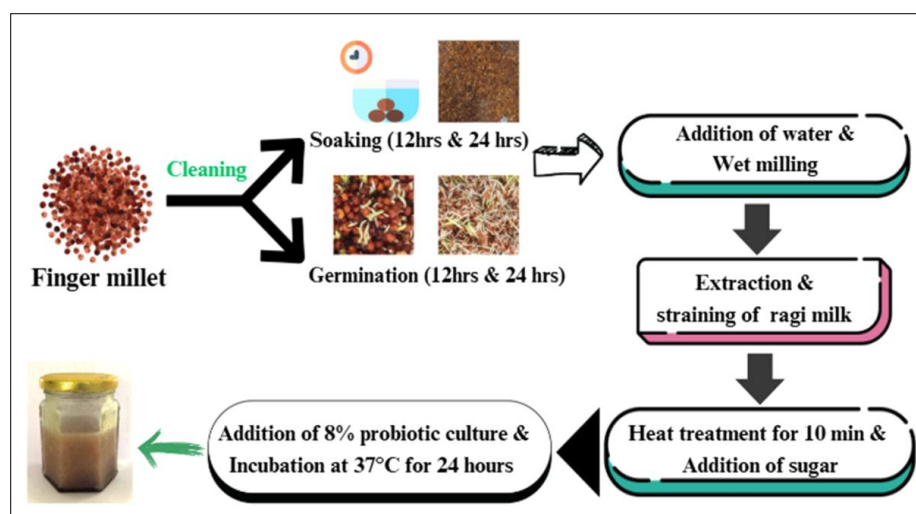


Fig 1: Flowchart of beverage formulation.

Sensory assessment of the beverage samples

Sensory evaluations of the beverage samples were conducted on the 1st and 7th day of storage of beverage samples stored in glass bottles under refrigeration. Sensory attributes such as appearance, aroma, consistency, taste, mouth feel and overall acceptability of the samples were evaluated by semi trained panelists by adopting a 9 point hedonic scale method. All the samples were prepared on the same date and stored in glass bottles at 4°C. Each sample was served in 50 ml portion in plastic cups and presented to the panelists in a balanced random order. All sensory evaluations were carried out in separate booths and saline water was offered as a palette cleanser along with a ranking questionnaire for the beverage samples. The sensory evaluation card is shown in Fig 2.

Statistical analysis

All the tests were performed in three replications and data were transferred to KAU Grapes Software for statistical analysis (Gopinath *et al.*, 2020). Kruskal Wallis test was

Table 1: Treatment details of the beverage samples.

Treatments	Variations
C	No soaking / germination
S ₁	Soaking (12 hrs)
S ₂	Soaking (24 hrs)
S ₃	Germination (12 hrs)
S ₄	Germination (24 hrs)

performed to determine the statistical difference of the sensory assessment data. CRD analysis was performed on all other physico- chemical and microbial data to determine the significant difference between each sample.

RESULTS AND DISCUSSION

Physico-chemical parameters of the probiotic millet beverage samples

The data present in the Table 2 shows the various physico-chemical properties of finger millet based probiotic beverage over a storage period of 7 days. The optimum pH for probiotic beverages typically ranges between 4.0-4.5 as this pH balances probiotic viability and sensory appeal of the product. This pH range offers a tangy taste to the beverage formulations and also helps in preventing the growth of spoilage microorganisms and pathogens. Fermentation process help in reducing the pH value of finger millet based probiotic beverages below 4.5 by production of organic acids and all beverage formulations exhibited a decrease in pH throughout fermentation and storage period (Yulia *et al.*, 2024). The pH values of the treatments ranges from 4.41-4.50 on day 1 and 3.98-4.01 on day 7. It is revealed from the table that pH level of the treatments decreased and total acidity level of the treatments increased during storage and pH values of all the samples were found around 4. The highest pH was found in control treatment 4.5 and the lowest was found in treatment S₃ 3.96. The pH evaluation results showed an increased acid

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SENSORY EVALUATION CARD

Product: Probiotic beverages

Date:

Name:

Signature:

Attributes	A	B	C	D	E
Appearance					
Aroma					
Consistency					
Taste					
Mouth feel					
Overall acceptability					

9- point Hedonic Scale

- 1- Dislike extremely
- 2- Dislike very much
- 3- Dislike moderately
- 4- Dislike slightly
- 5- Neither dislike nor like
- 6- Like slightly
- 7- Like moderately
- 8- Like very much
- 9- Like extremely

Fig 2: Sensory evaluation card.

content for all the treatments over the fermentation period (Muncey and Hekmat, 2021). Total acidity of probiotic beverages is a key physico-chemical parameter, typically expressed as a percentage of lactic acid equivalents and influences the taste, microbial stability and overall quality of probiotic beverages. Total acidity of the beverage formulations can be varied according to the type of beverage, probiotic starter culture and fermentation conditions. The total acidity of the treatments ranged from 0.18-0.20 (day 1) and rose up to a range of 0.21-0.24 (day 7). This significant decrease in pH and increase in acidity were observed due to production of organic acids and other metabolite production by the probiotic bacteria. These results are similar to the results observed in the studies of Shukla and Kushwaha (2017) and Hatami *et al.* (2023). The specific gravity of the beverage treatments ranged from 0.99-1.04 and no significant difference was found during the end of the storage. This result was supported by the findings of Hossain *et al.* (2020). Specific gravity of any beverage can be affected by solid content, sugar content, fermentation and storage duration. Furthermore, the total soluble solids (TSS) which are measured using the refractometer ranged from 5.43 °Brix-5.87 °Brix and however, it got lowered to 4.90 °Brix-5.40 °Brix after a period of 7 days. There was a significant difference in TSS °Brix value on day 1 and day 7. The TSS content of the probiotic beverages depends on the sugar, dietary starch and dietary protein. The conversion of carbohydrates into lactic acid by

probiotic bacteria lowers the total solid content in probiotic beverage formulations (Meera *et al.*, 2021). A slight decrease in TSS content during storage post fermentation may be due to the utilization of sugars and other metabolic byproduct of the probiotics in the beverage formulations and this report agrees with the result of Adebayotayo and Akpeji (2016).

Microbial monitoring of the beverage samples

The total viable count of finger millet based probiotic beverages fermented for 24 hours was examined on the 1st and 7th day of storage. This evaluation of probiotic cell viability began after the 24 hour incubation period of the fermentation process. As per the regulatory guidelines probiotic beverage formulations should typically contain a minimum of 10^6 - 10^9 CFU per ml of live microorganisms at the time of consumption in order to consider it as a probiotic and achieve health benefits. However the prepared beverage formulations maintained this safe standard throughout the storage period of seven days. The total viable count of beverage samples ranged from 9.03- 9.08 CFU/ml and is depicted in Table 3. There was a steady increase in the microbial growth in the first day and towards end of the storage, a slight decline in the viable count was seen. The viability count of the beverage formulations depends on factors like fermentation time, fermentation temperature, storage temperature and packaging material. The average counts of *Lactobacillus acidophilus* and *Streptococcus*

Table 2: Physicochemical parameters of the beverage samples.

Treatments	pH		Total acidity (%)		Specific gravity		TSS (°Brix)	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
C	4.50±0.01 ^a	4.01±0.01 ^a	0.18±0.00 ^e	0.21±0.00 ^c	0.99±0.01 ^b	1.00±0.01 ^c	5.43±0.35	4.90±0.10 ^b
S ₁	4.48±0.01 ^a	3.98±0.01 ^{bc}	0.19±0.00 ^d	0.23±0.00 ^b	1.03±0.01 ^a	1.03±0.01 ^{ab}	5.70±0.10	5.40±0.10 ^a
S ₂	4.46±0.02 ^b	4.00±0.01 ^{ab}	0.19±0.00 ^b	0.23±0.00 ^b	1.04±0.01 ^a	1.03±0.01 ^{ab}	5.77±0.15	5.40±0.10 ^a
S ₃	4.43±0.02 ^c	3.96±0.02 ^c	0.19±0.00 ^c	0.24±0.00 ^a	1.03±0.02 ^a	1.04±0.01 ^a	5.77±0.06	5.33±0.15 ^a
S ₄	4.41±0.01 ^d	3.98±0.01 ^{bc}	0.20±0.00 ^a	0.24±0.00 ^a	1.03±0.01 ^a	1.03±0.01 ^b	5.87±0.06	5.20±0.10 ^a
CD	0.02	0.02	0	0	0.02	0.01	-	0.2
SE(m)	0.01	0.01	0.00	0.00	0.01	0.00	0.10	0.06
CV(%)	0.27	0.32	0.72	0.99	1.28	0.67	3.17	2.15

Table 3: Total viable count of the beverage samples.

Treatment	<i>Lactobacillus acidophilus</i>		<i>Streptococcus thermophilus</i>		Viability %	
	Day 1 CFUml ⁻¹	Day 7 CFUml ⁻¹	Day 1 CFUml ⁻¹	Day 7 CFUml ⁻¹	<i>L. acidophilus</i> CFUml ⁻¹	<i>S. thermophilus</i> CFUml ⁻¹
C	9.03±0.01 ^c	8.72±0.01 ^c	9.01±0.00 ^d	8.38±0.04 ^d	96.53±0.03 ^c	93.01±0.42 ^d
S ₁	9.07±0.01 ^{ab}	8.87±0.02 ^a	9.03±0.00 ^b	8.67±0.02 ^b	97.79±0.18 ^a	96.00±0.22 ^b
S ₂	9.08±0.01 ^a	8.89±0.02 ^a	9.04±0.00 ^a	8.74±0.03 ^a	97.90±0.10 ^a	96.69±0.33 ^a
S ₃	9.06±0.01 ^b	8.81±0.02 ^b	9.02±0.00 ^c	8.57±0.02 ^c	97.28±0.06 ^b	95.06±0.15 ^c
S ₄	9.07±0.01 ^{ab}	8.82±0.03 ^b	9.02±0.00 ^c	8.63±0.02 ^b	97.20±0.20 ^b	95.63±0.23 ^b
CD	0.01	0.03	0	0.05	0.24	0.53
SE(m)	0.00	0.01	0.00	0.01	0.08	0.17
SE(d)	0.01	0.01	0	0.02	0.11	0.24
CV(%)	0.09	0.20	0.02	0.30	0.14	0.30

thermophilus in the finger millet-based probiotic beverages showed no statistically significant differences among the samples, as all treatments contained similar ranges of probiotic bacteria. Considering the results it can be concluded that probiotic bacterial strains *Lactobacillus acidophilus* and *Streptococcus thermophilus* thrive equally well in all the beverage formulations, irrespective of the treatment variations. In contrast, the beverage samples showed a low probiotic density of the probiotic strain *S. thermophilus* in comparison to the probiotic density of strain *L. acidophilus*. Nevertheless, the probiotics contained in the beverage formulations were 109CFU per serving, they were deemed to be able to benefit the consumer (Liew *et al.*, 2022). It is possible that the dietary fibre, oligosaccharides and resistant starch found in finger millets will act as prebiotics and aid in the development of probiotics. These results are in agreement with the results reported from the study of (Sertovic *et al.*, 2019; Mahapatra and Das, 2022).

The beverage treatments were subjected to microbial studies for analyzing the total bacterial count, total coliform count and total fungal count during the storage period of 7 days and are represented in Table 4. The finger millet based probiotic beverage samples were free from coliform and fungal count throughout the storage period of 7 days. The total bacterial count was not detected in the day 1 but on

day 7 it ranged from 4.35-4.39 CFUml⁻¹. Similar results were reported by (Ghosh *et al.*, 2015; Mahejibin *et al.*, 2021 and Sharma *et al.*, 2020).

Sensory assessment of the beverage samples

The sensory characteristics of different developed beverage treatments are presented in Table 5 and 6. The sensory profile was evaluated in terms of the attributes appearance, aroma, consistency, taste and mouth feel of the beverage samples. The organoleptic evaluation of beverage samples on the 1st day revealed an overall acceptability in the range of 6.7-8.7, whereas on day 7 it ranged between 5.8-7.7. Among the treatments S₃ attained highest overall acceptability score (8.7) followed by S₄ (8.6), S₂ (8.0), S₁ (7.3) and least score of (6.7) by the control sample. The treatments S₃ and S₄ obtained a similar score for consistency, taste and mouthfeel (8.7), followed by S₂, S₁ and Control. The prepared beverage's appearance received positive ratings across all treatments, which indicates enhanced product's visual enticement with highest value of 8.7 on the day 1 reduced to 7.7 on day 7 for S₃. The aroma had a sensory score of 8.7 on day 1 for treatment S₄ which got reduced to 7.6 on day 7. However, a significant difference was found in the case of appearance, aroma and overall acceptability among treatments. The distribution of high sensory scores of each attributes suggests that, finger millet based probiotic beverages are

Table 4: Microbiological evaluation of beverage samples.

Treatment	Total bacterial count (TBC) CFUml ⁻¹		Total fungal count (TFC) CFUml ⁻¹		Total coliform count (TCC) CFUml ⁻¹	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
C	ND	4.36±0.06	ND	ND	ND	ND
S ₁	ND	4.35±0.03	ND	ND	ND	ND
S ₂	ND	4.39±0.05	ND	ND	ND	ND
S ₃	ND	4.38±0.03	ND	ND	ND	ND
S ₄	ND	4.38±0.04	ND	ND	ND	ND
CD	ND	-	ND	ND	ND	ND
MSE	ND	0.00	ND	ND	ND	ND
SE(m)	ND	0.02	ND	ND	ND	ND
SE(d)	ND	0.03	ND	ND	ND	ND
CV(%)	ND	0.94	ND	ND	ND	ND

Table 5: Sensory assessment of beverage samples day 1.

Treatments	Day 1					
	Appearance	Aroma	Consistency	Taste	Mouth feel	Overall acceptability
C	7.0 ^a	6.2 ^a	6.8 ^a	6.8 ^a	6.8 ^a	6.7 ^a
S ₁	7.7 ^{ab}	7.2 ^b	7.2 ^a	7.3 ^a	7.2 ^a	7.3 ^b
S ₂	8.0 ^b	8.0 ^c	8.2 ^b	8.1 ^b	7.9 ^b	8.0 ^c
S ₃	9.0 ^c	8.7 ^d	8.7 ^c	8.7 ^c	8.7 ^c	8.7 ^d
S ₄	8.4 ^d	8.4 ^{cd}	8.7 ^c	8.7 ^c	8.7 ^c	8.6 ^d
χ ² value	80.988	96.480	89.830	87.575	91.082	100.050
p_value	0	0	0	0	0	0

Since the P-value is <0.05, there is a significant difference between at least a pair of treatments. Groups with same letters are not significantly different at 5% level. Value indicates mean score of sensory panel.

Table 6: Sensory assessment of beverage samples day 7.

Treatments	Day 7					
	Appearance	Aroma	Consistency	Taste	Mouth feel	Overall acceptability
C	5.8 ^a	5.8 ^a	5.8 ^a	5.8 ^a	5.8 ^a	5.8 ^a
S ₁	7.2 ^b	7.2 ^{bc}	7.2 ^{bc}	7.1 ^b	7.2 ^{bc}	7.2 ^b
S ₂	7.2 ^b	6.9 ^b	7.0 ^b	7.3 ^b	7.0 ^b	7.1 ^b
S ₃	7.7 ^c	7.6 ^c	7.6 ^d	8.0 ^c	7.6 ^c	7.7 ^c
S ₄	7.6 ^c	7.3 ^{bc}	7.4 ^{cd}	7.4 ^b	7.4 ^c	7.4 ^c
χ^2 value	67.087	55.366	70.727	62.805	69.834	76.018
p_value	0	0	0	0	0	0

Since the P-value is <0.05, there is a significant difference between at least a pair of treatments. Groups with same letters are not significantly different at 5% level. Value indicates mean score of sensory panel

in an acceptable range for a period of 7 days except the control sample. Positive acceptance of the sensory characteristics of S₁, S₂, S₃ and S₄ treatments during storage time was reported by the sensory panelists. Throughout the storage period, the S3 treatment maintained a higher score while the control treatment maintained a lower score for every attribute. It was evident from the sensory assessment data over the period of storage, the consumer approval criteria for every sample showed a declining tendency. The results of sensory assessment is in line with research result obtained by Hossin *et al.* (2021).

CONCLUSION

The inclusion of probiotic foods as a part of diet claims numerous health benefits to the consumers. The present study investigated the potential of utilizing finger millet as the main substrate in formulating gluten and dairy free probiotic functional beverages. The pretreatments such as soaking and germination of finger millet grains and incorporation of *Lactobacillus acidophilus* and *Streptococcus thermophilus* probiotic cultures significantly enhanced the beverage's sensory attributes, shelf life and overall acceptability. The results demonstrated that finger millet based probiotic functional beverages exhibit desirable appearance, aroma, consistency and taste while maintaining an adequate microbial viability over the storage period, without any growth of pathogenic or spoilage microorganisms. There upon finger millet based probiotic beverages can serve as a promising addition to the functional beverage market catering to both the health-conscious consumers and those seeking novel dietary options.

ACKNOWLEDGEMENT

The present study was supported by College of Agriculture, Vellayani, Kerala Agricultural University, Thiruvananthapuram.

Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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