

## Standardizing the Fermentation Process for Developing Oyster Mushroom Beverage

PRIYANKA MULAGE<sup>1</sup>, N. UMASHANKAR<sup>2</sup>, SUVARNA V. CHAVANAVAR<sup>3</sup>, R. MUTHURAJU<sup>4</sup>, USHA RAVINDRA<sup>5</sup>, MOHAN CHAVAN<sup>6</sup> AND M. B. DARSHAN<sup>7</sup>

<sup>1,2,3&4</sup>Department of Agricultural Microbiology, <sup>5</sup>Department of Food Science and Nutrition,

<sup>6</sup>Department of Agricultural Plant Biotechnology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065

<sup>7</sup>ICAR-AICRP on Post Harvest Engineering and Technology, UAS, GKVK, Bengaluru - 560 065

e-Mail : priyamulage122@gmail.com

### AUTHORS CONTRIBUTION

PRIYANKA MULAGE :

Conduct of experiment,  
analysis of data and  
Preparation of manuscript;

N. UMASHANKAR :

Conceptualization, design,  
guidance, supervision and  
reviewing of manuscript;

SUVARNA V. CHAVANAVAR ;

R. MUTHURAJU ;

USHA RAVINDRA ;

MOHAN CHAVAN &

M. B. DARSHAN :

Facilitated the setup of the  
experimental and planning  
of the experimental design

**Corresponding Author :**

PRIYANKA MULAGE

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### ABSTRACT

Mushroom beverages are emerging as new-age functional drinks that capitalize on the health-promoting properties of mushroom, offering a natural and nutritious alternative to traditional fermented beverages. This study aimed to develop a novel fermented beverage using grey oyster mushroom (*Hypsizygus ulmarius*) paste as the primary ingredient combined with water and 20 per cent sugar across all treatments. The fermentation process was standardized using *Lacto bacillus delbrueckii* 2775, a lactic acid bacterium with probiotic properties. The study examined the effects of varying quantities of oyster mushroom (10g, 20g, 30g and 40g) and different fermentation intervals on the pH, total soluble solids (TSS), titratable acidity (TA) and sensory attributes of the resulting beverages. The optimal formulation was achieved with 20g of oyster mushroom substrate fermented for four days and inoculated with 6 per cent *Lacto bacillus delbrueckii*. This combination resulted in the most favorable pH level (4.13), total soluble solids (12.25 °Brix) and titratable acidity (1.08%). Additionally, it received the highest sensory score of 13.84 on the fourth day of fermentation. These results underscore the potential of this optimized fermentation process for producing high-quality oyster mushroom beverage. This development could lead to an innovative, probiotic-rich functional drink that combine delightful flavors with substantial health benefits, meeting the growing demand for natural and nutritious alternative in the beverage market.

**Keywords :** Fermentation, *Lacto bacillus delbrueckii*, Mushroom beverage

MUSHROOM is a large visible macro fungus with a distinctive fruiting body, easily spotted and picked by hand whether it is epigeous or hypogeous. Mushrooms have been widely used as food and very often as delicious and nutritious food (Vincenti *et al.*, 2013). Approximately 14,000 described species out of an estimated 1.5 million fungi worldwide produce fruiting bodies large enough to be classified as mushrooms (Chang, 2006).

Mushroom production has witnessed phenomenal growth globally with annual output surpassing 40 million metric tons. Asia leads this sector, accounting for approximately 82.8 per cent of global production with China as the largest producer and consumer at over 41 million metric tons annually (FAOSTAT, 2022). Mushroom cultivation in India has advanced notably since the 1960s, reaching an annual production of around 2,42,900 tons with a recent

growth rate of 4.3 per cent (Sharma *et al.*, 2023). Primary varieties include button, oyster and milky mushrooms. Karnataka is emerging as an important contributor, providing about 4.5 per cent of India's mushroom output due to its favorable climate and availability of agricultural waste. Although the industry remains under developed, there is potential for growth by adopting improved cultivation practices and raising farmer awareness of the economic benefits of mushroom farming. Initiatives promoting sustainable practices and market development further enhance Karnataka's ability to meet the rising demand for nutritious food (ICAR, 2024).

Mushrooms are consumed globally for their nutritional and medicinal benefits and serve as a sustainable bioconversion tool, transforming waste into valuable resources. Despite India's favorable agro-climate, abundant agro-waste, low-cost labor and rich fungal diversity, mushroom cultivation remains under developed (Thakur, 2020). Mushrooms are nutritional power house providing low fat, high protein and a wealth of vitamins, essential minerals, trace elements and dietary fibers. They are also renowned for their medicinal properties containing anti-inflammatory compounds such as polysaccharides, terpenoids and phenolic compounds. Recently, mushrooms have gained recognition as a source of nutraceuticals, offering antioxidants, anticancer properties, prebiotic effects, immunomodulating benefits, cardiovascular support, antimicrobial effects and antidiabetic properties. Most mushroom products are considered safe and can provide significant health benefits on regular consumption (Khan *et al.*, 2013). As the world's population grows and per capita arable land diminishes compounded by rapid urbanization, industrialization, climate change and rising demand for quality functional foods, the focus must shift towards secondary agriculture and innovative crops like mushrooms (Kirk *et al.*, 2008).

Probiotics are defined as live microbial food supplements that provide beneficial effects to consumers when administered in adequate amounts (FAO, 2002). The regular intake of probiotics improves intestinal function improving the immune

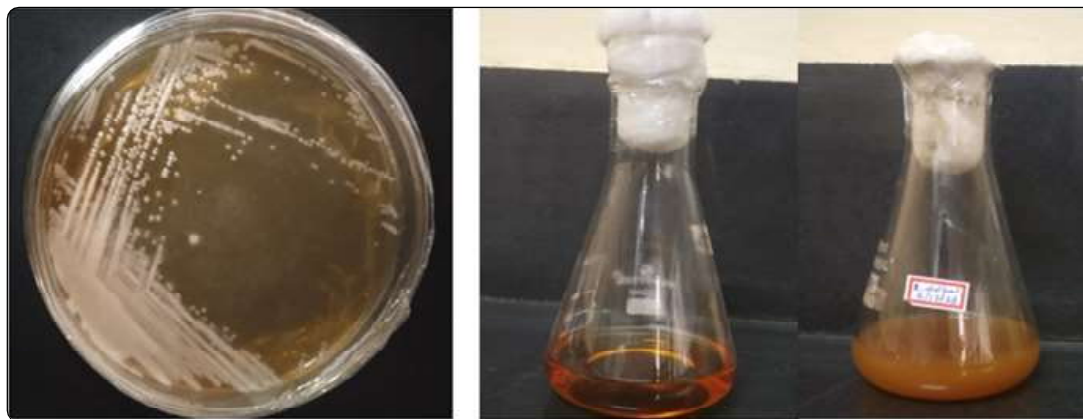
system and inhibiting pathogens. The most widely consumed probiotics are bacteria such as *Lacto bacillus* and *Bifidobacterium* (De Vrese and Schrezenmeir, 2008). Presently non-dairy probiotic products are gaining demand because some people have lactose intolerance (Prado *et al.*, 2010 and Pampangouda *et al.*, 2021). *Lacto bacillus delbrueckii*, a Gram-positive lactic acid bacterium is a popular probiotic organism in the fermentation process. The inclusion of *L. delbrueckii* in fermentation process of mushroom beverages can enhance the organoleptic characteristics, nutritional value, flavor and exhibit probiotic effects that strengthen the immune system and improve gut microbiome balance, contributing to the development of novel and health-promoting *Lacto bacillus* fermented drinks (Yang *et al.*, 2024).

The low shelf life of mushrooms is a constraint for their wide range of use to overcome this constraint the product has to be processed into a more durable product so that it will add value to the mushroom. One innovative approach is the development of a mushroom-based fermented beverage, which currently lacks availability in the market, hence, the present study focused on developing and standardizing mushroom beverage using probiotic *Lacto bacillus delbrueckii* in the study. The inclusion of probiotics not only enhances the nutritional profile of the beverage but also promotes gut health, improves digestion and adds functional benefits that cater to health-conscious consumers. This dual advantage of mushroom nutrition and probiotic properties could make the beverage an appealing option in the health drink market.

## MATERIAL AND METHODS

### Collection and Maintenance of Probiotic Culture

Lactic acid bacteria were obtained from the National Centre of Microbial Resources (NCMR) in Pune (Maharashtra) specifically *Lacto bacillus delbrueckii* 2775 was used in this study. The probiotic cultures were revived on De Man, Rogosa and Sharpe (MRS) agar medium (Plate 1). These cultures were then preserved in slants and glycerol stocks and stored at appropriate temperatures for further use.

Plate 1 : Pure cultures of *Lacto bacillus delbrueckii*

### Ingredients Used in the Experiment

**Oyster Mushroom** : The grey oyster mushroom was cultivated in the mushroom laboratory, Advance Centre for Skill Development in Mushroom Production Technology, Department of Agricultural Microbiology, UAS, GKVK, Bengaluru (Yohannes *et al.*, 2020). The mushroom beverage was prepared using freshly harvested mushrooms as a raw material for beverage preparation.

### Standardization of Fermented Oyster Mushroom Beverage

**Preparation of Fermented Mushroom Beverage** : Freshly harvested oyster mushrooms were initially washed with distilled water to remove surface impurities and dirt. The mushrooms were then cut into small pieces and a paste was prepared using a mixer. To standardize the formulation, 100 ml of water was added to varying quantities of oyster mushroom paste (10 g, 20 g, 30 g and 40 g) for optimization. Fermentation intervals were adjusted across a range of one to five days with each treatment containing 20 per cent of sugar. The mixture was pasteurized at 62.28 °C for 30 minutes to ensure microbial safety, after which a 6 per cent of *Lacto bacillus delbrueckii* starter culture was inoculated to initiate fermentation. Following the designated fermentation intervals (1 to 5 days), the resulting oyster mushroom beverage was stored at 4 °C to maintain quality and shelf life (Pampangouda *et al.*, 2021).

**Experiment Setup** : The experiment was conducted for standardization of mushroom quantity (10, 20, 30 and 40 g of mushroom) and fermentation duration (1, 2, 3, 4 and 5 days of fermentation) for oyster mushroom beverage preparation using *Lacto bacillus delbrueckii*. The results of the standardization of mushroom quantity and fermentation duration for the preparation of fermented mushroom beverage and its biochemical properties and organoleptic characteristics were analyzed.

### Biochemical Analysis

**pH, Total Soluble Solids (TSS) and Total Titrable Acidity (TA) %** : The pH was analyzed using a pH meter (AOAC, 2005). The total soluble solids (TSS) were measured using a digital hand refractometer and expressed as °Brix. Erma hand refractometer was used in the range of 0 to 32 °Brix. A drop of the sample was placed on the prism and the reading was recorded in °Brix. Total titrable acidity was determined as percent lactic acid by the titration method standard protocol (Ranganna, 1986) using 0.1 N NaOH and phenolphthalein as an indicator.

**Organoleptic Evaluation** : The sensory score of standardized fermented oyster mushroom beverage was evaluated (Plate 2) at regular intervals using a 20-point hedonic scale by semi-trained panel members considering mainly appearance, color, aroma, taste and overall acceptability etc. (Qiu *et al.*, 2022).

**Experimental Design** : The single factor study was designed using a Completely Randomized Block

Design arranged with 5 repetitions and assessed by analysis of variance (ANOVA) with the help of Opstat 2.0 software. The Duncan test was used for multiple comparisons and the level of statistical significance was set at  $p \leq 0.05$  (Duncan, 1955).

## RESULTS AND DISCUSSION

### Standardization of Oyster Mushrooms and duration of Fermentation for Developing Oyster Mushroom Beverage

The study standardized oyster mushroom quantities and fermentation durations with 20 per cent sugar added consistently to assess the efficiency of *Lactobacillus delbrueckii* 2775 in fermenting an oyster mushroom beverage. Results showed that different mushroom quantities and fermentation times significantly impacted the process. Optimal fermentation occurred with specific combinations leading to favorable changes in pH, TSS and titratable acidity. Sensory evaluation indicated that certain combinations had higher acceptability, demonstrating the potential of *Lactobacillus delbrueckii* 2775 in producing a quality fermented beverage.

### Biochemical Properties of Fermented Oyster Mushroom Beverage

*pH* : The initial pH of oyster mushroom juice was 6.43. Throughout the five-day fermentation process, a significant reduction in pH was observed across all treatments reflecting the metabolic activity of *Lactobacillus delbrueckii* that fermented sugars into lactic acid.

In Treatment 1 ( $T_1$ ), the pH decreased significantly from 6.13 (1<sup>st</sup> day) to 4.21 (5<sup>th</sup> day). Treatment 2 ( $T_2$ ) exhibited a decrease from 6.01 (1<sup>st</sup> day) to 3.96 (5<sup>th</sup> day). Similarly, Treatment 3 ( $T_3$ ) showed a substantial drop in pH from 5.87 (1<sup>st</sup> day) to 3.87 (5<sup>th</sup> day) while, treatment 4 ( $T_4$ ) demonstrated the most pronounced decrease with the pH falling from 5.83 (1<sup>st</sup> day) to 3.53 (5<sup>th</sup> day) (Table 1). These results indicate a statistically significant decline in pH across all treatments with the most significant reduction observed in Treatment 4.

The observed decrease in pH was indicative of the effective fermentation process, where *Lactobacillus delbrueckii* converts the sugars in the mushroom juice into lactic acid thus lowering the pH, this reduction highlights the importance of both substrate quantity and fermentation duration in achieving optimal acidity levels in fermented beverages. The significant drop in pH was consistent with the known capability of lactic acid bacteria (LAB) to produce lactic acid which not only enhances the flavor profile but also contributes to the preservation of the product by inhibiting spoilage microorganisms (Dhananjaya *et al.*, 2021 and Laikhuramand Vijayalaxmi, 2022).

The final pH values observed in this study ranging between 3.53 and 4.21 fall within the optimal pH range for LAB activity, which typically lies between 4.0 and 5.0. This lower pH not only enhances the sensory qualities of the beverage but also improves its shelf stability by creating an environment that was less favorable for pathogenic and spoilage organisms (Ramesh *et al.*, 2020).

**TABLE 1**  
**Effect of pH on fermentation of oyster mushroom beverage using *Lactobacillus delbrueckii***

Treatment details	1 <sup>st</sup> DOF	2 <sup>nd</sup> DOF	3 <sup>rd</sup> DOF	4 <sup>th</sup> DOF	5 <sup>th</sup> DOF
$T_1$ 10 g mushroom	6.13 <sup>a</sup>	5.99 <sup>a</sup>	5.52 <sup>a</sup>	4.50 <sup>a</sup>	4.21 <sup>a</sup>
$T_2$ 20 g mushroom	6.01 <sup>b</sup>	5.71 <sup>b</sup>	5.31 <sup>b</sup>	4.09 <sup>b</sup>	3.96 <sup>b</sup>
$T_3$ 30 g mushroom	5.87 <sup>c</sup>	5.62 <sup>bc</sup>	4.98 <sup>c</sup>	3.76 <sup>c</sup>	3.87 <sup>c</sup>
$T_4$ 40 g mushroom	5.83 <sup>c</sup>	5.55 <sup>c</sup>	4.76 <sup>d</sup>	3.65 <sup>d</sup>	3.53 <sup>d</sup>

Note : DOF- Days of Fermentation

**TABLE 2**  
**Effect of TSS (°Brix) on fermentation of oyster mushroom beverage using *Lactobacillus delbrueckii***

Treatment details	1 <sup>st</sup> DOF	2 <sup>nd</sup> DOF	3 <sup>rd</sup> DOF	4 <sup>th</sup> DOF	5 <sup>th</sup> DOF
T <sub>1</sub> 10 g mushroom	19.30 <sup>a</sup>	18.28 <sup>a</sup>	15.53 <sup>a</sup>	13.65 <sup>a</sup>	12.08 <sup>a</sup>
T <sub>2</sub> 20 g mushroom	19.14 <sup>b</sup>	17.10 <sup>b</sup>	13.26 <sup>b</sup>	11.68 <sup>b</sup>	11.23 <sup>b</sup>
T <sub>3</sub> 30 g mushroom	18.97 <sup>c</sup>	16.58 <sup>c</sup>	12.68 <sup>c</sup>	10.05 <sup>c</sup>	9.86 <sup>c</sup>
T <sub>4</sub> 40 g mushroom	18.78 <sup>d</sup>	16.11 <sup>d</sup>	12.11 <sup>d</sup>	9.35 <sup>d</sup>	8.93 <sup>d</sup>

Note : DOF- Days of Fermentation

**Total Soluble Solids (°Brix)** : The initial Total Soluble Solids (TSS) of the oyster mushroom beverage was 0 °Brix. However, following the addition of 20 per cent sugar to all treatments, the TSS increased to 21 °Brix before fermentation. In Treatment 1 (T<sub>1</sub>), the total soluble solids (TSS) decreased from 19.30 °Brix on the 1<sup>st</sup> day to 12.08 °Brix by the 5<sup>th</sup> day. Treatment 2 (T<sub>2</sub>) showed a similar reduction with TSS dropping from 19.14 °Brix (1<sup>st</sup> day) to 11.23 °Brix (5<sup>th</sup> day). Treatment 3 (T<sub>3</sub>) experienced a notable decline in TSS, from 18.97 °Brix (1<sup>st</sup> day) to 9.86 °Brix (5<sup>th</sup> day). Treatment 4 (T<sub>4</sub>) demonstrated the most pronounced decrease with TSS falling from 18.78 °Brix on the 1<sup>st</sup> day to 8.93 °Brix by the 5<sup>th</sup> day (Table 2). These results under score a statistically significant reduction in TSS across all treatments with Treatment 4 exhibiting the most substantial decrease. As fermentation progressed, a gradual and statistically significant decrease in TSS was observed across all treatments attributed to the metabolic activity of *Lactobacillus delbrueckii*.

The TSS measurements were crucial for understanding the concentration of soluble compounds in the fermented juice. The significant decline in TSS across all treatments reflects the efficiency of *Lacto bacillus delbrueckii* in fermenting the added sugars, resulting in a beverage with reduced sweetness and enhanced acidity. This change not only influences the sensory attributes of the beverage but also contributes to its preservation, as lower sugar levels and increased acidity create an environment less favorable to spoilage organisms. Thus, the reduction in TSS during fermentation was a key indicator of successful fermentation aligning

with the desired characteristics of a fermented oyster mushroom beverage.

The observed decrease in TSS was consistent with the expected outcomes of lactic acid bacteria (LAB) fermentation where sugars are converted into lactic acid and other metabolites. This conversion reduces the sugar content and TSS of the beverage (Rodriguez *et al.*, 2021).

**Titration acidity (%)** : The titratable acidity (TA) levels increased over the fermentation days, showing LAB's effectiveness in acidifying oyster mushroom juice, which was a desirable trait for fermented beverages. The initial titratable acidity (TA) of the mushroom juice was 0.15 per cent. TA values increased with higher quantities of oyster mushroom and longer fermentation times. In Treatment 1 (T<sub>1</sub>), the TA rose significantly from 0.35 per cent on the 1<sup>st</sup> day to 1.03 per cent by the 5<sup>th</sup> day. Treatment 2 (T<sub>2</sub>) exhibited an increase in TA from 0.48 % (1<sup>st</sup> day) to 1.24 per cent (5<sup>th</sup> day). Likewise, Treatment 3 (T<sub>3</sub>) showed a substantial TA increase from 0.51 per cent (1<sup>st</sup> day) to 1.36 per cent (5<sup>th</sup> day). Treatment 4 (T<sub>4</sub>), however, demonstrated the highest increase with TA rising from 0.59 per cent on the 1<sup>st</sup> day to 1.42 per cent by the 5<sup>th</sup> day (Table 3).

The TA was an important indicator of the acid content in fermented products reflecting both the consumability and organoleptic qualities of the food. The rise in TA during fermentation might be due to the production of organic acids, particularly lactic acid by LAB. This increase in acidity was essential for the safety and stability of fermented foods as it enhances

**TABLE 3**  
**Effect of TA (%) on fermentation of oyster mushroom beverage using *Lactobacillus delbrueckii***

Treatment details		1 <sup>st</sup> DOF	2 <sup>nd</sup> DOF	3 <sup>rd</sup> DOF	4 <sup>th</sup> DOF	5 <sup>th</sup> DOF
T <sub>1</sub>	10 g mushroom	0.35 <sup>c</sup>	0.49 <sup>b</sup>	0.72 <sup>d</sup>	0.93 <sup>c</sup>	1.03 <sup>d</sup>
T <sub>2</sub>	20 g mushroom	0.48 <sup>b</sup>	0.51 <sup>b</sup>	0.86 <sup>c</sup>	1.10 <sup>b</sup>	1.24 <sup>c</sup>
T <sub>3</sub>	30 g mushroom	0.51 <sup>b</sup>	0.69 <sup>a</sup>	0.91 <sup>b</sup>	1.29 <sup>a</sup>	1.36 <sup>b</sup>
T <sub>4</sub>	40 g mushroom	0.59 <sup>a</sup>	0.71 <sup>a</sup>	0.99 <sup>a</sup>	1.36 <sup>a</sup>	1.42 <sup>a</sup>

Note : DOF- Days of Fermentation

preservation by lowering the pH thereby inhibiting the growth of spoilage organisms and pathogens (Ganzle, 2015). In the context of mushroom beverages, maintaining the right balance of acidity was crucial for consumer acceptance and product quality. The observed rise in TA during the fermentation process underscores the role of LAB in producing a beverage with desirable acidity and improved shelf stability.

### Organoleptic Characterization of Fermented Oyster Mushroom Beverage

The overall acceptability of the oyster mushroom beverage was based on sensory evaluation and it varied across different treatments throughout the fermentation process. During the fermentation process of the oyster mushroom beverage, sensory scores varied significantly across treatments. On Day 1, Treatment 4 (T<sub>4</sub>) received the highest sensory score at 10.40, while Treatment 1 (T<sub>1</sub>) had the lowest score at 8.68. This trend continued on Day 2, with T<sub>4</sub> again achieving the highest score of 10.86 and T<sub>1</sub> the lowest at 10.46. By Day 3, T<sub>4</sub> retained the top score, reaching 12.20, whereas T<sub>1</sub> had the lowest score of 11.36. On Day 4, Treatment 2 (T<sub>2</sub>) emerged with the highest sensory score of 12.82, and T<sub>4</sub> dropped to the lowest at 10.69. Finally, on Day 5, T<sub>1</sub> recorded the highest score of 10.95 with T<sub>4</sub> showing the lowest score at 9.63 (Fig. 1). These results indicate fluctuations in sensory acceptance based on the fermentation days and treatment applied with T<sub>4</sub> generally performing well in the early stages but showing a decline in sensory acceptability as fermentation progressed.

The organoleptic characteristics of probiotic fermented milk was significantly influenced by both

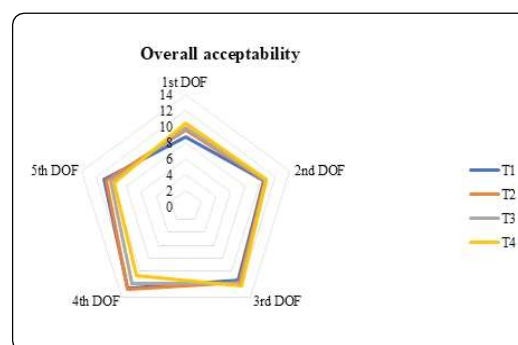


Fig. 1 : Effect of varied quantities of oyster mushroom on overall acceptability of oyster mushroom beverage during different intervals of fermentation with *Lacto bacillus delbrueckii*

the fermentation time and the specific LAB strain used (Conti-Silva and De Souza-Borges, 2019). The sensory quality of fermented beverage was closely tied to the metabolic activities of LAB which generate various volatile compounds that enhance flavor complexity (Silva *et al.*, 2024). However, the sensory quality of fermented products can deteriorate due to the accumulation of undesirable metabolites particularly with extended fermentation periods. This is consistent with the observed decline in sensory scores for Treatment 4 (T<sub>4</sub>) on the 5<sup>th</sup> day of fermentation indicating that prolonged fermentation can negatively impact product quality. Therefore, careful monitoring of fermentation duration is crucial to maintaining the desired sensory attributes and overall quality of the product (Ganzle, 2015).

The standardization and development of a novel fermented beverage using grey oyster mushroom and *Lactobacillus delbrueckii* have demonstrated promising potential in creating a functional drink that

offers both health benefits and appealing sensory attributes. The optimal formulation consisting of 20g of oyster mushroom substrate fermented for four days resulted in favorable pH, TSS and TA levels along with the highest sensory scores. These findings highlight the effectiveness of LAB in enhancing the nutritional and sensory qualities of mushroom-based beverages. This approach paves the way for innovative, probiotic-rich drinks that meet the growing consumer demand for natural and nutritious alternatives in the beverage market.

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