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Priyanka Mulage
Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, G.K.V.K, Bengaluru, Karnataka, India

N Umashankar
Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, G.K.V.K, Bengaluru, Karnataka, India

Suvarna V Chavannavar
Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, G.K.V.K, Bengaluru, Karnataka, India

Muthuraju R
Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, G.K.V.K, Bengaluru, Karnataka, India

Usha Ravindra B
Department of Food Science and Nutrition, University of Agricultural Sciences, G.K.V.K, Bengaluru, Karnataka, India

Mohan Chavan
Department of Agricultural Plant Biotechnology, University of Agricultural Sciences, G.K.V.K, Bengaluru, Karnataka, India

Darshan MB
Assistant Research Engineer, ICAR-AICRP on Post Harvest Engineering and Technology, University of Agricultural Sciences, G.K.V.K, Bengaluru, Karnataka, India

Corresponding Author:
Priyanka Mulage
Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, G.K.V.K, Bengaluru, Karnataka, India

Optimizing the fermentation process for oyster mushroom beverage preparation with *Lactobacillus plantarum*

Priyanka Mulage, N Umashankar, Suvarna V Chavannavar, Muthuraju R, Usha Ravindra B, Mohan Chavan and Darshan MB

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Abstract
Mushrooms are fungi with a high nutritional value and mushroom based beverages are emerging as a dynamic category of functional drinks, leveraging the health-enhancing properties of mushrooms to offer a wholesome and natural alternative to traditional fermented beverages. This study set out to develop a unique fermented drink 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 optimized using *Lactobacillus plantarum* 2975, a probiotic lactic acid bacterium known for its beneficial properties. The effects of varying oyster mushroom quantities (10 g, 20 g, 30 g and 40 g) and different fermentation durations on key parameters such as pH, total soluble solids (TSS), titratable acidity (TA) and sensory qualities of the beverage were thoroughly examined. The most effective formulation was found to be 20 g of oyster mushroom paste, fermented for four days with a 6 per cent *Lactobacillus plantarum* inoculation. This combination resulted in optimal pH (3.98), TSS (11.01 °Brix) and TA (1.21 %), along with the highest sensory score of 13.15 on day four of fermentation. These results emphasize the potential of this refined fermentation process to create a premium oyster mushroom beverage. This innovative development offers a probiotic-rich, flavorful drink that not only delivers remarkable health benefits but also meets the growing consumer demand for natural and nutritious alternatives in the ever-expanding beverage market.

Keywords: Fermentation, *Lactobacillus plantarum*, mushroom beverage, oyster mushroom

Introduction
Mushrooms, a type of macro fungus, have long been valued for their distinctive fruiting bodies and are widely recognized for their nutritional and medicinal properties. Of the approximately 1.5 million fungal species globally, around 14,000 produce fruiting bodies large enough to be classified as mushrooms (Chang, 2006) [2]. Mushroom production has surged globally, exceeding 40 million metric tons annually, with Asia, especially China, leading the market. In India, mushroom cultivation has grown significantly since the 1960s, reaching 242,900 tons annually, with a 4.3% growth rate (FAOSTAT, 2022) [7]. Key varieties include button, oyster and milky mushrooms, with Karnataka contributing 4.5 % of India's total output, supported by favorable climate and agricultural waste availability. While the sector remains underdeveloped, there is significant growth potential through improved cultivation practices and awareness of the economic benefits of mushroom farming (ICAR, 2024) [10].

Mushrooms are considered a nutritional powerhouse, rich in protein, vitamins, minerals, trace elements, and dietary fibers, with low fat content. They also possess medicinal properties due to compounds like polysaccharides, terpenoids and phenolic compounds, which have anti-inflammatory, antioxidant, anticancer, antimicrobial, and antidiabetic effects (Khan *et al*., 2013) [11]. Given the challenges posed by population growth, decreasing arable land, urbanization, and climate change, the focus is shifting toward secondary agriculture and innovative crops like mushrooms (Kirk *et al*., 2008) [12].

Probiotics, defined as live microorganisms that confer health benefits when consumed adequately, are gaining popularity. The most commonly consumed probiotics include

(*Lactobacillus* and *Bifidobacterium* species (De Vrese and Schrezenmeir, 2008) [4]. The demand for non-dairy probiotic products is rising, especially for individuals with lactose intolerance Prado *et al*., 2010) [16]. *Lactobacillus plantarum*, a Gram-positive lactic acid bacterium, is frequently used in fermentation processes. When incorporated into mushroom beverages, *L. plantarum* can enhance the product's organoleptic characteristics, nutritional value, flavor and provide probiotic benefits that support immune function and gut health, contributing to the development of novel, health-promoting fermented mushroom drinks (Yang *et al*., 2024) [2]. Due to the short shelf life of mushrooms, processing them into more durable products is essential for broader use. One innovative approach is the development of a mushroom-based fermented beverage, which is currently underrepresented in the market. This study aims to develop and standardize a mushroom beverage fermented with probiotic *Lactobacillus plantarum*. The inclusion of probiotics not only improves the nutritional profile but also promotes gut health, enhances digestion, and adds functional benefits, making the beverage an appealing option in the growing health drink market.

Materials and Methods

Ingredients used in the experiment

Oyster mushroom: The grey oyster mushroom was grown in the Mushroom Laboratory at the Advanced Centre for Skill Development in Mushroom Production Technology, Department of Agricultural Microbiology, UAS, GKVK, Bengaluru. Freshly harvested mushrooms served as the raw material for preparing the mushroom beverage.

Probiotic culture

Collection and maintenance of *Lactobacillus plantarum* (probiotic) culture used in the study

The *Lactobacillus plantarum* 2975 was obtained from the National Centre for Microbial Resource (NCMR) in Pune (Maharashtra) and was used in this study. The culture was revived on De Man, Rogosa and Sharpe (MRS) agar medium (Plate 1).

Plate 1: Pure culture of *Lactobacillus plantarum*

Preparation of fermented oyster mushroom beverage

Preparation of starter cultures of *Lactobacillus plantarum*

A loopful of *Lactobacillus plantarum* was transferred to MRS broth and incubated at 37°C for growth. The *Lactobacillus plantarum* culture with a concentration of $6x10^8$ CFU/mL, was then used to inoculate prepared oyster mushroom juice at a concentration of 6 per cent.

Preparation of starter culture in oyster mushroom juice

The grey oyster mushroom juice was prepared using 10 g of oyster mushroom paste, 5 per cent sugar and 100 mL of water was added which were then autoclaved to eliminate microbial contaminants. Following sterilization, 6 per cent of the *Lactobacillus plantarum* 2975 inocula was added and the mixture was incubated overnight to promote fermentation. The resulting activated culture of oyster mushroom juice had 10^7 CFU/mL and was subsequently utilized in the study (Plate 4).

Standardization of mushroom quantity and fermentation duration for oyster mushroom beverage

Preparation of substrate

The freshly harvested oyster mushrooms were selected and washed with distilled water to remove impurities and dirt and then mushrooms were cut into small pieces. The mushroom paste was made using a blender and 100 mL of water was added.

Experiment setup

The present study aimed to standardize the quantity of oyster mushrooms (10 g, 20 g, 30 g and 40 g) and evaluate the fermentation process at different intervals (1st, 2nd, 3rd, 4th, and 5th day). For all treatments, 100 mL of water was used as a base and mushroom juice was extracted and adjusted to 21 °Brix using a hand refractometer by adding 20 per cent sugar. The prepared juice was then filled into 150 mL glass bottles and pasteurized at 62.8 °C for 30 minutes. Following pasteurization, the juice was inoculated with *Lactobacillus plantarum* 2975 at a 6 per cent inoculum rate and left to ferment for 1 to 5 days. Samples were collected for further analysis, including pH, TSS, TA and sensory evaluation using a 20-point hedonic scale (Pampangouda *et al*., 2021) [15].

The present study was made to standardize oyster mushroom quantity *i.e*., 10 g, 20 g, 30 g and 40 g and kept at different intervals of fermentation *i.e*., 1, 2, 3, 4 and 5th day of fermentation. For all the treatments 100 mL of water was used as common and from the respective treatments, mushroom juice was extracted and adjusted to obtain 21 °Brix using a hand refractometer by adding 20 per cent sugar. The prepared juice was filled to 150 mL glass bottle and pasteurized at 63 °C for 30 minutes. The juice was inoculated with *Lactobacillus plantarum* 2975 as a starter culture at the rate of 6 per cent and kept for fermentation. Fermentation was carried out for 1, 2, 3, 4 and 5 days of fermentation (Plate 5). The samples were used for further analysis and evaluation. The observations were recorded *viz.*, pH, TSS, TA and sensory evaluation was done using 20-point hedonic scale (Pampangouda *et al*., 2021) [15].

Table 1: Experiment setup to standardize mushroom quantity and fermentation duration for oyster mushroom beverage using *Lactobacillus plantarum*

Treatment details
T_1 = *Lactobacillus plantarum* + 10 g of Mushroom
T_2 = *Lactobacillus plantarum* + 20 g of Mushroom
T_3 = *Lactobacillus plantarum* + 30 g of Mushroom
T_4 = *Lactobacillus plantarum* + 40 g of Mushroom

Note: Number of treatments - 4, Number of replications - 5, Design - CRD

Biochemical analysis

pH

The pH of the synbiotic oyster mushroom beverage samples was determined using a digital pH meter. The pH meter was standardized with buffer solutions of different pH (4.0, 7.0 and 10.0) were used to calibrate the instrument. Each sample was replicated three times and its mean value was taken as the pH of the sample.

Total soluble solids (°Brix)

Total soluble solid (TSS) was measured using a hand refractometer. Two drops of oyster mushroom beverage were placed on the glass panel of the hand refractometer for TSS measurement. It was expressed in degree Brix (Ranganna, 1995) [18].

Titrable acidity (Srivastava and Kumar, 1993)

Fresh filtered homogenized juice (10 mL) was made up to 100 mL with distilled water. This prepared solution (10 mL) was titrated against 0.1N NaOH solution with phenolphthalein as an indicator. The appearance of a light pink color was an end-point that quantifies the NaOH required to neutralize the sample. Then titrable acidity was calculated and expressed as per cent. The amount of titrable acidity present in 100 mL of sample was calculated as follows.

$$\text{Titrable acidity (\%)} = \frac{\text{Titre value x N of NaOH x Equivalent Wt. of acid x100}}{\text{Volume of sample taken x Vol. of aliquot taken x 1000}}$$

Organoleptic evaluation

The fermented synbiotic beverages developed from oyster mushroom were evaluated by selected five panel members with 20 point hedonic scales. The score of various treatments with respect to appearance, color, aroma, bouquet, vinegar, total acidity, sweetness, body, flavor, astringency and overall acceptability given by various panelists were pooled and the mean score for overall acceptability was worked out using 20 point hedonic scale.

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) [6].

Results and Discussion

Standardization of process parameters for mushroom beverage preparation using *Lactobacillus plantarum*

The experiment was conducted to know the mushroom quantity (10, 20, 30 and 40 g of mushroom) to be used and the duration of fermentation (1, 2, 3, 4 and 5 days of fermentation) to be maintained for oyster mushroom beverage preparation using *Lactobacillus plantarum*. The results of the mushroom quantity and fermentation duration for the preparation of fermented mushroom beverage was standardized based on the biochemical properties and organoleptic properties of the oyster mushroom beverage.

Biochemical properties of fermented mushroom beverage preparation

pH

In Treatment 1 (T_1) use of 10 g mushroom, the pH decreased significantly from 6.01 (1st day), 5.78 (2nd day), 5.48 (3rd day), 4.21 (4th day) and 4.02 (5th day). Treatment 2 (T_2) use of 20 g mushroom, exhibited a pH decrease from 5.90 (1st day), 5.53 (2nd day), 5.12 (3rd day), 3.98 (4th day) to 3.90 (5th day). Similarly, Treatment 3 (T_3) use of 30 g mushroom, showed a substantial drop in pH from 5.79 (1st day), 5.38 (2nd day), 4.97 (3rd day), 3.85 (4th day) to 3.77 (5th day) while treatment 4 (T_4) use of 40 g mushroom, observed the most pronounced decrease with the pH falling from 5.45 (1st day), 5.24 (2nd day), 4.81 (3rd day), 3.79 (4th day) to 3.68 (5th day). These results indicate a statistically significant decline in pH across all treatments, with the most significant reduction observed in Treatment 4 (Table 2).

A significant decrease in pH during fermentation is attributed to the accumulation of H^+ ions released by *Lactobacillus plantarum*, which converts sugars in the mushroom juice into lactic acid (Hutkins and Nannen, 1993; Kozarski *et al*., 2015) [9, 13]. In this study, pH decreased progressively over five days of fermentation, with a more pronounced drop observed as substrate concentration increased. This reduction in pH reflects the metabolic activity of the lactic acid bacteria, which fermented sugars into lactic and other acids. These findings align with Cheng *et al*. (2021) [3], who noted that higher substrate levels accelerate acid production due to increased microbial activity, highlighting the connection between substrate availability and fermentation efficiency.

Total soluble solids (°Brix)

The initial Total Soluble Solids (TSS) of the oyster mushroom beverage was 0.14 °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_1), the TSS decreased from 19.24 °Brix (1st day), 18.04 (2nd day), 15.94 (3rd day), 12.52 (4th day) to 10.95 °Brix (5th day). Treatment 2 (T_2) showed a comparable reduction with TSS dropping from 19.02 °Brix (1st day), 16.96 (2nd day), 13.13 (3rd day), 11.01 (4th day) to 9.46 °Brix (5th day). Treatment 3 (T_3) experienced a significant decline in TSS from 18.86 °Brix (1st day), 16.43 (2nd day), 12.51 (3rd day), 9.96 (4th day) to 8.33 °Brix (5th day). Similarly, Treatment 4 (T_4) showed the most pronounced decrease with TSS falling from 18.74 °Brix (1st day), 15.42 (2nd day), 11.98 (3rd day), 8.86 (4th day) to 7.42 °Brix (5th day) (Table 2).

The results show a significant reduction in Total Soluble Solids (TSS) across all treatments, with Treatment 4 exhibiting the most substantial decrease. As fermentation progressed, a consistent decline in TSS was observed, attributed to the metabolic activity of *Lactobacillus plantarum* (Di Cagno *et al*., 2013). The decrease in TSS reflects the efficiency of LAB in fermenting sugars, leading to a less sweet, more acidic beverage. This change not only impacts sensory attributes but also contributes to preservation, as lower sugar levels and increased acidity deter spoilage organisms (Kumoro *et al*., 2015) [14]. Thus, the reduction in TSS during fermentation indicates successful fermentation, consistent with LAB's conversion

of sugars into lactic acid and other metabolites (Rodriguez *et al*., 2021) [19].

Titratable acidity (%)

The initial value of TA of mushroom juice was 0.03 %. The TA values increased with increasing substrate quantity and the duration of fermentation. In Treatment 1 (T_1), the TA increased significantly from 0.41 % (1st day), 0.51 % (2nd day), 0.84 % (3rd day), 1.06 % (4th day) to 1.11 % (5th day). Treatment 2 (T_2) exhibited increased TA from 0.54 % (1st day), 0.59 % (2nd day), 1.01 % (3rd day), 1.21 % (4th day) to 1.28 % (5th day). Similarly, Treatment 3 (T_3) showed a substantially increased TA from 0.56 % (1st day), 0.76 % (2nd day), 1.26 % (3rd day), 1.34 % (4th day) to 1.37 % (5th day) while, treatment 4 (T_4) resulted the most pronounced increased with the TA from 0.61 % (1st day), 0.81 % (2nd day), 1.31 % (3rd day), 1.39 % (4th day) to 1.43 % (5th day) (Table 2).

The TA was an important indicator of the organic 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 by utilization of sugars present in juice particularly lactic acid by LAB (Yang *et al*., 2024) [22]. This increase in acidity was essential for the safety and stability of fermented foods as it enhances preservation by lowering the pH thereby inhibiting the growth of spoilage organisms and pathogens. In the context of mushroom beverages, maintaining the right balance of acidity was crucial for consumer acceptance and product quality (Ganzle, 2015) [8]. 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. The TA levels increased over the fermentation days showing the effectiveness of LAB in acidifying oyster mushroom juice, which was a desirable trait for fermented beverages (Xiang *et al*., 2020).

The results of the biochemical parameters of the oyster mushroom beverage using LAB strain *viz*., *Lactobacillus plantarum* have to be correlated with the results obtained in the organoleptic characterization to conclude what quantity of mushrooms to be used and what duration has to be maintained for the production of the oyster mushroom beverage.

Organoleptic characterization of fermented mushroom beverage using *Lactobacillus plantarum*

The overall acceptability of the oyster mushroom beverage was based on sensory evaluation using 20 point hedonic scale and it varied across different treatments throughout the fermentation process. On the 1st day of fermentation, Treatment 4 (T_4) received the highest sensory score (10.58), while Treatment 1 (T_1) had the lowest score (8.84). By the 2nd day, T_4 maintained the highest score (11.02) and T_1 again had the lowest score (10.56). On the 3rd day, T_3 achieved the highest score (12.31), whereas T_4 had the lowest (11.77). However, on the 4th day, Treatment 2 (T_2) emerged with the highest sensory score (13.15), while T_4 recorded the lowest (12.14). Finally, on the 5th day of fermentation, T_1 received the highest sensory score (10.44), with T_4 having the lowest (7.99) (Table 3-7). These results indicate fluctuations in sensory acceptance based on the fermentation days and treatment applied, T_4 generally performed well in the early stages but showed a decline in sensory acceptability as fermentation progressed *i.e*., on the 5th day of fermentation (Fig 1).

The variation in sensory scores across treatments indicates that fermentation conditions, substrate composition and microbial strains significantly impact the outcomes. While Treatment 4 (T_4) showed initial promise, it was more susceptible to over-fermentation, whereas Treatments 1 and 2 displayed better consistency or improvement over time. This highlights the importance of optimizing fermentation conditions to enhance sensory propertie. The sensory quality of fermented beverages is closely linked to the metabolic activities of LAB, which produce volatile compounds that enhance flavor (Xu *et al*., 2005) [21]. However, extended fermentation can lead to undesirable metabolites, as seen in the decline of T_4 sensory scores on day 5, demonstrating that fermentation duration must be carefully monitored to maintain product quality (Ganzle, 2015) [8].

The above experiment results revealed that the oyster mushroom beverage from *Lactobacillus plantarum* has the best with T_2 treatment having 20 g of oyster mushroom with 4 days of fermentation having an overall acceptability of 13.15.

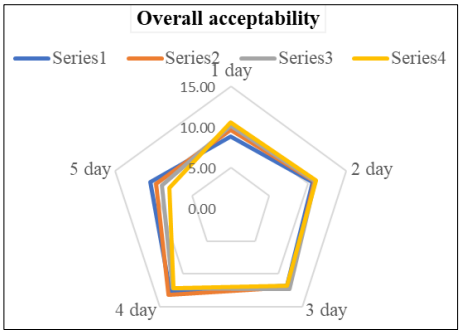

Fig 1: Overall acceptability of fermented mushroom beverage at different intervals of fermentation using *Lactobacillus plantarum*

Table 2: Effect of *Lactobacillus plantarum* on change in pH, TSS and TA during mushroom beverage fermentation process

Treatments	Fermentation duration (Days)														
	1			2			3			4			5		
	pH	TSS (°Brix)	TA (%)	pH	TSS (°Brix)	TA (%)	pH	TSS (°Brix)	TA (%)	pH	TSS (°Brix)	TA (%)	pH	TSS (°Brix)	TA (%)
T_1- *L. p.* + 10 g of Mushroom	6.01^a	19.24^a	0.41^c	5.78^a	18.04^a	0.51^d	5.48^a	15.94^a	0.84^d	4.21^a	12.52^a	1.06^d	4.02^a	10.95^a	1.11^d
T_2- *L. p.* +20 g of Mushroom	5.90^a	19.02^b	0.54^b	5.53^b	16.96^b	0.59^c	5.12^b	13.13^b	1.01^c	3.98^{ab}	11.01^b	1.21^c	3.90^b	9.46^b	1.28^c
T_3- *L. p.* +30 g of Mushroom	5.79^{ab}	18.86^c	0.56^b	5.38^c	16.43^c	0.76^b	4.97^c	12.51^c	1.26^b	3.85^b	9.96^c	1.34^b	3.77^c	8.33^c	1.37^b
T_4- *L. p.* + 40 g of Mushroom	5.45^b	18.74^d	0.61^a	5.24^d	15.42^d	0.81^a	4.81^d	11.98^d	1.31^a	3.79^b	7.86^d	1.39^a	3.68^d	7.42^d	1.43^a

Note: Means followed by different superscripts in column differ significantly at 5 % level of significance as per DMRT analysis.
For all the treatments 100 mL water was used as common
L. p. - *Lactobacillus plantarum*

Table 3: Effect of *Lactobacillus plantarum* on organoleptic characteristics of mushroom beverage on the first day of fermentation process

Treatments	Appearance (2)	Colour (2)	Aroma (2)	Bouquet (2)	Acidity (2)	Sweetness (2)	Body (2)	Astringency (2)	Flavour (2)	Quality (2)	Overall acceptability (20)
T_1- *L. p.* + 10 g of Mushroom	1.05	1.06^{b}	0.54^{d}	1.08^{b}	0.50^{c}	1.10^{ab}	1.00^{c}	1.00^{c}	0.54^{c}	0.97^{c}	8.84^{c}
T_2- *L. p.* +20 g of Mushroom	1.10	1.24^{a}	0.63^{d}	1.08^{b}	0.52^{c}	1.08^{b}	1.26^{b}	1.13^{b}	0.60^{b}	1.03^{b}	9.67^{b}
T_3- *L. p.* +30 g of Mushroom	1.14	1.20^{a}	0.69^{b}	1.15^{a}	0.56^{b}	1.14^{a}	1.39^{a}	1.18^{a}	0.62^{ab}	1.09^{a}	10.16^{a}
T_4- *L. p.* + 40 g of Mushroom	1.18	1.24^{a}	0.93^{a}	1.18^{a}	0.69^{a}	1.03^{c}	1.41^{a}	1.19^{a}	0.63^{a}	1.10^{a}	10.58^{a}

Note: Means followed by different superscripts in column differ significantly at 5 % level of significance as per DMRT analysis
For all the treatments 100 mL water was used as common
L. p. - *Lactobacillus plantarum*

Table 4: Effect of *Lactobacillus plantarum* on organoleptic characteristics of mushroom beverage on the second day of fermentation process

Treatments	Appearance (2)	Colour (2)	Aroma (2)	Bouquet (2)	Acidity (2)	Sweetness (2)	Body (2)	Astringency (2)	Flavour (2)	Quality (2)	Overall acceptability (20)
T_1- *L. p.* + 10 g of Mushroom	1.23	1.19	0.87^{c}	1.23^{c}	0.75^{d}	1.06^{a}	1.26^{c}	1.21^{ab}	0.65^{c}	1.11	10.56^{c}
T_2- *L. p.* +20 g of Mushroom	1.22	1.23	0.93^{b}	1.24^{bc}	0.77^{c}	1.04^{b}	1.34^{b}	1.23^{a}	0.69^{b}	1.09	10.78^{b}
T_3- *L. p.* +30 g of Mushroom	1.25	1.24	0.94^{b}	1.26^{b}	0.85^{b}	0.98^{c}	1.37^{a}	1.20^{bc}	0.72^{a}	1.10	10.91^{ab}
T_4- *L. p.* + 40 g of Mushroom	1.24	1.25	1.02^{a}	1.31^{a}	0.89^{a}	0.96^{d}	1.33^{b}	1.18^{c}	0.73^{a}	1.10	11.02^{a}

Note: Means followed by different superscripts in column differ significantly at 5 % level of significance as per DMRT analysis
For all the treatments 100 mL water was used as common
L. p. - *Lactobacillus plantarum*

Table 5: Effect of *Lactobacillus plantarum* on organoleptic characteristics of mushroom beverage on the third day of fermentation process

Treatments	Appearance (2)	Colour (2)	Aroma (2)	Bouquet (2)	Acidity (2)	Sweetness (2)	Body (2)	Astringency (2)	Flavour (2)	Quality (2)	Overall acceptability (20)
T_1- *L. p.* + 10 g of Mushroom	1.28	1.29	1.23^{c}	1.39^{b}	0.98^{c}	0.94^{a}	1.38^{bc}	1.26^{b}	0.89^{c}	1.19^{b}	11.83^{bc}
T_2- *L. p.* +20 g of Mushroom	1.30	1.32	1.28^{b}	1.42^{a}	1.04^{b}	0.92^{b}	1.41^{ab}	1.28^{b}	0.93^{b}	1.20^{ab}	12.10^{ab}
T_3- *L. p.* +30 g of Mushroom	1.30	1.32	1.31^{a}	1.44^{a}	1.11^{a}	0.89^{c}	1.43^{a}	1.31^{a}	0.99^{a}	1.21^{a}	12.31^{a}
T_4- *L. p.* + 40 g of Mushroom	1.28	1.28	1.23^{c}	1.39^{b}	1.13^{a}	0.84^{d}	1.38^{c}	1.26^{b}	0.87^{d}	1.11^{c}	11.77^{c}

Note: Means followed by different superscripts in column differ significantly at 5 % level of significance as per DMRT analysis
For all the treatments 100 mL water was used as common
L. p. - *Lactobacillus plantarum*

Table 6: Effect of *Lactobacillus plantarum* on organoleptic characteristics of mushroom beverage on the fourth day of fermentation process

Treatments	Appearance (2)	Colour (2)	Aroma (2)	Bouquet (2)	Acidity (2)	Sweetness (2)	Body (2)	Astringency (2)	Flavour (2)	Quality (2)	Overall acceptability (20)
T_1- *L. p.* + 10 g of Mushroom	1.32	1.31	1.39^{b}	1.50^{ab}	1.19^{b}	0.81^{b}	1.46	1.32^{b}	1.12^{b}	1.28^{a}	12.70^{b}
T_2- *L. p.* +20 g of Mushroom	1.32	1.33	1.51^{a}	1.53^{a}	1.31^{a}	0.75^{c}	1.47	1.36^{a}	1.29^{a}	1.28^{a}	13.15^{a}
T_3- *L. p.* +30 g of Mushroom	1.31	1.32	1.39^{b}	1.48^{b}	1.15^{c}	0.60^{d}	1.45	1.32^{b}	1.08^{c}	1.13^{c}	12.23^{c}
T_4- *L. p.* + 40 g of Mushroom	1.30	1.29	1.26^{c}	1.36^{c}	1.09^{d}	0.89^{a}	1.44	1.30^{b}	1.03^{d}	1.18^{b}	12.14^{c}

Note: Means followed by different superscripts in column differ significantly at 5 % level of significance as per DMRT analysis
For all the treatments 100 mL water was used as common, *L. p.* - *Lactobacillus plantarum*

Table 7: Effect of *Lactobacillus plantarum* on organoleptic characteristics of mushroom beverage on the fifth day of fermentation process

Treatments	Appearance (2)	Colour (2)	Aroma (2)	Bouquet (2)	Acidity (2)	Sweetness (2)	Body (2)	Astringency (2)	Flavour (2)	Quality (2)	Overall acceptability (20)
T_1- *L. p.* + 10 g of Mushroom	1.30	1.29	1.05^{a}	1.06^{a}	1.20^{c}	0.38^{a}	1.39	1.30	0.51^{a}	0.96^{a}	10.44^{a}
T_2- *L. p.* +20 g of Mushroom	1.30	1.29	0.89^{b}	0.78^{b}	1.24^{b}	0.36^{b}	1.39	1.30	0.36^{b}	0.78^{b}	9.69^{b}
T_3- *L. p.* +30 g of	1.30	1.29	0.61^{c}	0.52^{c}	1.26^{ab}	0.32^{c}	1.38	1.30	0.33^{c}	0.67^{c}	8.98^{c}

Mushroom											
T_4- *L. p.* + 40 g of Mushroom	1.30	1.29	0.31^d	0.33^d	1.28^a	0.21^d	1.38	1.30	0.21^d	0.38^d	7.99^d

Note: Means followed by different superscripts in column differ significantly at 5 % level of significance as per DMRT analysis
For all the treatments 100 mL water was used as common
L. p. - *Lactobacillus plantarum*

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