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Oyster Mushroom Powder Incorporation in Doogh: A Novel Approach to Boost Probiotic Stability

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ABSTRACT

Background and Objective: The worldwide demand for healthy functional beverages has prompted researchers to enhance the probiotics and benefits of traditional fermented drinks. Doogh is one of the most popular dairy drinks among Iranians and Turkish, which is yogurt-based. High phase separation and limited viability of the probiotics due to the acidic condition are two important challenges of this product. The present study aimed to investigate the physicochemical properties of doogh and the stabilizing and protective effects of oyster mushroom powder (*Pleurotus ostreatus*) on increasing the viability of probiotics in this traditional Iranian drink.

Materials and Methods: The scope of the present work was to investigate the acidity, physical stability (syneresis), viability, and total population of probiotics, as well as the sensory properties of the doogh beverage (color, aroma, texture and taste), after 14 days of storage at 4°C when it is prepared with different concentrations of mushroom powder. Accordingly, yogurts were produced with probiotic strain *Lactobacillus acidophilus* and mixed yogurt culture (Y_X11) comprising *Lactobacillus delbrueckii* subsp. Bulgaricus and Streptococcus thermophilus. For preparing the doogh samples, water, salt, mint powder, and oyster mushroom powder (0, 0.5, 0.75, and 1 g/L) were mixed. All measurements were done after 1, 7, and 14 days of doogh storage based on the pioneer studies.

Findings: The findings indicated that the higher concentration of mushroom powder provided superior protection for *L. acidophilus* and higher lactic acid bacteria count throughout storage; however, it diminished all the sensory attributes. Furthermore, applying 0.5% mushroom powder in a doogh formulation kept the product stable, reduced the pH decline rate, and controlled the flavor and aroma desirable after 14 days. The sensory properties of doogh samples within 7 days of storage were within acceptable limits; however, after 14 days, the sample containing 0.5% oyster mushroom powder had a higher score for color and in-mouth texture than the others. Therefore, it is possible to find an optimum concentration and method for adding oyster mushroom powder to the doogh beverages and use their health-beneficial properties in this dairy drink.

Conclusion: The addition of edible mushroom powder in most of the treatment levels improved the physical stability (syneresis), probiotic viability, and overall nutritional value of doogh samples after 14 days of storage at 4°C. However, the sensory properties after extended storage need further optimization in formulation. As probiotic viability is one of the most important challenges in functional probiotic drinks, especially dairy beverages, finding new modifications that can overcome the defects of this formulation can be insightful enough to develop innovative probiotic doogh drinks.

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افزودن پودر قارچ صدفی به دوغ: رویکردی نوین برای افزایش پایداری پروبیوتیک

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چکیده

هدف پژوهش: تقاضای جهانی برای نوشیدنی‌های سالم و فراسودمند، پژوهشگران را بر آن داشته است تا پروبیوتیک‌ها و فواید نوشیدنی‌های تخمیری سنتی را ارتقا دهند. دوغ یکی از محبوبترین نوشیدنی‌های لبنی در میان ایرانیان و ترکهاست که بر پایه ماست تهیه می‌شود. نرخ بالای دوفاز شدن و بقای محدود پروبیوتیک‌ها به‌دلیل شرایط اسیدی، دو چالش مهم برای تهیه این محصولبه شمار می‌رود. مطالعه حاضر با هدف بررسی ویژگی‌های فیزیکوشیمیایی دوغ و اثرات پایدارکننده و محافظتی پودر قارچ صدفی (*Pleurotus ostreatus*) بر افزایش بقای پروبیوتیک‌ها در این نوشیدنی سنتی ایرانی انجام شد.

مواد و روش‌ها: هدف از مطالعه حاضر بررسی میزان اسیدیته، پایداری فیزیکی، میزان زنده‌مانی و جمعیت کلی پروبیوتیک‌ها و همچنین ویژگی‌های حسی نوشیدنی دوغ (رنگ، عطر، بافت و طعم) پس از ١٤ روز نگهداری در دمای ٤ درجه سانتی‌گراد، هنگام تهیه با غلظت‌های مختلف پودر قارچ بود. به این منظور، ماست‌ها با سویه پروبیوتیک *Lactobacillus acidophilus* و کشت مخلوط ماست (Y_X11) شامل *Lactobacillus delbrueckii* subsp. bulgaricus و *Streptococcus thermophilus*تهیه شد. برای آماده‌سازی نمونه‌های دوغ، آب، نمک، پودر نعناع و پودر قارچ صدفی در غلظت‌های ۰ ، ۰/۵ ، ۰/۷۵ و ۱ گرم بر لیتر با هم مخلوط گردید. کلیه اندازه‌گیری‌ها بر اساس مطالعات پیشین، پس از ۱ ، ۷ و ١٤ روز نگهداری دوغ انجام شد.

یافته‌ها: یافته‌ها نشان داد که غلظت بالاتر پودر قارچ، محافظت بیشتری از *L. acidophilus* فراهم کرده و تعداد باکتری‌های اسید لاکتیک را در طول دوره نگهداری افزایش داد؛ با این حال، تمام ویژگی‌های حسی را کاهش داد. علاوه بر این، استفاده از ۰/۵ درصد پودر قارچ صدفی در فرمولاسیون دوغ، محصول را پایدار نگه داشته، نرخ کاهش pH را کاهش داد و طعم و عطر مطلوب را پس از ١٤ روز کنترل کرد. ویژگی‌های حسی نمونه‌های دوغ در طول ۷ روز نگهداری در محدوده قابل قبول قرار داشتند؛ با این حال، پس از ١٤ روز، نمونه حاوی ۰/۵ درصد پودر قارچ صدفی، نمره بالاتری در رنگ و بافت در دهان نسبت به سایر نمونه‌ها داشت.

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Introduction

Doogh is a traditional Iranian fermented dairy beverage with a high potential for being prepared with probiotic species. It has increased global popularity due to its health benefits, including gut health improvement, probiotic content, and unique organoleptic properties. This dairy drink can be produced by diluting yogurt with water and adding salt and herbs (Shahrajabian & Sun, 2023).

The presence of probiotics increases the nutraceutical properties of this product, and the prominent place of their action is the gastrointestinal tract, especially the colon (Ortiz-Lucas et al., 2013; Sebastián et al., 2019). Moreover, it has been proven that applying probiotics in foods and beverages can amplify health, prevent gastrointestinal disorders, and improve the immune system and anti-cancer properties, aligning with higher and better body metabolism (Nataraj et al., 2020). Therefore, it can convert the common doogh to a functional drink.

In today's world, mushrooms are vital to the human diet due to their unique and appropriate flavor and aroma (Radzki et al., 2023). Many various characteristics cause an increase in its application, like anti-inflammatory, antifungal, antidiabetic, and cytotoxic properties (M. Kosani et al., 2013). *Pleurotus ostreatus* is one of the most popular edible mushroom species, accounting for around 25% of cultivated mushrooms worldwide. It is very common in Asian countries (TiȚA et al., 2022). Therefore, adding this food component to a fermented drink might improve its functional properties and affect the probiotic's stability. Accordingly, the present study has aimed to explore the impact of edible mushroom powder and storage time on doogh's physicochemical, microbial, and sensory properties.

Materials and Methods

This study utilized two commercial bacterial cultures: a mixed yogurt culture (Y_X11) comprising *Lactobacillus delbrueckii* subsp. Bulgaricus, Streptococcus thermophilus, and a single-strain probiotic culture of *Lactobacillus acidophilus* (La-5). Both cultures were freeze-dried DVS-type strains from Chr. Hansen (Denmark). Additionally, Oyster mushrooms were obtained from a local market, and the mint powder was purchased from Golha Company (Tehran, Iran).

Preparation of mushroom powder

Oyster mushrooms were obtained from a local market and rinsed with cold water. The mushrooms were sanitized using a commercial vegetable sanitizer, and after drying the surface, the mushrooms were subjected to a blanching process involving immersion in 100°C water with 0.01% citric acid (Merck, Germany) and 3% salt for 3 minutes (Piskov et al., 2020). The blanching solution was discarded afterward, and the mushrooms were sliced thinly and uniformly. These slices were then placed in a vacuum oven (Pars Azma, Iran) set to 150°C, where they were dried under low-pressure conditions for 4 hours to prevent contamination. Once fully dehydrated, the dried mushrooms were ground and stored in sterile sealed dark containers at 4°C to preserve their quality until further use.

Proximate analysis

The crude lipid, crude fiber, and protein content of the samples were analyzed according to the methods of (Verma et al., 2024).

Determination of fat content

The fat content was measured using a solvent extraction approach. A precise 5 g of the dried sample was placed inside a thimble and introduced into an Automated Soxhlet system (Peco, Iran). The extraction process utilized ether (DR Mojallali, Iran) and was conducted over a period of one hour. Afterward, the ether was evaporated, and the residue collected in the flask was dried at 80–100°C in an oven. The dried residue was then cooled in a desiccator before being weighed to calculate the fat content (Verma et al., 2024), according to equation (1):

$$Fat\ content\ (\%) = \frac{(Final\ weight\ of\ the\ flask\ -\ Initial\ weight\ of\ the\ flask)}{Weight\ of\ the\ dried\ sample} \times 100 \quad (1)$$

Determination of Crude Fiber

The crude fiber content was determined through a gravimetric technique. The fat-free sample underwent acid hydrolysis, followed by alkali treatment. After the final filtration, the residue was dried and weighed. The dried sample was then incinerated in a muffle furnace until only a pale ash remained. Once cooled, the ash was weighed again (Verma et al., 2024). The percentage of crude fiber was calculated using equation (2):

(2)

$$Crude\ fiber\ (\%) = \frac{(Weight\ after\ drying\ -\ Weight\ after\ incineration)}{Original\ sample\ weight} \times 100$$

Determination of Protein

The protein content in the dried sample was determined using the Kjeldahl method, which quantifies protein as a function of total nitrogen. The sample underwent digestion in a Kjeldahl flask, followed by distillation using a standard Kjeldahl distillation setup (BonninTech, China) (Verma et al., 2024). The nitrogen content was calculated with equation (3):

(3)

$$Nitrogen\ (\%) = \frac{1.4 \times Normality\ of\ acid \times Titrant\ value}{sample\ weight} \times 100$$

Subsequently, the protein content was derived by multiplying the nitrogen percentage by the established nitrogen-to-protein conversion factor of 6.25, as shown in equation (4):

(4)

$$Protein\ (\%) = 6.25 \times Nitrogen\ content\ (\%)$$

Moisture Content Determination

The moisture content of the sample was determined by weighing 10 g of powdered material into a moisture cup, which was then placed in an oven (Pars Azma, Iran) set at 105°C. The sample was dried until a stable weight was achieved. After each drying cycle, the moisture cup was carefully transferred to a desiccator to cool, and then it was reweighed (Verma et al., 2024). The moisture content of the sample was calculated as the amount of moisture per 100 grams of material using equation (5):

(5)

$$Moisture\ (\%) = \frac{initial\ weight - final\ weight}{weight\ of\ sample} \times 100$$

Determination of Total Ash

The total ash content was determined by carefully placing approximately 5 g of the sample into a crucible. This crucible was then positioned on a clay pipe triangle and subjected to initial heating. Following the preheating, the crucible was transferred to a muffle furnace (Shimifann, Iran), where it was exposed to a temperature of 600°C for 4–5 hours. After the heating process, the crucible was allowed to cool to room temperature before being weighed. This procedure was repeated twice to ensure consistency in the results (Sunday et al., 2016). The resulting ash was light in color, ranging from white to pale grey. The percentage of ash was calculated using equation (6):

(6)

$$Ash\ (\%) = \left[\frac{weight\ of\ crucible\ before\ ashing - weight\ of\ crucible\ after\ ashing}{sampleweight}\right] \times 100$$

Total Carbohydrate Estimation

The available carbohydrate content in the sample was calculated using the method proposed by Sunday et al. (Sunday et al., 2016). This value was obtained by subtracting the individual contributions of crude protein, crude lipid, ash, and crude fiber from the total dry matter, according to equation (7):

(7)

$$Carbohydrate\ (\%) = 100 - (a + b + c + d)$$

Where:

A = the amount of crude protein

B = the amount of crude lipid

C = the amount of ash content

D = the amount of crude fiber

Starter cultures preparation

Homogenized milk was heated to 95°C for 15 minutes. To activate the mixed yogurt culture, a 50-unit package of the yogurt

starter culture was added to one liter of milk, and it dissolved entirely within 15 minutes at 20°C. The culture was then used at a 2.5% v/v concentration for inoculation. The single-strain probiotic culture (Chr. Hansen, Denmark) was aseptically introduced at a specific weight percentage.

Yogurt preparation

Cow's milk, containing 3.2% fat and 8.3% non-fat solids, was carefully pasteurized at 85°C for 30 minutes. Following pasteurization, the milk was allowed to cool to the optimal fermentation temperature of 37°C. At this point, the inoculation process began, adding yogurt starter culture at 0.2% (v/v) and the probiotic strain *Lactobacillus acidophilus* La-5 at 0.5% (w/v). The mixture was then incubated at 40°C, allowing fermentation to progress until the pH dropped to 4.3. After fermentation, the yogurt was promptly refrigerated at 4°C for storage (Taheri et al., 2009). Probiotic doogh samples were prepared using three different concentrations of edible mushroom powder (0.5%, 0.75%, and 1% w/v) and stored at 4°C for 14 days.

Producing Doogh samples

To produce doogh, the yogurt was diluted with drinking water (50% v/v), and NaCl (0.7% w/v) and powdered mint (1 g/L) were then added and stirred into the mixture. Probiotic doogh samples were prepared using three different concentrations of edible mushroom powder (0.5%, 0.75%, and 1% w/v) and stored at 4°C for 14 days. The doogh samples were further enriched with 0, 0.5, 0.75, and 1 g/L oyster mushroom powder. Finally, the samples were packaged in 250 mL PET bottles and stored at 4ºC for 14 days. Table 1 indicates the different formulations of probiotic doogh prepared with mushroom powder. It is worth noting that the sealed packages of mint powder were dried at 60°C again to prevent contamination and preserve the essential oil without damage.

Table 1. Different formulations of traditional Iranian doogh prepared with mushroom powder

Sample	Yogurt (v/v)	Water (v/v)	Salt(w/v)	Mint(g/L)	Mushroom powder (g/L)
Control	50	50	0.7	1	0
M-0.50	50	50	0.7	1	0.5
M-0.75	50	50	0.7	1	0.75
M-1.00	50	50	0.7	1	1

Measuring titrable acidity

To assess the acidity, 10 milliliters of the sample were meticulously measured and placed into a suitable beaker. Following this, 0.5 milliliters of phenolphthalein indicator (Merck, Germany) was introduced to the sample. The titration was performed with precision, using 0.1 N sodium hydroxide solution, slowly added until a delicate pink hue emerged, remaining stable for no less than 5 seconds (Eslami Moshkenani et al., 2015). The acidity was calculated based on equation (8) as below:

$$Acidity\ (\%) = \frac{N \times 0.009 \times 100}{V} \quad (8)$$

pH measurement

The pH of the samples was determined with utmost precision using a high-quality pH meter (Zagchemie, Iran). The sample was carefully transferred into a 30-milliliter beaker, where the pH meter electrode, having been thoroughly calibrated, was delicately immersed into the sample. To ensure accuracy, the sample temperature was maintained at a steady 20°C. Once the pH meter's readings reached a stable state, the precise pH value was recorded meticulously (Eslami Moshkenani et al., 2015).

Physical Stability

To assess the doogh's stability, 100 milliliters were carefully poured into a 100-milliliter graduated cylinder, and the doogh's initial height was meticulously recorded. The cylinder was then securely sealed with aluminum foil to prevent contamination. On the specified testing days, the doogh's height and any serum that

had separated were precisely measured (Ebrahimzadegan & Zomorodi, 2015). The stability percentage was then calculated using equation (9):

(9)

$$Stability\,(\%) = \frac{(height\ of\ th\ doogh - height\ of\ the\ serum)}{Initial\ height\ of\ the\ doogh}$$

Total Lactic Acid Bacteria Analysis

MRS agar (Lioflichem, Italy), a highly selective medium for isolating lactic acid bacteria, was meticulously prepared following the manufacturer's instructions and sterilized via autoclaving at 121°C for 20 minutes. After sterilization, the medium was allowed to cool to approximately 47°C, preserving bacterial cell viability during plating. Serial dilutions of the doogh samples were made using a sterile 0.1% peptone buffer (Quelab, UK) to achieve bacterial concentrations within a measurable range. For each dilution, 1 mL of the sample was aseptically transferred to sterile Petri dishes, then carefully adding 12–15 mL of the molten, cooled MRS agar. The plates were gently swirled to ensure uniform bacterial distribution throughout the medium. Once the agar had solidified at room temperature, the Petri dishes were inverted to prevent condensation and incubated (Shimi Azma, Iran) at 37°C for 24 hours. After the incubation period, bacterial colonies were enumerated using the Standard Plate Count (SPC) method, with only plates exhibiting 30 to 300 colonies being counted. The total number of lactic acid bacteria in the doogh sample was calculated based on the recorded colony counts and corresponding dilution factors (Sakul et al., 2024; Standards, 1387).

Viability of Lactobacillus acidophilus

To evaluate the viability of *L. acidophilus (La5)*, MRS-bile agar medium was utilized, meticulously prepared using MRS agar (Lioflichem, Italy) and %0.15 w/v bile sourced from Sigma-Aldrich (Reyde, USA). One milliliter of each sample was carefully diluted in 9 milliliters of sterile 0.1% peptone water (Quelab, UK) and thoroughly mixed using a vortex mixer to ensure even distribution. Serial dilutions were then performed to achieve the desired concentration. The viability of *L. acidophilus (La5)* was assessed by incubating the prepared plates aerobically at 37°C for 72 hours in a controlled environment (Shimi Azma, Iran), allowing sufficient time for bacterial growth and assessment (Sohrabvandi et al., 2012).

Sensory attributes

Given the well-known primary characteristics of doogh, a sensory evaluation session was conducted to assess attributes such as taste, color, texture, aroma, and overall acceptability using a 5-point hedonic scale with 12 panelists (6 men and 6 women). In this method, a score of 1 indicates the feature is unacceptable, while a score of 5 signifies the sample's complete desirability. The sensory evaluation results are reported in quantitative and qualitative formats (Eslami Moshkenani et al., 2015).

Statistical analysis

To investigate the effect of the probiotic strain Lactobacillus acidophilus and three levels of concentration of oyster mushroom powder added to fermented probiotic dairy drink (doogh), the analysis of variance (ANOVA) will be used on the samples in the SPSS statistical software. The means of the data will be compared using Duncan's test at a 95% confidence level within a completely randomized design. All tests will be conducted with two repetitions.

Results and Discussion

pH & Acidity

Table 2 indicates the changes in pH and acidity of doogh samples over 1, 7, and 14 days of storage at 4°C. Adding mushroom powder and storage time caused significant differences in these two parameters. pH decreased over time in all samples due to the increased microbial activity and the production of acidic compounds. On day 1, the 1.00% mushroom powder sample was significantly more acidic than the 0.75% sample ($P<0.05$). By day 14, the 0.75% sample maintained the highest pH value (4.13) compared to the other groups, indicating better stability.

Table 2. Results of the comparison of the mean pH in doogh samples containing mushroom powder based on storage time

Sample	Day 1	Day 7	Day 14
Control	4.60±0.00 [ab*]	4.36±0.00 [a]	4.09±0.00 [a]
M-0.50	4.60±0.00 [ab]	4.37±0.00 [ab]	4.11±0.00 [ab]
M-0.75	4.62±0.00 [b]	4.39±0.00 [b]	4.13±0.00 [b]
M-1.00	4.59±0.00 [a]	4.37±0.00 [ab]	4.11±0.00 [ab]

*The letters indicate significant differences between groups (P<0.05)

Table 3 presents the results of measuring the titrable acidity in doogh samples after 1, 7, and 14 days of storage at the constant temperature of 4°C. The more mushroom powder and extended storage, the higher the acidity in doogh samples (P<0.05). After one day of storage, the sample with the highest concentration of mushroom powder (1.00%) exhibited the highest acidity. In contrast, after 14 days, acidity levels reached 0.54 in the same mushroom powder concentration, compared to 0.47 in the control group. The activity of the lactic acid bacteria during the storage with mushroom powder caused an increase in acidity. This is due to the mushroom powder components, which serve as a nutrient medium for the probiotic bacteria, whose growth causes a lower pH in doogh samples.

Table 3. Results of the investigation of changes in the titratable acidity parameter in doogh samples based on the variables of mushroom powder concentration and storage time

Sample	Day1	Day7	Day14
Control	0.35±0.00 [a]	0.45±0.00 [a]	0.47±0.00 [a]
M-0.50	0.37±00 [a]	0.47±0.00 [b]	0.50±0.00 [b]
M-0.75	0.36±0.00 [a]	0.49±0.00 [c]	0.52±0.00 [c]
M-1.00	0.40±0.01 [b]	0.50±0.00 [d]	0.54±0.00 [d]

*The letters indicate significant differences between groups (P<0.05)

Proximate Composition of Oyster Mushroom Powder

Proximate analysis was conducted to determine the nutritional composition of oyster mushroom powder (*Pleurotus ostreatus*). The moisture content, crude protein, crude fat, crude fiber, and ash content were analyzed according to (Verma, Karakannavar, & Ashwini, 2023)(Verma et al., 2024). The results were expressed as percentages on a dry weight basis and are presented as means ± standard deviation.

The proximate analysis results of the oyster mushroom powder revealed a composition of 10.99±0.078% moisture, 21.80±0.141% crude protein, 1.03±0.014% crude fat, 16.10±0.071% crude fiber, 8.57±0.021% ash, and 41.51±0.064% carbohydrates. These values are consistent with those reported by Ofodile et al. (2020), who documented that dried *P. ostreatus* contained 11.20±0.47% moisture, 39.75±0.53% protein, 0.55±0.07% fat, 3.30±1.04% fiber, 8.65±0.52% ash, and 36.54±0.50% carbohydrates (Ofodile et al., 2020). Similarly, Biswas et al. (2019) reported 7.80% moisture, 14.35% protein, 1.10% fat, 7.60% ash, 2.12% crude fiber, and 67.03% carbohydrates in mushroom powder (Biswas et al., 2019).

The crude fiber content in this study (16.10±0.071%) aligns closely with the juice powder's fiber content (16.00±2.52%) as reported by Ofodile et al. (2020), while the carbohydrate content (41.51±0.064%) is comparable to the findings of Biswas et al. (2019), who reported 64.1% carbohydrates for dried mushrooms. These similarities indicate consistency across studies, despite

variations in drying methods, mushroom variety, or analytical conditions. Overall, the proximate analysis results are consistent with existing studies, reinforcing the potential use of *Pleurotus ostreatus* powder as a functional food ingredient that contributes to both nutritional benefits and food product stability.

Table 4. Proximate analysis of oyster mushroom powder. The values represent the mean of three replications ± standard deviation.

Components	Results (%)
Moisture	10.99±0.078
Crude Protein	21.80±0.141
Crude Fat	1.03±0.014
Carbohydrates	41.51±0.064
Crude fiber	16.10±0.071
Ash	8.57±0.021

Physical Stability

Syneresis, or whey separation, refers to liquid release from a product's gel structure, negatively impacting consumer acceptance. On day 1, the sample with 1.00% mushroom powder exhibited a serum phase height of 0.35 cm, while no whey separation was observed in other samples ($P<0.05$). After 7 days, significant differences emerged: The control sample had the lowest stability, 56.76% after 14 days of storage. The 0.50% mushroom powder sample demonstrated the highest stability, with values of 89.70% and 78.23% after 7 and 14 days, respectively, compared to the control. Overall, increased storage time resulted in lower physical stability (Table 5).

Table 5. Results of the investigation of physical stability in doogh samples based on the variables of storage time and mushroom powder concentration

Sample	Day1	Day7	Day14
Control	100.00±0.00 ^{a}	80.29 ± 0.21 ^{a}	56.76 ± 0.21 ^{a}
M-0.50	100.00±0.00 ^{a}	89.70 ± 0.21 ^{bc}	78.23 ± 0.28 ^{c}
M-0.75	100.00±0.00 ^{a}	91.17 ± 0.00 ^{c}	72.05 ± 0.35 ^{b}
M-1.00	97.94 ± 0.00 ^{b}	88.23 ± 0.00 ^{b}	73.82 ± 0.07 ^{b}

*The letters indicate significant differences between groups ($P<0.05$)

Therefore, the incorporation of mushroom powder, particularly at a 0.50% concentration, significantly reduced syneresis compared to the control, which was aligned with the findings of Radzki et al. (2023), who reported the improvement of fermented dairy products' stability with the inclusion of mushroom powder as a stabilizing agent (Radzki et al., 2023). The structural properties of the mushroom powder (i.e., polysaccharide content) may reinforce the gel matrix and inhibit whey separation, improving the overall oral texture and stability.

Viability of Microbial Species During Storage

Figure 1 presents the results of investigating the viability of lactic acid bacteria and Lactobacillus acidophilus in doogh samples over 1, 7, and 14 days of storage at a constant temperature of 4°C. The highest bacterial count of Lactic acid bacteria was observed after 7 days of storage, indicating a growth rate in the population of viable Lactic acid. The initial release of nutrients in the doogh samples may have occurred after 7 days, leading to an increase in bacterial survival before a subsequent decline. Applying 1% mushroom powder (w/v) in the formulation of doogh samples was the most effective treatment in maintaining bacterial viability, among other concentrations. Also, no significant decline was observed between days 7 and 14.

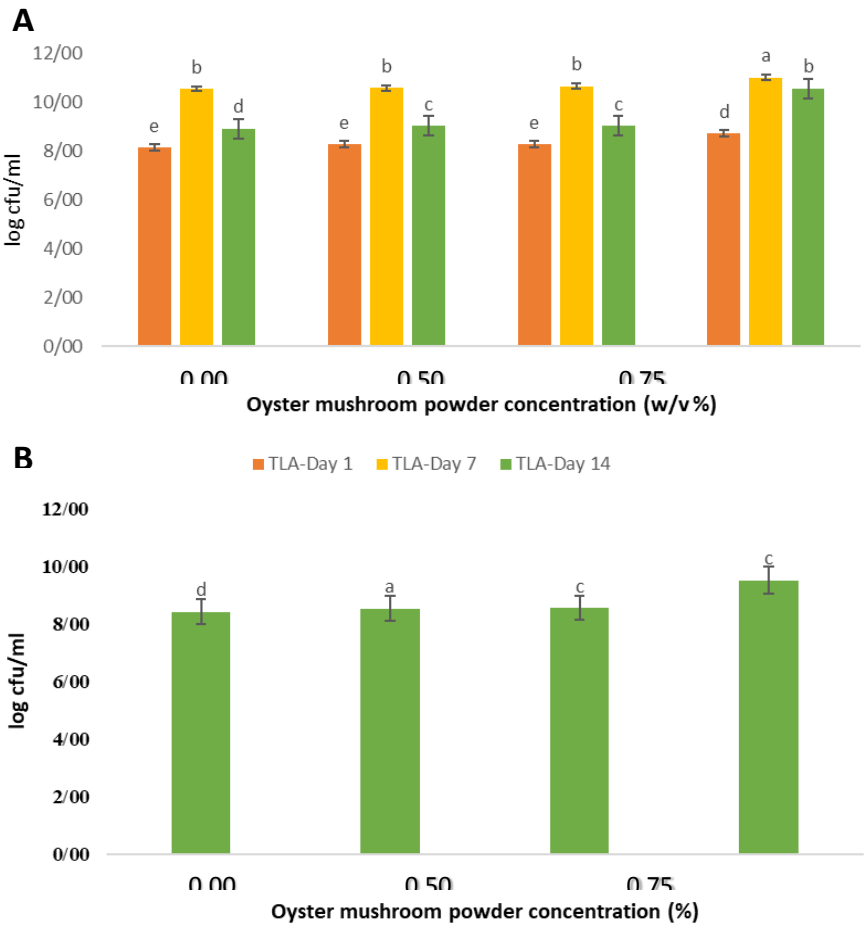

Figure 1. The survival rate of lactic acid and Lactobacillus acidophilus bacteria in doogh samples based on the variables of storage time and mushroom powder concentration in logarithmic units (cfu/ml): A) lactic acid bacteria after 1, 7, and 14 days of storage; B) Lactobacillus acidophilus after 14 days of storage

In Lactobacillus acidophilus population evaluation after 14 days of storage, samples containing 1% mushroom powder showed a significant increase in bacterial count (9.51 log CFU/ml) compared to the control (8.40 log CFU/ml) ($P<0.05$). Increasing mushroom powder concentration enhanced bacterial survival due to its protective and encapsulating effects against environmental and chemical stressors. The results were aligned with previous studies, highlighting the protective role of mushroom powders in fermented dairy products (Sarlak et al., 2017; Yu-Jin Choi et al., 2013). The protective effect of mushroom powder is likely due to its antioxidant properties, which help stabilize the microbial cells, particularly in acidic environments. The increased bacterial count over storage compared to the control group suggests that mushroom powder may also act as a prebiotic and stabilizing agent, contributing to microbial health and product quality (Michalska et al., 2025).

Sensory Evaluation

The sensory evaluation of doogh samples containing different concentrations of mushroom powder flavored with dried mint was assessed based on taste, flavor, texture, aroma, and color using a 5-point Hedonic scale. Figure 2 indicates the results of the panelists' scores recorded at sensory evaluation sessions. All samples were acceptable until 7 days of storage, and there was a significant decline in sensory attributes due to the storage impact ($P<0.05$). After 7 days of storage, the flavor

score decreased significantly by increasing the mushroom concentration.

The mean flavor score of doogh samples became less than 60% of the total score after 14 days of storage at a constant temperature of 4°C; the sample's acceptability was reduced because of the weak sensory perception and undesirable flavor.

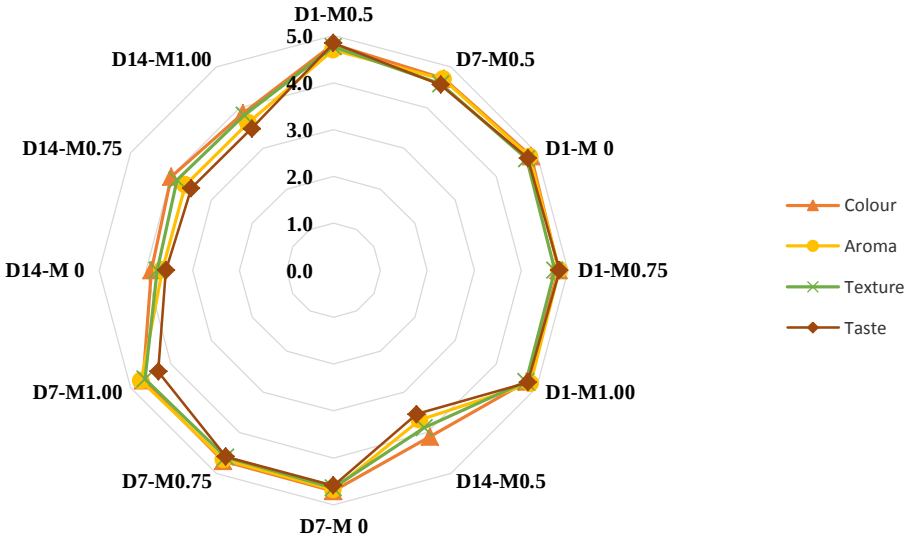

Figure 2. The average sensory evaluation scores of doogh samples containing different concentrations of edible mushroom powder over a 14-day storage period at the refrigerator (D: storage duration of doogh samples, M: concentration of mushroom powder)

Several publications corroborate the findings regarding the impact of mushroom powder on the stability and sensory attributes of fermented dairy products (Choudhury et al., 2013; Cruz-Martins et al., 2018; Davani-Davari et al., 2019; Dicks & Ellinger, 2020; Onuegbu et al., 2017). This was consistent with the findings of previous studies suggesting that the sensory appeal of probiotic products can be affected by extended storage and high concentrations of functionals (Sakul et al., 2024). This indicates that the main challenge of applying mushroom powder in doogh formulation is balancing the stability, nutritional, and sensory properties of doogh to maintain the acceptability and desirability of the product.

Principal Component Analysis (PCA)

PCA analysis was performed to identify the correlation between sensory characteristics and physicochemical properties of doogh samples. Four principal components were identified, explaining a cumulative variance of 90.64%, indicating strong correlations among the tested parameters. Table 7 shows the extraction coefficient of every parameter. Most of the studied parameters were extracted more than 80% except total lactic acid bacteria and stability after 14 days, which could be due to the higher differences of samples and heterogeny effect of mushroom concentration on the properties of doogh samples after 14 days at a constant temperature of 4°C.

Table 6. The extraction coefficients of the parameters under investigation in the current study in the factor correlation test

Test Parameters	Extraction Coefficient
pH1	0/898
Acidity1	0/995
pH7	0/936
Acidity7	0/958
pH14	0/989
Acidity14	0/984
Total Lactic Acid Bacteria1	0/677
Total Lactic Acid Bacteria7	0/854
Total Lactic Acid Bacteria14	0/983

Stability1	0/917
Stability7	0/866
Stability14	0/728
Lactobacillus Acidophilous 14	0/983
Colour	0/807
Aroma	0/993
Texture	0/985
Taste	0/855

The Pearson coefficients of correlated parameters are presented in Table 6. pH showed a significant positive correlation with sensory texture and stability. Similarly, acidity strongly correlated with stability (approx. 90%). The lactic acid bacteria population was moderately related to the aroma, suggesting a microbial influence on sensory quality.

Moreover, the significant correlation between bacterial counts and sensory properties mirrors the conclusions of the study of (Sarlak et al., 2017) noted that the stability of fermented dairy products could be improved without compromising sensory attributes when functional ingredients were added.

Accordingly, the spatial location of every parameter based on the extracted significant PCs in Figure 3 can indicate the possible correlation of parameters in a single group. Therefore, it is possible to arrange acidity, flavor, aroma, and stability after 14 days, and the total bacterial population in the first group has a positive correlation with PC1 and PC2 and a negative correlation with PC3. Also, Stability after 7 days and pH positively correlate with dual components of PC2 and PC3. The third possible group of attributes will contain color, texture, and stability on the first day, with a weak correlation with the color of the doogh samples.

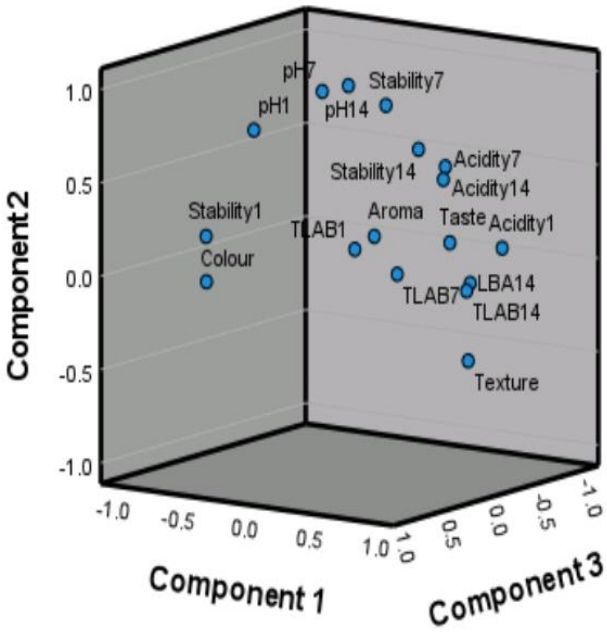

Figure 3. The spatial positions of the test parameters based on the three components obtained in the factor correlation test

Table 7 The Pearson coefficient determines the mutual relationship between the parameters under investigation in the current study

Correlation Matrix

	pH 1	Acidity1	pH7	Acidity7	pH14	Acidity14	Total LAB 1	Total LAB 7	Total LAB 14	Stability1	Stability7	Stability14	LBA 14	Color	Aroma	Texture	Taste
pH1	1/000	-0/522	0/826	-0/062	0/695	-0/058	0/018	-0/296	-0/566	0/632	0/332	-0/017	-0/559	0/391	-0/100	-0/727	-0/167
Acidity1		1/000	0/000	0/788	0/169	0/791	0/254	0/354	0/871	-0/931	0/381	0/533	0/891	-0/827	0/269	0/572	0/404
pH7			1/000	0/495	0/973	0/512	0/313	-0/069	-0/063	0/200	0/721	0/419	-0/042	0/043	0/167	-0/633	-0/093
Acidity7				1/000	0/618	0/986	0/516	0/368	0/807	-0/675	0/664	0/609	0/832	-0/465	0/369	0/020	0/022
pH14					1/000	0/658	0/334	0/003	0/063	0/074	0/853	0/610	0/093	-0/070	0/267	-0/563	-0/030
Acidity14						1/000	0/489	0/353	0/758	-0/629	0/760	0/733	0/789	-0/478	0/417	-0/008	0/060
Total LAB1							1/000	0/198	0/556	-0/147	0/195	0/220	0/520	-0/212	0/369	-0/291	-0/342
Total LAB7								1/000	0/449	-0/375	0/120	0/091	0/441	-0/248	-0/536	0/373	-0/180
Total LAB 14									1/000	-0/885	0/187	0/312	0/997	-0/607	0/287	0/419	0/031
Stability1										1/000	-0/107	-0/237	-0/895	0/724	-0/107	-0/707	-0/282
Stability7											1/000	0/907	0/234	-0/159	0/388	-0/366	0/006
Stability14												1/000	0/357	-0/336	0/550	-0/161	0/159
LAB 14													1/000	-0/605	0/312	0/411	0/062
Color														1/000	-0/033	-0/680	-0/648
Aroma															1/000	-0/378	0/071
Texture																1/000	0/489
Taste																	1/000

Therefore, the present study evaluated the impact of edible mushroom powder, specifically *Pleurotus ostreatus* (0.5%, 0.75%, and 1%), on a probiotic fermented dairy drink named Doogh over a 14-day storage at 4°C. The findings revealed that the incorporation of mushroom powder, particularly at a concentration of 0.5%, slows the pH decrease and acidity increase, enhancing the product's stability. Higher concentrations of mushroom powder, especially 1%, preserved the viability of probiotic bacteria, such as *Lactobacillus acidophilus* and other lactic acid bacteria, improving their survival during storage. The results were aligned with previous studies highlighting the protective role of mushroom powders in fermented dairy products (Sarlak et al., 2017; Yu-Jin Choi et al., 2013). The protective effect of mushroom powder is likely due to its antioxidant properties, which help stabilize the microbial cells, particularly in acidic environments. The increased bacterial count over storage compared to the control group suggests that mushroom powder may also act as a prebiotic and stabilizing agent, contributing to microbial health and product quality. Several publications corroborate the findings regarding the impact of mushroom powder on the stability and sensory attributes of fermented dairy products (Choudhury et al., 2013; Cruz-Martins et al., 2018; Davani-Davari et al., 2019; Dicks & Ellinger, 2020; Onuegbu et al., 2017).

Further comparison with the literature shows that the composition of *Pleurotus ostreatus* powder, including moisture (10.99%), protein (21.80%), fat (1.03%), and crude fiber (16.10%), is consistent with the findings of previous studies (Eslami Moshkenani et al., 2015; Taheri et al., 2009). These values confirm the nutritional value of mushroom powder, further supporting its use as a functional food ingredient or additive. The crude fiber and carbohydrate content were similar to the reports of (Ofodile et al., 2020) and (Biswas et al., 2019), reinforcing the potential for this mushroom variety to enhance the nutritional profile of functional beverages like doogh.

Additionally, the study investigated the whey separation in doogh samples. The incorporation of mushroom powder, particularly at a 0.50% concentration, significantly reduced syneresis compared to the control, which was aligned with the findings of (Radzki et al., 2023), who reported the improvement of fermented dairy products' stability with the inclusion of mushroom powder as a stabilizing agent. The structural properties of the mushroom powder (i.e., polysaccharide content) may reinforce the gel matrix and inhibit whey separation, improving the overall oral texture and stability. Moreover, the significant correlation between bacterial counts and sensory properties mirrors the conclusions of the study of (Sarlak et al., 2017) noted that the stability of fermented dairy products could be improved without compromising sensory attributes when functional ingredients were added. In terms of sensory evaluation, the mushroom powder had no significant impact on the sensory characteristics of the doogh after 7 days of storage. However, after 14 days, there was a notable decline in taste and flavor, particularly in samples with higher mushroom powder concentrations. This was consistent with the findings of previous studies suggesting that the sensory appeal of probiotic products can be affected by extended storage and high concentrations of functionals (Sakul et al., 2024). This indicates that the main challenge of applying mushroom powder in doogh formulation is balancing the stability, nutritional, and sensory properties of doogh to maintain the acceptability and desirability of the product.

Conclusion

In conclusion, adding edible mushroom powder improves the stability, probiotic viability, and overall nutritional value of doogh. However, the impact on sensory properties after extended storage highlights the need for further optimization in formulation and storage conditions to maintain the product's health benefits and sensory appeal. The results of this study provide valuable insights into the application of mushroom powder in

functional dairy products and its potential for large-scale industrial production. Future research should focus on balancing microbial viability, product stability, and sensory attributes for optimal results in industrial applications.

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