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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	Effects of *Lactarius hatsudake* fortification on physicochemical, microbiological and antioxidant properties of stirred-type yogurt during cold storage
Running Title (within 10 words)	Properties of LH-fortified yogurt
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Special remarks – if authors have additional information to inform the editorial office	
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Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This work was supported by grants from the Natural Science Foundation of Hunan Province (No. 2022JJ40013) and Scientific Research Foundation of Hunan Provincial Education Department (No. 23B0667) to Hanyu Zhu.
Author contributions (This field may be published.)	Conceptualization: Zhu H Data curation: Li G, Liu H Formal analysis: Liu H, Sun W Methodology: Yao X, Wu R, Hu J, Yang Q Validation: Li G, Sun W Investigation: Yao X Writing - original draft: Zhu H, Li G Writing - review & editing: Zhu H, Li G, Liu H, Sun W, Yao X, Wu R, Hu J, Yang Q
Ethics approval (IRB/IACUC) (This field may be published.)	This article does not require IRB/IACUC approval because there are no human and animal participants.

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Abstract

Food fortification is a cost-effective and efficient way to meet the needs of consumers looking for products with certain health benefits. *Lactarius hatsudake* (LH) is a wild and edible mushroom with nutritional and medicinal benefits, making it a promising natural, high-quality food option with multiple functions. During this research, yogurts that were stirred and enhanced with LH at concentrations of 0%, 0.5%, 1.5%, and 3.0% were prepared. The impact of LH on the color, number of viable lactic acid bacteria (LAB) cells, pH, titratable acidity (TA), syneresis, viscosity, texture, and antioxidant activity of the yogurt samples was assessed over a storage period of 28 d. The findings indicated that LH had a notable impact on the characteristics of yogurt samples over time, including decreased lightness, increased red-green and yellow-blue color values, decreased pH values, viscosity and cohesiveness, while increased viable LAB cells, the contents of TA, syneresis and consistency, and antioxidant activity. The yogurt fortified with 0.5% LH showed comparable textural parameter values and sensory scores to plain yogurt, indicating it could be the ideal amount for industrial production. These findings showed that LH improved the physicochemical, microbiological, and antioxidant properties of yogurt, suggesting the possibility of developing sustainable LH products in functional dairy products.

Keywords: *Lactarius hatsudake*; Stirred yogurt; Physicochemical and microbiological properties; Antioxidant activity; Sensory evaluation

Introduction

Lactarius hatsudake (LH), a valuable edible mushroom from the Russulaceae family, is found in the wild and is known for its natural health benefits (Shen et al., 2022; Wang et al., 2016). This ectomycorrhizal fungus is in a symbiotic relationship with Pinaceae or Quercus and can be found throughout Asia, Europe, and North America (Shen et al., 2022; Yang et al., 2022). LH's fruiting body can be consumed fresh, frozen, or processed form as mushroom oil (Shen et al., 2022). It is abundant in protein, polysaccharides, crude fiber, unsaturated fatty acids, nucleic acid derivatives, a variety of amino acids, vitamins, and essential nutrients (Zhu et al., 2023a). Besides its nutritional values, delicious taste, and aromatic flavor, LH has antitumor, antioxidation, and antiviral activities, and can alleviate diabetes symptoms, improve immune responses, and promote calcium absorption (Zhu et al., 2023a; Xiang et al., 2022). Thus, LH could be utilized as a promising natural high-quality food source with multiple functions.

The global increase in consumers' desire for functional foods that offer vital nutrients and promote good health is evident in recent times (Abdi-Moghadam et al., 2023). Therefore, the food industry has continuously strived to create functional food items that enhance physiological functions, boost nutritional content, and promote well-being (Savaiano and Hutkins, 2021). Fortification food with essential nutrients is a highly effective and economical strategy to enhance the nutritional value of diets and improve the overall health of the population (Hamed et al., 2021). Research has indicated that the addition of nutrients to food can have beneficial effects on health and can help prevent or treat certain illnesses (Ribeiro et al., 2021).

Yogurt is widely consumed as a popular fermented dairy product worldwide, serving as a primary source of probiotic bacteria and essential nutrients for humans (Kaur et al., 2020). Furthermore, yogurt can enhance the absorption of certain nutrients and their impact on health, solidifying its importance in a balanced diet (Abdi-Moghadam et al., 2023). Yogurt

consumption can lead to better health, such as lowering cholesterol absorption, improving digestion, strengthening the immune system, aiding in weight control, lowering the chances of breast and colorectal cancer, and enhancing cardiovascular, bone, and digestive health (Aleman et al., 2023; Rashwan et al., 2022; Savaiano and Hutkins, 2021). Adding nutrients to yogurt not only enhances its health benefits, but also meets consumer demand and boosts nutrient intake in daily diets (Hamed et al., 2021). Increasing numbers of functional ingredients from mushrooms and their extracts have been taken into consideration for incorporating into yogurt. Adding polysaccharides extracted from *Auricularia cornea* var. Li to set-type yogurt significantly improved its ability to retain water, viscosity, firmness, and cohesiveness, as well as the overall antioxidant power in vivo (Wang et al., 2022). Water-soluble polysaccharides extracted from the fruiting bodies of *Pleurotus ostreatus* increased the toughness, chewiness, and antioxidant power of yogurt (Radzki et al., 2023). Additionally, the mycelium of *Ganoderma lucidum*, *P. ostreatus*, and *P. eryngii* were utilized as alternative sources of tocopherol in yogurts to enhance their antioxidant properties (Bouzgarrou et al., 2018).

Our prior research discovered that the fruiting body of LH, gathered from the mountainous area of Nanyue in China, had high concentrations of total carbohydrate, protein, and essential elements, with low fat and calories (Zhu et al., 2023a), indicating its possible application as a functional food component. Hence, this study aimed to assess the practicality of using LH as a nutritious ingredient in the fortified yogurt. The stirred yogurts were prepared by incorporating different amounts of LH powder. Throughout a 28-d period of cold storage, the impact of LH on fortified yogurt was examined by observing alterations in color, viable lactic acid bacteria (LAB) cells, pH, titratable acidity (TA), rheological properties, texture, and antioxidant capacities.

Materials and Methods

Materials

The LH fruiting body sample was freeze-dried and then grounded with a plant pulverizer. After passed through a 120-mesh sieve, the LH powder was kept at a temperature of -18℃ for later use. The final moisture content of the dried LH powder was 12.34%. Based on our previous research, LH contained 66.43 ± 1.64 g/100 g dw of crude polysaccharides, 24.10 ± 1.21 g/100 g dw of protein, 2.50 ± 0.05 g/100 g dw of fat, and 6.97 ± 0.16 g/100 g dw of ash. The aqueous extract of LH had a total phenolic content (TPC) of 6.71 ± 0.53 mg gallic acid equivalent (GAE)/g dw (Zhu et al., 2023a).

Preparation of LH-enhanced yogurt

Cow milk (containing 3.2% milk protein, Mengniu, Inner Mongolia, China), sucrose (80 g/L), and starter culture (1 g/L) were utilized in the preparation of yogurts. Cow milk was mixed with sucrose and then heated to 85℃ for 30 min to sterilize. After cooling the pasteurized milk mixture to 42℃, it was then inoculated with a starter culture that included *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (Angel Yeast, Hubei, China). Afterwards, the mixture was left to ferment at 42℃ for 6 h before being cooled to 4℃. To create the LH-enriched yogurt, LH powder was mixed into plain yogurt at concentrations of 0.5%, 1.5%, and 3.0% (w/w), after it had been cooled. Following extensive blending, enriched flavored yogurt was distributed and kept in a dark environment at 4℃ for 28 d. The attributes and properties of yogurt samples were assessed three times during refrigeration time.

Determination of physicochemical and microbiological properties

Yogurts were analyzed for color, LAB density, pH, TA, viscosity, and texture on the 1st, 14th, and 28th days of refrigeration. Yogurt samples were analyzed for color using a colorimeter (Konica, Minolta, Chroma, Meter CR-400), with measurements taken for

lightness (L*), red-green (a*), and yellow-blue (b*) values. The TA was calculated by titrating 10 g of yogurt against the 0.1 mol/L NaOH solution, using phenolphthalein as an indicator, following the procedure described by Zhu et al. (2023b). The viscosity was determined by measuring it with 150 g of yogurt sample using a rotational viscometer spindle (DV-II, Brookfield, Middleboro, MA, USA) at a speed of 30 rpm for a duration of 1 min. The syneresis susceptibility of yogurt was assessed by centrifuging 20 g of samples at 5,000 × g for 5 min and then measuring the weight of the drained whey per 100 g of yogurt in the supernatant. Yogurts were analyzed for their texture properties including firmness, consistency, cohesiveness, and viscosity index using a TAXT Texture Analyzer (Stable Micro System, Godalming, Surrey, UK). The analysis was conducted with a backward extrusion test and a cylindrical probe diameter of 36 mm was utilized (Zhu et al., 2023b). Pre-test speed, test speed, post-test speed, trigger force, and distance were 1.0 mm/s, 1.0 mm/s, 2.0 mm/s, 10.0 g, and 10.0 mm, respectively. Viable cell count in yogurt was determined through serial dilution and spread plating methods, and reported as log CFU/g. Following the application of the diluted solution (100 μL) on De Man-Rogosa-Sharpe agar plates, they underwent anaerobic incubation at 37℃ for 72 h, with subsequent counting of plates showing 30-200 colonies.

The TPC and antioxidant assays

The TPC was determined using the Folin-Ciocalteu assay following the protocol outlined by Sheikh et al. (2023). To summarize, the yogurt was centrifuged at a speed of 12,000 × *g* for a duration of 10 min in order to gather the liquid portion. The supernatant was treated with Folin-Ciocalteu's reagent, followed by the addition of 2% Na_2CO_3 for a 30-min reaction period, and the absorbance was then measured at 760 nm. A calibration curve was created by preparing a solution of gallic acid (Sigma Aldrich, Co., St. Louis, MO, USA) with

concentrations ranging from 0 to 100 μg/mL. TPC values were indicated as the amount of GAE/g of yogurt.

Yogurt samples were used to measure antioxidant activity by assessing the scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radicals, following the method described in Zhu et al. (2023b). The soluble components in water were mixed with either DPPH-ethanol or ABTS solution and then assessed for absorbance at 517 nm or 734 nm. The L-ascorbic acid solution with different concentrations of 0-30 μg/mL was used to construct a standard curve. The yogurt samples' antioxidant properties were determined by analyzing a standard curve and were reported as μg of ascorbic acid/g of yogurt sample.

Sensory evaluation

The sensory evaluation of the yogurt samples was performed following the procedure of our previous procedure (Zhu et al., 2023b). In brief, twenty semi-trained panelists, including staff members and undergraduate students of the Department of Food Science and Technology, Hengyang Normal University, were served with the randomized samples of yogurt independently. The sensory parameters were evaluated by appearance, fermented odor, texture, taste quality, and overall acceptance based on a 9-point hedonic ranking scale (Costa et al., 2022). For each sensory perception, the average values were calculated based on the number of panelists values for each sample. The Institutional Review Board (IRB) of Hengyang Normal University approves sensory evaluation consent procedures (No. 2024LL008).

Statistical analysis

All experiments were conducted three times. Results were displayed as averages ± standard deviation. The characteristics of yogurt enhanced with varying levels of LH and

yogurt stored for different durations were examined through one-way ANOVA analysis conducted with IBM SPSS 24.0 software from IBM Corporation (Armonk, New York, USA). Duncan *post hoc* test was applied to determine significant differences at $p < 0.05$.

Results and Discussion

Effect of LH on yogurt color

The color of food products is an important factor in determining acceptance and palatability by consumers (Maruoka et al., 2023; Rashwan et al., 2022). The stirred yogurts fortified with 0%, 0.5%, 1.5%, and 3.0% of LH were prepared and thereafter stored at 4℃ for 28 d. In this study, the color parameters between yogurt samples enriched with different amounts of LH during storage were shown in Table 1, and they were significantly different ($p < 0.05$). We found that the yogurt sample turned brown-yellow after adding LH powder, and this color was visually positively correlated with the LH concentration and storage time (Fig. 1). The L* values of yogurt fortified with LH decreased significantly, while the a* and b* values increased significantly compared to yogurt without LH (control) ($p < 0.05$). Over a period of 28 d in cold storage, the L* values of yogurt samples fortified with LH decreased, while the a* and b* values increased in a concentration-dependent manner. In contrast, the color of the control samples remained relatively constant, indicating a significant impact of LH addition on color parameters. The high L* values of the control yogurt were primarily due to the presence of casein micelles and fat globules, which have the ability to reflect white light in the visible spectrum (Kaur et al., 2020). The changes in yogurt color could result from the pigments provided by LH. Cutting or breaking the fruiting bodies results in the formation of a milky juice that starts off carrot-colored but quickly darkens, eventually changing to a blue-green hue within minutes (Liu, 2007; Zhou and Liu, 2010). Besides, two azulene pigments, which are red-purple and purple, have been isolated from LH (Fang et al., 2006). Comparable findings were discovered in flavored yogurts enriched with additional

natural ingredients like *Grifola frondose* fruiting body (Aleman et al., 2023) and *Nelumbo nucifera* leaf (Kim et al., 2019).

Effect of LH on yogurt LAB cells

Delivering beneficial probiotics is one of the main attributes of yogurt, which confers health benefits to humans (Kaur et al., 2020). To produce desirable health benefits in the host, probiotics should be administered in adequate amounts to ensure sufficient numbers when they reach the large intestine (Jovanović et al., 2020). FAO/WHO recommends that probiotics in fermented beverages should have a viability level of at least 106–107 CFU/mL (or g) when consumed (Dimitrellou et al., 2019). Yet, the effectiveness of probiotics in yogurt is hindered by the rising levels of lactic acid, hydrogen peroxide, and bacteriocins as it is stored (Aleman et al., 2023). Therefore, it is difficult to keep large amounts of probiotics in yogurt while it is being stored, which poses a challenge in creating yogurt products. Mushrooms contain beneficial prebiotics like polysaccharides and fibers that can support the growth and function of helpful microorganisms, as discussed in recent studies (Kaur et al., 2020; Kulshreshtha, 2023; Shang et al., 2021). Adding mushrooms or extracts to yogurt is a key method to improve the survival of probiotics and effectively meet the need for probiotic benefits in the body.

The viable LAB cells in this study exceeded the minimum threshold in every yogurt group. Throughout the storage period, there were notable decreases in the viable LAB numbers in both the control and 3.0% LH-enhanced yogurt samples, with values ranging from 8.39 ± 0.04 log CFU/g to 7.97 ± 0.03 log CFU/g and from 8.67 ± 0.02 log CFU/g to 8.47 ± 0.01 log CFU/g ($p < 0.05$, Fig. 2A). The quantities of LAB concentrations in yogurt samples fortified with 0.5% and 1.5% LH showed a minor decrease with no notable variances after being stored in the refrigerator for 28 d ($p > 0.05$). Additionally, all yogurt samples containing LH had notably higher levels of viable LAB cells compared to the control group ($p < 0.05$). It

seemed that a relatively low dosage of LH (0.5%) could effectively promote the survival rate of LAB during cold storage and showed positive effects on maintaining probiotics in yogurt. LH contains abundant microbial nutrients like carbohydrates (66.43%), proteins (24.10%), and minerals (6.97%) (Zhu et al., 2023a), potentially enhancing the development of LAB in yogurt. Furthermore, the interaction of the peptides and amino acids in yogurt with the high content of carbohydrates in LH has synergistic effects to protect the beneficial probiotics (Zhu et al., 2023b). Likewise, yogurts enhanced with *G. frondose* (Aleman et al., 2023) and *Lentinula edodes* stipe powders (Zhu et al., 2023b) also favored the viability of LAB cells during storage.

Effect of LH on yogurt pH and TA

The pH and TA of yogurt are crucial factors that impact the overall quality of the product, as they can indicate the presence of microorganisms in a particular food and the effect of organic acids on taste (Rashwan et al., 2022). Herein, the pH of yogurt samples ranged from 4.30 to 4.73, and the TA ranged from 0.56% to 1.10% (Fig. 2). The values in fortified samples were generally within the desirable range for pH (3.24-4.59) and TA (0.72%-1.20%) of commercially available fermented milk as suggested by previous studies (Cho et al., 2020), which are important for consumer acceptance. Over time in storage, the pH levels of yogurt samples decreased as more LH was added, leading to an increase in their TA ($p < 0.05$, Fig. 2). This could be due to the abilities of prebiotics in LH to enhance the growth of starter cultures, leading to a quicker transformation of lactose into lactic acid. The lactic acid contributes to the yogurt formation through the coagulation of milk protein, and it is the main contributor to the typical acidic taste and odor of yogurt, which is positively responsible for giving yogurt a nice flavor (Montemurro et al., 2023; Kaur et al., 2020). By the conclusion of the storage period, a decline in pH and rise in TA were evident in all yogurt samples as shown in Fig. 2. This is caused by the post-acidification of LAB that occurs during storage

(Du et al., 2021). The highest TA value occurred in the 3.0%-LH fortified yogurt as predicted.

Effect of LH on yogurt viscosity and syneresis

The viscosity of yogurt is related to mouthfeel and may affect its appeal to consumers (Aleman et al., 2023). After the yogurt finished fermenting and forming a gel, the LH powder was mixed in, resulting in a smooth, thick, yet still pourable semi-solid with gentle mixing. The values of viscosity significantly decreased with increasing concentrations of LH and the extension of storage time ($p < 0.05$, Fig. 3A). The findings of Aleman et al. (2023) indicated that incorporating mushroom powder had an adverse impact on the viscosity of the yogurt, potentially caused by the mushroom particles disrupting the gel network within the yogurt matrix, leading to decreased viscosity. On the other hand, the addition of LH raised the growth of starter culture. According to reports, increased LAB levels may lead to the breakdown of milk solids or pH-related alterations in casein micelles, ultimately reducing gel strength (Kang et al., 2018), resulting in a decrease in yogurt viscosity. Likewise, these findings aligned with earlier studies indicating that the viscosity of yogurt decreased as more *L. edodes* stipe (Zhu et al., 2023b), *G. frondosa* (Aleman et al., 2023), and *P. ostreatus* polysaccharides (Radzki et al., 2023) were added.

Syneresis is considered another common defect in yogurt manufacturing (Kim et al., 2019). The network in acidic milk gel, which was produced by bacterial fermentation, could entrap serum inside (Rashwan et al., 2022), and syneresis occurs when serum drains from the yogurt gel. Consumer perception of yogurt may be impacted negatively by syneresis, which is a crucial factor influencing the stability and taste of yogurt (Du et al., 2021; Kwon et al., 2019). As shown in Fig. 3B, the control exhibited a rising trend in syneresis during refrigeration, and the addition of LH powder to the yogurt significantly increased it in a dose-dependent manner ($p < 0.05$). Possibly, the higher syneresis in yogurts fortified with LH may

be due to the yogurt's lower pH, which can reduce the stability of casein micelles and lead to whey separation (Cho et al., 2020). In addition, LH powder enhanced the growth of starter cultures in yogurt. The enzymatic action of the initial culture has the potential to degrade the protein structure, leading to a decrease in colloidal connections and a reduction in the size of milk protein aggregates, ultimately hindering the ability to retain water molecules (Dimitrova-Shumkovska et al., 2022). The weakening of the gel network in the yogurt caused it to lose its ability to retain whey within the gel structure, leading to liquid expulsion during storage (Dönmez et al., 2017).

Effect of LH on yogurt texture

Yogurt's gel structure is achieved through the aggregation of casein due to a decrease in pH and the bonding of caseins with denatured whey proteins through disulfide bonds (Rashwan et al., 2022). The addition of LH had a notable impact on the texture of the yogurt, as indicated by the texture profile analysis. It resulted in decreased cohesiveness and viscosity index, increased consistency, and comparable firmness when compared to the control ($p < 0.05$, Table 2). Typically, the firmness of yogurt is the primary factor used to evaluate its texture, referring to how well the product holds its shape when pressure is applied (Mousavi et al., 2019). The firmness of the control yogurt increased as it was stored, likely due to the rise in TA, leading to a contraction in gel structure and an increase in gel strength (Meena et al., 2022). Adding LH to the yogurt makes the firmness more stable, showing a slight decrease from the cold storage period of 1 d to 28 d ($p > 0.05$). The findings were consistent with the storage characteristics of set yogurt containing pineapple pomace powder (Meena et al., 2022).

The presence of LH led to higher consistency and lower cohesiveness values in yogurt during cold storage, with the effect being dependent on the dosage. The consistency of yogurt is a crucial factor that can enhance consumer approval (Jovanović et al., 2020). Except for the

0.05% LH-enhanced yogurt, which exhibited greater consistency than the 1.5% LH-enhanced yogurt, all yogurt samples fortified with LH powder had a higher consistency than the control, influenced by both the day and concentration factors (Table 2). The increased consistency of the enriched samples may be attributed to the synergy between polyphenols in LH and proteins in yogurt, which enhanced the fragile structure of the gel network within the yogurt mixture and was also potentially affected by the pH levels (Du et al., 2021). As a result, the weak post-acidification during cold storage might be responsible for the increase in consistency by strengthening protein-protein interactions and the formation of yogurt structure. Our findings are in agreement with the yogurts enriched with apple pomace (Jovanović et al., 2020) and mulberry pomace (Du et al., 2021) as beneficial components.

Cohesiveness refers to a product's ability to stick together, maintained by the inner bonds that uphold its form (Rashwan et al., 2022). Cohesiveness also relates to the extent of deformability of materials before they are broken down (Du et al., 2021). Therefore, assessing the texture of yogurt is crucial in determining its quality. Cohesiveness values among the control and LH-fortified yogurts showed the opposite results during the storage period. This might be due to the fermentation of the yogurt over time and the semi-solid structure’s breakdown into a liquid structure. The viscosity index results were in line with the viscosity data, indicating an intensified trend with a lower LH level. Specifically, the texture parameters of 0.5% LH-enhanced yogurt showed comparable values to the control after 1 d and 14 d of cold storage, indicating that this dosage could be ideal for large-scale manufacturing.

Antioxidant assays

Antioxidants help lower the chances of chronic illnesses by inhibiting or delaying oxidative harm to lipids, proteins, and nucleic acids, which is triggered by free radicals, unstable molecules within our bodies (Aramouni et al., 2023). Thus, enhancing yogurt with

natural antioxidants is a secure and efficient method to create antioxidant-packed functional yogurt that meets consumer expectations and enhances the health advantages of yogurt (Hamed et al., 2021).

In our study, the incorporation of LH in yogurt samples significantly increased the antioxidant activity and TPC compared to the control ($p < 0.05$, Table 3). The ABTS and DPPH radical scavenging activities were used to assess the overall antioxidant capacity of the yogurt samples. Due to the multifunctionality of most natural antioxidant compounds, it is not possible for a single method to completely elucidate the antioxidant activity of samples (Zhu et al., 2023a). Therefore, multiple antioxidant methods were employed to gain insight into the mechanisms of action. Table 3 summarized the DPPH and ABTS radical scavenging assays of yogurts, measured in ascorbic acid equivalents (AAE). The presence of LH in yogurt led to a notable rise in antioxidant activity compared to the control during the entire storage period ($p < 0.05$), with the 3.0% LH-enriched yogurt consistently showing the greatest ABTS and DPPH radical scavenging activity. Confirming earlier findings, LH may be viewed as a powerful radical scavenger, with LH water extracts showing AAE values of 158.62 ± 6.51 and 148.09 ± 1.34 mg ascorbic acid/g dw for DPPH and ABTS radical quenching, surpassing *Russula pseudocyanoxantha*, *Paraguayan Ganoderma* species, and *Cantharellus cibarius* (Zhu et al., 2023a). Additionally, yogurt itself contains antioxidant properties due to amino acids and bioactive peptides produced during fermentation by proteolytic degradation (Lee et al., 2021).

The TPC was measured in each yogurt sample over a period of 28 d, showing consistent trends with the ABTS and DPPH assays (Table 3). The minimal level of TPC in the control, which remained stable throughout storage, may be attributed to the presence of glucose (resulting from the breakdown of milk lactose) and amino acids (containing phenolic side chains) that disrupted the TPC reaction and measurement process (Dimitrova-Shumkovska et al., 2022). As it can be noticed in Table 3, there was a marked increase of TPC ($p < 0.05$) in

yogurt fortified with a relatively low concentration of LH (0.5%). Moreover, the LH powder led to a progressive increase in TPC in every enriched yogurt sample during storage, with the effect lasting until the end of the study ($p < 0.05$), indicating a beneficial impact on the antioxidant capacity of dairy items. It has been measured that the TPC of LH water extracts was 6.71 ± 0.53 mg GAE/g extract dw, and the TPC was positively and significantly correlated with its antioxidant capacities (Zhu et al., 2023a). The TPC obtained from LH is essential for improving the antioxidant strength of fortified yogurt and is advantageous for boosting the functionality of yogurt.

On the other side, extended refrigeration of the enriched samples for 14 d resulted in a comparable rise in TPC and antioxidant capacity in all LH-enriched yogurts, consistent with the findings of Dimitrova-Shumkovska et al. (2022). The rise in levels could potentially be attributed to the liberation of LH polyphenols from the yogurt mixture, possibly triggered by changes in pH, modifications in protein structure, or alterations in enzyme or bacterial activity (Meena et al., 2022). The reduction in total phenolic content of all samples after 28 d could be attributed to the growing interactions between milk proteins and polyphenols, as well as the breakdown of polymeric phenolics in the presence of LAB during storage (Kim et al., 2019).

Sensory analysis

Sensory evaluation is one of the crucial quality factors for consumers' food acceptance (Kim et al., 2023). Sensory tests were conducted for appearance, fermented odor, texture, taste quality, and overall acceptance of yogurt with different ratios of LH during storage (Fig. 4). Generally, the 0% fortified yogurt at the storage time of 1 d had the highest score of texture ($p < 0.05$), and the 0.5% fortified yogurt gained the maximum values of fermented odor and taste quality at 14 d ($p < 0.05$). LH is famous for its delicious taste, aromatic flavor, and nutritious values (Xiang et al., 2022). Therefore, the addition of LH significantly improved the fermented odor and taste quality ($p < 0.05$). But supplementation with LH at a

relative high level (e.g., 3%) had negative effects on these two sensory parameters. The higher scores of the appearance in the 0.5% and 1.5% fortified yogurt might be related to the color of LH, since colors such as orange or yellow are commonly related to vegetables or fruits (Aleman et al., 2023). On the other hand, the texture parameter decreased as the proportion of LH increased ($p < 0.05$), which was probably because of the lower viscosity and higher syneresis. Overall, the 0.5% LH-fortified yogurt exhibited similar sensory scores compared with the control, suggesting that this dose might be ideal for industrial production.

Conclusion

The current study revealed that the incorporation of LH in stirred-type yogurt improved the physicochemical, microbiological, and antioxidant properties. Yogurt enriched with LH led to lower lightness, pH values, and viscosity; higher red-green and yellow-blue color values, levels of TA and viable LAB cells, syneresis, and antioxidant activities against ABTS and DPPH radicals during cold storage when compared to control samples. According to texture analyses, the addition of LH reduced firmness, cohesiveness, and viscosity index, but increased consistency. The yogurt fortified with 0.5% LH showed comparable textural index values and sensory scores to the control yogurt at the cold storage times of 1 d and 14 d, indicating that this could be the ideal amount for large-scale manufacturing. Thus, LH may serve as a desirable component in producing nutritious yogurts that cater to the needs of health-conscious consumers and the dairy industry.

Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgements

This work was supported by grants from the Natural Science Foundation of Hunan Province (No. 2022JJ40013) and Scientific Research Foundation of Hunan Provincial Education Department (No. 23B0667) to Hanyu Zhu.

Author Contributions

Conceptualization: Zhu H. Data curation: Li G, Liu H. Formal analysis: Liu H, Sun W. Methodology: Yao X, Wu R, Hu J, Yang Q. Validation: Li G, Sun W. Investigation: Yao X. Writing - original draft: Zhu H, Li G. Writing - review & editing: Zhu H, Li G, Liu H, Sun W, Yao X, Wu R, Hu J, Yang Q.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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Tables and Figures

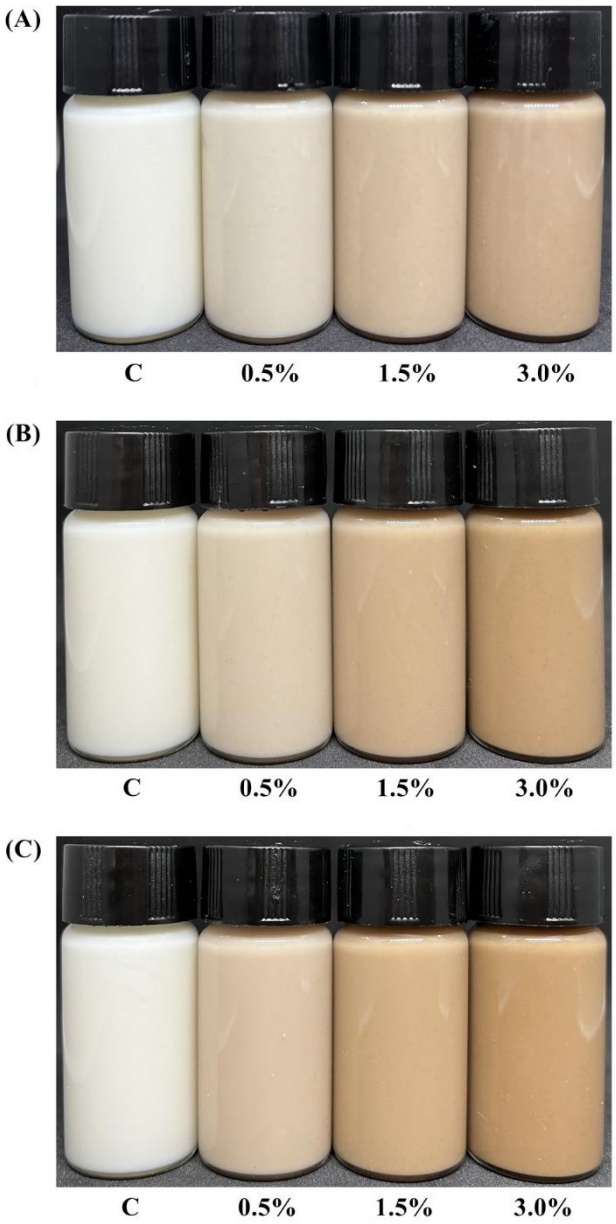

Fig. 1. Appearance of yogurt samples with different amounts of *Lactarius hatsudake* (LH) added at the storage times of 1 d (A), 14 d (B), and 28 d (C). C: the 0% fortified yogurt (as the control); 0.5%: 0.5% LH-fortified yogurt; 1.5%: 1.5% LH-fortified yogurt; 3.0%: 3.0% LH-fortified yogurt.

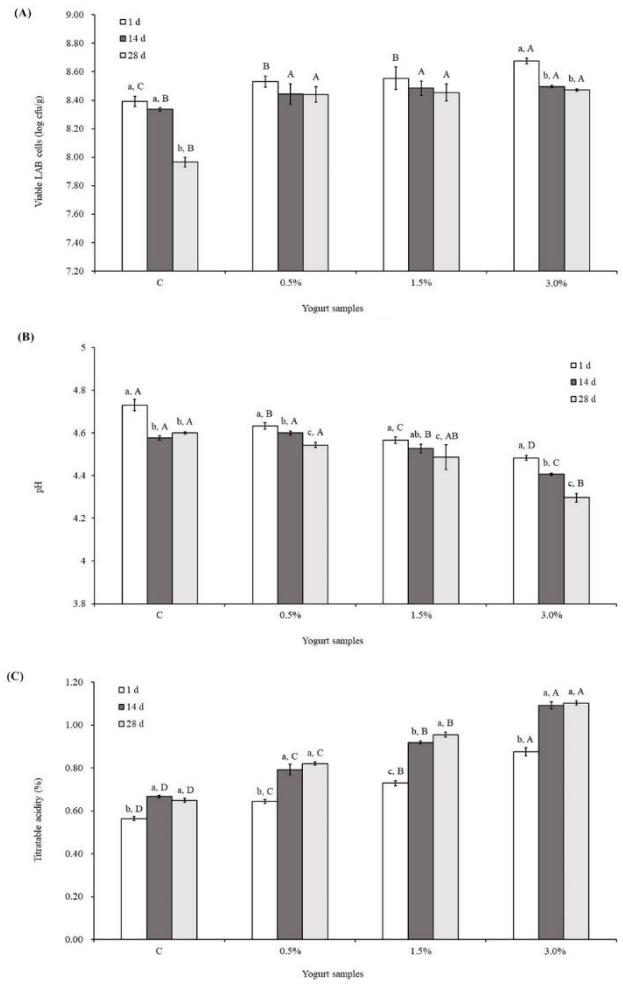

Fig. 2. The viable LAB cells (A), pH (B), and titratable acidity (C) of stirred yogurt fortified with different amounts of *Lactarius hatsudake* (LH) during cold storage. C: the 0% fortified yogurt (as the control); 0.5%: 0.5% LH-fortified yogurt; 1.5%: 1.5% LH-fortified yogurt; 3.0%: 3.0% LH-fortified yogurt. Different lowercase letter superscripts above the columns indicate statistically significant differences $p < 0.05$ between the yogurts at different storage times, and different capital letter superscripts above the columns indicate statistically significant differences $p < 0.05$ between the yogurts fortified with different amounts of LH (One-way Analysis of Variance followed by Duncan *post hoc* test).

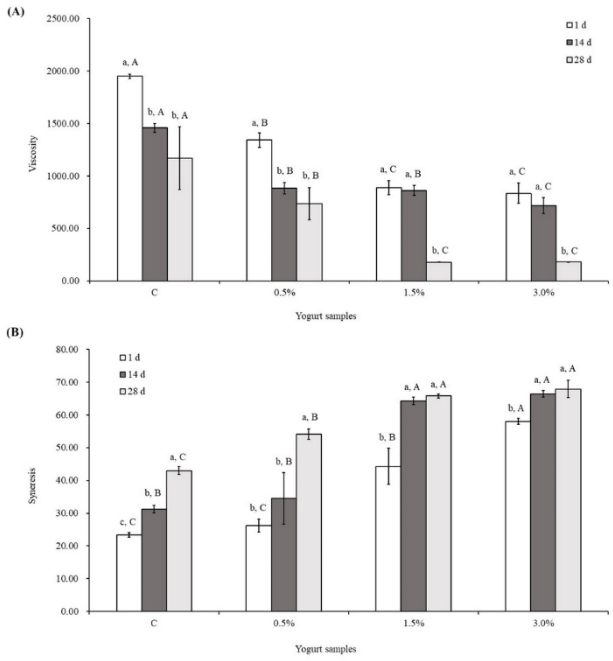

Fig. 3. The viscosity (A) and syneresis (B) values of stirred yogurt fortified with different amounts of *Lactarius hatsudake* (LH) during cold storage. C: the 0% fortified yogurt (as the control); 0.5%: 0.5% LH-fortified yogurt; 1.5%: 1.5% LH-fortified yogurt; 3.0%: 3.0% LH-fortified yogurt. Different lowercase letter superscripts above the columns indicate statistically significant differences $p < 0.05$ between the yogurts at different storage times, and different capital letter superscripts above the columns indicate statistically significant differences $p < 0.05$ between the yogurts fortified with different amounts of LH (One-way Analysis of Variance followed by Duncan *post hoc* test).

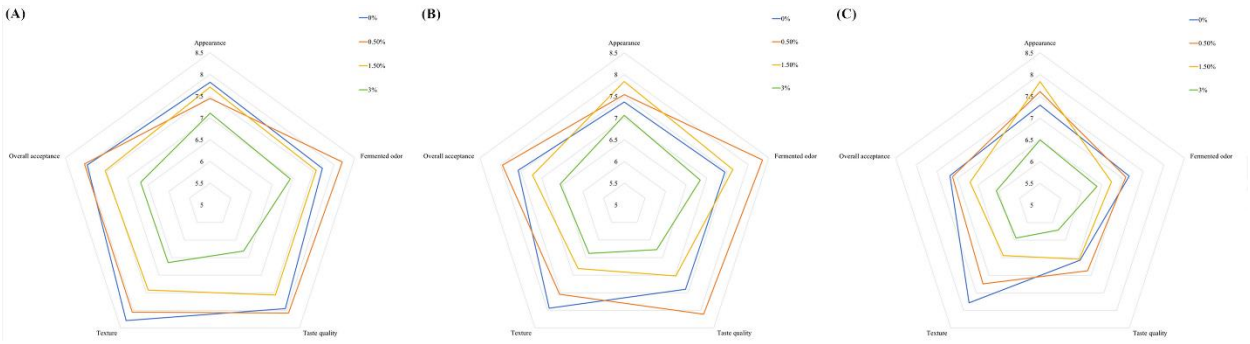

Fig. 4. Sensory analysis of stirred yogurt fortified with *Lactarius hatsudake* (LH) during cold storage. (A) Sensory analysis of yogurt samples stored at 4℃ for 1 d. (B) Sensory analysis of yogurt samples stored at 4℃ for 14 d. (C) Sensory analysis of yogurt samples stored at 4℃ for 28 d. C: the 0% fortified yogurt (as the control); 0.5%: 0.5% LH-fortified yogurt; 1.5%: 1.5% LH-fortified yogurt; 3.0%: 3.0% LH-fortified yogurt.

Table 1. Color analysis of the stirred yogurt incorporated with *Lactarius hatsudake* (LH) during the refrigerated storage

Parameter[1]	Treatment	Storage time (d)		
		1	14	28
L*	C	92.45 ± 0.43^{A}	93.06 ± 0.31^{A}	92.73 ± 0.90^{A}
	0.5%	$80.19 \pm 0.03^{a, B}$	$76.06 \pm 0.85^{b, B}$	$67.07 \pm 1.09^{c, B}$
	1.5%	$65.55 \pm 0.67^{a, C}$	$59.98 \pm 0.99^{b, C}$	$55.21 \pm 0.24^{c, C}$
	3.0%	$56.68 \pm 0.47^{a, D}$	$50.30 \pm 0.74^{b, D}$	$46.74 \pm 0.40^{c, D}$
a*	C	-1.71 ± 0.14^{D}	-1.91 ± 0.06^{D}	-1.85 ± 0.05^{D}
	0.5%	$2.58 \pm 0.10^{c, C}$	$3.84 \pm 0.27^{b, C}$	$7.13 \pm 0.12^{a, C}$
	1.5%	$5.54 \pm 0.09^{c, B}$	$7.75 \pm 0.14^{b, B}$	$9.28 \pm 0.16^{a, A}$
	3.0%	$6.52 \pm 0.05^{c, A}$	$8.50 \pm 0.08^{b, A}$	$8.87 \pm 0.20^{a, B}$
b*	C	$11.78 \pm 0.05^{a, D}$	$10.41 \pm 0.19^{b, D}$	$11.12 \pm 0.39^{a, C}$
	0.5%	$15.06 \pm 0.25^{c, C}$	$15.79 \pm 0.16^{b, C}$	$19.24 \pm 0.22^{a, B}$
	1.5%	$19.17 \pm 0.27^{c, B}$	$20.62 \pm 0.40^{b, B}$	$23.19 \pm 0.12^{a, A}$
	3.0%	$21.98 \pm 0.21^{b, A}$	$22.32 \pm 0.30^{b, A}$	$23.18 \pm 0.38^{a, A}$

All values are means ± SD (n = 3). Different lowercase letter superscripts in the same line indicate statistically significant differences $p < 0.05$ between the yogurts at different storage time (One-way Analysis of Variance followed by Duncan *post hoc* test). Different capital letter superscripts in the same column indicate statistically significant differences $p < 0.05$ between the yogurts fortified with different amounts of LH (One-way Analysis of Variance followed by Duncan *post hoc* test). [1]L* = lightness; a* = red-green color; b* = yellow-blue color. C: the 0% fortified yogurt (as the control); 0.5%: 0.5% LH-fortified yogurt; 1.5%: 1.5% LH-fortified yogurt; 3.0%: 3.0% LH-fortified yogurt.

Table 2. Texture profile analysis of *Lactarius hatsudake* (LH)-fortified yogurt during cold storage of 28 d

Parameter	Treatment	Storage time (d)		
		1	14	28
Firmness (g)	C	25.96 ± 0.97^{b}	$31.46 \pm 4.54^{a, A}$	$29.03 \pm 0.12^{ab, A}$
	0.5%	25.53 ± 0.54	26.08 ± 2.67^{AB}	24.97 ± 0.12^{B}
	1.5%	24.01 ± 0.83	24.45 ± 0.60^{B}	23.54 ± 0.93^{C}
	3.0%	26.24 ± 2.84	24.17 ± 0.81^{B}	25.61 ± 0.68^{B}
Consistency (g × s)	C	$115.80 \pm 2.22^{b, B}$	$126.14 \pm 3.03^{a, B}$	$125.14 \pm 0.62^{a, D}$
	0.5%	$116.37 \pm 2.17^{c, B}$	$125.29 \pm 3.47^{b, B}$	$138.02 \pm 0.56^{a, B}$
	1.5%	$118.59 \pm 5.45^{b, B}$	$133.23 \pm 2.91^{a, A}$	$129.40 \pm 1.52^{a, C}$
	3.0%	$137.66 \pm 1.85^{b, A}$	$132.30 \pm 1.51^{c, A}$	$142.95 \pm 1.55^{a, A}$
Cohesiveness (g)	C	9.36 ± 0.57^{b}	$12.22 \pm 0.55^{a, A}$	$12.66 \pm 0.62^{a, A}$
	0.5%	9.12 ± 0.80^{a}	$8.36 \pm 2.80^{ab, B}$	$6.09 \pm 0.48^{b, B}$
	1.5%	7.85 ± 1.34^{a}	$5.97 \pm 0.21^{b, B}$	$5.70 \pm 0.24^{b, B}$
	3.0%	8.00 ± 2.10	5.74 ± 0.31^{B}	5.50 ± 0.12^{B}
Viscosity index (g × s)	C	$13.98 \pm 1.56^{b, A}$	$20.17 \pm 0.93^{a, A}$	$21.80 \pm 0.49^{a, A}$
	0.5%	$12.40 \pm 5.62^{a, AB}$	$6.99 \pm 1.36^{ab, B}$	$5.19 \pm 0.55^{b, B}$
	1.5%	$9.27 \pm 2.94^{a, AB}$	$4.64 \pm 0.38^{b, C}$	$4.44 \pm 0.78^{b, B}$
	3.0%	$6.79 \pm 1.78^{a, B}$	$3.83 \pm 0.66^{b, C}$	$3.85 \pm 0.84^{b, B}$

All values are means ± SD (n = 3). Different lowercase letter superscripts in the same line indicate statistically significant differences $p < 0.05$ between the yogurts at different storage time (One-way Analysis of Variance followed by Duncan *post hoc* test). Different capital letter superscripts in the same column indicate statistically significant differences $p < 0.05$ between the yogurts fortified with different amounts of LH (One-way Analysis of Variance followed by Duncan *post hoc* test). C: the 0% fortified yogurt (as the control); 0.5%: 0.5% LH-fortified yogurt; 1.5%: 1.5% LH-fortified yogurt; 3.0%: 3.0% LH-fortified yogurt.

Table 3. Antioxidant assays and total phenolic content of *Lactarius hatsudake* (LH)-fortified yogurt during the cold storage

Antiradical assays	Treatment	Storage time (d)		
		1	14	28
AAE_{DPPH} (mg ascorbic acid/g) [a]	C	3.36 ± 0.60^{D}	2.95 ± 0.28^{D}	2.65 ± 0.11^{D}
	0.5%	$5.92 \pm 0.44^{b, C}$	$6.71 \pm 0.07^{a, C}$	$4.60 \pm 0.23^{c, C}$
	1.5%	$7.21 \pm 0.49^{ab, B}$	$8.50 \pm 0.48^{a, B}$	$5.99 \pm 1.01^{b, B}$
	3.0%	$8.93 \pm 0.33^{b, A}$	$10.04 \pm 0.12^{a, A}$	$8.08 \pm 0.33^{c, A}$
AAE_{ABTS} (mg ascorbic acid/g) [b]	C	$2.36 \pm 0.09^{a, D}$	$1.53 \pm 0.41^{b, D}$	$1.17 \pm 0.23^{b, D}$
	0.5%	6.50 ± 0.73^{C}	6.75 ± 0.44^{C}	6.03 ± 0.10^{C}
	1.5%	$8.67 \pm 0.23^{b, B}$	$10.38 \pm 0.34^{a, B}$	$7.11 \pm 0.27^{c, B}$
	3.0%	$10.58 \pm 0.19^{b, A}$	$11.98 \pm 0.29^{a, A}$	$10.03 \pm 0.15^{c, A}$
TPC (μg gallic acid/g) [c]	C	29.50 ± 4.71^{D}	24.82 ± 0.25^{D}	23.26 ± 2.89^{D}
	0.5%	$92.20 \pm 1.29^{b, C}$	$103.26 \pm 1.81^{a, C}$	$70.79 \pm 2.18^{c, C}$
	1.5%	$124.95 \pm 2.78^{b, B}$	$177.57 \pm 1.81^{a, B}$	$123.39 \pm 0.43^{b, B}$
	3.0%	$182.97 \pm 2.^{37b, A}$	$196.43 \pm 3.63^{a, A}$	$181.70 \pm 8.69^{b, A}$

All values are means ± SD (n = 3). Different lowercase letter superscripts in the same line indicate statistically significant differences $p < 0.05$ between the yogurts at different storage time (One-way Analysis of Variance followed by Duncan *post hoc* test). Different capital letter superscripts in the same column indicate statistically significant differences $p < 0.05$ between the yogurts fortified with different amounts of LH (One-way Analysis of Variance followed by Duncan *post hoc* test). [a] Ascorbic acid equivalent antioxidant capacity (AAE), 1,1-diphenyl-2-picrylhydrazyl (DPPH); [b] 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS); [c] total phenolic content (TPC). C: the 0% fortified yogurt (as the control); 0.5%: 0.5% LH-fortified yogurt; 1.5%: 1.5% LH-fortified yogurt; 3.0%: 3.0% LH-fortified yogurt.