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Research Article

Effect of High-Pressure Treatment on the Quality of a *Hericium erinaceus*: Millet Composite Beverage

Zhiguo Zhou,[1,2,3,4] Xiaolan Shang,[1,2,3,4] Zixin Wang,[1] Chengxi Sun,[1] and Lin Zhang[1,2]

[1]*College of Life Science, Langfang Normal University, Langfang 065000, China*
[2]*Technology Innovation Center for Utilization of Edible and Medicinal Fungi in Hebei Province, Edible and Medicinal Fungi Research and Development Center of Hebei Universities, Langfang 065000, China*
[3]*Langfang Key Laboratory of Microbial Fermentation, Langfang, China*
[4]*Langfang Key Laboratory of Food Nutrition and Safety, Langfang, China*

Correspondence should be addressed to Xiaolan Shang; iris381@163.com

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Hericium erinaceus-millet (HM) composite beverage was prepared by mixing *Hericium erinaceus* juice and millet juice and then subjected to high-pressure processing (HPP) of 300 MPa and 600 MPa at room temperature and thermal processing (TP) of 100°C for 3 min. The differences in pH, total soluble solids, amino acid nitrogen content, total colony numbers, sensory scores, and shelf life of HPP-treated and TP-treated samples stored at 4°C, 27°C, and 37°C for 80 days and the differences in volatile substances stored at 4°C and 27°C for 30 days were studied. The results showed that there was no significant difference in total soluble solids, amino acid nitrogen, and pH when comparing HPP- and TP-treated HM beverages. The order of HM beverages' shelf life following different treatments was as follows: TP-treated >600 MPa HPP-treated >300 MPa HPP-treated. When stored at 4°C, the shelf life of the three treatments was 63, 52, and 39 days, respectively. Compared with TP-treated beverages, HPP-treated beverages better retained their ester flavor compounds, especially ethyl acetate. Moreover, the main volatile compounds in TP-treated beverages changed more during storage than those in HPP-treated beverages. Overall, HPP-treated beverages had advantages in terms of flavor over TP-treated beverages.

1. Introduction

Hericium erinaceus is an edible fungus used as both a medicine and food; it is known for its delicious taste and nutritional quality [1]. It is high in protein and carbohydrates, containing 26.3 g of protein and 4 g of crude polysaccharide per 100 g [2]. In addition to its nutritional benefits and attractive taste, *Hericium erinaceus* benefits the internal organs, helps digestion, and has unique digestive-tract protection, conditioning, and repair functions. *Hericium erinaceus* polysaccharides can promote the growth of gastrointestinal probiotics, enhance human immunity, and resist gastrointestinal tumors [3, 4]. Vigna et al. [5] found that taking *Hericium erinaceus* orally can reduce the symptoms of depression, anxiety, and sleep disorders. Given its physiological effects, *Hericium erinaceus* has become an important raw material for food processing as well as health food research and development.

Millet, also known as foxtail millet, has high nutritional value. It contains many indispensable nutrients, such as carbohydrates, proteins, fats, vitamins, and minerals. It also has medicinal value [6, 7]. Particularly, millet protein has a complete range of amino acids, and it is rich in eight essential amino acids, which account for 41.9% of its total amino acids [8].

Currently, beverages sold on the market are mainly sterilized using thermal sterilization. This process can prolong the shelf life of beverages, but it also reduces their nutritional value and affects their volatile compounds [9–11]. In recent years, high-pressure sterilization methods

have started to be used in the beverage industry rather than thermal sterilization [12, 13]. High-pressure processing (HPP) refers to the use of 100–1000 MPa pressure to treat raw materials. As a nonthermal processing technology, it can inactivate microorganisms and/or enzymes under high pressure [14, 15]. Compared with traditional thermal processing (TP), the advantage of HPP is the retention of more nutrients and volatile compounds in food, especially volatile compounds. Zhu et al. [16] used different sterilization methods (conventional pasteurization, microwave sterilization, ultrasonic sterilization, and high-pressure sterilization) to treat cloudy apple juice and found that high pressure can effectively retain volatile compounds. Pan et al. [17] conducted a high-pressure treatment on Tainong mango juice and found that high-pressure treatment can better retain the volatile compounds in the juice.

Most of the research on fruits and vegetables under high pressure focuses on the direct high-pressure treatment of a single fruit or vegetable juices after juice extraction, such as pineapple fruit [18], hawthorn berry [19], and tomato juices [20]. However, there is limited research on the products that first extract different fruit or vegetable juices, then add additives for compounding, and then carry out a high-pressure treatment. In the present study, a previously prepared *Hericium erinaceus*-millet (HM) composite beverage was subjected to different pressure (300 and 600 MPa). The effects on the beverage were then compared with those arising from TP treatment. Specifically, the differences in pH, total soluble solids, amino acid nitrogen content, total colony numbers, sensory scores, shelf life, and volatile compounds in beverage were assessed following the various treatments. Overall, this research provides a scientific basis for the industrial production of high-pressure processed beverages.

2. Materials and Methods

2.1. Materials. *Hericium erinaceus* and millet were purchased from the local Walmart supermarket; amylase and cellulase were purchased from Xiaosheng Industrial Group Co., Ltd; and all other chemicals used in this study, which were of analytical grade, were purchased from Solabio Corporation.

2.2. Preparation of HM Composite Beverage. Dried *Hericium erinaceus* was crushed into powder using a pulverizer (RS-FS1801, Rongshida Group Co., Ltd., China). Water was added to give an *Hericium erinaceus* powder: water ratio of 1 : 40, and 0.3% cellulase was added for enzymolysis in a 50°C water bath for 2 h. After killing the enzyme with a 50°C water bath, the solution was filtered using four layers of gauze, and 0.04% diatomite was added for 2 days to obtain *Hericium erinaceus* juice. Using the aforementioned pulverizer, the millet was also crushed into powder. Water was added to produce a millet flour: water ratio of 1 : 40. The solution was heated to 80°C, and 0.6% amylase was added for enzymolysis for 1.5 h. After the enzyme was killed using boiling water, the solution was filtered using four layers of gauze, and 0.04% diatomite was added. The miscellaneous foam was removed, and the solution was maintained for 2 days to obtain millet juice. Subsequently, the *Hericium erinaceus* juice and millet juice were mixed using a ratio of 3 : 1, after which 10% white granulated sugar and 0.15% citric acid were added to every 100 mL of mixed solution to obtain the HM composite beverage. This was injected into high temperature- and high-pressure-resistant plastic (polyethylene terephthalate) bags. For HPP, high-pressure equipment (HPP.L1-600 MPa/2 L, TJHTSM Bioengineering Co., Ltd., China) was used to subject the HM beverage to high pressure of 300 and 600 MPa at room temperature for 10 min. The come-up time and release time were approximately 20 and 15 s, respectively. Water was used as the medium throughout the high-pressure treatment process. The quality of HPP-treated beverages was compared with that of TP-treated beverages (i.e., treated at 100°C for 3 min).

2.3. Storage Conditions. HM beverages not subjected to sterilization were used as a control group, whereas the HM beverages treated with HPP and TP represented the HPP and TP treatment groups, respectively. The samples were stored at 4°C, 27°C, and 37°C, respectively, and stored samples were tested at 0, 7, 14, 28, 42, 56, and 80 days to determine the changes in quality and shelf life of the beverages during storage.

2.4. Determination of pH and Total Soluble Solids. The pH values of the samples were measured at room temperature using a pH meter (PB-10, Sartorius, Germany) according to the method described in GB 5009.237–2016 "National Food Safety Standard Determination of Food pH." The total soluble solids (TSS) of samples were measured using a digital refractometer (RP-101, Atago, Tokyo, Japan) at ambient temperature (20°C) and expressed as Brix according to the method of Raj et al. [21].

2.5. Determination of Amino Acid Nitrogen Content. Amino acid nitrogen content was determined according to the method of Luan et al. [22]. First, 5 mL of the sample was diluted to 100 mL using water, and 20 mL of this solution was collected. To this, 60 mL of water was added, and the solution was titrated with 0.050 M sodium hydroxide standard solution until the pH, as indicated by an acidimeter, was 8.2. Subsequently, 10 mL of formaldehyde solution was added, the new solution was mixed well, and it was titrated with sodium hydroxide standard titration solution until the pH was 9.2. To conduct a blank reagent test, 80 mL of water was adjusted to pH 8.2 using 0.050 M sodium hydroxide standard solution, after which 10.0 mL of formaldehyde solution was added, and the solution was titrated to pH 9.2 using sodium hydroxide standard titration solution. The solution was continuously stirred during this process.

2.6. Determination of Total Colony Numbers. To determine total colony numbers, a method from GB 4789.2–2016

"National Food Safety Standard Food Microbiological Test for Total Colony Determination" was used with slight modifications. The sample was first diluted with sterile normal saline to an appropriate dilution using a tenfold gradient dilution method. Then, 15–20 mL of nutrient agar medium was added to a sterile Petri dish, and 1 mL of the sample solution was added to the same dish. After mixing evenly, the contents of the dish were cultured at 37°C in an incubator for 48 h. The total number of colonies was counted, and these results were expressed as CFU/mL.

2.7. Sensory Evaluation. To compare the sensory characteristics of each sample, sensory evaluations were conducted. Fifteen selected and trained personnel evaluated the appearance, color, flavor, taste, and general acceptability of the samples. The scores were given via quantitative descriptive analysis; they were recorded using a 100-point system in which appearance, color, flavor, taste, and general acceptability accounted for 20 points each. Each of the 15 personnel evaluated a sample three times. Before tasting each sample, the personnel were asked to gargle with purified water.

2.8. Determination of Shelf Life. Shelf life was determined according to the method of Buvé et al. [23] with slight modifications. The samples were stored in incubators (SPX-150B-Z, Shanghai Boxun Industrial Co., Ltd., China) with temperatures of 4°C, 27°C, and 37°C, respectively, and shelf life was determined at 0, 7, 14, 28, 42, 56, and 80 days. Detection indexes included sensory evaluation and the total number of colonies (according to the methods described above). After detection, the detected values of the various indexes were compared with the standard values. If the total number of colonies was within the required range and the sensory indexes exceeded the standard value range, this showed that sensory score was a key factor affecting the shelf life of the beverage. A sensory score of <80 was used as the end point of shelf life; that is, the shelf life of the beverage had been reached. Using Excel 2016, the sensory score (y) of beverages at each temperature under different treatment methods was regressed with storage time (x), and multiple regression equations were obtained. The sensory index standard value of 80 was used in the regression equation to obtain the shelf life of HM beverages at each temperature under different treatment methods.

2.9. Determination of Volatile Compounds

2.9.1. Sample Preparation. To determine the volatile compounds in beverages, the method of Guimarães et al. [24] was applied with slight modifications. First, a 5 mL sample was added to a 15 mL gas-phase headspace sample bottle, and the sample bottle was placed in a water bath with a constant temperature (55°C) that had been preheated for 20 min. A phase microextraction needle was inserted into the sample bottle, and the sample was extracted for 30 min. Subsequently, the needle was removed and inserted into the sample inlet of a GC–MS system (TRACE 1310, Thermo Scientific Co., Ltd., USA) for desorption at 250°C for 15 min. Samples used to determine volatile compounds were obtained using the same method applied when determining shelf life. Samples treated with 300 MPa HPP, 600 MPa HPP, and TP were stored at 4°C and 27°C, respectively, and the volatile compounds were determined at 0, 15, and 30 days for each treatment.

2.9.2. Chromatographic Conditions. The chromatographic column was TG-5 ms (30 m × 0.25 mm; 0.25 μm). The stationary phase was polyethylene glycol, and the carrier gas was high-purity helium with a flow rate of 1.0 mL/min. The temperature of the injection port was 250°C, and the split ratio was 5 : 1. The initial furnace temperature was 40°C, which was maintained for 3 min. The initial furnace temperature was first increased to 150°C at 4°C/min for 1 min and then to 250°C at 8°C/min for 6 min.

2.9.3. Mass Spectrum Conditions. For the adopted electronic ionization source, the electron energy was 70 EV, and the ion source temperature was 250°C. The interface temperature was 250°C, and the mass scanning range was 43–500 u. A standard tuning file was used, and data acquisition was conducted using full scanning mode. No solvent delay was applied.

2.10. Statistical Analysis. TSS, pH, amino acid nitrogen, total colony numbers, and sensory evaluation were statistically analyzed using Excel 2016 and SPSS 16.0. Principal component analysis (PCA) of volatile compounds was conducted using SIMCA 14.1 software.

3. Results and Discussion

3.1. Changes in TSS Content. The change in TSS content during storage is a key index used to measure the quality of various fruits and vegetables as well as their processed products [25]. As shown in Table 1, during storage at 4°C, HPP and TP had no significant effect on the TSS content of beverages ($P > 0.05$). Similar findings were reported by Subasi and Alpas [26] and Kebede et al. [27]. However, during storage at 27°C and 37°C, although the effect of HPP and TP on TSS content in the early stage of storage was not significant ($P > 0.05$), TSS content increased significantly in the later stage of storage ($P < 0.05$). This may have been due to the deterioration in beverage quality in the later stage, which is consistent with the results of the sensory evaluation (see Section 3.5).

3.2. Changes in pH Levels. As shown in Table 1, the different treatment methods had no significant effect on the pH of beverages ($P > 0.05$); however, pH decreased significantly as storage time was extended ($P < 0.05$). These results are consistent with those of Wu et al. [18] for the treatment of pineapple juice, and they may be attributable to the metabolism of microorganisms in composite beverages [18, 28].

Table 1: Changes in total soluble solids, pH, amino acid nitrogen content, total number of colonies, and sensory scores of samples during storage.

	Storage time (days)	Total soluble solids (Brix)			pH			Amino acid nitrogen content (g/100 mL)			Total number of colonies (CFU/mL)			Sensory scores		
		4°C	27°C	37°C	4°C	27°C	37°C	4°C	27°C	37°C	4°C	27°C	37°C	4°C	27°C	37°C
Control	0	10.00 ± 0.00^{Aa}	10.00 ± 0.00^{Ba}	10.00 ± 0.00^{Ba}	3.51 ± 0.00^{Aa}	3.51 ± 0.00^{Aa}	3.51 ± 0.00^{Aa}	0.011 ± 0.000^{Aa}	0.011 ± 0.000^{Aa}	0.011 ± 0.000^{Aa}	ND	ND	ND	100 ± 1^{Aa}	100 ± 1^{Aa}	100 ± 1^{Aa}
	7	9.75 ± 0.35^{Aa}	10.01 ± 0.01^{Ba}	9.95 ± 0.07^{Ba}	3.17 ± 0.03^{Ba}	3.20 ± 0.06^{ABa}	3.22 ± 0.06^{Ba}	0.025 ± 0.005^{Ba}	0.021 ± 0.000^{Ba}	0.018 ± 0.000^{Aa}	ND	$(3.15 \pm 0.07) \times 103^{Ab}$	$(2.30 \pm 0.00) \times 103^{Aa}$	93 ± 1^{Ba}	75 ± 0^{Bb}	69 ± 1^{Bc}
	14	9.85 ± 0.07^{Aa}	9.83 ± 0.04^{Ba}	10.00 ± 0.14^{Ba}	2.97 ± 0.10^{BCa}	2.57 ± 0.42^{Ba}	2.92 ± 0.02^{Ca}	0.032 ± 0.000^{Ba}	0.280 ± 0.000^{Ca}	0.030 ± 0.002^{Ba}	1.50 ± 0.71^{Ab}	$(1.80 \pm 0.00) \times 104^{Ab}$	$(3.50 \pm 0.14) \times 104^{Ab}$	72 ± 1^{Cb}	53 ± 1^{Cc}	50 ± 1^{Cb}
	28	9.60 ± 0.00^{Aa}	10.00 ± 0.14^{Ba}	10.10 ± 0.00^{Ba}	2.81 ± 0.08^{Ca}	2.81 ± 0.04^{Ba}	2.81 ± 0.04^{CDEa}	0.046 ± 0.005^{Ca}	0.420 ± 0.000^{Da}	0.035 ± 0.000^{BCa}	33.50 ± 2.12^{Ab}	$(2.35 \pm 0.07) \times 107^{Bb}$	$(2.85 \pm 0.07) \times 107^{Ab}$	63 ± 1^{Dd}	44 ± 1^{Db}	41 ± 1^{Dd}
	42	9.75 ± 0.07^{Aa}	9.15 ± 0.07^{Aa}	11.00 ± 0.00^{Cb}	2.85 ± 0.03^{Ca}	2.74 ± 0.06^{Ba}	2.68 ± 0.01^{Ea}	0.048 ± 0.002^{Ca}	0.051 ± 0.002^{Eb}	0.040 ± 0.002^{Ca}	$(1.89 \pm 0.02) \times 105^{Ab}$	$(3.35 \pm 0.21) \times 107^{Cb}$	$(4.15 \pm 0.49) \times 107^{Ab}$	49 ± 1^{Ed}	38 ± 1^{Ec}	33 ± 1^{Ec}
	56	9.85 ± 0.07^{Aa}	10.45 ± 0.07^{Cb}	8.30 ± 0.14^{Aa}	2.86 ± 0.08^{Ca}	2.77 ± 0.04^{Ba}	2.74 ± 0.05^{DEa}	0.062 ± 0.004^{Db}	0.048 ± 0.003^{Ea}	0.050 ± 0.001^{Da}	$(1.21 \pm 0.01) \times 106^{Bb}$	$(3.70 \pm 0.28) \times 107^{Cb}$	$(5.35 \pm 0.07) \times 107^{Ab}$	41 ± 1^{Fd}	31 ± 1^{Fc}	24 ± 1^{Fb}
	80	9.90 ± 0.00^{Aa}	10.80 ± 0.00^{Bb}	11.80 ± 0.14^{CBc}	2.92 ± 0.04^{Ca}	2.81 ± 0.01^{Bb}	2.87 ± 0.02^{CDa}	0.077 ± 0.001^{Ec}	0.076 ± 0.001^{Fc}	0.075 ± 0.004^{Ec}	$(3.45 \pm 0.07) \times 108^{Cb}$	$(3.20 \pm 0.00) \times 109^{Dc}$	$(7.70 \pm 0.28) \times 109^{Bb}$	26 ± 1^{Gd}	16 ± 1^{Gd}	6 ± 1^{Gc}
300 MPa	0	10.00 ± 0.00^{Aa}	10.00 ± 0.00^{Aa}	10.00 ± 0.00^{Aa}	3.50 ± 0.00^{Aa}	3.50 ± 0.00^{Aa}	3.50 ± 0.00^{Aa}	0.014 ± 0.000^{Aa}	0.014 ± 0.000^{Aa}	0.014 ± 0.000^{Aa}	ND	ND	ND	100 ± 0^{Aa}	100 ± 0^{Aa}	100 ± 0^{Aa}
	7	9.90 ± 0.14^{Aa}	9.95 ± 0.07^{Aa}	9.95 ± 0.07^{Aa}	3.14 ± 0.04^{Ba}	3.13 ± 0.04^{Ba}	3.16 ± 0.06^{Ba}	0.028 ± 0.000^{Ba}	0.021 ± 0.000^{Aa}	0.028 ± 0.000^{Bb}	ND	ND	ND	95 ± 1^{Ba}	92 ± 1^{Ba}	90 ± 1^{Bb}
	14	9.90 ± 0.00^{Aa}	9.95 ± 0.07^{Aa}	10.00 ± 0.14^{Aa}	2.92 ± 0.10^{BCa}	2.92 ± 0.03^{Ca}	2.90 ± 0.01^{Ca}	0.037 ± 0.002^{BCab}	0.315 ± 0.000^{BCa}	0.032 ± 0.000^{BCa}	ND	ND	ND	88 ± 1^{Ca}	88 ± 1^{Cb}	88 ± 1^{Ba}
	28	9.90 ± 0.00^{Aa}	10.05 ± 0.07^{Aa}	10.15 ± 0.07^{Aa}	2.77 ± 0.10^{Ca}	2.76 ± 0.06^{Da}	2.79 ± 0.04^{CDEa}	0.044 ± 0.002^{Ca}	0.035 ± 0.000^{BCa}	0.039 ± 0.000^{CDa}	ND	ND	1.50 ± 0.71^{ABa}	84 ± 0^{Dc}	85 ± 1^{Ca}	72 ± 0^{Cc}
	42	9.90 ± 0.00^{Aa}	10.05 ± 0.07^{Ab}	10.45 ± 0.07^{Ba}	2.87 ± 0.02^{Ca}	2.83 ± 0.04^{CDa}	2.71 ± 0.01^{Ea}	0.058 ± 0.003^{Dab}	0.037 ± 0.003^{Ca}	0.044 ± 0.002^{Dab}	ND	1.00 ± 0.00^{Aa}	5.00 ± 1.41^{Ba}	79 ± 1^{Ec}	74 ± 1^{Db}	65 ± 1^{Db}
	56	10.00 ± 0.00^{Aa}	10.10 ± 0.00^{Aa}	10.80 ± 0.00^{Cb}	2.79 ± 0.04^{Ca}	2.72 ± 0.04^{Da}	2.76 ± 0.04^{DEa}	0.042 ± 0.005^{Ca}	0.038 ± 0.004^{Ca}	0.059 ± 0.004^{Ea}	3.00 ± 0.00^{Ba}	4.50 ± 0.71^{ABa}	11.00 ± 1.41^{Ca}	74 ± 0^{Fc}	65 ± 1^{Eb}	58 ± 1^{Ea}
	80	9.93 ± 0.04^{Aa}	10.65 ± 0.07^{Ba}	11.50 ± 0.00^{Dab}	2.89 ± 0.03^{Ca}	2.86 ± 0.01^{CDb}	2.87 ± 0.04^{CDa}	0.044 ± 0.002^{Cb}	0.030 ± 0.002^{Ba}	0.030 ± 0.003^{Ba}	4.00 ± 1.41^{Ba}	8.50 ± 2.12^{Bb}	16.50 ± 2.12^{Ca}	64 ± 1^{Gc}	54 ± 1^{Fc}	42 ± 1^{Fb}
600 MPa	0	10.00 ± 0.00^{Aa}	10.00 ± 0.00^{Aa}	10.00 ± 0.00^{Aa}	3.51 ± 0.01^{Aa}	3.51 ± 0.01^{Aa}	3.51 ± 0.01^{Aa}	0.014 ± 0.000^{Aa}	0.014 ± 0.000^{Aa}	0.014 ± 0.000^{Aa}	ND	ND	ND	100 ± 1^{Aa}	100 ± 1^{Aa}	100 ± 1^{Aa}
	7	9.75 ± 0.35^{Aa}	9.95 ± 0.07^{Aa}	9.90 ± 0.14^{Aa}	3.18 ± 0.03^{Ba}	3.13 ± 0.07^{Ba}	3.17 ± 0.07^{Ba}	0.032 ± 0.005^{Ba}	0.037 ± 0.002^{Bb}	0.019 ± 0.002^{Aa}	ND	ND	ND	93 ± 0^{Ba}	93 ± 1^{Ba}	93 ± 1^{Ba}
	14	9.80 ± 0.00^{Aa}	9.90 ± 0.14^{Aa}	9.90 ± 0.00^{Aa}	2.98 ± 0.11^{BCa}	2.91 ± 0.01^{Ca}	2.91 ± 0.03^{Ca}	0.044 ± 0.002^{Cb}	0.039 ± 0.000^{BCa}	0.035 ± 0.000^{Ba}	ND	ND	ND	91 ± 1^{BCa}	89 ± 1^{Cb}	90 ± 1^{Ca}
	28	9.80 ± 0.00^{Aa}	10.00 ± 0.00^{Aa}	10.05 ± 0.07^{Aa}	2.80 ± 0.08^{CDa}	2.76 ± 0.06^{CDa}	$2.80 \pm 0.01C^{Da}$	0.049 ± 0.000^{Ca}	0.049 ± 0.000^{CDa}	0.042 ± 0.000^{BCa}	ND	ND	ND	89 ± 1^{CDb}	85 ± 1^{Da}	88 ± 1^{Ca}
	42	9.93 ± 0.04^{Aa}	10.10 ± 0.00^{Ab}	10.55 ± 0.07^{Ba}	2.77 ± 0.03^{CDa}	2.84 ± 0.04^{CDa}	2.73 ± 0.03^{Da}	0.071 ± 0.001^{Dc}	0.059 ± 0.004^{Db}	0.044 ± 0.002^{BCab}	ND	ND	ND	86 ± 1^{Db}	80 ± 0^{Ea}	75 ± 1^{Da}
	56	10.25 ± 0.35^{Aa}	10.45 ± 0.07^{Bb}	10.90 ± 0.00^{Cb}	2.73 ± 0.04^{Da}	2.69 ± 0.02^{Da}	2.68 ± 0.01^{Da}	0.039 ± 0.005^{BCa}	0.038 ± 0.004^{Ba}	0.050 ± 0.006^{Ca}	ND	ND	3.00 ± 1.41^{Ba}	79 ± 1^{Eb}	72 ± 1^{Fa}	58 ± 1^{Ea}
	80	9.90 ± 0.00^{Aa}	10.90 ± 0.00^{Cb}	11.15 ± 0.07^{Ca}	$2.87 \pm 0.02C^{Da}$	2.82 ± 0.02^{CDb}	2.93 ± 0.08^{Ca}	0.030 ± 0.002^{Ba}	0.038 ± 0.004^{Bab}	0.044 ± 0.003^{BCb}	2.50 ± 0.71^{Ba}	6.50 ± 2.12^{Bb}	8.00 ± 1.41^{Ca}	71 ± 1^{Fb}	60 ± 2^{Gb}	52 ± 1^{Fa}
Thermal processing (HP)	0	10.00 ± 0.00^{Aa}	10.00 ± 0.00^{Aa}	10.00 ± 0.00^{Aa}	3.50 ± 0.00^{Aa}	3.50 ± 0.00^{Aa}	3.50 ± 0.00^{Aa}	0.028 ± 0.000^{Aa}	0.028 ± 0.000^{Aa}	0.028 ± 0.000^{Aa}	ND	ND	ND	100 ± 0^{Aa}	100 ± 0^{Aa}	100 ± 0^{Aa}
	7	9.75 ± 0.35^{Aa}	10.00 ± 0.00^{Aa}	$10.03 \pm 0.04A^{Ba}$	3.17 ± 0.07^{Ba}	3.20 ± 0.06^{Ba}	3.17 ± 0.04^{Ba}	$0.031 \pm 0.006A^{Ba}$	0.039 ± 0.005^{Bb}	0.033 ± 0.002^{Ab}	ND	ND	ND	95 ± 0^{Ba}	93 ± 0^{Ba}	93 ± 1^{Ba}
	14	9.65 ± 0.35^{Aa}	10.00 ± 0.01^{Aa}	$9.80 \pm 0.00A^{Ba}$	2.98 ± 0.07^{BCa}	2.99 ± 0.11^{BCa}	2.98 ± 0.01^{Ca}	$0.035 \pm 0.000A^{Ba}$	0.042 ± 0.000^{BCa}	0.035 ± 0.000^{ABa}	ND	ND	ND	91 ± 1^{Ca}	92 ± 1^{Ba}	89 ± 1^{Ca}
	28	9.90 ± 0.00^{Aa}	10.05 ± 0.07^{Aa}	10.15 ± 0.07^{Ba}	2.81 ± 0.07^{Ca}	2.80 ± 0.04^{CDa}	2.77 ± 0.04^{Da}	0.042 ± 0.000^{Ba}	0.046 ± 0.000^{BCa}	0.039 ± 0.005^{ABCa}	ND	ND	ND	92 ± 0^{Ca}	87 ± 1^{Ca}	84 ± 1^{Db}
	42	10.25 ± 0.35^{Aa}	10.50 ± 0.00^{Bc}	11.00 ± 0.00^{Cb}	2.82 ± 0.03^{Ca}	2.84 ± 0.04^{CDa}	2.75 ± 0.01^{Da}	0.059 ± 0.004^{Cb}	0.051 ± 0.002^{Cb}	0.051 ± 0.002^{Db}	ND	ND	ND	88 ± 0^{Da}	82 ± 1^{Da}	76 ± 1^{Ea}
	56	10.25 ± 0.35^{Aa}	10.55 ± 0.07^{Bb}	11.05 ± 0.07^{Cb}	2.79 ± 0.02^{Ca}	2.73 ± 0.03^{Da}	2.77 ± 0.04^{Da}	$0.037 \pm 0.003A^{Ba}$	0.044 ± 0.002^{BCa}	0.046 ± 0.005^{BCDa}	ND	ND	ND	82 ± 1^{Ea}	70 ± 0^{Ea}	60 ± 1^{Fa}
	80	10.00 ± 0.00^{Ab}	10.90 ± 0.00^{Cb}	12.10 ± 0.14^{Dc}	2.96 ± 0.04^{Ca}	2.97 ± 0.02^{Ca}	2.91 ± 0.06^{Ca}	0.037 ± 0.002^{ABb}	0.041 ± 0.003^{BCb}	0.050 ± 0.001^{CDb}	ND	ND	1.50 ± 0.71^{Ba}	76 ± 1^{Fa}	66 ± 1^{Fa}	54 ± 1^{Ga}

Note: A–G: means within storage time with different superscript letters are significantly different ($P < 0.05$). a–d: means within processing method with different superscript letters are significantly different ($P < 0.05$). ND: colony count was not observed.

Table 2: Shelf life of samples under different treatment conditions.

(°C)		Control	300 MPa	600 MPa	TP
4°C	Regression equation	$y = -1.0887x + 96.34$	$y = -0.4129x + 96.329$	$y = -0.3294x + 97.35$	$y = -0.2844x + 98.182$
	Shelf life (d)	15	39	52	63
27°C	Regression equation	$y = -1.0797x + 83.119$	$y = -0.5633x + 97.902$	$y = -0.4688x + 97.84$	$y = -0.43x + 98.169$
	Shelf life (d)	3	31	38	43
37°C	Regression equation	$y = -1.1624x + 81.312$	$y = -0.6892x + 96.253$	$y = -0.6299x + 99.766$	$y = -0.5918x + 98.534$
	Shelf life (d)	1	23	31	31

3.3. Changes in Amino Acid Nitrogen Levels. With the extension of storage time, the protein in Hericium erinaceus and millet was gradually decomposed into amino acids [29]. Therefore, the amino acid nitrogen showed an upward trend in the samples treated with 300 MPa, 600 MPa, and TP. However, the content of amino acid nitrogen decreased in the later stage of storage, which may be due to oxidative degradation caused by being stored for too long [30]. Through data analysis of amino acid nitrogen of different treatments, the results showed that there was no significant difference in amino acid nitrogen between HPP- and TP-treated samples during storage at 4°C ($P > 0.05$), but a significant (but still relatively minor) difference was observed between these treatments following storage at 27°C and 37°C ($P < 0.05$). This showed that low temperature is conducive to the maintenance of beverage nutrients during storage [18].

3.4. Changes in the Total Number of Colonies. High pressure is widely used in the sterilization of various beverages, such as carrot-orange mixed juice [31], cloudy apple juice [32], and tomato juice [20]. Usaga et al. [33] found that 600 MPa HPP lasting for 3 minutes can be used in the commercial application of acidic fruit juices and beverages. In this paper, 300 and 600 MPa HPP were used to explore the sterilization effect of HM beverages with acidic characteristics treated under these two pressures for 10 minutes. The results showed that, without HPP or TP treatments, samples produced colonies after 7 days of storage. After 300 and 600 MPa treatments, the time taken to produce colonies was significantly prolonged, indicating that HPP was conducive to prolonging the shelf life of HM beverages [34]. Compared with HPP-treated beverages, TP-treated beverages produced fewer colonies during the same storage period, indicating that 100°C TP is more effective than HPP for sterilization of HM beverages.

3.5. Changes in Sensory Quality. The freshly prepared HM beverage was highly rated for sensory quality (sensory score: 100 out of 100). As storage time was extended, the sensory indexes showed a significant downward trend, especially for HM beverages without HPP or TP treatment. The sensory scores for HPP- and TP-treated HM beverages were higher in the early stage of storage, and the scores for HM beverages stored at low temperature were higher than those for HM beverages stored at high temperature. This was likely due to higher temperature accelerating the reaction speed of substances as well as the corruption and deterioration of the beverages [35].

3.6. Shelf Life of HM Beverages. As shown in Table 2, the shelf life of HM beverages differed under different treatments. The shelf life of HM beverages under TP treatment was the longest, followed by 600 MPa HPP-treated beverages, 300 MPa HPP-treated beverages, and the control group, which was consistent with the total number of colonies. Table 2 also shows that an increase in storage temperature gradually reduced the shelf life of HM beverages, which is consistent with the Arrhenius equation [36].

3.7. Changes in Volatile Compounds

3.7.1. Variety and Content Analysis of Volatile Compounds. Aroma is one of the sensory characteristics that affect the quality of beverages [11]. Therefore, it is very important to retain the aroma components in a beverage during processing. Table 3 shows the volatile compounds and relative contents in HM beverages, and Table 4 shows the types and contents of volatile compounds. Esters accounted for a large proportion of the volatile compounds (Table 4). Particularly, the content of ethyl acetate with fruit flavor [37] was rich; however, the content of ethyl acetate decreased as storage time increased, and it could not be detected in TP-treated samples stored at 27°C for 15 and 30 days. By contrast, ethyl acetate could be detected in HPP-treated samples, which was consistent with the findings of Wu et al. [18], who suggested that HPP-treated products retain esters better than TP-treated products. In total, 13 aldehydes were detected across all samples, among which nonanal and octanal with citrus and flower flavors [38] were rich. Aldehyde content showed an upward trend with the extension of storage time. In total, 27 alcohols were detected; among these, 2-ethylhexanol with an aromatic flavor [39] and octanol with a light flavor [40] were rich. Like aldehyde content, alcohol content increased overall as storage time increased.

3.7.2. PCA of Volatile Compounds in Different Samples. PCA is a method used to simplify the data, changing many interconnected original variables to a few orthogonal principal component variables. In an orthogonal transformation, a set of observations of possibly correlated variables is transformed into linearly uncorrelated variables, called principal components [41]. In this study, HM beverages contained many volatile compounds. PCA was used to distinguish the differences in volatile compounds among the three differently treated HM beverages during storage and to determine the components that play major roles. In total, 40 flavor components contained in more than three samples

Table 3: Volatile flavor components and relative contents in different beverage samples.

Volatile components	300HPP-4°C-0 d	600HPP-4°C-0 d	TP-4°C-0 d	300HPP-4°C-15 d	600HPP-4°C-15 d	TP-4°C-15 d	300HPP-4°C-30 d	600HPP-4°C-30 d	TP-4°C-30 d	300HPP-27°C-0 d	600HPP-27°C-0 d	TP-27°C-0 d	300HPP-27°C-15 d	600HPP-27°C-15 d	TP-27°C-15 d	300HPP-27°C-30 d	600HPP-27°C-30 d	TP-27°C-30 d
Ethyl acetate	55.89	56.39	49.66	53.33	56.68	45.29	37.29	42.73	17.08	50.73	58.26	49.66	38.88	34.76	ND	25.74	15.26	ND
Propyl acetate	12.86	12.42	9.00	13.26	9.31	16.45	4.57	7.32	0.91	11.59	11.02	18.30	3.98	ND	ND	3.61	9.42	ND
Butyl acetate	ND	ND	9.40	3.43	ND	ND	3.88	1.95	ND	ND	ND	ND	3.42	ND	ND	2.98	2.74	ND
Isoamyl acetate	ND	ND	ND	ND	ND	ND	ND	ND	0.91	ND	ND	ND	ND	ND	ND	ND	ND	0.89
Allyl isobutyrate	ND	ND	ND	ND	ND	ND	ND	0.90	ND	ND	ND	ND	ND	ND	ND	1.89	ND	ND
Di-tert-butyl dicarbonate	ND	ND	ND	ND	ND	ND	ND	0.64	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hexyl acetate	ND	ND	ND	ND	ND	ND	ND	ND	0.78	ND	ND	ND	ND	ND	ND	ND	ND	0.68
Octyl formate	ND	ND	ND	ND	ND	ND	0.77	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Butyl butyrate	ND	ND	ND	ND	ND	ND	0.74	ND	0.42	ND	ND	ND	ND	ND	ND	ND	ND	ND
Diisopropyl adipate	ND	ND	ND	ND	ND	ND	ND	ND	3.88	ND	ND	ND	ND	ND	ND	ND	ND	ND
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	ND	ND	ND	ND	ND	ND	3.46	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methyl pentanoate	0.41	ND	0.96	ND	ND	ND	ND	ND	ND	0.38	ND	ND	ND	ND	ND	ND	ND	ND
Methyl decanoate	ND	0.51	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(S)-Isopropyl lactate	ND	0.08	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Butyl isobutyrate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.39	ND
Propanoic acid, pentyl ester	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.14	ND	ND	ND	ND	ND
Methyl 2-hydroxydecanoate	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.50	ND	ND	ND	ND	ND	ND	ND	ND
Amyl formate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.13	ND	ND	0.14
Hexadecanoic acid, 15-methyl, methyl ester	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.64	ND	ND	ND	ND	ND	ND
3-Methyl-1-pentanal	ND	ND	ND	ND	ND	ND	ND	1.21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Isovaleral	ND	ND	ND	ND	ND	ND	ND	ND	1.58	ND	ND	ND	ND	ND	ND	ND	ND	1.74
Hexanal	ND	ND	ND	ND	10.06	20.61	1.67	6.72	17.08	ND	0.98	ND	ND	ND	21.75	1.55	3.08	18.04
2-Methylvaleraldehyde	ND	ND	ND	ND	1.40	ND	1.35	ND	ND	ND	ND	ND	ND	ND	ND	1.04	ND	ND
n-Octanal	2.60	2.62	ND	5.16	2.47	4.68	3.85	5.07	2.24	4.43	2.63	3.08	ND	ND	ND	1.96	3.02	1.96
Nonyl aldehyde	7.66	5.89	6.87	15.08	7.29	ND	6.29	13.65	13.32	10.34	5.16	6.81	4.16	2.91	2.58	1.88	1.90	11.69
2-Furaldehyde	ND	ND	1.45	ND	0.36	1.53	0.70	0.54	1.42	ND	ND	1.58	7.75	8.54	12.44	12.40	13.01	1.34
Decyl aldehyde	ND	1.12	ND	ND	1.06	ND	0.84	ND	1.30	ND	ND	ND	ND	ND	ND	ND	0.70	0.95
Benzaldehyde	0.53	ND	2.10	0.51	1.17	0.83	0.66	1.13	1.35	0.63	0.97	ND	2.43	ND	1.64	3.28	3.62	ND
Pentadecanal	ND	ND	ND	ND	ND	ND	1.85	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5-Methyl-2-furancarboxaldehyde	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.76	0.85	0.50	ND	ND	ND
Phenylacetaldehyde	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	5.45	ND	ND	ND	ND
Tridecanal	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.97	ND	ND
2,3-Hexanedione	1.11	ND	ND	ND	ND	ND	ND	ND	ND	1.58	ND	ND	ND	ND	ND	ND	ND	ND
6-Methyl-5-hepten-2-one	ND	0.97	ND	ND	ND	ND	1.07	ND	1.46	ND	1.00	ND	ND	ND	0.55	ND	ND	0.35
4-Methyl-2-hexanone	ND	ND	ND	ND	ND	ND	ND	0.78	ND	ND	ND	ND	ND	ND	ND	ND	0.62	1.28
1-Cyclopentyl-2-propanone	ND	ND	ND	ND	ND	1.14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acetone	0.67	ND	ND	0.82	0.40	ND	ND	ND	ND	ND	0.50	ND	ND	ND	ND	ND	ND	ND
2,3-Heptanedione	ND	ND	ND	ND	ND	0.28	1.79	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-Hexanone	ND	ND	0.41	ND	ND	ND	ND	ND	ND	0.65	0.34	ND	ND	ND	ND	ND	ND	1.32
3-Methyl-4-heptanone	ND	0.50	ND	ND	ND	ND	ND	ND	ND	ND	0.50	ND	ND	ND	ND	ND	ND	ND
2,5-Dimethyl-3-hexanone	0.53	0.52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(Z)-6,10-Dimethyl-5,9-undecadien-2-one	ND	0.78	ND	ND	ND	ND	ND	ND	0.78	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,3,3,5,5,6,6-Octamethyl-4-heptanone	ND	0.68	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6,10-Dimethyl-2-undecanone	ND	ND	ND	ND	ND	ND	0.54	ND	0.24	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-Methyl-2-heptanone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.89	ND	ND
Methoxyacetone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.61	0.54	ND	ND	ND
1-Hydroxy-2-propanone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.86	2.68	1.09	ND

Table 3: Continued.

Volatile components	300HPP-4°C-0 d	600HPP-4°C-0 d	TP-4°C-0 d	300HPP-4°C-15 d	600HPP-4°C-15 d	TP-4°C-15 d	300HPP-4°C-30 d	600HPP-4°C-30 d	TP-4°C-30 d	300HPP-27°C-0 d	600HPP-27°C-0 d	TP-27°C-0 d	300HPP-27°C-15 d	600HPP-27°C-15 d	TP-27°C-15 d	300HPP-27°C-30 d	600HPP-27°C-30 d	TP-27°C-30 d
2,4-Dimethyl-3-hexanone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.52	ND	ND	ND	ND	ND	ND
2(5H)-Furanone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.41	ND	0.38	0.51	ND	0.51
Cyclooctane-1,2-dione	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.26	ND	ND
2-Hydroxy-2-cyclopenten-1-one	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.78	0.62	1.54	ND	ND	13.73
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.52	ND	ND	2.50
2-Methyl-4-octanone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.68	ND	ND	ND	ND	7.58
1,3-Dihydroxy-2-propanone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	14.11	ND	1.68	ND
2-Hydroxy-gamma-butyrolactone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.52	2.57	ND	ND
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.61	3.11	7.02	2.49	1.18	2.93
3-Methyl-1-butanol	ND	ND	ND	ND	ND	ND	ND	1.23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Pentanol	ND	ND	ND	ND	ND	ND	0.24	ND	ND	ND	ND	ND	ND	7.72	ND	ND	ND	1.12
1-Hexanol	1.07	0.51	3.41	0.83	1.07	0.64	1.23	0.83	1.28	1.58	1.43	0.64	1.23	2.10	0.53	0.79	0.90	ND
2-Methyl-5-hexen-3-ol	ND	ND	ND	ND	ND	ND	0.40	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Ethyl-1-hexanol	1.62	1.61	1.25	0.90	1.29	0.88	3.93	1.46	20.39	1.70	0.88	ND	2.64	4.09	2.08	1.77	2.04	ND
(2S)-2-Methyl-1-butanol	ND	ND	ND	ND	ND	0.39	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Tridecyn-4-ol	ND	ND	3.08	0.54	ND	0.30	ND	0.38	ND	ND	ND	0.87	0.65	ND	ND	ND	0.59	ND
1-Octanol	1.68	1.24	1.35	1.35	1.10	1.02	2.71	2.12	3.38	ND	1.70	0.79	1.70	1.62	1.15	1.16	1.36	7.09
(3S)-3-Methyl-1-pentanol	ND	ND	ND	ND	0.11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Heptanol	ND	ND	ND	ND	ND	0.22	ND	ND	ND	1.42	ND	ND	ND	ND	ND	ND	ND	ND
3-Methyl-1-hexanol	1.27	ND	ND	0.54	ND	ND	ND	0.32	ND	1.18	ND	ND	ND	ND	ND	ND	ND	ND
1-Nonanol	ND	1.76	ND	1.15	0.81	1.34	2.03	1.07	ND	ND	1.95	4.34	8.53	ND	ND	ND	ND	ND
1-Dodecanol	ND	ND	ND	ND	ND	ND	2.73	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.67	ND
1-Decanol	ND	ND	ND	ND	ND	ND	ND	ND	1.35	ND	ND	ND	ND	ND	ND	1.01	ND	ND
1-(2-Furanyl)-3-butene-1,2-diol	ND	0.57	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Tetradecanol	ND	ND	ND	ND	ND	ND	ND	0.81	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Pentadecanol	ND	ND	ND	ND	ND	ND	0.81	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methyl-2-propanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.19	ND	ND	ND
3-Methyl-1-pentanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.63	0.43	ND	ND
2-Nonen-1-ol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.62	9.93	18.42	
1-Ethyl-cyclohexanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.40	ND	ND	0.32	ND
2,6-Dimethyl-3-heptanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.59	ND	ND	ND	ND	ND	1.12
3,3-Dimethyl-1-butanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.99	ND	ND	ND	ND	ND	ND	ND	ND
2-Furanmethanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.32	3.79	1.80	ND
5-Methylheptan-1-ol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.82	ND
Phenethyl alcohol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.50	ND	ND	ND	ND
Allyl alcohol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.61	2.40	ND	ND	ND	ND
Acetic acid	6.35	3.42	3.97	ND	3.41	2.50	8.22	4.39	3.96	6.81	6.02	4.35	8.57	13.38	12.36	11.18	11.95	2.93
1,1-Dimethyl ethyl butyric acid	ND	0.23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Butyric acid	ND	ND	ND	ND	ND	ND	ND	0.27	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Isovaleric acid	1.44	ND	2.87	0.46	0.47	0.44	1.21	0.63	ND	1.15	0.36	1.38	ND	ND	0.37	0.70	0.58	0.35
Isovaleric acid	ND	ND	ND	0.96	ND	ND	2.75	1.34	1.30	ND	ND	1.14	0.81	ND	0.95	1.76	1.55	0.96
Valeric acid	1.67	0.99	2.79	ND	0.81	0.78	ND	ND	ND	1.86	0.88	ND	ND	ND	ND	ND	ND	ND
2-Ethylhexanoic acid	1.10	ND	ND	0.46	ND	ND	ND	ND	ND	0.88	ND	ND	ND	ND	ND	ND	ND	ND
Octanoic acid	ND	ND	ND	ND	ND	ND	0.77	ND	ND	ND	ND	ND	ND	ND	ND	0.68	0.80	ND
2-Hydroxyisobutyric acid	ND	ND	ND	0.37	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nonanoic acid	ND	5.00	ND	ND	ND	ND	0.96	0.61	0.66	ND	3.00	3.29	ND	ND	0.39	0.69	1.16	0.37
Heptanoic acid	0.83	ND	ND	0.73	ND	0.66	0.69	ND	0.68	1.57	0.53	ND	ND	ND	ND	ND	ND	ND
Decanoic acid	ND	0.74	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-Dimethylbutyric acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.96	ND
Formic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.81	2.93	2.62	1.96	2.39	ND

Table 3: Continued.

Volatile components	300HPP-4°C-0 d	600HPP-4°C-0 d	TP-4°C-0 d	300HPP-4°C-15 d	600HPP-4°C-15 d	TP-4°C-15 d	300HPP-4°C-30 d	600HPP-4°C-30 d	TP-4°C-30 d	300HPP-27°C-0 d	600HPP-27°C-0 d	TP-27°C-0 d	300HPP-27°C-15 d	600HPP-27°C-15 d	TP-27°C-15 d	300HPP-27°C-30 d	600HPP-27°C-30 d	TP-27°C-30 d
2-Ethylbutyric acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.01	ND	ND	ND	ND
(Z)-5,5-Dimethyl-2-hexene	ND	0.32	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,7-Dimethyl-nonane	ND	0.92	ND	ND	ND	ND	ND	ND	1.55	ND	ND	ND	ND	ND	ND	ND	0.60	ND
2,5-Dimethyl-1-hexene	ND	ND	ND	0.11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Decene	ND	ND	ND	ND	ND	ND	ND	1.52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,6-Dimethyloctane	ND	ND	ND	ND	ND	ND	ND	ND	0.73	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-Methyl-1-heptene	ND	ND	0.08	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-Dimethylpentane	0.46	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.00	ND	ND	ND	ND	ND	ND	ND
(Z)-8-Methyl-2-decene	ND	ND	ND	ND	ND	ND	ND	0.37	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-Trimethyl-cyclopentane	ND	ND	0.38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hexadecane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.05	ND	ND
Propanoic acid, anhydride	0.31	0.20	ND	ND	0.43	ND	ND	ND	ND	ND	ND	ND	0.36	0.36	ND	ND	ND	ND
Propanoic acid, 2-methyl, anhydride	ND	ND	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methacrylic anhydride	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.31	ND	ND	ND	ND	ND	ND	ND
1-(2-Furanyl)-ethanone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.35	ND	ND	ND
2,5-Furandicarboxaldehyde	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.23	4.47	ND	1.72	1.70	ND
Tetrahydro-3-furanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.17	ND

Note: ND: volatile flavor components were not observed.

Table 4: The varieties and contents of volatile compounds in different beverage samples.

Samples	Esters		Aldehydes		Ketones		Alcohols		Acids		Hydrocarbons		Others	
	N	RC (%)	*N*	RC (%)	*N*	RC (%)	*N*	RC (%)	*N*	RC (%)	*N*	RC (%)	*N*	RC (%)
300HPP-4°C-0 d	3	69.16	3	10.79	3	2.31	4	5.65	3	11.39	1	0.46	1	0.31
600HPP-4°C-0 d	4	69.40	3	9.64	5	3.45	5	5.69	5	10.39	2	1.24	1	0.20
TP-4°C-0 d	4	69.01	3	10.42	1	0.41	4	9.09	3	9.63	2	0.47	1	1.00
300HPP-4°C-15 d	3	70.01	3	20.76	1	0.82	6	5.32	5	2.97	1	0.11	0	0.00
600HPP-4°C-15 d	2	65.99	7	23.80	1	0.40	5	4.38	3	4.69	0	0.00	1	0.43
TP-4°C-15 d	2	61.75	4	27.65	2	1.42	7	4.80	4	4.39	0	0.00	0	0.00
300HPP-4°C-30 d	6	50.70	8	17.20	3	3.39	8	14.10	6	14.61	0	0.00	0	0.00
00HPP-4°C-30 d	5	53.54	6	28.33	1	0.78	8	8.22	5	7.24	2	1.89	0	0.00
TP-4°C-30 d	6	23.96	7	38.28	3	2.48	4	26.41	4	6.59	2	2.28	0	0.00
300HPP-27°C-0 d	4	63.21	3	15.41	2	2.23	5	6.88	5	12.27	0	0.00	0	0.00
600HPP-27°C-0 d	2	69.28	4	9.74	4	2.34	4	5.97	4	10.80	1	1.00	1	1.31
TP-27°C-0 d	3	68.59	3	11.48	1	0.52	5	9.24	4	10.17	0	0.00	0	0.00
300HPP-27°C-15 d	3	46.28	4	15.11	4	5.47	6	16.35	3	12.19	0	0.00	2	4.59
600HPP-27°C-15 d	2	34.90	4	17.75	3	4.34	7	20.84	3	17.33	0	0.00	2	4.84
TP-27°C-15 d	1	0.13	5	38.91	9	32.03	6	11.90	5	16.68	0	0.00	1	0.35
300HPP-27°C-30 d	4	34.21	7	23.08	6	11.40	7	10.57	6	16.97	1	2.05	1	1.72
600HPP-27°C-30 d	4	27.81	6	25.33	4	4.56	8	18.44	7	20.40	1	0.60	2	2.87
TP-27°C-30 d	3	1.72	6	35.72	8	30.20	4	27.75	4	4.62	0	0.00	0	0.00

Note: *N*: number; RC: relative content.

Table 5: Contribution and cumulative contribution of principal components.

Principal component	Contribution rate (%)	Cumulative contribution rate (%)
1	28.5	28.5
2	22.5	51.0
3	14.9	65.9

were screened and used in PCA. The contribution rates of the first and second principal components were 28.5% and 22.5%, respectively; the cumulative contribution rates of the first three and first two principal components were 65.9% and 51.0%, respectively (Table 5). A two-dimensional scatter diagram for the first two principal components was produced, and the UV scaling method was used as a scaling method (Figures 1 and 2).

As shown in Figure 1, the volatile compounds of 300 MPa HPP-treated samples changed greatly when they were stored at 4°C for 30 days. The volatile compounds of samples treated with 600 MPa HPP and stored at 4°C for 15 and 30 days were similar. The samples treated with 300 MPa HPP and stored at 27°C for 0 and 15 days were in the diagonal position, indicating that the volatile compound composition with high content in 300 MPa HPP-treated samples stored at 27°C for 0 days was low in 300 MPa HPP-treated samples stored at 27°C for 15 days. Additionally, the volatile compound composition of the samples stored for 15 days was close to that of samples stored for 30 days. Similar results arose for samples stored at 27°C after the 600 MPa HPP treatment. The volatile compound components with high content in 600 MPa HPP-treated samples stored at 27°C for 0 days were low in 600 MPa HPP-treated samples stored at 27°C for 30 days. The volatile compounds of TP-treated samples differed according to whether the samples were stored at 4°C or 27°C.

In Figure 2, the abscissa represents the first principal component, and the component far from the abscissa played a major role in the first principal component. The ordinate represents the second principal component, and the component far from the ordinate played a major role in the second principal component. In this study, nonanal contributed the most to the first principal component, followed by 2-hydroxy-2-cyclopenten-1-one and ethyl acetate. The largest contribution to the second principal component was made by n-hexanal, followed by n-octanol and 3,7-dimethylnonane.

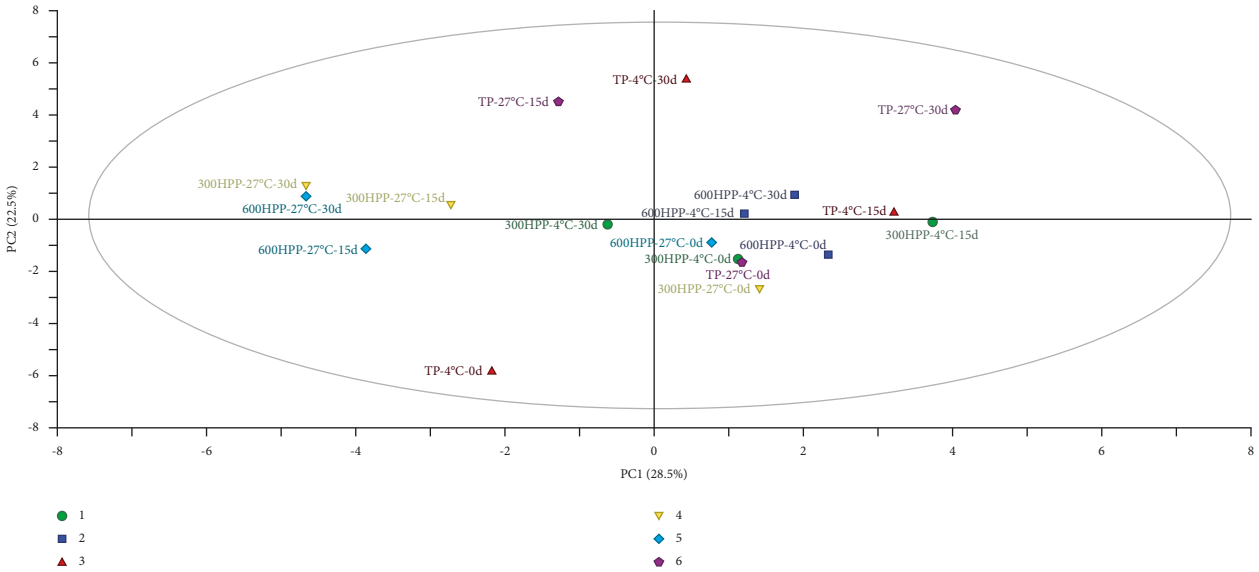

Figure 1: PCA score chart.

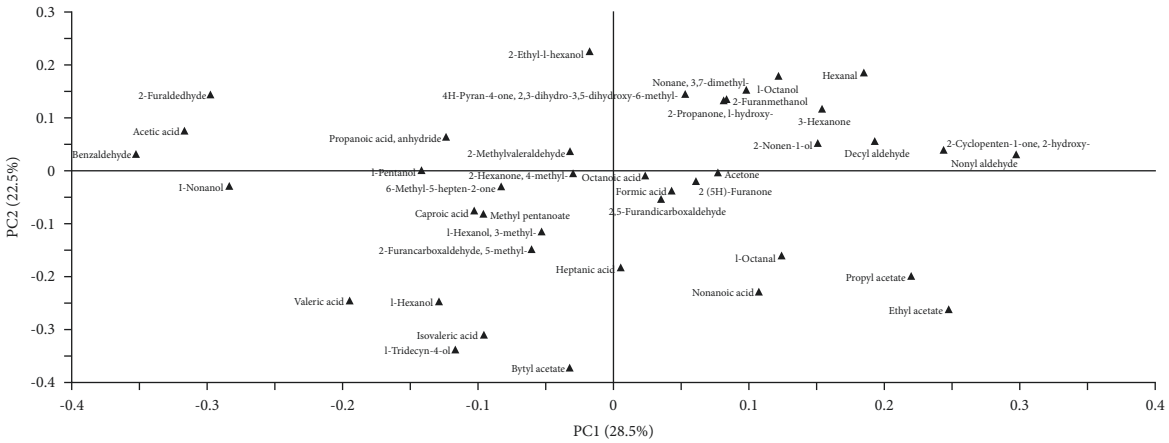

Figure 2: PCA loading plot.

4. Conclusions

There was no significant difference in soluble solid, amino acid nitrogen, and pH levels between HPP- and TP-treated HM beverages. Nevertheless, the colony count of HM beverages treated with TP was lower than that of HPP-treated beverages; nevertheless, the colony count for both treatments was within the standard range. The sensory score was a key factor associated with the shelf life of HM beverages. When the sensory score was <80, this was taken as the end point of shelf life. The order of shelf life for HM beverages subjected to different treatments was as follows: TP-treated >600 MPa HPP-treated >300 MPa HPP-treated. Esters were abundant volatile compounds in HM beverages, and HPP treatment retained more esters than those retained with TP. Indeed, TP treatment resulted in more changes to the main volatile compounds during storage than those detected following HPP. In terms of flavor, HPP-treated HM beverages were superior to TP-treated HM beverages. These findings show that high pressure is a promising processing technology, which can be applied to the products of composite beverages, and effectively protects the components of aromatic substances. This study provides some data to support the development of new compound beverage products.

Abbreviations

HM: *Hericium erinaceus*-millet
HPP: High-pressure processing
TP: Thermal processing
TSS: Total soluble solids
PCA: Principal component analysis.

Data Availability

The data that support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest .

Authors' Contributions

Xiaolan Shang and Zhiguo Zhou designed the experiment, contributed to the acquisition of data, and interpreted the data. Zixin Wang and Chengqian Sun contributed to analyses of soluble solids, amino acid nitrogen, pH, colony count, sensory quality, and shelf life. Lin Zhang contributed to analyses of volatile compounds.

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