International Food Research Journal 31(3): 647 - 657 (June 2024)
Journal homepage: *http://www.ifrj.upm.edu.my*

Changes in flavour- and taste-presenting substances in *Hypsizygus marmoreus* mushroom packaged in polypropylene nanofilm

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Article history

Received:
23 September 2023
Received in revised form:
20 March 2024
Accepted:
22 March 2024

Keywords

Hypsizygus marmoreus, quality deterioration, polypropylene nanofilm, taste active value, flavour and taste-presenting substances

Abstract

Hypsizygus marmoreus mushroom is known for its pleasant flavour and delicious taste. However, its flavour quality deteriorates rapidly during postharvest storage. In the present work, we explored the dynamic changes in flavour-presenting substances and taste components in *H. marmoreus* packaged in polyethylene nanofilm (0.05 mm) during low temperature storage (12 days). We used headspace solid-phase microextraction combined with gas chromatography-mass spectrometry to analyse the volatile flavour components; high performance liquid chromatography and liquid chromatography-mass spectrometry to analyse the non-volatile flavour (taste-presenting) substances (*e.g.*, soluble sugars, taste nucleotides, free amino acids, and organic acids); and the relative odour activity value (ROAV) and taste active value (TAV) methods to determine the flavour substances in fresh and stored *H. marmoreus*. Results revealed that *n*-octanal, 1-octene-3-one, and 1-octene-3-ol were the characteristic volatile flavour substances, and that *trans*-2-octenal was the key substance affecting the formation of volatile flavours in fresh *H. marmoreus*. *Trans,trans*-2,4-nonadienal, and 3-octanone were the key volatile substances that resulted in flavour deterioration. Among the non-volatile flavour substances, trehalose, 5'-GMP, glutamic acid, alanine, and arginine were the key taste-presenting substances. In addition, oxalic acid and lysine were the key taste substances after *H. marmoreus* deterioration. These results provided a theoretical basis for rapid quality detection, flavour identification, and shelf-life prediction of *H. marmoreus*.

DOI
https://doi.org/10.47836/ifrj.31.3.10

Introduction

Hypsizygus marmoreus mushroom is favoured by consumers due its unique seafood-like flavour (Chien *et al.*, 2016). However, its high-moisture content, fragile tissue, vigorous postharvest respiration, and lack of protective tissue on the epidermis make *H. marmoreus* prone to mechanical damage and microbial decomposition, resulting in flavour deterioration. This has become a problem that limits the development of the mushroom industry. Therefore, preservation technologies have been used to maintain the storage quality of mushrooms, including biological inhibitors, modified atmosphere, and composite preservation technologies (Wang *et al.*, 2011; Xia *et al.*, 2021).

Nanopackaging is an emerging packaging method. The application of nanotechnology in the field of food packaging has greatly solved problems of food quality, safety, and stability (Sharma *et al.*, 2017). Fang *et al.* (2016) prepared a nanocomposite film that can maintain the sensory quality of *Flammulina velutipes*. Yang *et al.* (2019) reported reduced energy metabolism, and prolonged storage period of *F. velutipes* when packaged in nanopackaging material. Zuo *et al.* (2021) studied the changes of gene expression in *F. velutipes* under nanopackaging, and reported that it could delay the quality deterioration of *F. velutipes* during storage.

The flavour of edible mushrooms is attributed to the presence of volatile and non-volatile flavour compounds. Volatile flavour compounds are mainly alcohol, aldehydes, acids, and esters, while non-volatile flavour compounds mainly include taste-presenting substances such as soluble sugars, nucleotides, free amino acids, and some organic acids (Pinho *et al.*, 2008). Studies have evaluated the changes in volatile flavour compounds during

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postharvest storage of edible mushrooms, such as *F. velutipes*, *Agaricus bisporus*, and *H. marmoreus* (Fang *et al.*, 2017; Yan *et al.*, 2020). However, there is no comprehensive analysis of the changes in both flavour- and taste-presenting substances during the quality deterioration of *H. marmoreus*.

Consumers generally judge the freshness of *H. marmoreus* based on sensory indicators such as colour, smell, and morphological ripeness (Harada *et al.*, 2003). However, the initial stage of deterioration cannot be reliably perceived by the human sensory system. Therefore, it is important to develop an accurate and rapid method to identify its quality deterioration. As a new, safe, non-toxic, and effective preservation technology, nanofilm packaging has been widely used in the preservation of other fruits and vegetables. In the present work, we used *H. marmoreus* packaged in nanofilm, and analysed the changes in volatile and non-volatile flavour compounds during low-temperature storage, by assessing the relative odour activity value (ROAV) and taste active value (TAV). Additionally, we evaluated the deterioration degree of mushrooms. The obtained findings would provide a theoretical basis for the rapid detection of *H. marmoreus* quality, and prediction of its shelf life.

Materials and methods

Materials

Sample preparation, treatment, and storage

Hypsizygus marmoreus was obtained from Zhangye Shennong Rare Mushroom Industry Co., Ltd. (Gansu, China), stored at 4°C for 2 h, and transported to the laboratory at low temperatures. The selected mushrooms were of similar stipe length, with a closed pileus, and without mechanical damages, pests, or diseases. Next, 250 g of mushrooms were accurately weighed for nanofilm packaging, divided into three parallel experiments, and stored at 4 ± 1°C. Samples were collected and analysed on days 0, 6, and 12.

Chemicals

Decanol (GCS grade) and methanol (GC grade) were purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. Soluble sugar standards such as mannitol and trehalose, ethanol (chromatographic grade), acetonitrile (chromatographic grade), organic acid standards such as oxalic acid, formic acid, malonic acid, citric acid, fumaric acid, and succinic acid, and flavour nucleotide standards such as 5'-AMP, 5'-CMP, 5'-GMP, 5'-UMP, and 5'-IMP were purchased from Shanghai Yuanye Biotechnology Co., Ltd. Amino acid mixed standard was purchased from Beijing Solarbio Science and Technology Co., Ltd.

Preparation of packaging materials

The nanofilm consisted of a biaxially stretchable polypropylene (BOPP; Shanghai Fuming New Material Technology Co., Ltd., China) with the following characteristics: thickness of 0.05 mm, tensile strength > 16 MPa, moisture permeability of 23 g $(m^2\ 24\ h)^{-1}$, oxygen permeability of 9,730 cm^2 $(M^2 \cdot 24\ h \cdot 0.1\ MPa)^{-1}$, light transmittance of 89%, and carbon dioxide transmittance of 20,010 cm^2 $(m^2 \cdot 24\ h \cdot 0.1\ MPa)^{-1}$. Polypropylene tray (180 × 120 × 30 mm) was provided by Zhucheng Wanrui Plastic Co., Ltd. (Shanghai, China).

Experimental methods

HS-SPME-GC-MS analysis

Headspace solid-phase microextraction combined with gas chromatography-mass spectrometry (HS-SPME-GC-MS) was performed for the analysis of volatile compounds (Laurienzo *et al.*, 2010). Under the protection of nitrogen stream, the extraction head was inserted into the injection port of the gas chromatograph for aging for 2 h, and the aging temperature was 250°C. The extraction head was aged for 0.5 h before each use to remove impurities remaining on it. The mushrooms were then ground in a mortar in the presence of liquid nitrogen. Next, 2.0 g of mushroom powder were transferred into 20-mL headspace vials followed by *n*-decyl alcohol. The vials were sealed with PTFE-silicone septa (Supelco, Bellefonte, PA, USA), and mixed by magnetic stirring. After placing the fibre into the vials, the vials were placed in a 40°C water bath for 30 min to extract volatile flavour compounds, and the end of the extraction head was then inserted into the injection port to conduct dissociation analysis of volatile compounds.

The analysis of volatile compounds was performed using a GC-MS system (TRACE 1310, Agilent Technologies, Santa Clara, CA, USA). Volatiles were separated using a capillary column (DB-WAX, Agilent Technologies, Santa Clara, CA, USA; 60 m × 250 μm × 0.25 μm). The oven temperature program was set at 40°C for 5 min, maintained isothermally for 7 min, raised to 180°C at

3°C/min, and maintained isothermally for 8 min. Helium was the carrier gas, and the flow rate was 1 mL·min^{-1} (Cui *et al.*, 2015). The temperature of the electron ionisation source was 250°C, and mass spectra were obtained by electronic impact at 70 eV. Temperatures of quadrupole and interface were 200 and 250°C, respectively. The data were collected at 1/scan over 50-350 U (Yang *et al.*, 2015).

Relative odour activity value analysis

Relative odour activity value (ROAV) of volatile compounds was calculated as described by Liu *et al.* (2019) using Eq. 1:

$$\mathrm{ROAV} \approx \frac{C_i}{C_{max}} \times \frac{T_{max}}{T_i} \times 100 \quad \text{(Eq. 1)}$$

where, C_i = concentration of a certain volatile compound in mushroom, C_{max} = concentration of the volatile component with the largest flavour contribution in the sample; T_{max} = threshold value of the volatile component with the largest flavour contribution in the sample, generally measured in water or in a simple matrix; and T_i = threshold value of a certain volatile compound.

Extraction and determination of soluble sugars

Ground *H. marmoreus* was frozen at -70°C. Extraction was carried out according to Tsai *et al.* (2008). The mushroom powder (0.1 g) was first extracted with 1.6 mL of 50% aqueous ethanol at 50°C for 30 min. The resulting suspension was centrifuged at 6,000 rpm for 15 min. The residue was re-extracted by the same method, and the supernatants were pooled. The supernatant was concentrated at 40°C under reduced pressure, and the solid residues were dissolved in acetonitrile (50%) to 1 mL. Soluble sugars were determined using HPLC (Agilent 1200, Agilent Technologies, Santa Clara, CA, USA) and a Sugar-D column (250 mm × 4.6 mm, 5 μm; Agilent Technologies, USA).

Extraction and determination of 5′-nucleotides

5′-Nucleotides were extracted and analysed as previously reported with slight modifications (Li *et al.*, 2018). Mushroom samples (1.0 g) were ultrasonically extracted with 4 mL of ultrapure water at 80°C for 15 min, cooled, and centrifuged at 5,000 rpm for 15 min. The residue was re-extracted using the same method, and the supernatants were pooled. The combined extract was passed through a 0.45-μm filter membrane (ANPEL Laboratory Technologies Inc., Shanghai) for HPLC analysis equipped with a variable wavelength detector (Agilent Technologies) and a UltimateAQ-C18 column (250 × 4.6 mm, 5 μm; Agilent Technologies).

Extraction and determination of free amino acids

Free amino acids were extracted and analysed following a previous method with slight modifications (Wang *et al.*, 2010). The mushroom sample (0.1 g) was extracted with 1 mL of 0.5 mol/L aqueous hydrochloric acid at 20°C for 20 min. The resulting suspension was centrifuged at 20,000 *g* for 20 min. Finally, 250 μL of the extraction supernatant was transferred to a liquid chromatography vial, and adjusted to 1 mL with 80% acetonitrile aqueous solution. The pooled extract was passed through a 0.22-μm filter membrane (ANPEL Laboratory Technologies Inc., Shanghai) for analysis by liquid chromatography-mass spectrometry (LC-MS, Agilent Technologies) equipped with an Infinity Lab Poroshell 120 HILIC-Z column (2.1 × 100 mm, 2.7 μm; Agilent Technologies).

Extraction and determination of organic acids

Organic acids were extracted and analysed following a previous method with slight modifications (Wu *et al.*, 2015). The mushroom sample (1.0 g) was extracted with 2 mL of 0.01 mol/L KH_2PO_4 (pH = 2.8) at 45°C for 30 min. The resulting suspension was centrifuged at 5,000 rpm for 15 min. Finally, the supernatant was transferred to an HPLC vial, and adjusted to 1 mL. The pooled extract was passed through a 0.45-μm filter membrane (ANPEL Laboratory Technologies Inc., Shanghai) for analysis by HPLC (Agilent Technologies) coupled to a Zorbax-EclipseXDB-C18 column (250 mm × 4.6 mm, 5 μm).

Taste active value analysis

Taste active value (TAV) of non-volatile compounds in mushrooms was calculated as reported by Chen and Zhang (2007) using Eq. 2:

$$TAV = \frac{C}{T} \quad \text{(Eq. 2)}$$

where, C = concentration of a certain non-volatile compound in mushroom, and T = threshold value generally measured in water or in a simple matrix. Compounds with TAV > 1 are considered to be active in food taste.

Statistical analysis

Statistical analysis was performed using Microsoft Office Excel 2019 and Heml 1.0.3.7. For multiple comparisons, the least significant differences (LSD) at a 95% confidence level was used. Data were expressed as mean ± standard deviation.

Results and discussion

Volatile flavour compounds

Heat map analysis of volatile flavour substances

To evaluate the changes in volatile flavour substances during storage, heat map cluster analysis was performed. A total of 66 volatile substances were detected during storage: 23 aldehydes, 22 alcohols, ten ketones, three esters, four carboxylic acids, and four furans (Figure 1). The rows in the figure indicate different types of volatile substances, and the column indicates different storage periods. The three different storage periods had obvious colour differences, suggesting that the content of volatile substances was significantly different among the storage periods. Clustering occurred at the minimum distance level at days 6 and 12, which indicated that the content of volatile substances on these days was very similar. As the Euclidean distance increased, day 0 began to cluster together. In terms of the area of the blue-purple region, day 12 = day 6 > day 0, indicating the type of volatile substances day 0 > day 6 = day 12. In terms of the three periods as a whole, *n*-octanal, 3-octanone, *trans*-2-nonanal, 1-octene-3-one, nonanal, octanol, *n*-octanol, and 1-octene-3-alcohol had darker colour, indicating that their content during storage was higher than that of other substances. This result was consistent with Kuka *et al*. (2014) on *Cantharellus cibarius*.

ROAV analysis of volatile flavour substances

The contribution of volatile compounds to aroma depends on their concentration and threshold level. It is inaccurate to define the contribution of the substance to aroma only based on the content of volatile substances (Liu *et al*., 2008; Burdock, 2009). Therefore, based on the concentration and threshold level, ROAV was calculated. ROAV was determined based on the average value of the samples in each storage period. Substances with ROAVs between 0.1 and 1 can modify flavour, while substances with ROAVs between 1 and 100 are key substances that affect flavour (Arshak *et al*., 2004). Table 1 shows that seven components had ROAVs between 1 and 100, and seven components had ROAVs between 0.1 and 1. On day 0, substances with ROAVs between 1 and 100 were *n*-octanal, *trans*-2-octenal, 1-octen-3-ol, and 1-octene-3-one; and those with ROAVs between 0.1 and 1 were *n*-hexanal, nonanal 3-octanone, and 2-pentyl furan. On day 6, the substances with ROAVs between 1 and 100 were *n*-octanal, nonanal, 1-octene-3-ol; and the substances with ROAVs between 0.1 and 1 were *trans*-2-octenal, *trans*,*trans*-2,4-nonadienal, 3-octanol, 3-octanone, and 2-pentyl furan. Finally, on day 12, the substances with ROAVs between 1 and 100 were *n*-octanal, *trans*,*trans*-2,4-nonadienal, 1-octen-3-ol, 3-octanone, and 1-octen-3-one; and those with ROAVs between 0.1 and 1 were nonanal, *trans*-2-octenal, 3-octanol, and 2-pentyl furan. It has been shown that 1-octe-*n*-3-ol, 1-octen-3-one, and 1-hexanol were the major compounds found in Portuguese chanterelle (Pinho *et*

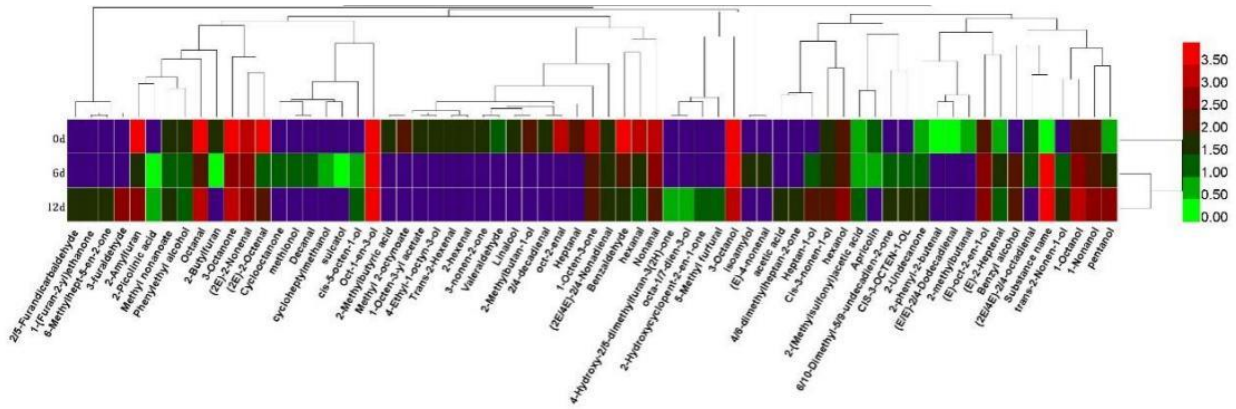

Figure 1. Heat map of volatile substances in samples. Blue-violet in the heat map indicates that flavour content is zero, and the colour changes from green to red indicates the change in flavour content from low to high.

Table 1. Changes in key volatile flavour substances in *H. marmoreus* packaged in nanofilm during storage.

CAS Number	Chemical name	Threshold	ROAV (0 d)	ROAV (6 d)	ROAV (12 d)	Odour description
66-25-1	*n*-Hexanal	4.5	0.19	0.03	0.01	Grassy, fishy
124-13-0	Octanal	0.7	2.37	4.76	2.89	Intense fruity
18829-55-5	(E)-2-Heptenal	13	0.02	0.01	0.00	Aromatic
124-19-6	Nonanal	1	0.79	1.22	0.51	Citrus, fishy
2548-87-0	*Trans*-2-octenal	3	2.47	0.12	0.90	/
100-52-7	Benzaldehyde	350	0.01	0.00	0.00	Bitter almond
18829-56-6	*Trans*-2-nonanal	ND	ND	ND	ND	/
5910-87-2	*Trans*,*trans*-2,4-nonadienal	0.062	0.47	0.90	1.02	Floral and fruity
71-41-0	*n*-Pentanol	4000	0.00	0.00	0.00	Slight
18409-17-1	*Trans*-2-octene-1-ol	ND	ND	ND	ND	/
111-27-3	*n*-Hexanol	250	0.00	0.00	0.01	Fruity
20296-29-1	3-Octanol	42	0.06	0.21	0.73	Mushroom, milk
3391-86-4	1-Octene-3-ol	1	8.93	14.18	62.74	Mushroom
111-87-5	*n*-Octanol	110	0.00	0.01	0.04	Oily, citrus
143-08-8	1-Nonanol	50	0.00	0.01	0.02	Rose
10340-23-5	*Cis*-3-nonen-1-ol	ND	ND	ND	ND	/
60-12-8	Phenylethanol	86	0.00	0.00	0.00	Rose
106-68-3	3-Octanone	21.4	0.20	0.67	2.08	Ketone, waxy
4312-99-6	1-Octene-3-one	0.005	100.00	100.00	100.00	Soil, mushroom
2516-97-4	Methanesulfonylacetic acid	ND	ND	ND	ND	/
1731-84-6	Methyl nonanoate	ND	ND	ND	ND	Fruit wine and coconut-like
3777-69-3	2-Pentyl furan	5	0.26	0.21	0.30	Grassy, metallic

ND: substances that were not analysed in the calculation of ROAV since the threshold could not be accessed; /: no related reports. Odour description was adapted from Wang *et al.* (2010).

al., 2008). Ketones and alcohols were recognised as the predominant volatile compounds in fresh *F. velutipes*, and 3-octanone and 3-octanol were the most abundant (Fang *et al*., 2017).

During the whole storage period, the ROAVs of *n*-octanal, 1-octene-3-one, and 1-octene-3-ol ranged between 1 and 100, suggesting that they were characteristic flavour substances in *H. marmoreus*. However, the ROAV of *trans*-2-octenal in the early storage period (0 day) was 1 - 100; therefore, *trans*-2-octenal affected the flavour in fresh *H. marmoreus*. The content of *trans*-2,4-nonadienal and 3-octanone increased significantly in the late storage period, and their ROAVs ranged between 1 and 100. Therefore, these compounds contributed to flavour deterioration in *H. marmoreus* mushrooms.

Non-volatile flavour substances

Content of sweet substances

Soluble sugars are the main sweet substances in edible fungi. Their types and contents greatly affect the taste of edible fungi (Chiang *et al.*, 2006). Figure 2 shows that the main sweet substances in *H. marmoreus* were trehalose followed by a small amount of mannitol, consistent with the results reported by Sławińska *et al.* (2020). During the early (day 0), mid-term (day 6), and late (day 12) storage periods, the total content of sweet substances increased at first, and subsequently decreased, which was mainly caused by changes in trehalose. Water loss in *H. marmoreus* mushroom could have stimulated the synthesis of trehalose. Trehalose then formed a film on the cell surface to effectively protect

proteins. A decrease in trehalose could have been due to *H. marmoreus* deterioration (Liu *et al.*, 2019). At the end of storage, trehalose and mannitol decreased from 70.70 and 2.14 mg·g^{-1} to 51.03 and 0.26 mg·g^{-1}, respectively, and the total sugar content decreased from 72.84 to 51.29 mg·g^{-1}. Trehalose content was relatively high, and the most representative sweet substance. Therefore, *H. marmoreus* deterioration can be determined by measuring the changes in trehalose content.

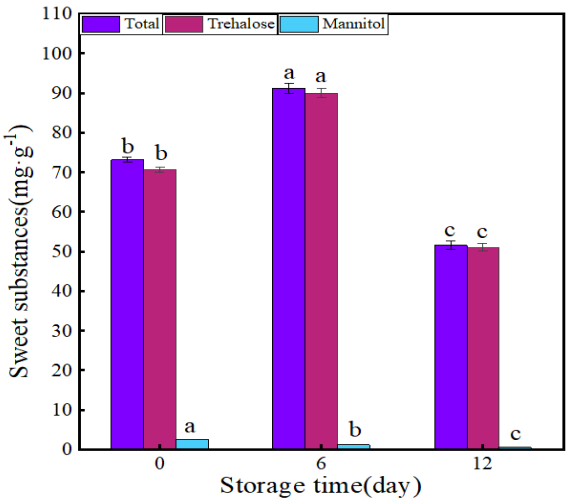

Figure 2. Changes in contents of sweet substances in *H. marmoreus* packaged in nanofilm during storage. Vertical lines represent standard deviation (± SD); values with different lowercase letters are significantly different (p < 0.05). Significant differences are between groups.

Content of taste-presenting nucleotides and changes in TAV

Taste-presenting nucleotides are important flavour substances in *H. marmoreus*, because they exert an umami taste (Samarasiri and Chen, 2022). 5'-UMP, 5'-IMP, and 5'-GMP are nucleotides that have strong taste (Wang *et al.*, 2014). In the present work, the most predominant flavouring nucleotides were 5'-AMP, 5'-GMP, and 5'-UMP (Table 2). Among them, 5'-AMP has sweet taste, and can effectively reduce the bitterness in *H. marmoreus* (Leksrisompong *et al.*, 2012). 5'-GMP has meaty taste and stronger umami taste than MSG. The total content of taste nucleotides increased with storage (Table 3). TAVs of 5'-GMP and 5'-AMP were > 1, indicating that both substances had significant contribution to taste. The increase in 5'-GMP during storage could have been attributed to the decomposition of nucleic acids, and the synthesis of nucleotides. The TAV of 5'-GMP was the highest, indicating that 5'-GMP contributed the most to taste. Consequently, changes in 5'-GMP can be used as a rapid detection index for mushroom deterioration.

Changes in free amino acid content and TAV

Amino acids are important taste substances, usually with sour, bitter, or sweet taste. Compounds containing sulphur atoms would naturally have sulphur flavour (Shallenberger, 1993). Among them, tyrosine, glutamic acid, alanine, phenylalanine, aspartic acid, glycine, and their corresponding sodium salts contributed to umami taste. Therefore, they are referred to as taste amino acids. Eleven amino acids were detected, among which threonine, alanine, glutamic acid, and arginine had pleasant taste, while other amino acids had unpleasant taste (Table 4). There is also a synergistic interaction between sweet amino acids and IMP, and the presence of IMP increases sweetness (Kawai *et al.*, 2002). Arginine is abundant in many types of seafood, and provides pleasant taste rather than bitterness for these products (Spurvey *et al.*, 1998). During the whole storage process, the total amount of amino acids showed a decreasing trend, which could have been due to its participation in the body's metabolism, and was continuously decomposed. At the beginning of storage (day 0), amino acids with higher content were lysine, phenylalanine, threonine, alanine, glutamic acid, and arginine. In the middle of storage (day 6), the content of sweet amino acids decreased, while the content of bitter amino acids increased. At the end of storage (day 12), except for phenylalanine, the content of other sweet amino acids continued to decrease, while the content of bitter amino acids increased further. Table 5 shows that the 11 amino acids had TAVs < 1, indicating that the amino acids did not contribute much to the overall taste of *H. marmoreus* (Kato *et al.*, 1989). Alanine, glutamic acid, and arginine, which produce pleasant sensations, had TAVs > 0.1, indicating that they contributed to the flavour of *H. marmoreus* more than the other amino acids, and with prolonged storage, their TAV gradually decreased, indicating flavour deterioration.

Lysine is a bitter amino acid, and in the late storage period (day 12), its TAV was > 0.1, which was higher than that in the early storage period (day 0). Lysine had greater contribution to flavour than other amino acids in the late storage period (Van,

Table 2. Changes in 5'-nucleotide content (μg·g^{-1}) in *H. marmoreus* packaged in nanofilm during storage.

Taste-presenting nucleotide	0 d	6 d	12 d
5'-CMP	105.58 ± 0.36^{c}	205.67 ± 3.5^{b}	301.3 ± 1.87^{a}
5'-UMP	308.93 ± 0.38^{c}	686.47 ± 7.93^{b}	714.12 ± 2.2^{a}
5'-GMP	226.76 ± 0.61^{c}	499.83 ± 5.35^{b}	622.9 ± 1.68^{a}
5'-IMP	54.01 ± 1.73^{c}	105.9 ± 3.64^{b}	131.52 ± 2.63^{a}
5'-AMP	685.18 ± 3.29^{b}	635.07 ± 26.58^{c}	822.48 ± 38.49^{a}
Total	1380.46 ± 2.59^{c}	2132.94 ± 32.88^{b}	2592.32 ± 31.43^{a}

Table 3. Changes in TAV of 5'-nucleotides in *H. marmoreus* packaged in nanofilm during storage.

Taste-presenting nucleotide	Threshold* (μg/mL)	TAV (0 d)	TAV (6 d)	TAV (12 d)
5'-CMP	ND	/	/	/
5'-UMP	ND	/	/	/
5'-GMP	125	1.81	4.00	4.98
5'-IMP	250	0.22	0.42	0.53
5'-AMP	500	1.37	1.27	1.64

*: recognition threshold of 5'-nucleotide in water; ND: relevant literature has not been consulted, so 5'-CMP and 5'-UMP are not discussed in this article. Threshold values were adapted from Chiang *et al.* (2006).

Table 4. Changes in free amino acids content (μg·g^{-1}) in *H. marmoreus* packaged in nanofilm during storage.

Free amino acid	0 d	6 d	12 d
Glutamate (Glu)	95.13 ± 0.14^{a}	75.05 ± 0.60^{b}	16.4 ± 1.57^{c}
Alanine (Ala)	64.64 ± 0.38^{a}	40.77 ± 0.99^{b}	34.6 ± 1.31^{c}
Arginine (Arg)	52.20 ± 1.23^{a}	36.92 ± 0.35^{b}	35.3 ± 0.53^{b}
Lysine (Lys)	41.59 ± 1.08^{b}	35.54 ± 0.55^{c}	54.6 ± 0.52^{a}
Phenylalanine (Phe)	24.14 ± 0.45^{a}	16.18 ± 0.25^{c}	20.3 ± 0.13^{b}
Threonine (Thr)	20.86 ± 0.40^{a}	13.58 ± 0.34^{c}	17.0 ± 0.32^{b}
Tyrosine (Tyr)	10.74 ± 0.81^{b}	10.10 ± 0.14^{b}	12.3 ± 0.32^{a}
Isoleucine (Ile)	7.50 ± 0.10^{a}	4.78 ± 0.15^{c}	5.75 ± 0.19^{b}
Leucine (Leu)	4.50 ± 0.13^{a}	2.86 ± 0.07^{c}	3.50 ± 0.07^{b}
Valine (Val)	4.16 ± 0.39^{a}	3.43 ± 0.05^{b}	4.07 ± 0.24^{a}
Methionine (Met)	1.33 ± 0.05^{a}	1.15 ± 0.05^{b}	0.82 ± 0.03^{c}
Total	326.79	240.36	185.18

Table 5. Changes in TAV value of free amino acids in *H. marmoreus* packaged in nanofilm during storage.

Free amino acid	Taste attribute	Threshold (μg/mL)	TAV (0 d)	TAV (6 d)	TAV (12 d)
Glutamate (Glu)	Umami (+)	300	0.317	0.250	0.055
Alanine (Ala)	Sweetness (+)	600	0.108	0.068	0.058
Arginine (Arg)	Sweetness, bitterness (+)	500	0.104	0.074	0.070
Lysine (Lys)	Bitterness (-)	500	0.083	0.071	0.11
Phenylalanine (Phe)	Sweetness (+)	900	0.024	0.018	0.023
Threonine (Thr)	Sweetness (+)	2600	0.008	0.005	0.007
Tyrosine (Tyr)	Bitterness (-)	910	0.012	0.011	0.014
Isoleucine (Ile)	Bitterness (-)	900	0.008	0.005	0.006
Leucine (Leu)	Bitterness (-)	1900	0.002	0.002	0.002
Valine (Val)	Sweetness, bitterness (-)	400	0.010	0.009	0.010
Methionine (Met)	Bitterness, sulphur (-)	300	0.004	0.004	0.003

+: pleasant taste, -: unpleasant taste. Taste attributes were adapted from Chen and Zhang (2007) and Wang *et al.* (2014).

2015). In summary, alanine, glutamic acid, and arginine were the key amino acids in fresh *H. marmoreus*, and lysine was the key amino acid following deterioration. Therefore, changes in these amino acids can be used as indicators of *H. marmoreus* deterioration.

Changes in organic acid content and TAV

Organic acids participate in several physiological and biochemical reactions in fruits and vegetables. The type and content of organic acids are also important factors affecting the quality and flavour of edible fungi (Li *et al.*, 2014). In the present work, we measured oxalic and fumaric acids (Figure 3). During storage, oxalic acid decreased at first, and subsequently increased ($p < 0.05$). During early and mid-storage periods, oxalic acid was involved in metabolism. At the end of the storage period, the quality of mushrooms decreased, and the content of oxalic acid increased due to microbial decomposition. Fumaric acid increased, and subsequently decreased during storage, but the total content showed a slow increase, consistent with the results of Jabłońska-Ryś *et al.* (2022). Figure 4 shows that TAV of oxalic acid in the early and late storage period was > 1, indicating that oxalic acid had significant contribution to the flavour of *H. marmoreus* during these two periods. TAV in the middle storage period was < 1, indicating that oxalic acid had little effect on the flavour of *H. marmoreus*. Fumaric acid had high threshold value. The TAV of each period of storage was < 1; therefore, fumaric acid had little contribution to the flavour of *H. marmoreus*. It can thus be concluded that the content of oxalic acid can be used as an indicator of *H. marmoreus* deterioration.

Conclusion

The present work demonstrated that octanal, 1-octene-3-one, and 1-octene-3-ol were the main volatile flavour substances, and *trans*-2-octenal was the key substance that affected the formation of volatile flavours in fresh *H. marmoreus*. *Trans,trans*-2,4-nonadienal, and 3-octanone were the key volatile substances involved in flavour deterioration. Among the non-volatile flavour substances, trehalose, 5'-GMP, glutamic acid, alanine, arginine, and oxalic acid were the most predominant in fresh *H. marmoreus*. Lysine was the main non-volatile flavour ingredient of *H. marmoreus* after deterioration. Therefore, the deterioration of *H. marmoreus* can be quickly predicted by the change in these compounds, and the establishment of a rapid detection tool / technique.

Acknowledgement

The authors are grateful for the financial support received from the Gansu Province Balanced Transfer Payment for Strong Science and Technology Subsidy (grant no.: NXKJ2023-02), the National Key Research and Development Program of China (grant

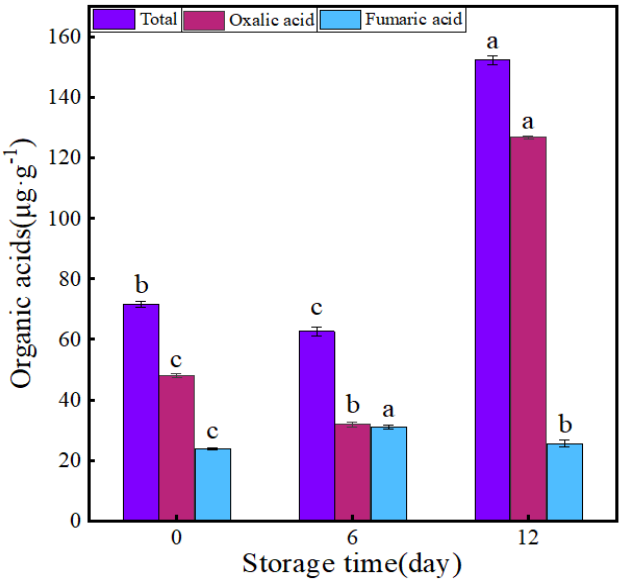

Figure 3. Changes in organic acids content in *H. marmoreus* packaged in nanofilm during storage. Vertical lines represent standard deviation (± SD); values with different lowercase letters are significantly different ($p < 0.05$). Significant differences are between groups.

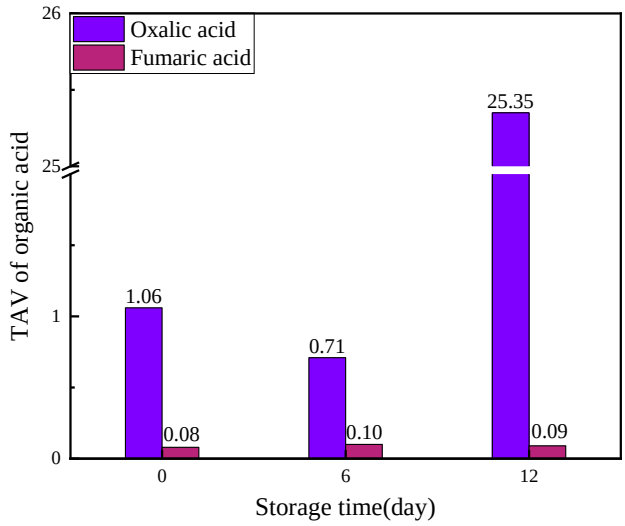

Figure 4. Changes in TAV of organic acids in *H. marmoreus* packaged in nanofilm during storage. Thresholds was referred to Van (2015); threshold of oxalic acid is 45 μg·g^{-1}, threshold of fumaric acid is 300 μg·g^{-1}.

no.: 2018YFD0400205), and the Gansu Province Science and Technology Major Projects (grant no.: 21ZD4NA016). The authors also thank the International Science Editing (http://www.internationa lscienceediting.com) for editing this manuscript.

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