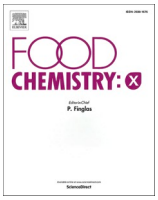
Food Chemistry: X 20 (2023) 101029

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effects of adding milk to fermented black mulberry (*Morus nigra* L.) juice on its antioxidant activity in C2C12 cells and changes in volatile flavor compounds during storage

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ARTICLE INFO

Keywords:
Antioxidant
Fermented black mulberry juice
Fruit milk
Storage
Volatile flavor compounds

ABSTRACT

This study assessed the impact of milk on the bioactive compounds, physicochemical properties, antioxidant activity, ROS inhibition, and volatile flavor compounds of fermented black mulberry juice (FBMJ). Firstly, the results showed that 25% concentration of milk was the most suitable for preparing FBMJ-Milk. Compared to the control group, the addition of milk significantly increased the SOD activity and antioxidant capacity, as well as enhanced the total phenolic content (TPC) and SOD storage stability. Secondly, HS-SPME-GC–MS combined with OPLS-DA analysis identified 49 compounds in FBMJM, including 12 esters, 6 acids, 1 ketone, 2 aldehydes, 19 alcohols and 9 other compounds. During the storage, the levels of ethyl ester compounds decreased significantly, while the degradation of ester produced some acid and alcohol compounds. The findings revealed that the addition of milk was beneficial for maintaining the antioxidant stability of FBMJM during storage and enhancing the richness of product flavor.

1. Introduction

Juice-flavored milk refers to milk mixed with fruit juice, which offers properties beyond nutritional purposes as they contain one or more compounds that provide enhanced functionality in the body, promoting well-being and health or reducing the risk of several diseases. Due to the well-known antioxidant properties and health benefits of milk, juice-and-milk-based beverages have been widely accepted. (Salvia-Trujillo, Morales-de la Peña, Rojas-Graü, & Martín-Belloso, 2011). Indeed, research has indicated that flavored milk plays an important role in the overall healthy dietary patterns of children and adolescents due to its rich nutritional content and appealing taste (Ricklefs-Johnson & Pikosky, 2023). However, it is worth noting that currently available commercially flavored dairy products often contain excessive amounts of sugar and additives for flavor purposes. While the addition of sugar may promote overall milk consumption, this strategy can have adverse effects on calorie intake and potentially contribute to childhood and adolescent obesity(Patel, Moghadam, Freedman, Hazari, Fang, & Allen, 2018).

It is crucial to research a beverage that is uniquely flavored, low in calories, and has strong antioxidant properties. To achieve this goal, researchers have combined fruit juice, fruit peel, and other fruit residues with probiotics and milk to produce yogurt. The resulting yogurt not only exhibits strong stability but also demonstrates higher antioxidant activity. However, the unique texture of yogurt inherently limits its ability to contain a high level of fruit juice. As a result, its antioxidant capacity may be relatively limited (Ge et al., 2022).

Therefore, we aim to develop a fermented fruit juice flavored milk with pre-fermentation technology, which involves fermenting the juice before adding milk. This not only helps to preserve the fermented flavor but also enhances the enrichment of bioactive compounds, thereby boosting antioxidant activity. Over the past decade, research on the combination of fruit-milk has primarily focused on process optimization, protein properties (Khulal, Ghnimi, Stevanovic, Rajkovic, & Cirkovic

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https://doi.org/10.1016/j.fochx.2023.101029
Received 1 September 2023; Received in revised form 15 November 2023; Accepted 22 November 2023
Available online 26 November 2023

Velickovic, 2021), antioxidant, and bioavailability studies (Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2014). Further, numerous studies have shown that the phenolic compounds in plants have a high binding affinity for proteins, carbohydrates, and lipids (Cianciosi et al., 2022). The addition of fermented fruit juice may have a negative impact on the sensory and physical properties of milk, and the feasibility of using it as a supplement needs to be carefully assessed (Iriondo-DeHond, Blázquez-Duff, del Castillo, & Miguel, 2020). Furthermore, although researchers have extended the stability and shelf life (Islam et al., 2023) of fruit juice through fermentation and increased its antioxidant activity and unique flavor, it remains unclear whether these advantages can be carried over to fruit milk or what flavor and physicochemical properties may arise from the addition of fermented fruit juice to milk.

In this study, to investigate the effects of fermented fruit juice on milk, we chose black mulberry (*Morus nigra* L.) as the research material for fermentation. The research group optimized the fermentation process of black mulberry juice in the preliminary stage and obtained fermented black mulberry juice (FBMJ) with enhanced antioxidant and bioactive content (Lv et al., 2022). Prepare FBMJM by adding milk of different concentrations to FBMJ. Firstly, we studied the influence of different milk concentrations on the bioactive compounds and physicochemical properties of FBMJM. Then, we established an oxidative stress model using hydrogen peroxide-induced C2C12 cells to evaluate the ability of FBMJM to protect cells from oxidative damage and its intracellular antioxidant activity. Furthermore, the changes in volatile flavor compounds in FBMJM during storage were detected using HS-SPME-GC–MS. The differences in volatile components in FBMJM at different storage times were analyzed using orthogonal partial least squares discriminant analysis (OPLS-DA). We dynamically monitored the physicochemical properties and bioactive substance content of FBMJM during storage and conducted a correlation analysis with the volatile compound content. This study explores the rationality and applicability of FBMJM to determine whether it is a stable, nutritionally rich innovative food product.

2. Materials and methods

2.1. Materials

Fresh black mulberries (*Morus nigra* L.) were sourced from Kuqa, Xinjiang, the entire experimental process used the same batch of black mulberries. The bacterial strains were obtained from China General Microbiological Culture Collection Center (Beijing, China). Milk was purchased from Liyuan supermarket in Urumqi (Xinjiang Uygur Autonomous Region, China). C2C12 cells were obtained from Procell Life Science & Technology Co., Ltd. (Wuhan, China). The assay kits for superoxide dismutase (SOD), DPPH(1,1-diphenyl-2-picrylhydrazyl) free radical scavenging capacity, total antioxidant capacity (ABTS(2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) method), and total antioxidant capacity (FRAP method) were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Ethanol, gallic acid, sodium hydroxide, dioctyl alcohol, and Folin–Ciocalteu phenol reagent were acquired from Tianjin Shengao Chemical Reagent (Tianjin, China). All reagents used in the experiment were of analytical grade unless otherwise specified.

2.2. Preparation of fermented black mulberry juice with milk

The FBMJ was prepared based on the fermentation process previously discussed (Lv, et al., 2022). Purple ripe black mulberries were selected, abnormal or the diseased fruits were removed, the selected fruits were homogenized at 25 °C. Subsequently, add 0.2 % pectinase, 0.1 % cellulase, and 0.1 % hemicellulase (v/v) (Novozymes®,Ltd.,Beijing, China) separately to the homogenized mulberries. Then, the enzymatic hydrolysis was carried out at 55℃ for 3 h. After that, adjust the soluble solids content to 18.0° Brix by adding sucrose. The enzymes are inactivated at 85 °C for 30 min, the homogenized mulberry hydrolysate was inoculated at 37 °C. The total bacterial density in black mulberry juice was 6×10^6 CFU/mL. *Lactobacillus paracei*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp., *Bifidobacterium animalis* subsp., and *Lactobacillus fermentum* were mixed in the ratio of 27.96 %, 15.37 %, 16.64 %, 5.12 %, 15.83 %, and 19.08 %. The mixed cultures with the juice substrate were maintained in a closed ambient for anaerobic fermentation. FBMJ-milk (FBMJM) sample was prepared in sterilized conical bottles. 50 mL of FBMJ was added to each bottle with 0 %, 5 %, 15 %, 25 %, 35 %, and 45 % milk (*v/v*), which was thoroughly dispersed using a homogenizer and pasteurized at 65℃ for 30 min to prepare the FBMJM. The control group (FBMJD) changed the milk to dd water, and the other conditions remained unchanged. After being sterilized and canned, the samples are stored at 4℃ for further analysis.

2.3. Bioactive compounds

Total phenolic content (TPC) was measured by the methods reported by Rosl et al. (Mohd Rosli et al., 2022) with modifications. A series of preprocessing steps were performed to remove the oligosaccharides from the sample. TPC was calculated using the standard curve equation. The activity of superoxide dismutase activity (SOD) was determined following the manufacturer's protocols.

2.4. Measurement of physicochemical indicators

The pH measurements of the samples were conducted using a pH meter (IS 128; Shanghai Marker Instrument Technology Co., Ltd., Shanghai, China).

The method described by Amirdivani and Baba (Amirdivani & Baba, 2011) was employed to determine the total titratable acidity (TTA) with slight modifications. Initially, 2.5 g of FBMJM was mixed with 12.5 mL of distilled water in a homogenizer. Then, three to four drops of 1 % phenolphthalein were added, and the mixture was titrated with 0.1 mol/L sodium hydroxide (NaOH) while continuously stirring until a uniform pink color was obtained. Record the volume of NaOH used. The TTA was calculated as follows:

$$\text{Acidity } (^{\circ}T) = V_{\text{NaOH}} \times 40 \tag{1}$$

The method for detecting the total nitrogen content (TNC) in the sample is based on the Kjeldahl method specified in the national standard "GB5009.5-2016″ (China). Accurately measure 15 mL of sample, and transfer it into a dry 500 mL Kjeldahl flask. Add 0.4 g of copper sulfate, 6 g of potassium sulfate, and 20 mL of sulfuric acid for digestion in a digestion furnace. Then, the diluted sample is transferred to an automatic Kjeldahl nitrogen analyzer. Prior to use, the analyzer is prepared by adding sodium hydroxide solution (400 g/L), standard hydrochloric acid solution (0.05 mol/L), and boric acid solution (20 g/L) containing mixed indicator A (Combine 2 parts of methyl red ethanol solution (1 g/L) with 1 part of methylene blue ethanol solution (1 g/L) before use.).

The total nitrogen content in the sample is calculated using Eq. (2),

$$X = \frac{(\nu_1 - \nu_2) \times c \times 0.0140}{m \times \nu_3/100} \times F \times 100 \tag{2}$$

X: The content of total nitrogen in the sample (g/100 g); ν_1:The volume of standardized hydrochloric acid titrant consumed by the sample solution (mL); ν_2: The volume of standardized hydrochloric acid titrant consumed by the blank solution (mL); *c*: The concentration of the standardized hydrochloric acid titrant (mol/L); 0.0140: The mass of nitrogen equivalent to 1.0 mL of hydrochloric acid [c(HCl) = 1.000 mol/L] titrant (g); *m*: The mass of the sample (g); ν_3: The volume of the neutralizing solution absorbed (mL); F: The coefficient for converting nitrogen to protein. For pure milk, F is 6.38, and for fruit milk, *F* is 6.25;

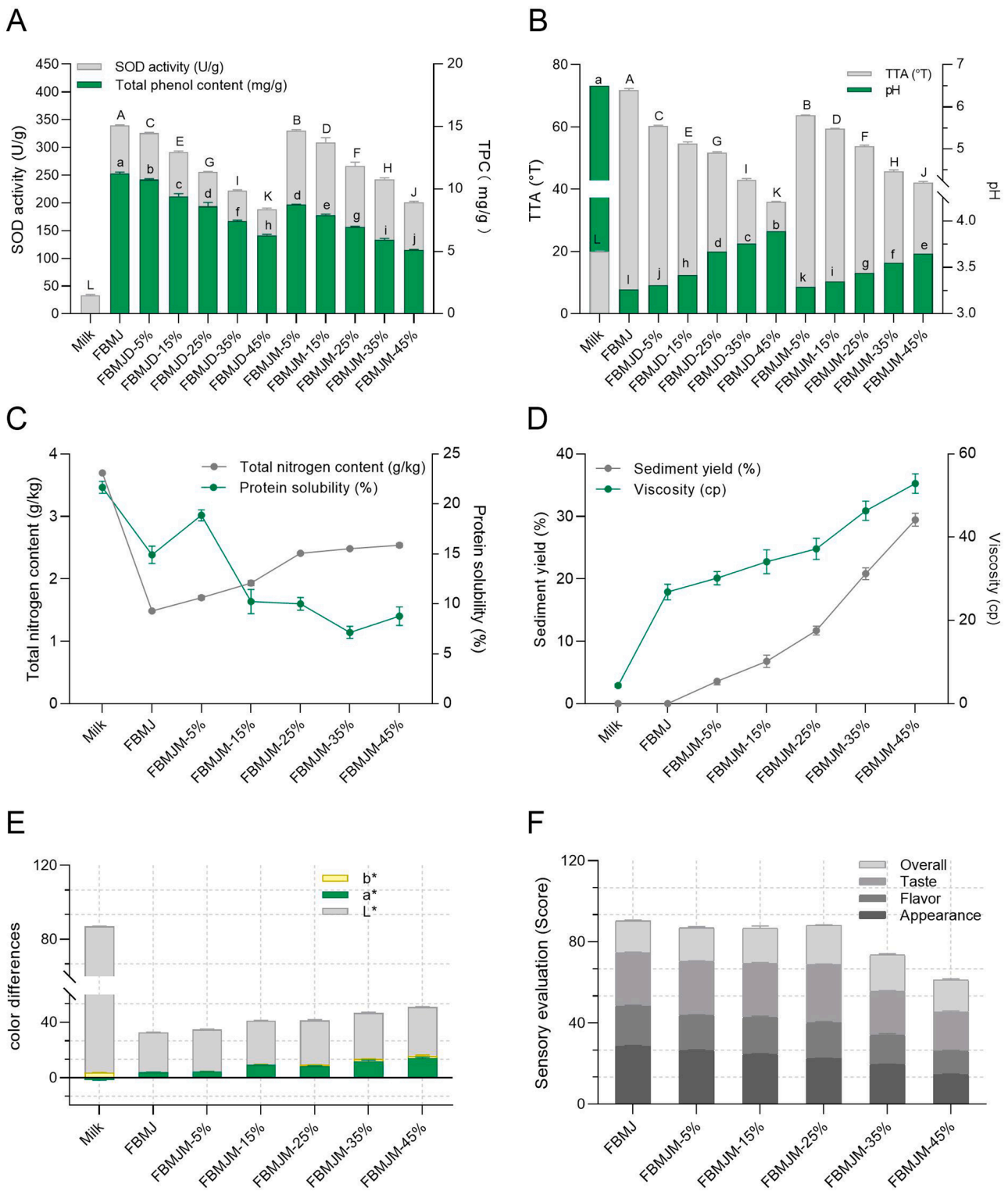

Fig. 1. Bioactivity (A, total phenol content (TPC) and superoxide dismutase (SOD) activity); Physicochemical indexes (B, total titratable acid (TTA) and pH; C, total nitrogen content (TNC) and protein solubility; D, viscosity value and precipitation rate; E, color differences; F, sensory evaluation) of FBMJM and FBMJD (C-F only detect the indicators of FBMJM).

100: Conversion factor.

The protein solubility detection method refers to the national standard "GB 19541–2017" (China). Samples were solubilized 10 % (v/v) in potassium hydroxide solution (2 g/L). The samples were centrifuged at 2,700 r/min for 10 min and the supernatant obtained was collected. After centrifugation, measure the total nitrogen content in the potassium hydroxide solution (W1). Simultaneously measure the total nitrogen content (W2) in the sample. The result was expressed as % of soluble protein.

The stability was measured according to Stoker's principle (Schkoda, Hechler, & Kessler, 1999). Further, 10 mL of the sample was added into a graduated centrifuge tube and centrifuged at 3500 rpm for 30 min. The

upper-layer solution was discarded. Then, the lower-layer sediment (wet residue) was weighed, and the sediment yield (SY) was calculated as follows:

$$\text{Sediment yield (\%)} = \text{Sediment mass (g)/10 mL sample mass (g)} \times 100 \quad (4)$$

The viscosity value of the sample was measured using a viscometer (Rapid-20; Bosin, Shanghai, China). The sample was left to stand in a 25℃ water bath for 30 min prior to measurement. Then, the sample was placed in the viscometer, and the viscometer temperature was set to 25℃ and the rotation speed to 60 rpm.

The color evaluation of each sample is performed using a colorimeter (DS-700D, Hangzhou Color Spectrum Technology Co., LTD., Hangzhou, China) calibrated with a white calibration plate.

Sensory evaluation is an essential component in assessing the quality and acceptability of food products. In this study, the methods reported by Liu et al. (Liu, Sheng, Li, Zhang, Tang, & Shan, 2022) were used to conduct a sensory evaluation of the FBMJM samples. Training and evaluation were carried out following the guidelines set by the International Organization for Standardization to ensure the accuracy and consistency of the evaluation process (I.S.O., 1993). The results of the sensory evaluation are presented in supplementary Table T1, providing a comprehensive overview of the sensory attributes of the FBMJM samples.

The DPPH, ABTS and FRAP detection methods are conducted following the instructions provided by the manufacturer's assay kits.

2.5. Cell culture and treatment

The C2C12 myoblasts used in this study were derived from mice. They were cultured in a medium containing high-glucose DMEM (Gibco, NY, USA), which was supplemented with 10 % fetal bovine serum (Hyclone, UT, USA) and 1 % penicillin/streptomycin antibiotics (Gibco, NY, USA). The cells were incubated in a controlled environment, with a temperature of 37℃ and 95 % air and 5 % CO_2 composition.

2.6. Cell viability assay

Using H_2O_2 of different concentrations, cells were treated for 12, 24, and 48 h, and it was determined that treating cells with 500 μmol/L H_2O_2 for 24 h reached the optimal induction time and concentration. Normal group experiments were conducted at various concentrations of FBMJD and FBMJM (0, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 10 mg/mL medium) for 24 h to assess the cellular viability using the CCK-8 kit. In the drug group, the cells were exposed to different concentrations of FBMJD and FBMJM (0, 0.25, 0.5, 1, 2, 4, 8, and 10 mg/mL medium) for 24 h, followed by treatment with 500 μmol/L H_2O_2 for 24 h. The effectiveness of similar concentrations was demonstrated in previous studies, which guided the selection of concentration and time ranges for FBMJ and FBMJM in this study. After incubating the cells in 10 μL of CCK-8 solution at 37 °C for 90 min, the absorbance was measured using a microplate reader at 450 nm (Synergy H4, BioTek, VT, USA).

2.7. Evaluation of intracellular antioxidant activity

Intracellular ROS generation was evaluated by staining the cells with DCFH-DA (BL714A; Biosharp, Anhui, China). The cells treated with samples of varying concentrations were incubated with a fluorescent dye, DCFH-DA (at a final concentration of 10 vM), at 37 °C in the dark for 30 min. Subsequently, the cells were analyzed by flow cytometry (CytoFLEX; Beckman Coulter, CA, USA).

2.8. Analysis of volatile flavor compounds

The volatile flavor compounds were analyzed based on the methodology proposed by Whitener et al. (Beckner Whitener et al., 2015) using SPME combined with GC–MS technology.

Further, 2.0 g of the sample was weighed, placed in a 20-mL SPE bottle, and sealed. Next, 1 μL of internal standard (1000 times diluted 2-octanol) was added. The sample was then thermally balanced at 60℃ on the SPE platform for 30 min. Simultaneously, the SPE head was aged at 250℃ at the inlet of the gas chromatograph for 30 min. After aging, the SPE head was inserted into the SPE bottle at a depth of about 1 cm. The SPE handle was pressed to push out the extraction fiber, which was absorbed for 30 min and then removed. The samples were analyzed by GC–MS at 250℃ for 5 min. They were examined by GC–MS (7890A-5975C; Agilent Technologies, CA, USA) using the Agilent MassHunter Qualitative Analysis software (Agilent) and the mass spectra library (linear retention indexes) of the National Institute of Standards and Technology (version 11; NIST/EPA/NIH, USA).

2.9. Statistical analysis

The data were presented as mean ± standard deviation (SD) ($n = 3$). Analysis of variance and Duncan's multiple range tests were performed using SPSS 20.0 (SPSS Science, Chicago, USA). Comparison between homogeneous sample groups was assessed through *t*-test at 95 % significance level. All figures were created using GraphPad Prism v8.0.2 (GraphPad, CA, USA).

3. Results

3.1. Changes in the SOD activity and TPC of different contents of FBMJM

Plants produce phenolic compounds that possess the ability to precipitate proteins, have antioxidant activity, and are responsible for imparting astringency and bitter taste in many foods (Cianciosi, et al., 2022). Superoxide dismutase (SOD) is an important component of the human antioxidant system, and has the ability to efficiently and specifically remove superoxide anion radicals. The TPC of the samples decreased with the increase in the addition of milk (Fig. 1A). It is possible that the decrease in total phenolic content (TPC) is due to non-specific binding between proteins and phenols. It is worth noting that compared to the FBMJD group, the FBMJM group has lower TPC content but significantly higher SOD content, which is attributed to the presence of 33.42 U/g of SOD activity of milk (Fig. 1A).

3.2. Changes in physicochemical indexes of different contents of FBMJM

By conducting tests on the physicochemical parameters of FBMJM, it ensures that the product meets quality requirements and ensures food safety for consumers. Additionally, the test results of these physicochemical parameters also help researchers adjust production processes and improve product formulations.

The pH value of fruit-flavored milk can reflect its acidity or alkalinity, which may be related to the product's taste and stability. The changes in TTA and pH values of FBMJM are shown in Fig. 1B. Compared to FBMJ, the TTA value of FBMJM45% decreased from 71.86°T to 42.15°T, and the pH increased from 3.26 to 3.65. Compared to the FBMJD group, FBMJM had a higher pH value. This indicates that the addition of milk has a significant impact on the acidity of the sample and may alter the chemical composition of FBMJ (S. Li, Ye, & Singh, 2021).

Measuring the protein content in fruit milk provides information on the nutritional composition, quality characteristics, and food safety of the product. As shown in Fig. 1C, the protein content of milk, FBMJ, and 25 % FBMJM are 3.49, 1.48, and 2.4 g/kg, respectively. Adding milk to FBMJ increased its protein content. As the milk content increased to 25 %, the protein solubility decreased rapidly from 18.86 to 9.99. However, both the protein content and solubility have reached the standards set by Chinese flavored dairy product enterprises.(Q/AWXW 0002 S-2022).

It is generally believed that the texture and viscosity of food are the

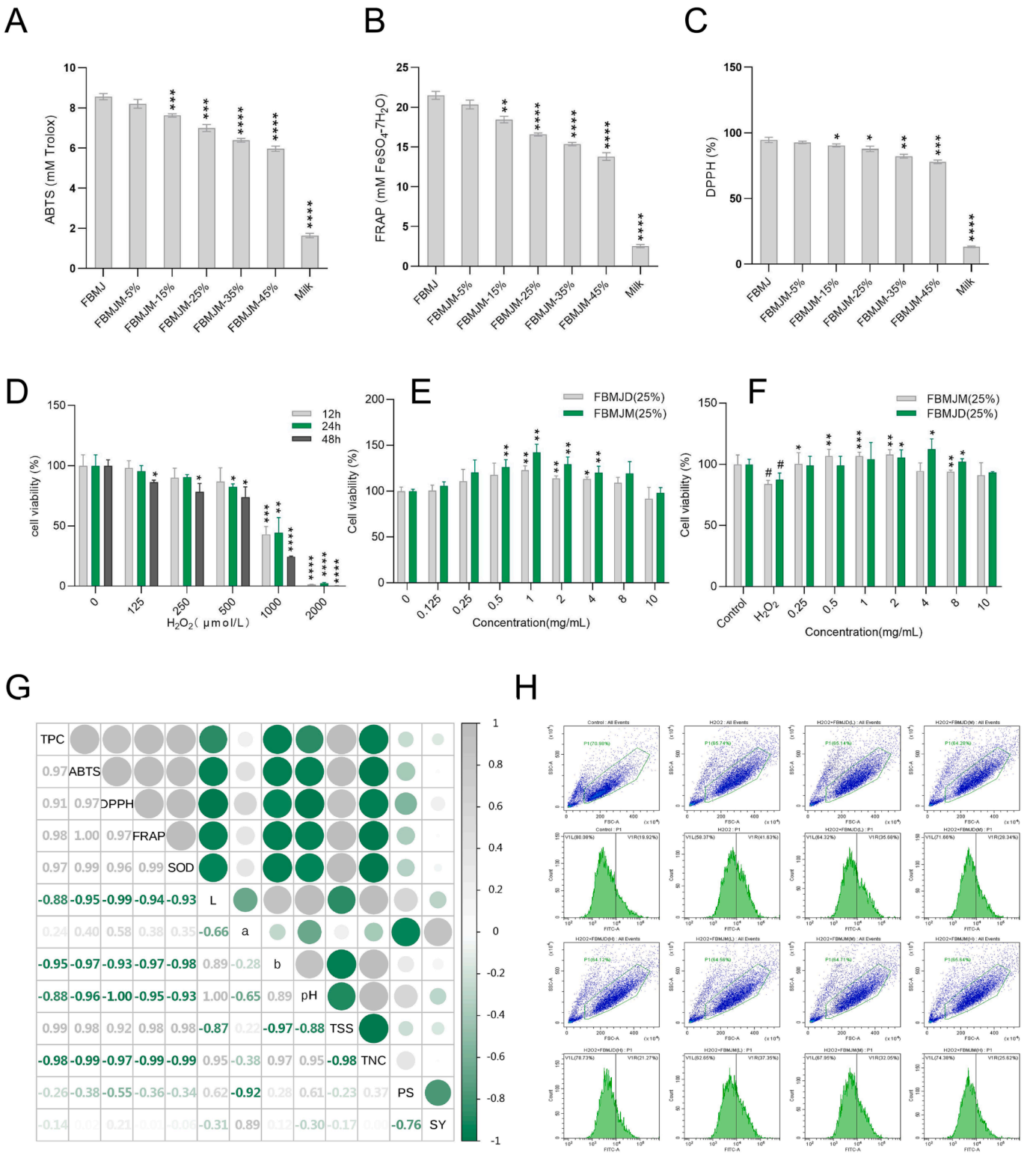

Fig. 2. A-C, antioxidant activity of FBMJM; D, Establishment of oxidative stress model of C2C12 cells induced by H_2O_2; E, Effects of FBMJM and FBMJD on cell viability of normal cells; F, The effects of FBMJM and FBMJD on the survival rate of 500 μmol/L H_2O_2-oxidatively stimulated C2C12 cells compared with the control group (# $p < 0.05$) and damaged group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.); G, The relationship between the antioxidant activity and bioactive compound content of FBMJM.; H, The inhibitory effect of FBMJM and FBMJD on H_2O_2-induced intracellular ROS production in C2C12 cells. The intracellular ROS production was determined using the DCFH-DA method with flow cytometry.

primary determining factors for consumer acceptance and preference for food and beverages. The viscosity and sedimentation yield of FBMJM gradually increased with an increase in the addition of milk (Fig. 1D). The sediment yield amount of FBMJM25% was 11.71 %. The stability of milk was significantly influenced by hydrogen ions in the environment. At the aforementioned pH value, the stability of milk deviated from the protein isoelectric point, which could cause protein coagulation and precipitation (Aydogdu, O'Mahony, Huppertz, Magan, & McCarthy,

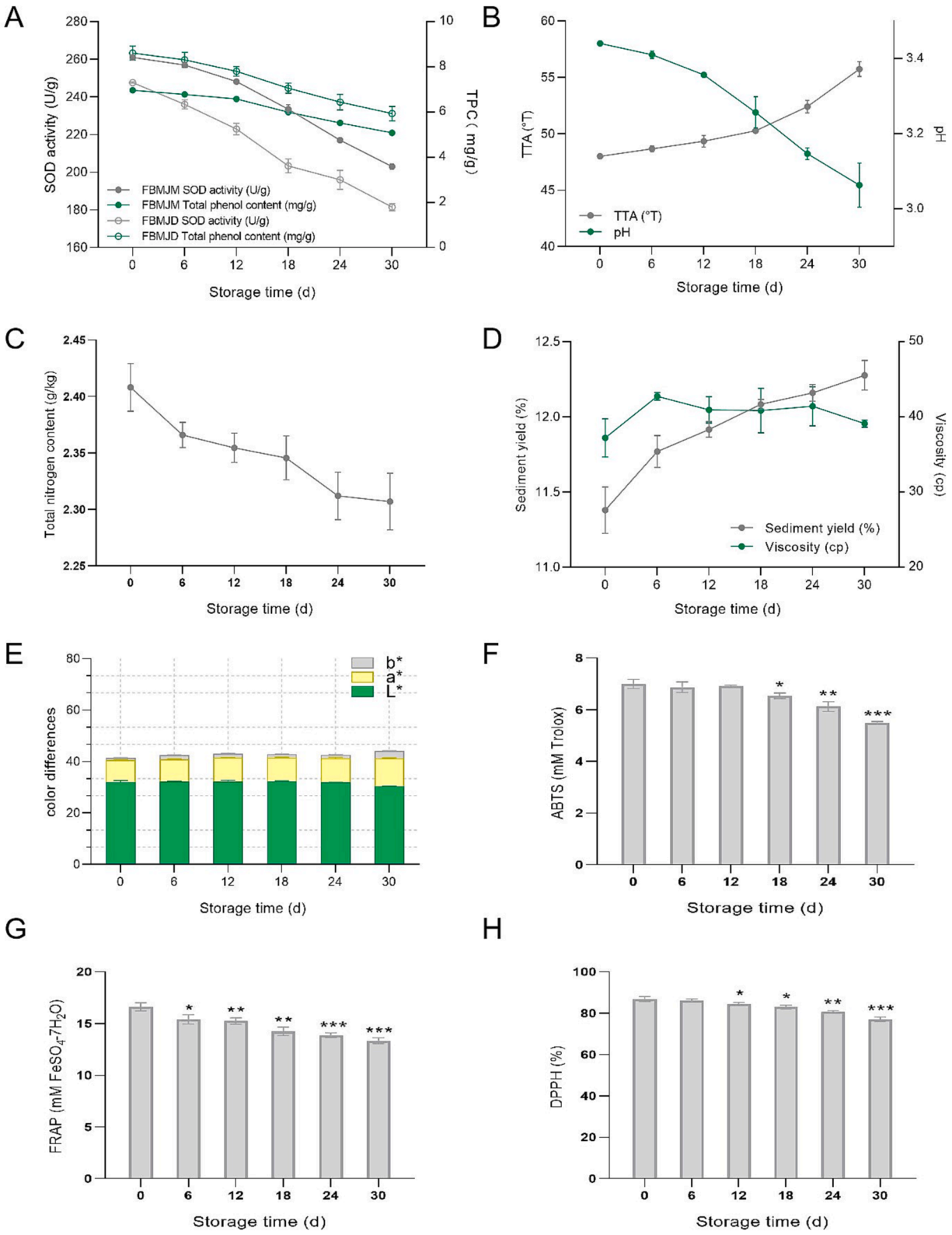

Fig. 3. The effects of different storage times on the bioactive substances (A, total phenol content (TPC) and superoxide dismutase (SOD) activity), chemical properties (B, total titratable acid (TTA) and pH; C, total nitrogen content (TNC), physical (D, viscosity value and stability; E, color differences) and antioxidant ability (F, total antioxidant capacity(ABTS); G, ferric ion reducing antioxidant power (FRAP); H, DPPH radical scavenging activity) of FBMJM during storge. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.).

2023). Protein precipitation can increase viscosity. The viscosity of FBMJM25% is 37.22 cp, which is higher compared to pure milk and FBMJ. This phenomenon also occurs when combining plant-based materials such as mulberries (Du et al., 2021) with milk. Combining FBMJ with milk significantly increases the viscosity. Appropriately increasing the viscosity of fruit-flavored milk not only enhances consumer sensory experience but also contributes to the stability of FBMJM (Wu et al., 2023).

The detection of color difference in food can assess its appearance quality, optimize production processes, and detect the freshness and deterioration of the product. The color difference values of all samples are shown in Fig. 1E. In the samples, milk has the lowest a* value of −1.61, while the highest L* and b* values are 87.08 and 3.68, respectively. For FBMJ, the L*, a*, and b* values are 32.7, 3.89, and 0.02, respectively. As the amount of milk added increases, the L*, a*, and b* values all increase. Samples with higher milk proportions appear brighter and show more redness and yellowness. The sensory evaluation of FBMJM was the highest at 25 % (Fig. 1F). Therefore, further research on FBMJM selected samples prepared with the addition of 25 % milk.

Based on the results of the correlation analysis (Fig. 2G), protein solubility is positively correlated with acidity and negatively correlated with sedimentation. The addition of FBMJ to milk lowers its pH value, leading to a decrease in protein solubility and the occurrence of sedimentation. This, in turn, affects the viscosity and color difference of the fruit and milk beverage. The presence of protein and colored suspensions in the sedimentation increased the brightness of the FBMJM.

3.3. Antioxidant activity

3.3.1. Changes in the antioxidant activity of different contents of FBMJM

As shown in Fig. 2, we conducted tests on ABTS, FRAP, and DPPH of FBMJM. All three antioxidant tests showed the same trend, which is that the higher the FBMJ content, the stronger the antioxidant activity. The milk had an ABTS activity of 1.63 mM Trolox, FBMJ had 8.55 mM Trolox, and the sample with 25 % milk added had 6.99 mM Trolox. Although milk does not contain TPC, it still exhibits certain antioxidant activity, which may be attributed to the active peptides and bioactive enzymes present. The overall trend of FRAP was similar to that of ABTS. The oxidative activity is related to the presence of polyphenols, which is why the research results show a similar increasing trend as observed in TPC. The correlation analysis study (Fig. 2G) indicates a strong correlation ($p < 0.05$) between the antioxidant capacity and TPC content, which is consistent with the findings of Zhang et al's (2023a) study on the antioxidant activity of oat phenolic compounds.

3.3.2. Effects of FBMJM on H_2O_2-induced cytotoxicity in C2C12 cells

In complex food processing, the interaction between phenolic compounds and proteins is always closely related to the functionality of the food (Cianciosi, et al., 2022). Therefore, for a more comprehensive study of the antioxidant effects of FBMJM in cells, we chose to induce oxidative stress in C2C12 cells using H_2O_2 and established FBMJD as a control to test whether milk affects the antioxidant activity of FBMJ.

As shown in Fig. 2E, in the normal cell group, FBMJM concentrations below 4 mg/mL did not exhibit significant toxic effects on the cells and even had a certain degree of growth-promoting effect ($P < 0.05$). When the concentration of FBMJM was 1 mg/mL, cell viability increased by 42.27 %. Treatment of the model group with 500 μmol/L H_2O_2 for 24 h resulted in a significant decrease in cell viability by 17.56 % ($P < 0.05$). In the drug group, the researchers observed a dose-dependent increase in cell viability in the drug-treated group (Fig. 2F). Comparing the drug groups with the model group, we observed that the concentration of 2 mg/mL FBMJD and 1 mg/mL FBMJM led to a significant increase in cell viability by 20.71 % ($P < 0.05$) and 27.56 % ($P < 0.001$), respectively. These findings indicated that FBMJM displayed superior efficacy compared with FBMJD in safeguarding C2C12 cells from oxidative stress (Ju et al., 2021).

3.3.3. Effect of FBMJM on intracellular ROS levels in C2C12 cells

In cells, reactive oxygen species (ROS) can cause damage to essential biomolecules such as lipids, proteins, and DNA, leading to mitochondrial dysfunction and cell death. We used flow cytometry to detect the production of intracellular ROS to gain a more detailed understanding of the protective effects of FBMJ against oxidative stress.

Treatment with 500 μmol/L H_2O_2 significantly increased ROS levels compared with the control group. However, the presence of FBMJD and FBMJM reversed this trend in a dose-dependent manner. Flow cytometry analysis revealed that the H_2O_2 group exhibited 2.09 times higher ROS fluorescence compared with the control group. In contrast, the high-dose group of FBMJD showed a 1.62-fold decrease in ROS fluorescence compared with the H_2O_2 group, while the high-dose group of FBMJM displayed a 1.96-fold decrease, highlighting the stronger antioxidant effect of FBMJM (Fig. 2H). Importantly, the addition of 25 % milk did not diminish the antioxidant activity of FBMJM. Previous studies also demonstrated the potential to enhance the antioxidant capacity of milk by incorporating plant-based ingredients (Ahmad et al., 2020). This finding further supported the effectiveness of this approach.

3.4. Effects of different storage times on FBMJM

3.4.1. Effects of different storage times on the SOD activity and TPC in FBMJM

The instability of polyphenolic substances can lead to browning and oxidation of the sample, and their composition and content can also cause changes in the color and flavor of the sample. Furthermore, TPC is positively correlated with antioxidant activity, so studying the changes in TPC is essential for the quality research of FBMJM. The bioactive components content declined as the storage time increased (Fig. 3A). The TPC of FBMJM decreased by 1.88 mg/g within 30 days. Although FBMJD had a higher initial TPC, its content decreased at a faster rate during storage compared with FBMJM, with a total decrease of 2.68 mg/g in TPC within 30 days, which was 1.42 times the decrease in FBMJM. These results were consistent with the findings of Zarif et al (Zarif, Shabbir, Shahid, Noor, & Imran, 2023), who found that protein bodies improve the digestive stability and bioavailability of β-carotene. Some studies have shown that protein particles, as a common delivery system, can effectively improve the stability and bioavailability of phenolic compounds and mask their bitterness (Zhang, Cheng, Wang, & Fu, 2020).

SOD in fruit milk effectively removes free radicals and other substances. Essentially, SOD is a protein that is susceptible to degradation by temperature, oxygen concentration, pH value, and presence of metal ions (Zhu, Yuan, Guo, Luo, Liu, & Miao, 2023). In FBMJM, the SOD activity gradually decreased during storage by 4.1 U/g (Fig. 3A), with the slowest decline occurring in the initial 6 days. The most rapid decline occurred in the SOD activity by 16.47 U/g between the 18th and 24th days. The correlation analysis results (Fig. 5) indicate that SOD activity and TPC are strongly negatively correlated with pH value, sedimentation rate, a*, and b*. Therefore, the possible reason is that during the storage process of FBMJM, the content of antioxidants such as vitamins and total phenolics decreases, accelerating the degradation of SOD enzyme (Wang, Yin, Ao, Yin, Ren, & Lu, 2022). The decline in the SOD activity followed a logarithmic trend, with a total decrease of 58.04 U/g over the 30-day storage period. Moreover, the decline in the SOD activity in FBMJM was slower ($P < 0.01$) than that in FBMJD.

3.4.2. Effects of different storage times on the physical and chemical properties of FBMJM

The changes in the levels of TTA and pH during refrigeration are shown in Fig. 3B. The acidity increased significantly starting from the 18th day of refrigeration, indicating a decrease in freshness. Compared with freshly prepared FBMJM, the acidity increased by 7.73°T on the 30th day of storage. Similarly, the pH value reached its lowest point of 3.06 on the 30th day.The acidity of the fruit milk changed due to the

Table 1
Volatile compounds identified in milk, FBMJ and FBMJM using headspace solid phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC–MS). (0 day, fresh FBMJM, 6 day − 30 day means the FBMJM storage 6–30 days).

No.	Compound	CAS	RT/min	Concentration (μg/mL)							
				Milk	FBMJ	FBMJM					
						0 day	6 day	12 day	18 day	24 day	30 day
Ester											
1	12,15-Octadecadiynoic acid, methyl ester	57156–95-3	22.67	0.0015 ± 0.0005	0.0008 ± 0.0005b	0.0002 ± 0.0001b	0.0003 ± 0.0001b	0.0002 ± 0.0001b	0.0259 ± 0.0223a	0.0002 ± 0.0001b	0.0002 ± 0.0001b
2	1-Butanol, 2-methyl-, acetate	624–41-9	3.38	n.d.	0.0346 ± 0.0194abc	0.0421 ± 0.0045ab	0.0421 ± 0.0045ab	0.0366 ± 0.0134abc	0.0459 ± 0.0123a	0.0255 ± 0.0055bc	0.0223 ± 0.002c
3	Allyl methallyl ether	14289–96-4	5.68	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0003 ± 0.0002
4	Benzeneacetic acid,3-tetradecyl ester	88336–99-6	22.24	0.0005 ± 0.0001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5	Benzoic acid, ethyl ester	93–89-0	20.97	n.d.	0.0006 ± 0.0001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6	Carbonic acid, eicosyl vinyl ester	2243791–78-6	20.97	n.d.	0.0003 ± 0.0001a	0.0002 ± 0.0001ab	0.0001 ± 0.0001b	n.d.	n.d.	n.d.	n.d.
7	Methyl propionate	554–12-1	3.34	n.d.	n.d.	n.d.	0.028 ± 0.0132a	0.0246 ± 0.001ab	0.0266 ± 0.0113a	0.0181 ± 0.0025ab	0.0208 ± 0.0031ab
8	Decanoic acid, ethyl ester	110–38-3	30.93	n.d.	0.0018 ± 0.0001	0.0013 ± 0.0006	n.d.	n.d.	n.d.	n.d.	n.d.
9	Ethyl Acetate	141–78-6	3.35	n.d.	n.d.	n.d.	0.027 ± 0.0189a	0.0222 ± 0.002ab	0.0256 ± 0.0116a	0.0172 ± 0.0036ab	0.0217 ± 0.0049ab
10	Formic acid, heptyl ester	112–23-2	11.96	n.d.	0.0025 ± 0.0019a	0.0005 ± 0.0001b	0.0003 ± 0.001b	0.0004 ± 0.0003b	0.0004 ± 0.0002b	0.0002 ± 0.0002b	n.d.
11	Hexanoic acid, 2,4-dimethyl-, methyl ester	14251–44-6	22.17	n.d.	0.0045 ± 0.0001b	0.0373 ± 0.0169a	n.d.	n.d.	n.d.	n.d.	n.d.
12	Hexanoic acid,2-phenylethyl ester	6290–37-5	25.11	n.d.	0.0003 ± 0.0001b	0.0005 ± 0.0001a	n.d.	n.d.	n.d.	n.d.	n.d.
13	Methyl 10,12-heptadecadiynoate	120650–77-3	13.98	0.0034 ± 0.0009a	n.d.	n.d.	0.0003 ± 0.0001b	0.0002 ± 0.0001b	0.0002 ± 0.0001b	0.0001 ± 0.0001b	0.0001 ± 0.0001b
14	Nonanoic acid, ethyl ester	139556–87-9	22.17	n.d.	0.005 ± 0.001b	0.0351 ± 0.0177a	n.d.	n.d.	n.d.	n.d.	n.d.
15	Nonanoic acid,5-methyl-, ethyl ester	116530–40-6	30.93	n.d.	0.0018 ± 0.0001a	0.0013 ± 0.0006b	n.d.	n.d.	n.d.	n.d.	n.d.
16	Octanoic acid, ethyl ester	5129–53-3	22.15	n.d.	0.0045 ± 0.0001a	0.0367 ± 0.017b	n.d.	n.d.	n.d.	n.d.	n.d.
17	Oxirane-2-carboxylic acid, ethyl ester	4660–80-4	6.06	n.d.	0.0003 ± 0.0002b	0.0011 ± 0.0002a	0.0011 ± 0.0002a	0.0004 ± 0.0001b	0.0002 ± 0.0001bc	0.0002 ± 0.0001bc	0.0002 ± 0.0001bc
18	Undecanoic acid, ethyl ester	627–90-7	30.94	n.d.	0.0016 ± 0.0007a	0.0016 ± 0.0002a	n.d.	n.d.	n.d.	n.d.	n.d.
Acid											
19	Acetic acid	64–19-7	3.8	n.d.	n.d.	0.0222 ± 0.0023 cd	0.0192 ± 0.0023d	0.035 ± 0.0013a	0.0262 ± 0.0057bc	0.0224 ± 0.0028 cd	0.029 ± 0.0036b
20	Acetic acid, anhydride with formic acid	2258–42-6	5.36	n.d.	n.d.	n.d.	n.d.	n.d.	0.0165 ± 0.0151a	0.0223 ± 0.0024a	0.0243 ± 0.0029a
21	Alanine	56–41-7	2.52	0.0008 ± 0.0001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22	Cystine	56–89-3	9.41	0.001 ± 0.0001a	n.d.	0.0009 ± 0.0002a	n.d.	n.d.	n.d.	n.d.	n.d.
23	Glycine,N-(*N*-L-alanylglycyl)	3146–40-5	17.96	0.0009 ± 0.0001a	n.d.	0.0007 ± 0.0002a	n.d.	n.d.	n.d.	n.d.	n.d.
24	L-Lactic acid	50–21-5	2.03	n.d.	0.0322 ± 0.0023a	0.0222 ± 0.0081ab	0.015 ± 0.0162ab	0.0215 ± 0.0074ab	0.0215 ± 0.0074ab	0.0352 ± 0.0328a	0.0219 ± 0.003ab
25	Methyltartronic acid	595–48-2	2.9	n.d.	0.4058 ± 0.098d	0.4862 ± 0.2734 cd	0.7858 ± 0.0587bc	1.3664 ± 0.1713a	0.9359 ± 0.2559b	0.8244 ± 0.0729b	0.8369 ± 0.0728b
26	*N*-Methylglycine	107–97-1	27.19	0.0002 ± 0.0001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
27	Pterin-6-carboxylic acid	948–60-7	17.66	0.0044 ± 0.0023a	0.0018 ± 0.0006b	0.0009 ± 0.0002b	0.0002 ± 0.0001b	0.0003 ± 0.0001b	0.0005 ± 0.0002b	0.0004 ± 0.0001b	0.0002 ± 0.0001b
Ketone											
28	2-Heptanone	110–43-0	34.51	0.0195 ± 0.0011	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
29	2-Hexanone, 4-methyl	105–42-0	8.57	0.0195 ± 0.0011	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30	8-Hydroxy-2-octanone	25368–54-1	8.63	0.009 ± 0.0024	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

(*continued on next page*)

Table 1 (*continued*)

No.	Compound	CAS	RT/ min	Concentration (μg/mL)							
				Milk	FBMJ	FBMJM					
						0 day	6 day	12 day	18 day	24 day	30 day
31	Acetoin	513–86-0	3.8	n.d.	0.0861 ± 0.0177a	0.0312 ± 0.025c	0.0206 ± 0.0013 cd	0.0563 ± 0.0044b	0.0207 ± 0.0115 cd	0.0114 ± 0.0036 cd	0.0146 ± 0.002 cd
Aldehyde											
32	8-Octadecenal	56554–94-0	15.63	0.0027 ± 0.0005	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
33	Benzaldehyde,2,4-dimethyl	15764–16-6	23	n.d.	0.0018 ± 0.0003bc	0.0003 ± 0.0001 cd	0.0049 ± 0.0004a	0.0024 ± 0.0008b	0.0048 ± 0.0019a	0.0028 ± 0.0006b	0.0025 ± 0.0005b
34	Dodecanal	112–54-9	17.85	n.d.	0.0009 ± 0.0002	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
35	Nonanal	124–19-6	17.85	n.d.	0.0009 ± 0.0002a	0.0009 ± 0.0003a	n.d.	n.d.	n.d.	n.d.	n.d.
Alcohol											
36	(3-Methyl-oxiran-2-yl)-methanol	872–38-8	3.23	n.d.	0.1012 ± 0.0105a	0.0369 ± 0.023bc	0.0086 ± 0.0109c	0.0725 ± 0.0511ab	0.004 ± 0.0036c	0.0157 ± 0.001c	0.0058 ± 0.0033c
37	3-Methyl-2-butanol	598–75-4	3.79	n.d.	0.045 ± 0.0495a	0.0017 ± 0.0004b	0.0021 ± 0.0006b	0.0105 ± 0.0079ab	0.0008 ± 0.0003b	0.0019 ± 0.0001b	0.0012 ± 0.0002b
38	1,2-Propanediol	57–55-6	2.08	n.d.	0.0889 ± 0.0209ab	0.0534 ± 0.0419bc	0.0335 ± 0.0019 cd	0.0957 ± 0.0308a	0.0232 ± 0.0018 cd	0.0256 ± 0.0077 cd	0.0214 ± 0.0169 cd
39	2-Pentanol	6032–29-7	5.04	n.d.	n.d.	n.d.	n.d.	n.d.	0.0026 ± 0.0041b	0.0003 ± 0.0001ab	0.0043 ± 0.0006a
40	1,3-Butanediol	107–88-0	6.8	n.d.	0.0054 ± 0.0009b	0.0025 ± 0.0001c	0.003 ± 0.0004c	0.0154 ± 0.001a	0.0031 ± 0.0015c	0.0017 ± 0.0007 cd	0.0008 ± 0.0001de
41	10-Azido-1-decanethiol	57395–48-9	7.74	n.d.	0.0014 ± 0.0011abc	0.0017 ± 0.0001ab	0.0012 ± 0.0002bc	0.0015 ± 0.0009abc	0.0006 ± 0.0002 cd	0.0024 ± 0.0005a	n.d.
42	1-Butanol, 3-methyl	123–51-3	4.1	n.d.	1.1603 ± 0.0974a	0.7088 ± 0.136b	0.4528 ± 0.1031d	1.007 ± 0.1741a	0.4643 ± 0.1453 cd	0.3812 ± 0.0931d	0.6818 ± 0.0354bc
43	1-Hexadecanol,2-methyl	2490–48-4	26.73	n.d.	0.0003 ± 0.0001bc	0.0002 ± 0.0001bc	0.0001 ± 0.0001 cd	n.d.	0.0006 ± 0.0002a	0.0003 ± 0.0001b	n.d.
44	1-Hexanol	111–27-3	7.71	n.d.	n.d.	0.0009 ± 0.0001 cd	0.0013 ± 0.0003bcd	0.0041 ± 0.0014a	0.002 ± 0.0003bc	0.0015 ± 0.0001bc	0.0026 ± 0.0013b
45	1-Hexanol, 5-methyl	627–98-5	8.11	n.d.	0.0059 ± 0.0009a	0.0006 ± 0.0001 cd	0.0007 ± 0.0001 cd	0.0012 ± 0.0003c	0.0007 ± 0.0001 cd	0.0045 ± 0.0005b	n.d.
46	1-Pentanol	71–41-0	4.1	n.d.	0.9496 ± 0.451b	0.7088 ± 0.136bc	0.4529 ± 0.1032c	1.007 ± 0.1741a	0.4643 ± 0.1453c	0.3812 ± 0.0931 cd	0.6239 ± 0.1007bc
47	1-Pentanol, 3-methyl	589–35-5	7.75	n.d.	n.d.	n.d.	n.d.	n.d.	0.0006 ± 0.0006b	0.2575 ± 0.2415a	0.0006 ± 0.0003b
48	2-Butanol, 3-methyl	598–75-4	3.85	n.d.	0.0008 ± 0.0003b	0.0004 ± 0.0003bc	0.002 ± 0.0007a	n.d.	0.0004 ± 0.0002bc	0.0003 ± 0.0001bc	n.d.
49	2-Heptanol,6-amino-2-methyl	372–66-7	4.39	0.003 ± 0.0005	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
50	2-Hexanol, 3-methyl	2313–65-7	4.97	n.d.	0.0005 ± 0.0003b	0.0018 ± 0.0012ab	0.0006 ± 0.0002b	0.0008 ± 0.0005b	0.0003 ± 0.0003b	0.0031 ± 0.0027a	0.0009 ± 0.0003ab
51	2-Hexyl-1-octanol	19780–79-1	35.13	n.d.	0.0003 ± 0.0001b	0.0002 ± 0.0001b	0.0002 ± 0.0001b	0.0003 ± 0.0002b	0.0041 ± 0.0006a	0.0001 ± 0.0001b	n.d.
52	Benzenemethanol	100–51-6	16.87	0.006 ± 0.0022a	0.0014 ± 0.0007b	0.0005 ± 0.0001b	0.0002 ± 0.0001b	0.0004 ± 0.0002b	0.0002 ± 0.0001b	0.0005 ± 0.0002b	0.0003 ± 0.0001b
53	*cis*-p-Mentha-2,8-dien-1-ol	425394–92-9	16.87	0.0002 ± 0.0001b	0.0004 ± 0.0002a	0.0003 ± 0.0001ab	n.d.	n.d.	n.d.	n.d.	n.d.
54	Cyclobutanol	2919–23-5	4.7	0.0016 ± 0.0001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
55	Ethanol	64–17-5	2.9	n.d.	0.694 ± 0.3535bc	0.4863 ± 0.2734c	0.7939 ± 0.0532bc	1.3579 ± 0.1688a	0.9429 ± 0.2664b	0.8283 ± 0.081bc	0.923 ± 0.0581b
56	Isopinocarveol	6712–79-4	16.44	n.d.	0.0005 ± 0.0001a	0.0002 ± 0.0001b	n.d.	n.d.	n.d.	n.d.	n.d.
57	Phenylethyl Alcohol	60–12-8	18.45	n.d.	0.0096 ± 0.0014a	0.0103 ± 0.0004a	0.0046 ± 0.0005c	0.0078 ± 0.0004b	0.0052 ± 0.0013c	0.0052 ± 0.0001c	0.0049 ± 0.0002c
58	Propylene Glycol	123120–98-9	3.33	n.d.	0.0488 ± 0.0091	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
59	*Threo*-3-bromo-2-pentanol	159475–12-4	2.7	1.0353 ± 0.0405	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
60	*trans*-2-Undecen-1-ol	75039–84-8	17.85	n.d.	0.0008 ± 0.0001a	0.0006 ± 0.0001b	n.d.	n.d.	n.d.	n.d.	n.d.
Others											
61	(2-Aziridinylethyl) amine	4025–37-0	2.39	0.0935 ± 0.0054	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
62	1,2,5-Oxadiazole	288–37-9	3.34	n.d.	n.d.	n.d.	n.d.	0.0181 ± 0.0017a	0.009 ± 0.0002b	0.0187 ± 0.0011a	n.d.

(*continued on next page*)

Table 1 (*continued*)

No.	Compound	CAS	RT/ min	Concentration (μg/mL)							
				Milk	FBMJ	FBMJM					
						0 day	6 day	12 day	18 day	24 day	30 day
63	1,3,5-Cycloheptatriene	544–25-2	18.45	n.d.	0.0087 ± 0.0017a	0.0087 ± 0.0008a	0.0085 ± 0.0002a	0.0085 ± 0.0002a	n.d.	n.d.	n.d.
64	1-Heptene, 6-methyl	5026–76-6	8.11	n.d.	n.d.	n.d.	n.d.	n.d.	0.0009 ± 0.0001a	0.0003 ± 0.0001c	0.0005 ± 0.0001b
65	1-Iodo-2-methylundecane	73105–67-6	25.84	n.d.	n.d.	0.0002 ± 0.0001b	0.0002 ± 0.0001ab	0.0002 ± 0.0001b	0.0002 ± 0.0001b	0.0002 ± 0.0001b	0.0003 ± 0.0001a
66	1-Octene, 3-methyl	13151–08-1	8.01	n.d.	0.0011 ± 0.0004a	0.0011 ± 0.0001a	n.d.	n.d.	n.d.	n.d.	n.d.
67	2-(1-Cyclopentenyl) furan	115754–78-4	23.04	n.d.	0.0005 ± 0.0002a	0.0004 ± 0.0002a	n.d.	n.d.	n.d.	n.d.	n.d.
68	dl-Allo-cystathionine	535–34-2	8.44	n.d.	n.d.	0.0003 ± 0.0001c	0.0002 ± 0.0001c	0.0004 ± 0.0002bc	0.0002 ± 0.0001c	0.0005 ± 0.0001b	0.0014 ± 0.0001a
69	dl-Phenylephrine	1477–63-0	11.71	0.0003 ± 0.0001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
70	Formamide	75–12-7	27.19	n.d.	0.0037 ± 0.0011a	0.0043 ± 0.0004a	0.0018 ± 0.0002b	0.0021 ± 0.0005b	n.d.	n.d.	n.d.
71	Mesitylene	108–67-8	12.76	n.d.	n.d.	n.d.	n.d.	0.0004 ± 0.0002b	0.0004 ± 0.0004b	0.0009 ± 0.0001a	0.0004 ± 0.0001b
72	Naphthalene,1,2,3,4-tetrahydro-1,1,6-trimethyl	475–03-6	22.67	n.d.	0.0003 ± 0.0001bc	0.0003 ± 0.0001b	0.0002 ± 0.0001bc	n.d.	0.0002 ± 0.0001c	0.0008 ± 0.0001a	n.d.
73	Octadecane, 6-methyl	10544–96-4	17.68	0.0016 ± 0.0004a	n.d.	0.0002 ± 0.0001b	0.0002 ± 0.0001b	0.0002 ± 0.0001b	0.0001 ± 0,0001b	0.0001 ± 0.0001b	n.d.
74	p-Cymene	99–87-6	16.86	n.d.	n.d.	n.d.	0.0002 ± 0.0001ab	0.0003 ± 0.0001a	0.0002 ± 0.0001ab	0.0002 ± 0.0001b	0.0002 ± 0.0001ab
75	Tetracontane,3,5,24-trimethyl	55162–61-3	25.84	0.0014 ± 0.0005a	0.0002 ± 0.0001b	0.0008 ± 0.0011ab	n.d.	n.d.	n.d.	n.d.	n.d.
76	2-Formylhistamine	959055–98-2	2.71	1.0412 ± 0.0425	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
77	4-Fluorohistamine	56–92-8	4.27	0.0029 ± 0.0026	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
78	Dextroamphetamine	51–64-9	2.58	0.0021 ± 0.0018	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

decomposition of dietary compounds such as fat and protein during storage (Vigolo, Niero, Penasa, & De Marchi, 2022).

The variation in TNC during refrigeration is shown in Fig. 3C. The protein content decreased continuously with increasing storage time. In the first 6 days, it decreased by 0.04 g/kg and then decreased by 0.06 g/kg in the following 24 days. Proteins are prone to degradation during storage, which also leads to the formation of new flavor substances (T. Ma et al., 2022).

The viscosity of the FBMJM remained relatively stable under 4℃ storage conditions. This is similar to the findings of Nan Li et al (N. Li, Choi, Vuia-Riser, Carter, Drake, & Zhong, 2022) regarding lemon-flavored milk. Under storage conditions at 4℃, lemon-flavored fruit milk beverage exhibits lower viscosity and stronger stability. Compared to yogurt, FBMJM, as an acidic fruit juice-flavored beverage, possesses lower viscosity characteristics. This feature makes it more suitable for further development into a low-viscosity beverage, especially for elderly individuals or consumers with swallowing difficulties (Garin, De Pourcq, Martín-Venegas, Cardona, Gich, & Mangues, 2014). At the same time, the sediment yield varied significantly (Fig. 3D). The sediment yield of the sample increased from 11.38 % to 12.28 % after 30 days of storage. The increase in sediment yield was related to the colloidal stability and acidity changes of the sample (O'Connell & Fox, 2010). The increase in sedimentation is also significantly negative correlated with the TPC (Fig. 5). Phenolic compounds can interact with hydrophilic residues in proteins, such as hydroxyl groups and tyrosine residues, through hydrogen bonds or van der Waals forces. These interactions may alter the structure and solubility of proteins, leading to the loss of protein solubility and the formation of precipitates (Cianciosi, et al., 2022).

L* initially increases and then decreases within 30 days of storage, while a* and b* gradually increase (Fig. 3E), consistent with the phenomenon observed in lotus seed juice (Zhang et al., 2023b). The increase in brightness of FBMJM is due to the sedimentation of colored suspended particles. As non-enzymatic browning reactions occur in the later stages of storage, the color gradually darkens and the brightness decreases. The stability of a* and b* is maintained, thanks to the low pH environment of FBMJM, which plays an important role in preserving the color stability of its anthocyanins and flavonoids.

3.4.3. *Effects of different storage times on the antioxidant activity of FBMJM*

Specifically, the ABTS index (Fig. 3F) decreased significantly from 6.9943 mM Trolox after 30 days of storage to 5.48 mM Trolox ($P < 0.001$). The initial FRAP (Fig. 3G) of FBMJM was 16.6171 mM $FeSO_4{\cdot}7H_2O$, which decreased significantly on the 30th day ($P < 0.001$). DPPH (Fig. 3H) decreased significantly from 86.84 % on the 30th day to 77.01 % ($P < 0.001$). These antioxidant indicators show a decrease during the storage process, but compared to pure milk, they remain at a relatively high level on the 30th day (Fig. 2A – C). The antioxidant capacity was related to the total phenol and active enzyme content in FBMJM. Therefore, the data for TPC and SOD activity (Fig. 3A) exhibited similar trends.

3.5. *Effect of different storage times on the FBMJM volatile compounds*

Most flavor compounds undergo significant changes during storage, which can result in differences in the acceptability of a given product. Volatile flavor component analysis was conducted on milk, FBMJ, and FBMJM during a 30-day storage period, When the VIP value exceeds 1, the variable plays an important role in the OPLS-DA discriminant process (Giannetti, Boccacci Mariani, Mannino, & Marini, 2017). Further,

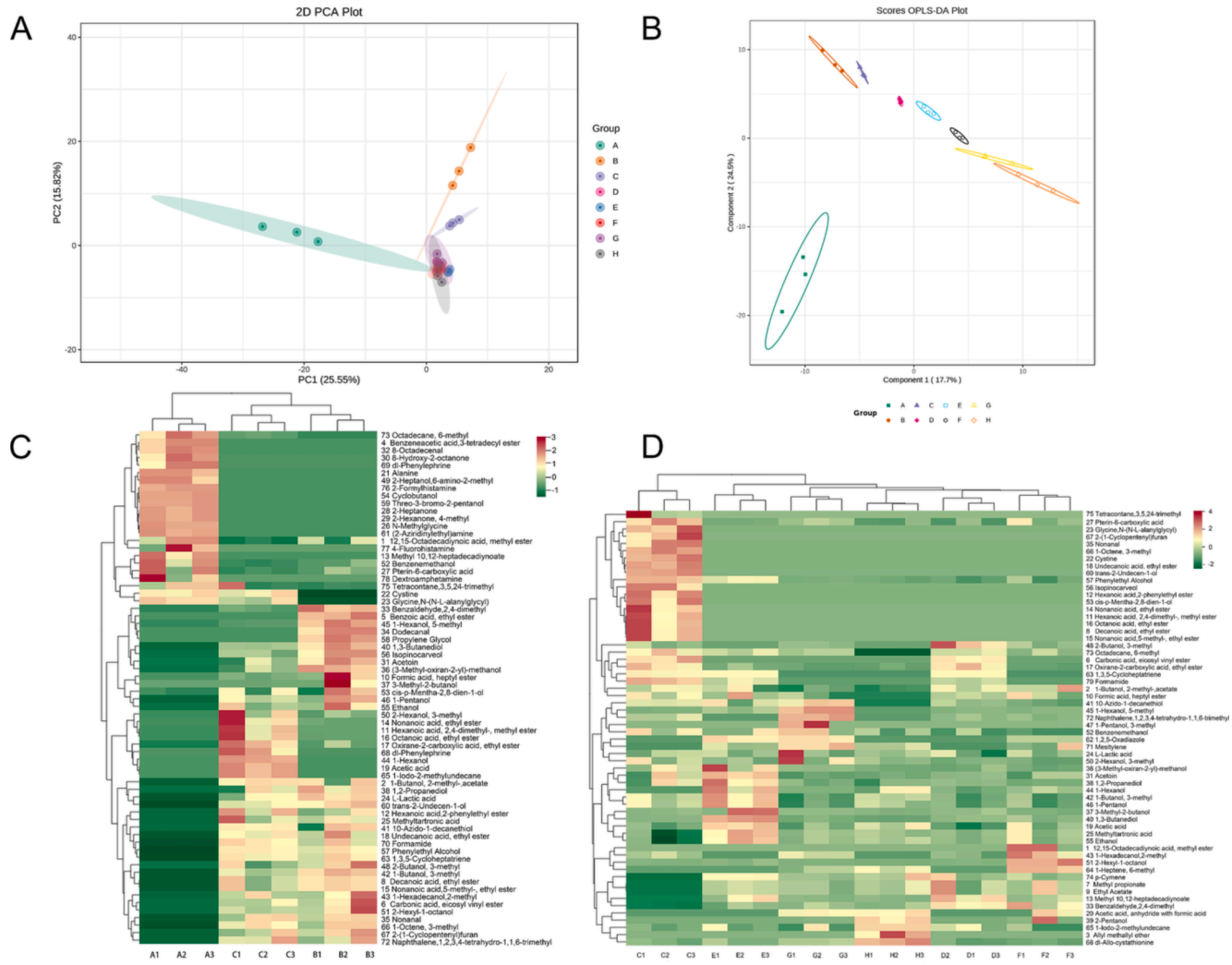

Fig. 4. Principal component analysis (PCA) score plots (A), OPLS-DA score plots (B) from the milk (Group A), FBMJ(Group B) and FBMJM(Group C–H) samples during storage, Heatmap and hierarchical cluster analysis of volatiles compounds in jmilk (Group A), FBMJ(Group B) and FBMJM(Group C–H) samples during storage (C, Group A–C; D, Group C–H). Rows and columns indicate volatiles and group information, respectively. Green to red shading corresponds to the abundance of volatiles, shifting from low to high. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

78 volatile compounds were screened and identified, as depicted in Table 1. Among all samples, 18 esters, 9 acids, 4 ketones, 4 aldehydes, 25 alcohols, and 18 other compounds were identified. In fresh FBMJM, 49 compounds were identified (12 esters, 6 acids, 1 ketone, 2 aldehydes, 19 alcohols, and 9 other compounds). Mixing fermented juice with milk to prepare fruit milk increased the abundance of flavor components in the product.

In the OPLS-DA plot, the components were more easily distinguishable (Fig. 4B, A–4H). Meanwhile, the permutation test results for the OPLS-DA model are $Q^2 = 0.941$, $p < 0.005$, and $R^2Y = 0.984$, $p < 0.005$. This indicates that the model has strong explanatory and predictive power, making the results highly reliable. The cluster analysis (Fig. 4C) results demonstrated that the nonvolatile components of milk, FBMJ, and FBMJM were clearly categorized into three groups. The relative distance of the storage time from day 0 indicates the effect of different storage times on the volatile compounds in the sample. (Leng, Hu, Cui, Tang, & Liu, 2021).

The volatile flavor components of FBMJM underwent significant changes during the 30-day storage period (Fig. 4D). The concentrations of various esters ($P < 0.05$) significantly decreased during storage. The phenomenon is consistent with the findings in loquat (Huang et al., 2023) during storage. The compounds that undergo significant changes are primarily some ethyl esters (Table 1), such as benzoic acid, ethyl ester; decanoic acid, ethyl ester; nonanoic acid, ethyl ester; nonanoic acid, 5-methyl, ethyl ester; octanoic acid, ethyl ester and undecanoic acid, ethyl ester. This kind of compound has a low odor threshold and has a significant impact on the core flavor components of mulberries (Meng, Imamura, Katayama, Obata, & Sugawara, 2017). Correlation analysis (Fig. 5) shows that the degradation of esters results in an increase in acid and alcohol compounds, such as 1-hexanol; 1-pentanol; ethanol; methyltartronic acid and acetic acid (Kaprasob, Kerdchoechuen, Laohakunjit, Sarkar, & Shetty, 2017). Meanwhile, a small amount of new ester content gradually increases during this process, such as methyl propionate; ethyl acetate and methyl 10,12-heptadecadiynoate, and these compounds are negatively correlated with acid and alcohol, which may be due to the fact that acetic anhydride and formic acid can react with alcohols to generate esters (Xu et al., 2022), and these components can increase their flavor at low concentrations, but will become the main contributing factors to fruit spoilage after exceeding the threshold (D. Ma et al., 2023). Two core flavor compounds in mulberries, namely nonanal (green) and 2-heptanone (fruit), disappear during the storage process (Calín-Sánchez, Martínez-Nicolás, Munera-Picazo, Carbonell-Barrachina, Legua, & Hernández, 2013). Furthermore, some aldehydes and terpenoids, such as 1-heptene, 6-methyl and mesitylene, were newly produced during storage, which showed a significant negative correlation with carbonic acid, eicosyl

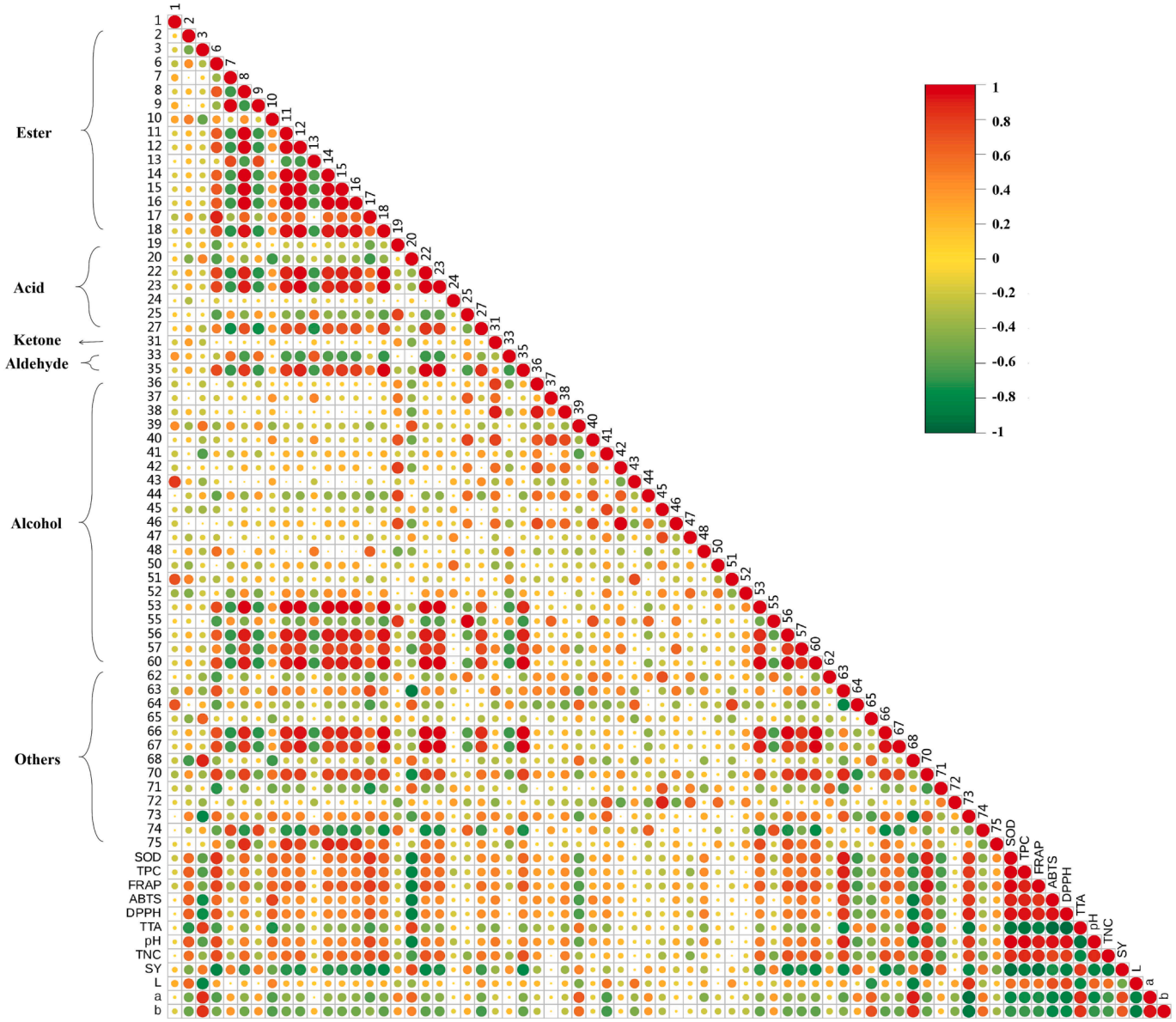

Fig. 5. Lower triangular heat map represents pairwise correlation analysis between oxidation resistance and flavor compounds during FBMJM storage. Each square represents the Spearman's rank correlation coefficient at a significance level of $p \leq 0.05$. Positive correlations ($r > 0.5$) are shown with an orange-red scale. Negative correlations ($r < -0.5$) are shown with a green scale. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

vinyl ester and oxirane-2-carboxylic acid, ethyl ester.

4. Conclusions

This study demonstrates that using fermented fruit juice to prepare flavored milk by mixing with milk is feasible, based on the physicochemical properties, efficacy, and flavor components of fruit milk. The study shows that FBMJM containing 25 % milk has the best sensory evaluation, and the protein solubility and precipitation rate are also within an acceptable range. Compared to FBMJ diluted with water (FBMJD), FBMJM showed significantly improved antioxidant activity ($P < 0.01$). However, during storage, the levels of SOD, TPC, and antioxidant activity decreased over time. Additionally, the sediment yield of FBMJM increased on the 6th day of storage, but it did not have a significant impact on viscosity. This is beneficial for preparing FBMJM as a low-viscosity acid beverage for consumers with swallowing difficulties. The content of bioactive components and antioxidant activity decreased with increasing storage time, and the TPC reduction rate of FBMJM was slower than that of FBMJD. This suggests that adding milk improves the stability of phenolic compounds during storage, slowing down their degradation. GC–MS results showed significant differences between FBMJM and FBMJ and milk. During storage, volatile flavor compounds gradually decrease. Among them, the main degradation occurred in ester compounds, resulting in the production of acids and alcohols. Ethyl acetate and ethanol significantly increased during the storage process and gave rise to new terpenoid compounds and aldehydes, such as 1-heptene, 6-methyl, and mesitylene. In addition, this study has limitations as it lacks specific analysis of the interaction between proteins and phenolic compounds. In the next step, molecular methods can be used to study the interaction mechanisms between protein molecular weight, amino acid composition, phenolic compound composition in FBMJM.

CRediT authorship contribution statement

Mingshan Lv: Conceptualization, Methodology, Writing – original draft. **Xiaolu Liu:** Data curation, Software. **Keping Chen:** Data curation, Software, Writing – review & editing. **Aihemaitijiang Aihaiti:** Conceptualization, Writing – review & editing, Supervision. **Ruxianguli maimaitiyiming:** Methodology, Validation, Investigation. **Jun Xing:** Writing – review & editing. **Xuefeng Yin:** Writing – review & editing. **Li Zheng:** Writing – review & editing. **Fangfang Jiao:** Writing – review & editing. **Liang Wang:** Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

This research was funded by Research on Key Technologies for Refined Deep Processing of Xinjiang characteristic subsidiary agricultural products, Autonomous Region Key Research and Development Program Sub-project (2022B02026-3), Excellent doctoral Scientific Research and Innovation Program of Xinjiang University (XJU2022BS052) and Xinjiang Autonomous Region Graduate Innovation Project (XJ2023G030).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101029.

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