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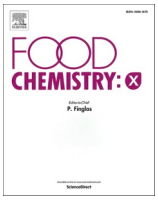

Multidimensional flavor analysis of Yongchun aged vinegar: Impact of aging on quality and flavor profile

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Abstracts.

Yongchun aged vinegar (YCAV), produced through traditional semi-solid-state fermentation with red yeast rice, undergoes significant physicochemical and sensory transformations during aging. This study explores the quality and flavor dynamics of YCAV aged for 3, 5, 8, and 10 years using sensory evaluation and multidimensional analytical techniques. Results show that aging increases total soluble solids (TSS), color difference, and glycine content, while decreasing arginine levels. Acetic acid and esters were the dominant volatile compounds, with red yeast rice enhancing color depth and umami characteristics. TSS and volatile flavor compounds (VFCs) have been identified as key quality indicators for vinegar through regression model analysis. The research results provide a theoretical basis for rapid quality evaluation of the vinegar industry.

1. Introduction

Vinegar, a globally cherished condiment, is traditionally produced through alcoholic and acetic fermentation of cereals, vegetables, or fruits. Its flavor complexity arises from raw materials, fermentation processes, and aging, with regional practices yielding distinct varieties such as traditional Chinese cereal vinegar, Japanese black vinegar, and balsamic vinegar (Chen et al., 2016; Zhou et al., 2020). The organoleptic quality of vinegar hinges on a balance of volatile and non-volatile compounds. Acetic acid (dominant sourness), ethyl acetate (fruity notes), and tetramethyl-pyrazine (nutty aromas) are key flavor contributors, while non-volatile components, such as organic acids and amino acids, play pivotal roles in modulating taste balance and mouthfeel. For instance, organic acids like lactic and citric acid synergistically soften the sharpness of acetic acid, enhancing overall palatability (Gao et al., 2021), while amino acids such as glycine (sweetness) and arginine (bitterness) dynamically shift during aging, reducing astringency and improving consumer acceptability (Li et al., 2023). Prolonged aging induces oxidation, esterification, and Maillard reactions, which lead to the accumulation of organic acids, esters, and melanoid pigments. These changes contribute to both a richer taste complexity and deeper color (Ho et al., 2017; Yang et al., 2024). While each of these factors plays a distinct role in the overall flavor profile of vinegar, there are few comprehensive, multi-dimensional evaluations that integrate their combined effects.

Red yeast rice (*Monascus purpureus*) is integral to producing YCAV. Red yeast rice plays a dual role: it imparts a characteristic reddish hue and introduces bioactive metabolites with antioxidant and cardioprotective properties (Banach et al., 2022; Lee et al., 2023).

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Critically, red yeast rice derived organic acids, amino acids, and pigments synergistically modulate umami, kokumi, and color during aging (Li et al., 2023). Despite these potentials, the role of red yeast rice in overall quality and its interaction with aging-induced physicochemical changes are poorly understood, limiting industrial optimization of YCAV quality.

Flavor assessment of vinegar traditionally combines sensory evaluation with instrumental analysis. Sensory evaluation provides a more sensitive indication of the flavor profile of vinegar and consumer preferences (Zhang et al., 2008). The instrumental analysis of physicochemical indicators such as pH, total acidity (TA), ΔE, and TSS serve as a basis for quantifiable judgments of fermentation kinetics and flavor quality (Andreou et al., 2023; Sun et al., 2023). Advanced techniques such as gas chromatography–mass spectrometry (GC–MS) and electronic nose (*E*-nose) enable precise profiling of volatile compounds, whereas high-performance liquid chromatography (HPLC) and electronic tongue (*E*-tongue) decode non-volatile components (Jiang et al., 2019; Ríos-Reina et al., 2020). Despite these tools, translating multidimensional evaluation indicators into rapid industrial metrics remains challenging.

To better understand the overall quality of vinegar, this study integrated sensory evaluation with multidimensional quality testing methods (HPLC, GC–MS, E-nose and E-tongue) to analyze physicochemical parameters and sensory evaluation of YCAV samples of 3, 5, 8, and 10 years old from two major manufacturers (F1 and F2). By correlation analysis and quality regression model construction of multidimensional quality indicator data, the study attempted to reveal the complementary roles of nonvolatile flavors, volatile odors, and red yeast rice of YCAV, the contribution of aging to the overall quality, and at the same time, to optimize and simplify the overall quality evaluation indicators. These findings provide new insights into the flavor profile of aged vinegars and could enhance rapid quality control in industrial production.

2. Materials and methods

2.1. Vinegar samples

Bottled YCAV samples aged 3, 5, 8, 10 years, produced by two large-scale manufacturers (designated as factory 1 and 2 or F1 and F2) with a long history of production in Yongchun County, Quanzhou City, Fujian Province, China, were purchased from their commercial outlets. Both manufacturers, located in the core processing area of the vinegar industry in Yongchun County, employ traditional semi- solid-state fermentation methods involving the addition of red yeast rice, with slight variations in specific yeast strains and fermentation conditions based on the influence of their respective traditional processes. YCAV samples of different age and brand for analysis were purchased in commercial packages of 12 bottles (500 mL/bottle) in complete cardboard boxes. To ensure the consistency and comparability of the experimental results, five bottles from the same aging year but different production batches and ex-factory date were randomly selected and three batches were selected for each sample for parallel analysis.

2.2. Chemicals standards and reagents

Analytical-grade chemicals and reagents were sourced as follows: acetic acid (95 %) from Xilong Science Co. Ltd., China; ethanol (95 %) from Sinopharm Chemical Reagent Co. Ltd., China; sodium hydroxide, 2,4-dinitrofluorobenzene, anhydrous ethanol, sodium bicarbonate, potassium dihydrogen phosphate, sodium acetate, and sodium dihydrogen phosphate (95 %) from Sinopharm Chemical Reagent Co. Ltd., China; phenolphthalein from Ron Reagent, China; phosphoric acid from Aladdin, China; and high-purity acetonitrile and methanol (chromatographic grade) from Sigma, Germany.

2.3. Sensory evaluation

A sensory evaluation was conducted by a panel of 20 individuals (eight males and twelve females) aged 20 to 45, comprising Food Science students and quality control staff with over a year of training in evaluating fermented product flavors. The evaluation followed a slightly modified version of previously described protocols (Zhang et al., 2023). Calibration solutions included ethyl acetate, guaiacol, acetic acid, glucose, dietary salt, L-alanine, and quinine hydrochloride as reference standards. Vinegar samples were pre-warmed in a 40 °C water bath for 20 min, and 6 mL aliquots were presented to panelists randomly for odor assessment and intensity scoring in a temperature-controlled laboratory (25 °C). The ratings were based on a 5-point scale, where 1 indicated very weak and 5 indicated very strong flavor. All panelists participated with informed, written consent (Zhuang et al., 2016). These findings solely reflect experimental data and do not constitute commercial evaluation of any brand.

2.4. Analysis of physical and chemical indicators

The pH, total acidity (TA), and total soluble solids (TSS) of the samples were measured using ISO 1842-1991, ISO 750-1981, and ISO 2173-2003 methods, respectively. The ΔE (color difference) chromaticity was analyzed following a slightly modified method previously described by Andreou et al. (Andreou et al., 2023). Instruments were calibrated and maintained at 25 ± 2 °C before pH and TSS measurements. TA was assessed by titration and reported as mg/kg of acetic acid. For ΔE measurement, 25 mL of each vinegar sample was diluted 1:1 with distilled water and filtered using filter paper (20 μm). A reference solution was prepared by dissolving 1.75 g of glacial acetic acid in 50 mL of distilled water. A spectrophotometer (TS7700 Spectrophotometric Colorimeter, Shenzhen SUNSHI Technology Co.) was used to measure L*, a*, and b* values, with ΔE calculated as $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]\hat{}(1/2)$ (Andreou et al., 2023). All analyses were performed in triplicate.

2.5. Detection of organic acid and amino acid levels

Organic acids (OAs) were analyzed using HPLC (EasySep-1020, Shanghai, China) following a slightly modified version of a previously described method (Liu et al., 2019). The HPLC system was equipped with a UV detector set to 210 nm and an Agilent TC-C18 column (4.6 mm inner diameter × 250 mm length, 5 μm particle size). Briefly, 5 mL of each vinegar sample was evaporated at 85 °C in a water bath, redissolved in 5 mL of 80 % ethanol, and centrifuged at 3000 rpm for 15 min. The supernatant was evaporated, re-dissolved in 1 mL of mobile phase solution, centrifuged again at 3000 rpm for 15 min, filtered through a 0.22 μm microporous membrane (BS-PES25–22-S, BioSharp, Beijing, China), and stored at 4 °C for further analysis. The mobile phase comprised methanol and 0.05 mol/L sodium dihydrogen phosphate in a ratio of 2:98 (*v*/v) adjusted to pH 2.5. The HPLC conditions included a column temperature of 25 °C, a flow rate of 1 mL/min, an injection volume of 20 μL, and detection at 214 nm. External standards such as citric acid, DL-malic acid, lactic acid, acetic acid, tartaric acid, L-pyroglutamic acid, oxalic acid, and fumaric acid were used for qualitative and quantitative analysis, with a standard curve prepared from serial dilutions filtered through 0.22 μm membranes.

Amino acids (AAs) levels were measured by HPLC, based on a previously described method with slight modifications (Li et al., 2023). Briefly, 1.0 mL of each sample was mixed with 3 mL of anhydrous ethanol and centrifuged at 4000 rpm for 5 min. Next, 500 μL of the sample was combined with 250 μL each of PITC-acetonitrile and triethylamine-acetonitrile solutions and incubated at room temperature for 1 h. After derivatization, 2 mL of n-hexane was added, vortexed for 1 min, and allowed to stand for 10 min. The lower layer was collected and filtered through an organic membrane for HPLC analysis using the

EasySep® 1020. The mobile phase A comprised 0.1 mol/L sodium acetate (pH 6.5) and acetonitrile (95:5, *v*/v), while mobile phase B consisted of acetonitrile and water (4:1, v/v). The HPLC conditions included a column temperature of 40 °C, a flow rate of 1 mL/min, an injection volume of 5 μL, and detection at 254 nm. Seventeen external amino acid standards, including aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, and cystine, were used for quantification. The standards were serially diluted, filtered through 0.22 μm membranes, and used to prepare a standard curve for analysis.

2.6. Detection of volatile flavor compounds using HS-SPME/GC–MS

VFCs in vinegar samples were analyzed using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS), or HS-SPME-GC–MS, following a slightly modified previously described method (Sun et al., 2023). Briefly, vinegar samples were diluted 10-fold with distilled water, and 6 mL was transferred to a headspace flask. NaCl (1.5 g) and 10 μL of 100 ppm octanediol (internal standard) were added, and the flask was sealed with a PTFE spacer. The mixture was stirred at 600 rpm on a magnetic stirrer (85–2 Digital Display, Shanghai Shuangjie Experimental Equipment Co, China) in a 50 °C water bath for 10 min to reach equilibrium. The SPME fiber (65 μm PDMS/DVB, Supelco, USA) was exposed to the headspace at 40 °C for 40 min and promptly analyzed using GC–MS (QP2010plus, Gas Chromatograph-Mass Spectrometer, Shimadzu, Kyoto, Japan). The analysis utilized an Rtx-5MS capillary column (60 m × 0.32 mm × 0.25 μm (Restek Corporation, Bellefonte, PA, USA) with the inlet port set at 250 °C for 5 min. The temperature program began at 40 °C, held for 3 min, and increased to 220 °C at a rate of 4 °C/min, before being held for 5 min. The mass spectrometry used an EI source at 70 eV with a scanning range of *m/z* 35–450. The VFCs were identified using a combination of NIST 20 library searches, relative retention index (RI) comparisons (calculated from n-alkanes), and characteristic ion analysis. Quantification was performed using the internal standard and normalization methods.

2.7. Determination of characteristic aroma components via ROAV analysis

The relative odor activity value (ROAV) was used to identify the characteristic aroma components of YCAV at different aging stages. Compounds with ROAV $\geq$1 were considered characteristic aroma compounds. ROAV was calculated using the formula:

ROAV = Mass concentration / Odor threshold.

Odor threshold values were obtained from relevant literature sources (Zhang et al., 2023).

2.8. Aroma and taste profile detection using E-nose and E-tongue

Aroma profiles of vinegar samples were analyzed using an electronic nose (*E*-nose, PEN3, Airsense, Germany) equipped with a 10-sensor metal oxide sensor (MOS) array, each responsive to specific aroma components (Li et al., 2023). The E-nose detection method followed a previously described method with slight modifications (Guo et al., 2020). Briefly, vinegar samples were diluted 10-fold with distilled water, and 6 mL was transferred to 20 mL headspace vials, which were equilibrated at room temperature for 30 min before connection to the E-nose. The internal flow rate was 200 mL/min, with a detection period of 180 s per sample to ensure sensor signal stabilization. After each measurement, the detector chamber was flushed with clean air for 80 s to reset sensor signals to baseline.

The taste profile was evaluated using an electronic tongue (*E*-tongue, SA402B, Insent, Japan) with seven sensors (CA0, GL1, C00, AE1, CT0, AAE, Aftertaste-B) targeting sourness, sweetness, bitterness, astringency, saltiness, richness, and aftertaste. The detection method followed a slightly modified version of a previously described method (Gao et al., 2021). Briefly, vinegar samples were diluted 1000-fold with distilled water, and 50 mL of each sample was placed in a 300 mL beaker and equilibrated at 25 ± 1 °C for 30 min. The samples were then analyzed using the E-tongue, with a data acquisition period of 3 min per sample. Sensors were cleaned and reactivated between measurements.

2.9. Statistical analysis

Principal component analysis (PCA) and radar plots were generated using Origin Pro2022b (Origin Lab Inc., Hampton, MS, USA). Correlation analysis and sensory evaluation heatmaps were created in R Studio (RStudio Team, 2015). Partial least squares discriminant analysis (PLS-DA) was performed using SIMCA 14.1 (Umetrics, Umea, Sweden), while thermograms were plotted with Tbtools (version 1.082, Toolbox for Biologists, China). Additional statistical analyses and mathematical regression were conducted using SPSS 22.0 (SPSS Inc., USA). One-way ANOVA was applied to compare group means, with statistical significance set at $p < 0.05$.

3. Results and discussion

3.1. Sensory evaluation

The sensory characteristics of the vinegar samples were assessed using quantitative descriptive analysis, focusing on odor, taste, and color. Nine sensory attributes were evaluated, including sourness, sweetness, umami, saltiness, bitterness, kokumi, color, ester smell, and burnt smell (Fig. 1). Among these, sourness, kokumi, and color were identified as the dominant sensory features. Kokumi, a term introduced by Japanese scientist Ueda, describes a sensation of mouthfulness, continuity, and thickness(Chang et al., 2022).

The sensory ratings for F1 vinegar samples at various ages indicated that sourness, kokumi, and color were the most prominent attributes, with bitterness receiving the lowest overall score (Fig. 1a). Notably, sourness, sweetness, umami, kokumi, and color scores increased progressively with aging. Ester smell and bitterness initially showed an upward trend before decreasing, peaking in the F1–8 sample. Conversely, the burnt flavor decreased initially, followed by an increase, with F1–8 recording the lowest score for burnt flavor (Fig. 1a). For the F2 vinegar samples, the sensory profile across different ages shows higher scores for ester smell, sourness, saltiness, bitterness, kokumi, and color, while sweetness and umami were rated lower (Fig. 1b). Kokumi and color scores consistently increased with aging, whereas sourness peaked at five years. The bitterness trend mirrored that seen in F1 samples, showing an initial rise followed by a decline. F2–3 exhibited the highest score for burnt flavor and the lowest for sweetness and umami (Fig. 1b). The acceptability comparison between the two vinegar brands (Fig. 1c), indicated that F1 samples had generally higher acceptability scores than F2 samples, with acceptability improving steadily with aging. Due to the content of sweet-tasting glycine (Gly) increased over time, while the content of bitter-tasting arginine (Arg) decreased. This trend aligns with the sensory evaluation findings, suggesting that aging reduces bitterness and enhances sweetness, thereby improving the overall acceptability of the vinegar. These observations are consistent with prior research that reported the influence of aging on enhancing desirable sensory attributes in vinegar (Andreou et al., 2023).

3.2. Changes in physical and chemical indicators

The flavor profile of vinegar, particularly sourness, is influenced by key physicochemical parameters such as pH, total acidity (TA), color, and total soluble solids (TSS), which serve as essential indicators of product quality. As shown in Fig. 2a, the pH of both vinegar brands generally increased with prolonged aging, reaching its highest value at the 10-year mark. Notably, F2 samples consistently exhibited lower pH

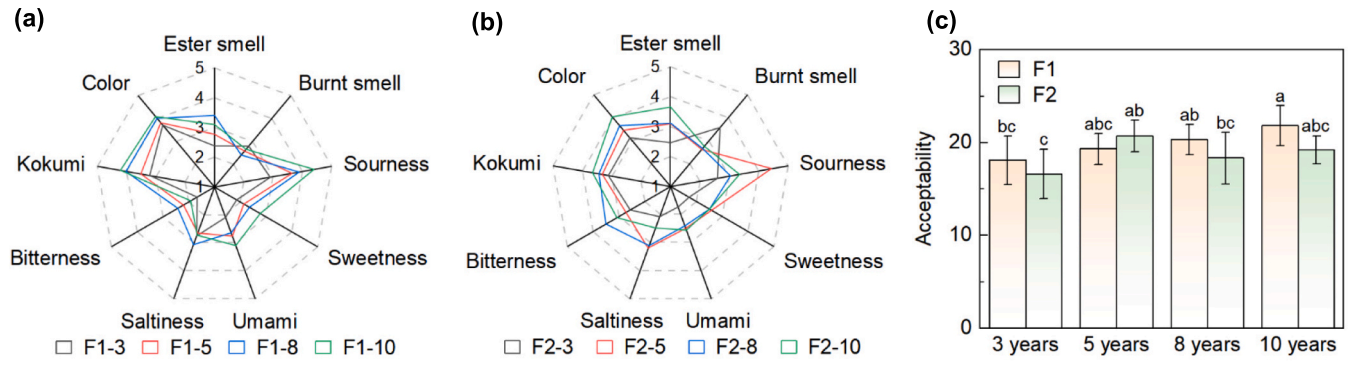

Fig. 1. Sensory evaluation and flavor profiles of YCAV samples from two companies (F1 and F2) at various aging stages. (a and b) Sensory evaluation radar chart, (c) Acceptability scores bar chart.

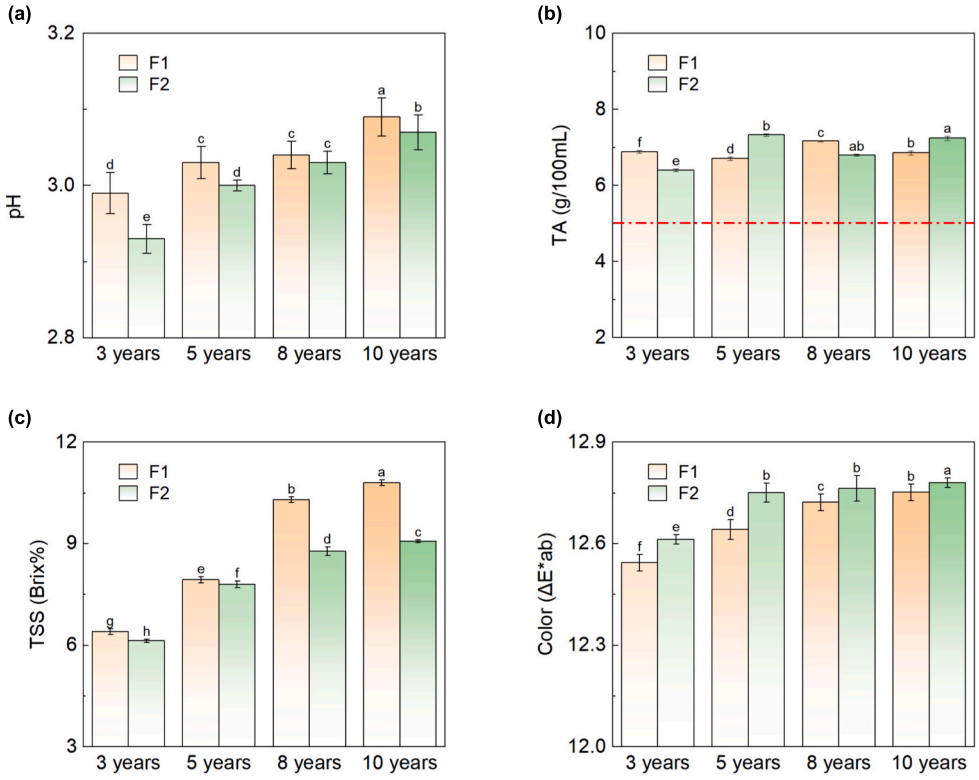

Fig. 2. Physicochemical indicators of two brands of YCAV samples (F1 and F2). (a) pH distribution, (b) Percentage total acid (%TA), (c) Total soluble solids (TSS), (d) Color.

levels compared to F1 samples throughout the aging process. Interestingly, previous research on Chinese Zhejiang vinegar, which underwent a shorter fermentation period of 90 days, documented a progressive pH decrease over time (Zhang et al., 2023). The differences between our findings and the previous study are likely due to the extended aging duration in our study. Prolonged aging can drive dynamic changes in ion balance and small molecule interactions, initially lowering the pH but gradually leading to an increase as equilibrium is established (Consonni et al., 2008). Similar patterns have been reported in the acid content of Cantonese-style rice vinegar (Sun et al., 2023) and Zhenjiang aromatic vinegar (Huang et al., 2022), supporting the notion that long-term aging induces complex chemical transformations that modulate acidity and flavor development.

Total acidity (TA) is a critical quality indicator for commercial vinegar. According to Chinese National Standards (GB/T 26531–2011), the TA of vinegar should be no less than 5.0 g/100 mL. As shown in Fig. 2b, both vinegar brands consistently exceeded this threshold, with TA levels surpassing 6.0 g/100 mL throughout the aging process. TA fluctuated within a ± 5 % range but exhibited an overall gradual increase over time. This pattern is consistent with previous studies on Zhejiang vinegar, where TA increased more sharply during shorter fermentation periods (Zhang et al., 2023). The slower, steadier rise in TA observed in our samples may be attributed to the extended aging period, during which ongoing microbial activity and slow acid formation contribute to the progressive accumulation of organic acids.

TSS in vinegar, composed mainly of sugars, organic acids, and amino acids, helps balance the sharp acidity and enhance palatability (Chen et al., 2012). As shown in Fig. 2c, TSS levels in both F1 and F2 samples increased with aging, reaching the highest concentrations in the 10-year samples. This result is consistent with observations in balsamic vinegar

aged in wooden barrels, where water evaporation and concentration effects contribute to higher TSS in later stages (Andreou et al., 2023). The linear regression equations relating TSS to aging, i.e., $Y_{F1} = 1.557 \ln(X) + 4.965$ and $Y_{F2} = 0.979\ ln(X) + 5.495$, showed high correlation coefficients ($R^2 = 0.9504$ and 0.9108, respectively). These data suggest that TSS and regression models can be reliable indicators for assessing the impact of aging on vinegar quality.

Chromaticity, influenced by soluble solids, exhibited an upward trend over time for both vinegar brands, with the darkest samples observed at 10 years of aging (Fig. 2d). Regression analyses of △E chromaticity against aging, i.e., $Y_{F1} = 0.0704\ ln(X) + 12.489$ and $Y_{F2} = 0.0567\ ln(X) + 12.572$, demonstrating strong correlations ($R^2 = 0.9529$ and $R^2 = 0.925$, respectively). The darkening of vinegar during aging is attributed to polymerization reactions, such as the Maillard reaction, which generates melanoid pigments responsible for the characteristic deep reddish-brown hue (Andreou et al., 2023). Additionally, red yeast rice, a raw material used in the production of these vinegars, likely contributes to the observed coloration (He et al., 2022). Red yeast rice serves as a natural source of food-grade pigments (Agboyibor et al., 2018), with pigmented rice varieties from different regions displaying diverse shades of red, orange, and yellow (Liu et al., 2018). The high regression coefficients suggest that the inclusion of red yeast rice complements the chromaticity changes during aging. Overall, these findings highlight the significant impact of aging on pH, TSS, and △E chromaticity, underscoring its role in altering the physicochemical properties of vinegar.

3.3. Taste quality analysis

The sensory attributes of vinegar, including bitterness, sweetness, and saltiness, significantly influence its overall taste. Key contributors to these attributes, i.e., organic acids and amino acids, were profiled using HPLC and an *E*-tongue to better understand their role in taste quality.

Organic acids play a critical role in shaping the taste of vinegar, with acetic acid being the main component responsible for its characteristic flavor. However, a blend of other organic acids contributes to a balanced sourness while reducing the sharpness of acetic acid, producing a smoother taste (Gao et al., 2021). HPLC analysis of the two vinegar brands identified eight organic acids: oxalic, tartaric, pyruvic, L-malic, lactic, acetic, citric, and succinic acids (Table 1, Fig. 3a). The F1 samples contained a higher cumulative content of organic acids compared to the F2 samples, although both brands showed a decline in organic acid concentration with aging. Five organic acids, i.e., L-malic, citric, acetic, pyruvic, and lactic acids (Fig. 3b), emerged as significant markers (VIP > 1), making these potentially useful for differentiating between aging stages of vinegar and product branding. These results are consistent with the study by Gao et al. who identified similar organic acids (i.e., acetic acid, lactic acid, succinic acid, and tartaric acid) as key indicators of taste quality in rice vinegar (Gao et al., 2021).

Amino acids are fundamental to the taste profile of vinegar, contributing flavors such as umami, sweetness, and bitterness depending on their chemical properties. Seventeen amino acids were detected in the two vinegar brands (Table 1, Fig. 3c), with the F1 samples showing higher overall amino acid content than the F2 samples. Notably, seven amino acids, i.e., Met, Asn, Thr, His, Ser, Val, and Cys, were identified as significant markers (VIP > 1) for differentiating between brands and aging stages (Fig. 3d). This observation is consistent with previous findings where amino acids, such as Glu, Ala, Val, and Arg were highlighted as important contributors to aged rice vinegar flavor (Gao et al., 2021).

The *E*-tongue system, known for its reliability and objectivity, and its ability to mimic human taste perception and identify flavor profile differences across brands and aging stages (Zhou et al., 2024) was also used for the vinegar flavor analysis. As shown in Fig. 4, the F1 samples exhibited significantly higher salty taste compared to the F2 samples ($p \leq 0.05$), while other sensory responses were largely similar between the two brands. Both sourness and saltiness decreased with aging for both brands, a finding different from recent studies where shorter fermentation periods showed increased acidity and reduced bitterness in early stages (Gao et al., 2021). However, these results are consistent with a recent study, where decreasing acidity and increasing TA were observed in aged Zhejiang vinegar, highlighting the role of extended aging (Zhang et al., 2023). Therefore, the observed changes suggest that the salty taste detected by the E-tongue could be a potential marker for distinguishing

Table 1
Quantitative table of organic acids and amino acids in vinegar.

NO.	Compounds	RT	Organic acids content (mg/mL)							
			F1–3	F1–5	F1–8	F1–10	F2–3	F2–5	F2–8	F2–10
1	Oxalic acid	2.848	0.61 ± 0.04^{c}	0.80 ± 0.05^{b}	0.73 ± 0.01^{b}	0.48 ± 0.01^{d}	0.74 ± 0.03^{b}	0.78 ± 0.06^{b}	1.12 ± 0.09^{a}	0.56 ± 0.04^{c}
2	Tartaric acid	3.353	0.82 ± 0.03^{b}	0.72 ± 0.01^{c}	0.94 ± 0.08^{a}	0.96 ± 0.06^{a}	0.58 ± 0.01^{d}	0.18 ± 0.01^{g}	0.34 ± 0.02^{f}	0.45 ± 0.01^{e}
3	Pyruvic acid	4.03	0.25 ± 0.02^{d}	0.19 ± 0.01^{e}	0.08 ± 0.01^{g}	0.05 ± 0.01^{h}	0.59 ± 0.02^{a}	0.43 ± 0.01^{b}	0.36 ± 0.01^{c}	0.16 ± 0.01^{f}
4	L-Malic acid	4.365	0.26 ± 0.02^{e}	0.22 ± 0.02^{e}	0.44 ± 0.04^{c}	0.59 ± 0.05^{b}	0.73 ± 0.02^{a}	0.57 ± 0.02^{b}	0.32 ± 0.02^{d}	0.14 ± 0.01^{f}
5	Lactic acid	5.505	10.32 ± 0.84^{a}	9.04 ± 0.59^{b}	8.92 ± 0.54^{b}	8.86 ± 0.58^{b}	6.48 ± 0.62^{ab}	6.76 ± 0.09^{c}	6.76 ± 0.52^{c}	6.40 ± 0.16^{c}
6	Acetic acid	5.783	16.57 ± 0.63^{a}	16.12 ± 0.92^{a}	15.90 ± 1.58^{a}	15.85 ± 0.71^{a}	9.54 ± 0.13^{b}	9.50 ± 0.02^{b}	9.46 ± 0.74^{b}	9.19 ± 0.02^{b}
7	Citric acid	8.491	1.39 ± 0.13^{e}	1.71 ± 0.05^{ab}	1.72 ± 0.09^{a}	1.76 ± 0.15^{a}	1.57 ± 0.01^{bcd}	1.64 ± 0.1^{abc}	1.53 ± 0.05^{cde}	1.48 ± 0.01^{de}
8	Succinic acid	10.11	5.34 ± 0.26^{e}	4.93 ± 0.03^{ab}	4.73 ± 0.37^{a}	4.67 ± 0.26^{a}	3.53 ± 0.06^{bcd}	3.50 ± 0.25^{abc}	2.82 ± 0.22^{cde}	3.55 ± 0.07^{de}
NO.	Compounds	RT	Amino acids content (mg/L)							
1	Arg	20.653	146.5 ± 7.0^{a}	142.6 ± 8.2^{a}	140.9 ± 7.8^{a}	139.2 ± 11.3^{a}	63.6 ± 2.3^{b}	60.6 ± 5.8^{b}	58.3 ± 0.02^{b}	57.2 ± 2.1^{b}
2	Thr	20.901	30.9 ± 2.6^{c}	26.9 ± 2.1^{d}	32.9 ± 2.1^{c}	31.4 ± 2.9^{c}	32.5 ± 2.5^{c}	53.7 ± 3.8^{a}	25.1 ± 0.2^{d}	49.0 ± 0.2^{b}
3	Ala	21.634	192.4 ± 13.8^{a}	188.5 ± 2.7^{a}	187.1 ± 9.5^{a}	187.1 ± 10.4^{a}	79.6 ± 1.3^{b}	74.0 ± 1.6^{b}	74.6 ± 2.9^{b}	76.9 ± 1.3^{b}
4	Pro	22.04	50.5 ± 3.5^{a}	41.7 ± 2.2^{c}	45.1 ± 2.2^{b}	41.6 ± 2.3^{c}	18.3 ± 0.8^{de}	15.5 ± 0.01^{e}	17.5 ± 0.3^{de}	19.8 ± 0.8^{d}
5	Leu	32.211	83.9 ± 2.8^{a}	79.7 ± 6.8^{ab}	78.9 ± 5.6^{ab}	78.2 ± 2.6^{b}	40.2 ± 1.9^{c}	35.33 ± 0.4^{cd}	30.1 ± 1.0^{d}	31.7 ± 3.1^{d}
6	Val	28.224	63.2 ± 1.1^{ab}	59.5 ± 0.8^{bc}	58.5 ± 3.7^{bc}	64.6 ± 5.0^{a}	43.1 ± 1.9^{de}	40.3 ± 0.2^{e}	44.4 ± 2.6^{d}	46.1 ± 0.7^{d}
7	Ser	15.733	49.6 ± 2.0^{a}	35.1 ± 1.0^{b}	29.2 ± 1.4^{c}	29.3 ± 1.8^{c}	23.1 ± 1.6^{d}	28.7 ± 0.5^{c}	22.2 ± 1.3^{d}	27.9 ± 2.6^{c}
8	Glu	8.391	92.9 ± 0.6^{a}	92.7 ± 8.1^{a}	86.5 ± 8.5^{a}	86.8 ± 6.0^{a}	21.0 ± 1.6^{b}	20.3 ± 1.5^{b}	20.9 ± 0.4^{b}	20.6 ± 0.6^{b}
9	Ile	31.831	45.0 ± 1.8^{a}	42.9 ± 1.2^{a}	42.6 ± 3.2^{a}	41.7 ± 2.8^{a}	27.5 ± 1.9^{b}	23.2 ± 1.1^{c}	23.3 ± 2.2^{c}	27.8 ± 2.6^{b}
10	Phe	34.326	44.0 ± 2.8^{c}	41.7 ± 1.6^{c}	39.2 ± 2.6^{d}	39.1 ± 0.7^{d}	33.5 ± 0.1^{e}	29.4 ± 1.2^{f}	36.7 ± 1.6^{d}	30.6 ± 0.4^{f}
11	Gly	16.78	42.4 ± 0.3^{b}	60.2 ± 5.0^{a}	57.0 ± 3.5^{a}	56.9 ± 3.9^{a}	21.0 ± 0.3^{c}	17.1 ± 0.3^{cd}	16.5 ± 1.1^{d}	15.7 ± 0.1^{d}
12	Cys	29.828	39.0 ± 0.4^{e}	40.3 ± 2.4^{de}	43.1 ± 1.3^{d}	40.4 ± 1.8^{de}	55.4 ± 0.4^{b}	51.0 ± 2.3^{c}	60.6 ± 1.2^{a}	57.8 ± 3.8^{ab}
13	Asn	6.783	19.6 ± 1.8^{e}	26.6 ± 2.4^{c}	41.1 ± 0.5^{a}	34.1 ± 1.4^{b}	29.4 ± 1.2^{c}	22.2 ± 2.2^{de}	26.9 ± 2.5^{c}	23.6 ± 0.6^{d}
14	Met	29.454	18.9 ± 1.3^{b}	17.3 ± 1.1^{b}	13.0 ± 0.9^{c}	13.5 ± 1.0^{c}	23.5 ± 0.3^{a}	23.7 ± 1.1^{a}	24.2 ± 1.4^{a}	4.7 ± 0.1^{d}
15	Tyr	27.153	13.8 ± 0.5^{d}	13.5 ± 0.7^{d}	14.1 ± 1.4^{d}	13.0 ± 0.4^{d}	28.2 ± 2.7^{a}	20.6 ± 0.7^{c}	21.4 ± 1.6^{bc}	23.3 ± 2.3^{b}
16	Lys	35.321	10.6 ± 0.6^{d}	9.5 ± 0.2^{d}	4.6 ± 0.1^{e}	9.6 ± 0.5^{d}	20.3 ± 1.1^{c}	23.8 ± 1.9^{b}	28.3 ± 2.1^{a}	20.8 ± 1.9^{c}
17	His	18.655	9.74 ± 0.33^{e}	13.7 ± 1.0^{b}	10.3 ± 0.6^{de}	11.7 ± 0.8^{c}	16.3 ± 0.4^{a}	14.2 ± 0.7^{b}	16.3 ± 0.5^{a}	11.1 ± 0.3^{cd}

[a] Different letters in the same row indicate significant difference ($p < 0.05$).

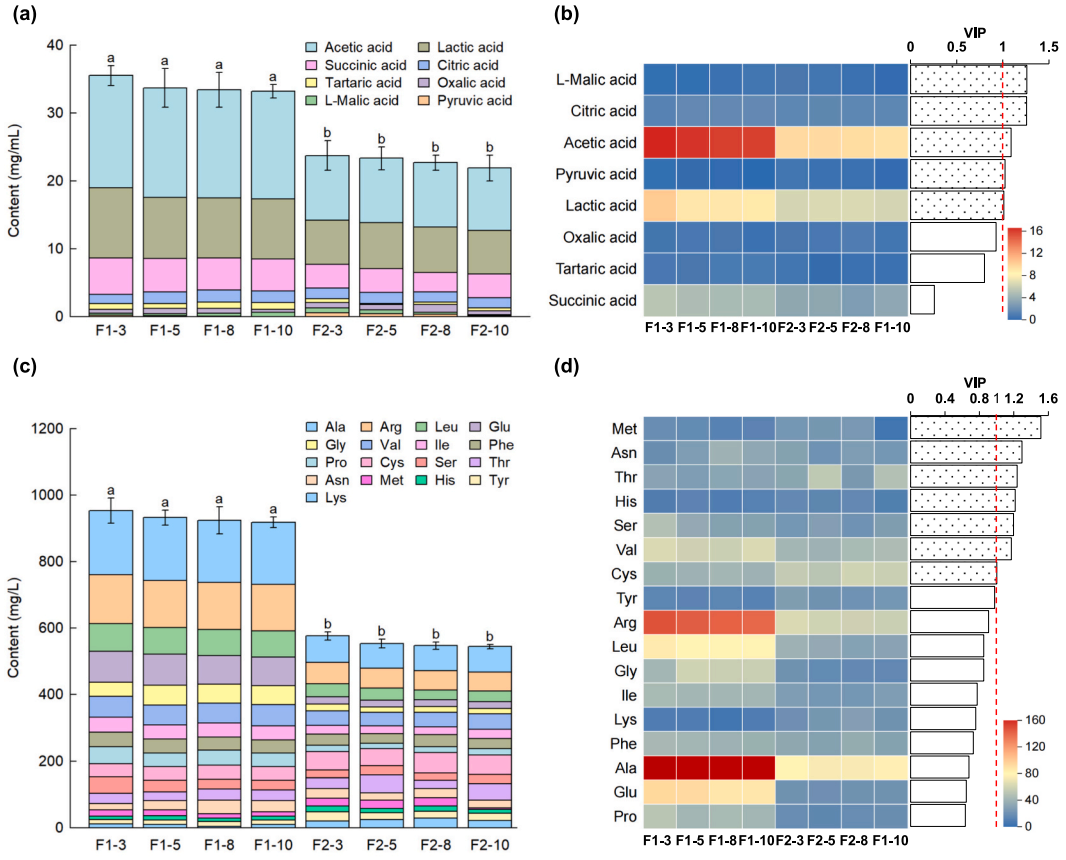

Fig. 3. Organic acid and amino acid profile of two brands of YCAV samples (F1 and F2). (a) Organic acids profile, (b) Organic acid contents heatmap and PLS-DA VIP values diagram, (c) Amino acids profile, (d) Amino acids contents heatmap and PLS-DA VIP values diagram.

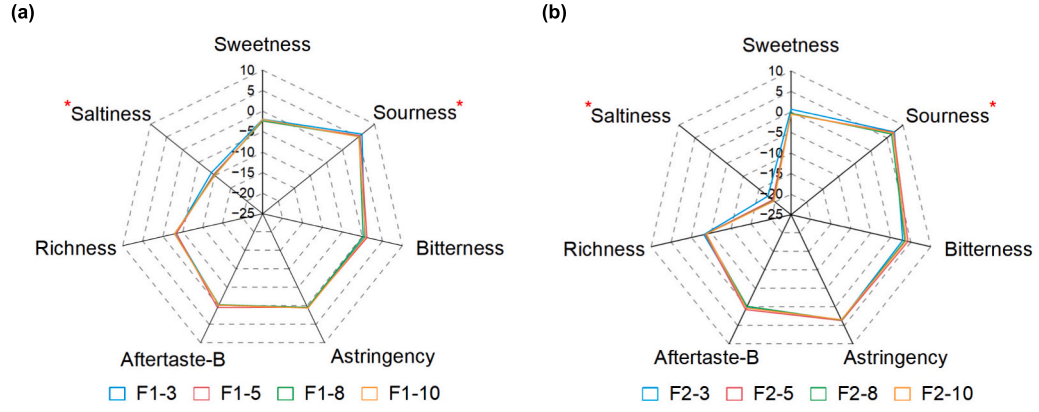

Fig. 4. Distribution of *E*-tongue response values of two brands of YCAV samples (F1 and F2) at different ages. Electronic tongue radar chart of (a) F1 samples, and (b) F2 samples.

between brands and aging periods.

3.4. Volatile flavor quality analysis

The perception of vinegar's volatile flavor properties significantly impacts consumer appeal, yet analyzing these substances is challenging due to their low concentration and volatility. Advanced methods such as HS-SPME-GC/MS and the E-nose, which simulates human olfactory response, provide reliable tools for precise detection and analysis (Xia et al., 2024).

Using the HS-SPME-GC/MS method, 24 VFCs were identified across two brands of YCAV, including nine esters, eight acids, three alcohols, and four miscellaneous compounds (Supplementary Table). Esters were confirmed as the dominant aromatic compounds, consistent with previous findings in YCAV (Zhang et al., 2023). Compounds with an odor activity value (ROAV) $\geq$ 1 were classified as odor-active compounds (OACs), which significantly contributed to the flavor profiles. Comparative analysis revealed 12 OACs (Table 2), with an upset plot (Fig. 5a) illustrating shared and unique OACs between the two brands. Six OACs were common to both brands, i.e., acetic acid, 3-methyl-butanoic acid, ethyl phenylacetate, phenethyl acetate, dibutyl phthalate, and acetoin. Acetic acid was identified as the primary flavor compound, consistent

Table 2
Table of odor active compounds and ROAV values in vinegar.

NO.	Compounds	Odor characteristic	Threshold (μg/L)	Odor Activity Values (ROAVs)							
				F1–3	F1–5	F1–8	F1–10	F2–3	F2–5	F2–8	F2–10
1	Acetic acid	Vinegar	14.4	277	3217	1154	1513	–	3700	646	993
2	3-Methyl-butanoic acid	Cheesy, fatty	148	5	4	5	8	2	4	4	3
3	Octanoic acid	Cheesy	1057	–	–	–	–	1	1	2	2
4	Isobutyl acetate	Fruity	66	6	1	2	3	–	–	–	–
5	Ethyl phenylacetate	Honey, rose	148.2	≤1	–	1	1	2	1	–	2
6	Phenethyl acetate	Rose smell	909	19	3	15	13	2	3	2	3
7	Dibutyl phthalate	Faint odor	30	8	1	1	13	≤1	13	48	30
8	Phenethyl alcohol	Rose smell	5100	2	2	2	3	–	–	–	–
9	Benzaldehyde	Bitter, cherry	426	2	≤1	2	2	–	–	–	–
10	Acetoin	Buttery, creamy	800	–	–	–	≤1	≤1	7	3	2
11	Trimethyl-pyrazine	Nutty, musty	460	–	–	–	–	–	2	–	2
12	Tetramethyl-pyrazine	Nutty	22.8	–	–	–	–	1	40	25	32

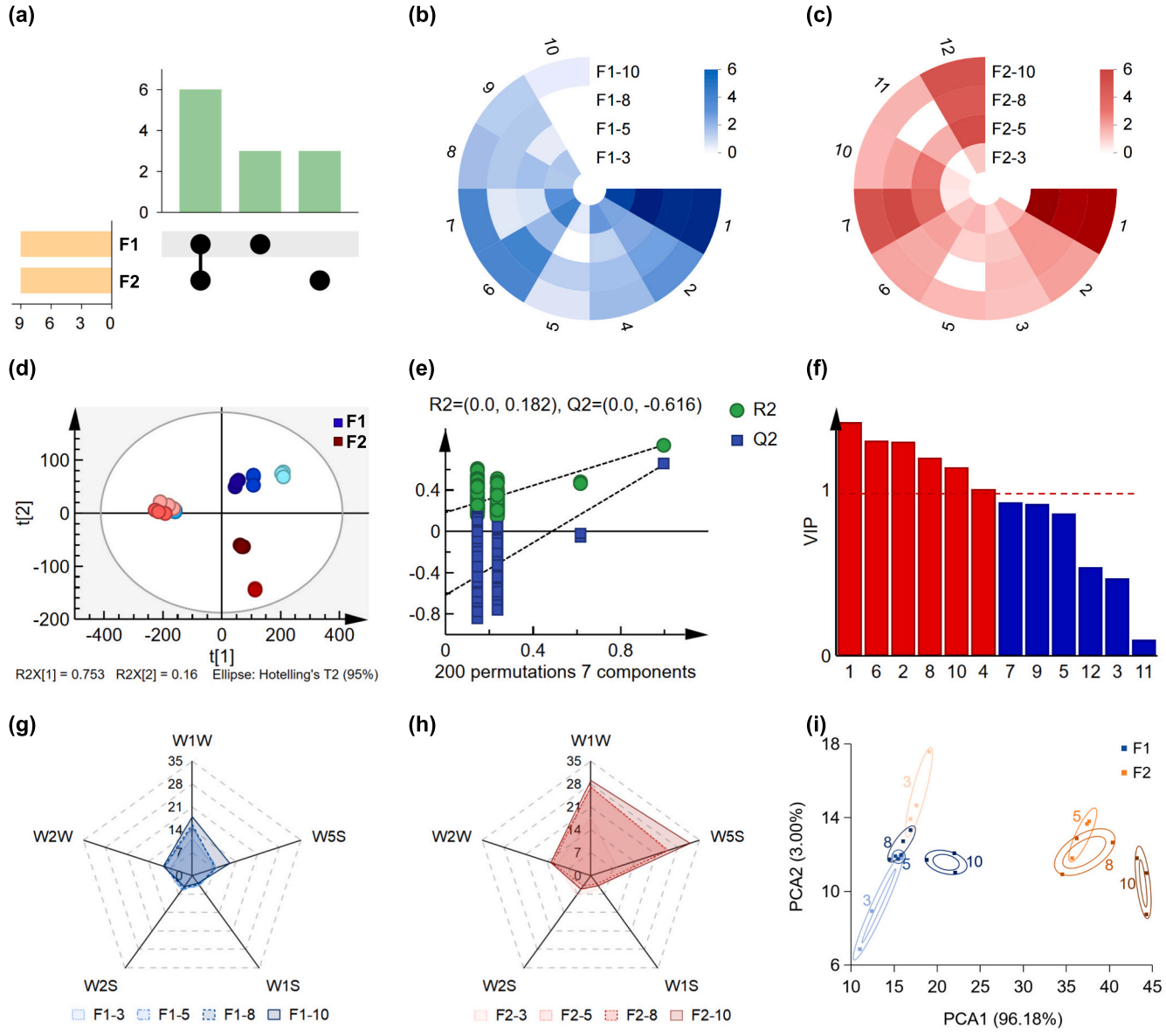

Fig. 5. Distribution of volatile flavor compounds in two brands of YCAV samples at different ages. (a) Up-Set plot of ROAVs compounds, ROAV calorific value plots for (b) F1 samples and (c) F2 samples, (d) PLS-DA score plot for ROAVs compounds, (e) Plot of permutation tests of ROAVs compounds (f) PLS-DA VIP values diagram of ROAVs compounds, Electronic nose radar chart of (g) F1 samples, and (h) F2 samples, (i) PCA analysis plot.

with findings in Shanxi aged vinegar (Zhou et al., 2020). Similarly, 3-methyl-butanoic acid, prominent in Zhenjiang aromatic vinegar, and compounds such as ethyl phenylacetate and acetoin, found in Cantonese-style rice vinegar and aged Hebei vinegar (Al-Dalali et al., 2022; Sun et al., 2023), were also detected. Acetoin has been recognized as a key flavor contributor in Shanxi aged vinegar (Zhang et al., 2023). Phenethyl acetate, identified in both vinegar brands, and dibutyl phthalate, previously reported in small amounts in Sichuan Baoning and Shanxi aged vinegars (Kuang et al., 2020), added complex sensory attributes, including cheesy, honey, rose, faint odor, and buttery notes. Distinct OACs were unique to each brand, serving as potential odor fingerprint identifiers for differentiation. F1 contained isobutyl acetate, phenethyl alcohol, and benzaldehyde, while F2 featured octanoic acid, trimethyl-pyrazine, and tetramethyl-pyrazine. These unique compounds highlight the distinct flavor profiles of the two vinegar brands, contributing to their individual sensory characteristics.

Aging influenced OACs' flavor intensity as depicted in Fig. 5b. In F1 samples, acetic acid demonstrated the highest intensity, peaking in 5-year aged samples, a trend similar to Zhenjiang aromatic vinegar, which initially increases and then declines over time (Zhou et al., 2020). The intensities of 3-methyl-butanoic acid and phenethyl acetate, with their characteristic cheesy and ester-like scents, rose progressively with aging, consistent with their role in other regional vinegars, i.e., Zhenjiang aromatic vinegar and Cantonese style rice vinegar (Sun et al., 2023; Zhou et al., 2020). Acetoin appeared only in 10-year aged F1 vinegar, aligning with the patterns seen in Cantonese-style rice vinegar (Sun et al., 2023). For F2 samples, acetic acid was also dominant, peaking at 5 years, while the intensity of 2-phenylethyl ester gradually increased with aging, a trend consistent with observations in Sichuan Baoning vinegar, where it was identified as key OAC (Kuang et al., 2020). These data underscore that OAC variation with aging could serve as an indicator for assessing vinegar age.

The results of OAV-based PLS-DA analysis of the vinegar samples are shown in Fig. 5. The contribution of PC1 and PC2 after dimensionality reduction in the loading diagram of Fig. 5d is 75.3 % and 16 %, respectively, which together amount to 91.3 % representing the main characteristic information of vinegar samples. The vinegar samples of the two brands were clustered into one family in the figure, and the projections on the PC1 axis could be significantly distinguished, indicating that the odor active compounds were the identification factors for the differences in vinegar brands, and the odor active compounds unique to each of the two brands were the key odor fingerprint markers. In Fig. 5e, the replacement test ($n = 200$), $R^2 < 0.4$ and $Q^2 < 0.05$, indicated that the PLS-DA model was successfully fitted, and the results of the statistical analysis model were reliable. Fig. 5f acetic acid, phenethyl acetate, 3-methyl-butanoic acid, phenethyl alcohol, acetoin, Isobutyl acetate were VIP different volatile flavor compounds in the vinegar samples.

The *E*-nose provided rapid and effective monitoring of the odor profile differences between brands and aging stages. Significant variations ($P \leq 0.05$) were observed in the response signals of W1W, W2W, W1S, W2S, and W5S detectors, which are sensitive to various compounds, including sulfides, aromatic compounds, methyl compounds, alcohols, aldehydes, ketones, and nitrogen oxides. Radar plots in Fig. 5g and Fig. 5h revealed that the F1 samples had higher response signals from the W1W, W5S, and W2W detectors compared to F2 ($P \leq 0.01$). Within the same brand, response differences between W1W and W5S increased with aging ($P \leq 0.05$), highlighting these detectors' sensitivity to aging-related changes. Principal component analysis (PCA) of the E-nose data (Fig. 5i) showed that PC1 captured 96.18 % of the sample information, allowing clear differentiation between brands. Similar findings were reported by Gao et al., who noted dynamic E-nose response changes during the 28 days of YCAV fermentation, particularly in W5S and W1S signals, which initially rose and then declined (Gao et al., 2022), consistent with the aging trends observed in this study. Thus, the E-nose proved effective in distinguishing between vinegar brands and aging stages.

3.5. Correlation between sensory attributes and multidimensional indicators

To evaluate the comprehensive quality of vinegar flavors, multidimensional analysis was performed using physical and chemical indicators such as pH, total acidity (TA), color difference (ΔE), total soluble solids (TSS), and quality indicators including organic acids (OA), amino acids (AA), and volatile flavor compounds (VFC). A primary concern for researchers and quality control professionals is understanding the correlation between these measurements and their relative importance in describing overall flavor quality. This study employed Mantel analysis to explore these correlations and used a stepwise regression model to quantify the contribution of each indicator to the overall flavor profile, which could aid in the identification of key quality indicators and streamlining quality assessments in production. The Mantel analysis results are depicted in Fig. 6, with a heat map illustrating correlations between the multidimensional indicators and their weighting in overall flavor quality. TSS showed a significant positive correlation with pH and ΔE, while ΔE was negatively correlated with OA and AA, indicating that TSS can serve as a practical proxy for detecting OA and AA levels in vinegar. In the sensory evaluation network shown on the left of Fig. 6, red lines indicate positive correlations between sensory attributes and test results, while blue lines indicate negative correlations. The line thickness represents the strength of the correlation. TSS exhibited a strong correlation with sensory attributes such as ester smell, sourness, sweetness, umami, kokumi, and color. VFCs were closely linked to burnt smell and sourness, suggesting that both TSS and VFCs could serve as reliable indicators for assessing overall vinegar flavor and quality.

To validate the importance of key indicators influencing vinegar acceptability, a stepwise regression model was developed, with vinegar acceptability as the dependent variable (Y) and multidimensional indicators as independent variables (X). The optimal regression equation was determined to be $Y = 0.601X_{TSS} + 0.4317X_{VFC} + 12.645$. This analysis identified total TSS and VFC as the most critical factors influencing the comprehensive flavor quality of aged vinegar, corroborating results from the Mantel analysis. The flavor quality of vinegar is shaped by factors such as geographical environment, raw material composition, and brewing process. Notably, the four renowned Chinese vinegars, i.e., Shanxi aged vinegar, Zhenjiang fragrant vinegar, Sichuan Baoning vinegar, and Yongchun aged vinegar (YCAV), exhibit significant variations in physicochemical properties and flavor profiles (Zhang et al., 2023). This study of two YCAV brands from the same geographical region revealed distinct compositional differences, highlighting the nuanced influences of production processes. TSS have been identified as a major quality determinant in sweet potato vinegar (Chen et al., 2019), while VFC are considered a key differentiating factor among China's famous vinegars (Guo et al., 2023). These findings align with the present study, underscoring the pivotal roles of TSS and VFC in vinegar quality assessment. Collectively, TSS and VFC serve as effective indicators for rapid quality evaluation and production control in vinegar manufacturing. This study contributes valuable insights into the flavor quality of YCAV and its compositional determinants.

4. Conclusion

This study comprehensively explored the sensory characteristics, physicochemical properties, taste profiles, and volatile flavor compounds of two aged vinegar brands (F1 and F2) of YCAV over a 10-year period. The findings demonstrate that prolonged aging significantly enhances YCAV quality by modulating key attributes such as sourness, kokumi, and color, while progressively reducing bitterness. Notably, sensory evaluation revealed that F1 samples exhibited higher overall acceptability, which aligned with the increased glycine and decreased

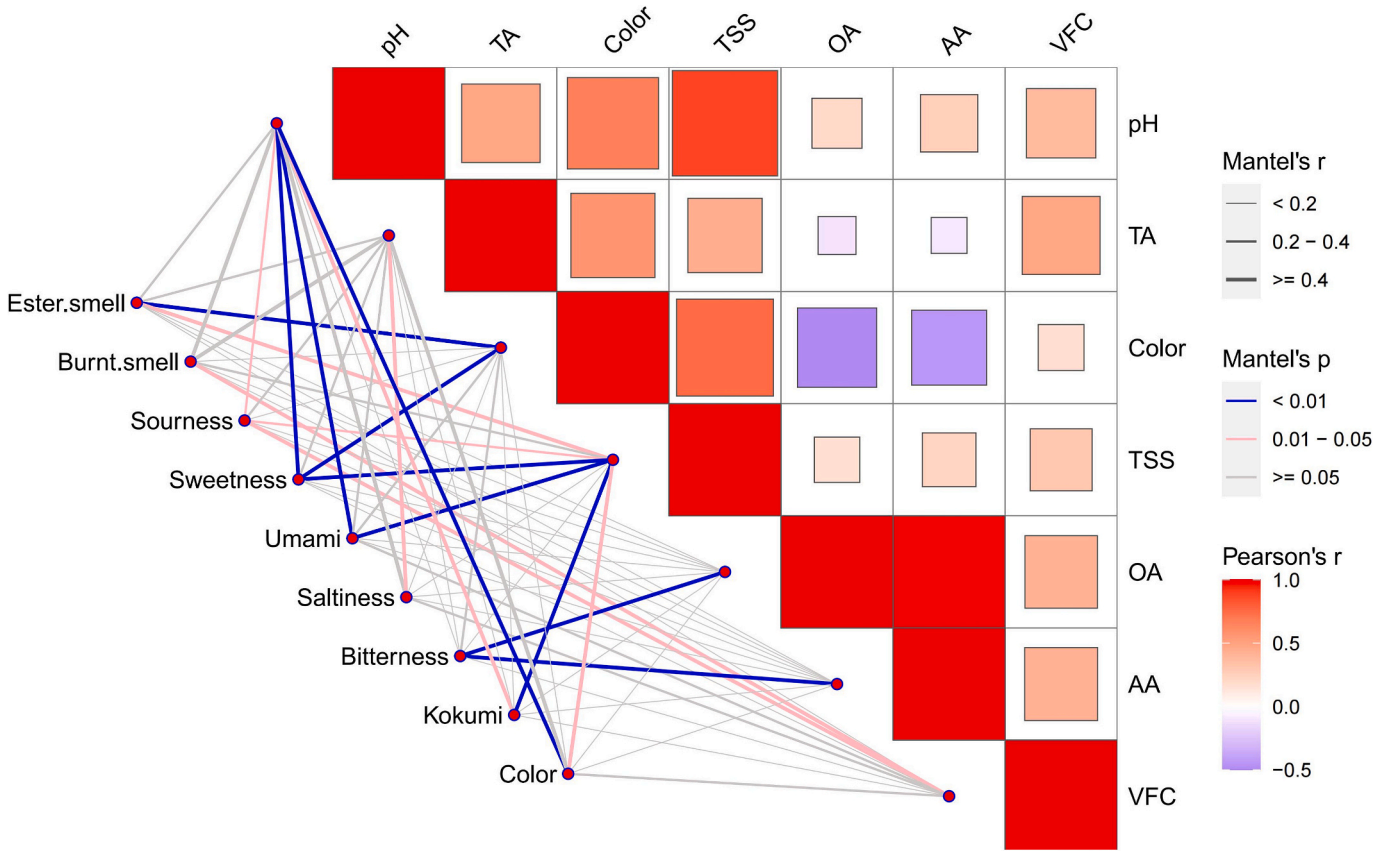

Fig. 6. Mantel analysis of the correlation between sensory attributes and multidimensional testing indicators of two brands of YCAV samples at different ages.

arginine content over time, contributing to enhanced sweetness and reduced bitterness.

The physicochemical analysis highlighted dynamic changes during aging, including a gradual pH increase, stable yet rising TA, and elevated TSS. These changes reflect ongoing chemical transformations, likely influenced by microbial activity and organic acid accumulation. Chromaticity analysis of YCAV indicated that color deepened with age, driven by Maillard reactions and pigment contributions from red yeast rice, with regression models confirming strong correlations between aging and color changes.

Taste quality analysis of YCAV via HPLC and *E*-tongue systems revealed that key organic acids (e.g., acetic, lactic, and citric acids) and amino acids (e.g., Met, Thr, and Val) shape the evolving flavor profile. While organic acid content declined with aging, contributing to a smoother sourness, amino acid profiles shifted to favor sweet and umami-enhancing compounds. The salty taste detected by the E-tongue emerged as a potential marker for distinguishing brands and aging stages.

Volatile flavor analysis using HS-SPME-GC/MS and E-nose identified 24 VFCs and 12 key OACs (ROAV $\geq$1), with esters and acids dominating the aroma profile. Distinct OACs for each brand of YCAV served as unique aromatic fingerprints, while shared compounds like acetic acid, 3-methyl-butanoic acid, and phenethyl acetate intensified with aging, enriching the overall sensory complexity. The E-nose further confirmed brand- and age-specific differences in volatile compounds, underscoring its utility for rapid aroma profiling.

Overall, these findings provide valuable insights into the intricate chemical and sensory transformations occurring during vinegar aging. The study not only reinforces the relationship between aging and improved sensory quality of YCAV but also highlights potential biomarkers for age and brand differentiation. These results could guide vinegar manufacturers in optimizing aging processes and establishing quality standards to meet consumer preferences for high-quality aged vinegar. In light of this, future research and practice may consider exploring microbial-driven flavor transformations and refining predictive models for vinegar quality optimization.

Ethical statement

This study was approved by ethical permission of Jimei University, China. All the sensory evaluations were carried out with the understanding and written consent of the judging panel.

CRediT authorship contribution statement

Haocheng Wei: Supervision, Project administration, Methodology, Funding acquisition. **Yijun Chen:** Writing – original draft, Visualization, Validation, Software, Data curation. **Houmei Shen:** Writing – original draft, Visualization. **Junyin Deng:** Formal analysis, Data curation. **Shuangshuang Zhang:** Formal analysis, Data curation. **Shen Yang:** Methodology, Data curation. **Liangze Fang:** Resources. **Chuanbo He:** Visualization, Software. **Jude Juventus Aweya:** Writing – review & editing, Funding acquisition. **Qingbiao Li:** Supervision, Conceptualization. **Hui Ni:** Writing – review & editing, Methodology.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102373.

Data availability

Data will be made available on request.

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