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Effect of caramel colors addition on the color, physicochemical properties, and flavor of Huangjiu

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ABSTRACT

Caramel color is a food colorant widely used in the fermented beverage industries. In this context, this study investigated the effects of caramel color on the quality of Huangjiu in terms of color, physicochemical properties, sensory characteristics, and the release of volatile organic compounds (VOCs). The results showed that Class IV caramel colors with the highest color intensity had the lowest coloring ability in Huangjiu due to precipitation. Caramel colors significantly affected the total sugar, titratable acidity, and amino nitrogen of Huangjiu. Sensory analyses indicated that the three classes of caramel colors significantly affected 10 sensory attributes and the aroma release of Huangjiu. The headspace concentrations of the 40 VOCs in Huangjiu changed significantly. Principal component analysis (PCA) and cluster analysis showed that Classes I and III caramel colors had similar effects on the VOCs in Huangjiu. Partial least-squares discriminant analysis (PLS-DA) showed that 9 compounds made important contributions to distinguishing different classes of caramel-colored Huangjiu samples. This study provides a theoretical foundation for the scientific application of caramel color in Huangjiu and other fermented food industries.

1. Introduction

Color is an important indicator of food quality that affects consumer acceptance and appetite. Caramel color is one of the world's most widely used food colorants, with good water solubility and strong coloring capacity, which is vital in many food products such as soft drinks, alcoholic beverages, condiments, and baked goods (Liang et al., 2019; Vollmuth, 2018). Caramel color is a complex mixture of compounds produced using carbohydrates as the main raw material, which can be divided into four classes based on whether ammonium or sulfite compounds are used as catalysts during production: Class I (plain caramel), no ammonium or sulfite compounds are used; Class II (caustic sulfite caramel), only sulfite compounds are used; Class III (ammonia caramel), only ammonium compounds are used; and Class IV (sulfite ammonia caramel), both ammonium and sulfite are used (Scotter, 2011). The four classes of caramel colors have distinct properties in terms of color, pH, and composition due to differences in the production processes (Scotter, 2011).

Huangjiu (Chinese rice wine) is one of the oldest alcoholic beverages in the world, and is popularly consumed in China. The typical manufacturing process for Huangjiu involves material preparation, simultaneous saccharification and fermentation, filtration, sterilization, and aging (Liu et al., 2021). In the absence of colorant additions, newly brewed Huangjiu is usually light yellow in color, which gradually deepens during aging (Wang, 2007). However, the aging process of Huangjiu takes several years and is susceptible to environmental factors

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(Wang et al., 2023), which makes it difficult to control the color stability of different batches of Huangjiu. Therefore, caramel color is widely used as a colorant in the Huangjiu industry to ensure consistent color quality of the product. According to the Chinese National Food Safety Standard for Uses of Food Additives (GB2760–2014), the colorants legally used in Huangjiu are Classes I, III, and IV caramel colors.

Although caramel color is used to color foods, it inevitably changes their nature. Caramel color molecules carry a net electrical charge (Chen and Gu, 2014; Myers and Howell, 1992; Scotter, 2011), which may interact with proteins, phenols, and other substances in Huangjiu, resulting in changes in its stability (Wang and Wang, 2006). Caramel color contains low-molecular-weight compounds such as pyrazines, furans, aldehydes, and ketones, which may produce flavors such as nutty aroma and bitterness and affect the flavor of Huangjiu (Chen and Gu, 2014; Li et al., 2021). In addition, high-molecular-weight polymers, such as melanoidins, in caramel colors may interact with VOCs in Huangjiu. Caramel color has been reported to reduce the release of VOCs and the overall aroma perception in foods such as coffee and wine, a phenomenon known as aroma or flavor binding (Gigl et al., 2021, 2022; Hofmann et al., 2001; Ortega-Heras and González-Sanjosé, 2009). Due to differences in the preparation process and composition of the three classes of caramel colors, their effects on the quality of Huangjiu may also be different. It has been reported that the addition of caramel colors may cause turbidity in Huangjiu and alter the release of aroma compounds. However, the effects of caramel colors on the color, physicochemical properties, and sensory characteristics of Huangjiu, as well as the differences in the effects of different caramel color classes on Huangjiu, have not yet been reported (Liu et al., 2020).

In this context, the main objectives of this study were to first analyze the differences in coloring ability among different caramel color classes in Huangjiu and their underlying reasons, secondly to explore the effect of caramel color addition on the physicochemical properties and sensory characteristics of Huangjiu, and then to evaluate the divergent effects of different caramel color classes on the release of VOCs in Huangjiu, and further clarify the differences in VOC characteristics between samples. Finally, multivariate statistical analysis was used to identify the potential markers for distinguishing the classes of caramel colors added to Huangjiu. The findings of this study provide a robust theoretical foundation for the scientific application of caramel color in Huangjiu and other fermented food industries.

2. Materials and methods

2.1. Reagents and chemicals

All standards of chromatographic grade were purchased from J&K Chemical Ltd. (Beijing, China). n-Alkanes (C_7–C_{30}) were purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China) and were of chromatographic grade. Analytical-grade ethanol, lactic acid, and sodium chloride (NaCl) were purchased from China Pharmaceutical Corporation (Shanghai, China).

2.2. Sample preparation

Huangjiu samples were provided by a Huangjiu company in Shaoxing, Zhejiang, China. Through the investigation of Huangjiu companies, 9 caramel color samples commonly used in the Huangjiu industry were purchased from three different manufacturers, which were Sethness Roquette Food Ingredients (Lianyungang) Co., Ltd. (Lianyungang, China), Aipu Food Industry Co., Ltd. (Shanghai, China) and Weifang Hengtai Food Co., Ltd (Weifang, China). Class I caramel color products were Sethness YT90, SB245 and SRC-YT25. Class III caramel color products were Hengtai Brewing Type I, Aipu BC-007 and Sethness SRC-3212. Class IV caramel color products are Aipu DS-036, Hengtai ZS-500B and Sethness SRC4430.

Huangjiu samples without caramel color were used as controls. After company investigation, caramel colors were added to Huangjiu at the proportions of 0.03%, 0.10%, and 0.20% (w/v) commonly used in the Huangjiu industry to compare the differences in the effects of different addition amounts. Among them, the differences between the three classes of caramel colors were compared at the proportion of 0.20% (w/v).

2.3. Color analysis

Caramel color is expressed by the color intensity, red index, and yellow index (Sengar and Sharma, 2014). Aqueous solutions of 0.10% (w/v) caramel color were taken and their absorbances at 610 nm, 510 nm, and 460 nm were measured in a 1 cm cuvette and their color intensity was calculated as follows:

$$\textit{Color intensity}(\mathrm{EBC}) = (A_{610} \times 20000)/\mathrm{C} \tag{1}$$

where the C value depends on the class of caramel color, that is, 0.053 for Class I, 0.076 for Class III, and 0.085 for Class IV. Red index and yellow index were calculated as follows:

$$\textit{Red index} = 10 \times lg(A_{510}/A_{610}) \tag{2}$$

$$\textit{Yellow index} = 10 \times lg(A_{460}/A_{610}) \tag{3}$$

The color of Huangjiu was indicated by CIELab color parameters (Han et al., 2021; Zhang et al., 2020). The transmission mode was set using an UltraScan Pro1166 high-precision spectrophotometer (HunterLab, Reston, VA, USA) with a light source D65 and an observation angle of 10°. Huangjiu samples were placed in a 5 × 3 × 1 cm glass cuvette, and L* (brightness), a* (red/green value) and b* (yellow/blue value) were measured, and C* (chromaticity), h* (Hue angle), and ΔE (total color difference) were subsequently calculated. L*values ranged from 0 (black) to 100 (white). a* indicates the degree of red (+) or green (-), and b* indicates the degree of yellow (+) or blue (-). h* is a red-yellow hue at 0–90°, and a yellow-green hue at 90–180°. ΔE indicates the overall difference in color between samples, and ΔE values > 2.8 in wine matrices is visually perceptible by the human eye (Zhang et al., 2019). C* , h , and ΔE values can be calculated according to the following formulas (Han et al., 2021; Zhang et al., 2019):

$$C^* = \left[(a^*)^2 + (b^*)^2\right]^{1/2} \tag{4}$$

$$h^* = \arctan(b^*/a^*) \tag{5}$$

$$\Delta E = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2} \tag{6}$$

Meanwhile, the browning index (BI) of Huangjiu was calculated based on the L* , a* and b* values with reference to the method reported in (Gu et al., 2023).

2.4. Turbidity analysis

The transmittance of the Huangjiu was determined using a spectrophotometric method (Su et al., 2017). The spectra of the clarified Huangjiu samples were scanned in the range 380–800 nm. A wavelength of 800 nm, at which all the samples showed almost no absorption, was chosen as the measurement wavelength. Huangjiu was vortexed and immediately measured at 800 nm using ultrapure water as a reference to characterize the degree of turbidity.

2.5. Physicochemical properties analysis

Organic acids, amino acids and small peptides, and carbohydrates are the main components of Huangjiu, and their contents can be approximated by titratable acidity (TA), amino nitrogen (AN), and total sugar (TS) respectively. Their determination is carried out by titration method in accordance with the Chinese standard analytical method for

Huangjiu GB/T 13662–2018, in which the TA and TS contents were measured in equivalents of lactic acid and glucose, respectively.

2.6. *Sensory evaluation*

Sensory evaluation of the caramel color and Huangjiu samples was performed using a Quantitative descriptive analysis (Kreutzmann et al., 2007). The sensory evaluation team comprised 10 experienced Huangjiu evaluators (six females, four males; 22–55 years in age). All evaluators gave informed consent before each sensory evaluation and approved by Jiangnan University Medical Ethics Committee (JNU202309011RB12). They had received sufficient professional training, including an understanding of descriptors, wine tasting, and the use of scales. The sensory panel discussed and reached a consensus on a glossary including 11 aromas (nutty, fruity, vanilla, acidic, mushroom, Qu aroma, yeasty, caramel-like, smoky, floral, and coconut-like) and 5 taste attributes (sour, sweet, bitter, umami, and astringent). Approximately, 30 mL of Huangjiu were placed in a tasting glass with a random three-digit code and presented to the panelists in random order. Descriptors were quantified using a 10 cm unstructured linear scale ranging in intensity from 0 (not perceptible) to 10 (strongly perceptible).

2.7. *Volatile organic compounds (VOCs) analysis*

VOCs analysis of Huangjiu was performed using headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) (Zhao et al., 2022; Liu et al., 2019, 2021). Briefly, 2 mL of the Huangjiu sample, 4 mL of ultrapure water, 2 g of sodium chloride, and 10 μL of internal standard (101.8 mg/L 2-octanol solution in ethanol) were added to a 20 mL vial fitted with a PTFE/silicone septum. After sample equilibration (5 min at 50 °C), VOCs were extracted exposing the 50/30 μm DVB/CAR/PDMS fiber (Supelco Inc., Bellefonte, PA, USA) in the vial headspace for 50 min at 50 °C, keeping the vial under orbital shaking at 250 rpm. The fiber was then desorbed in the GC injection port (splitless mode) at 250 °C for 5 min

The GC-MS analysis was performed using a TRACE 1300 gas chromatograph equipped with an ISQ 7000 mass spectrometer (Thermo Scientific, Waltham, MA, USA). In the GC system, a TG-WAXMS column (30 m × 0.25 mm × 0.25 μm, Thermo Scientific) was used with temperature program started at 40 °C for 2 min and increased to 230 °C with a ramp of 5 °C/min, remained for 10 min. High-purity helium was used as the carrier gas at a flow rate of 1.0 mL/min. Electron ionization at 70 eV was utilized as the ionization source with a 33–350 mass unit range. The temperatures of ion source and transfer line were 280 °C and 240 °C, respectively.

VOCs identification was based on a comparison of the mass spectra and retention index (RI) with those of authentic standards. The RIs of unknown compounds were calculated from the RI based on a series of standard n-alkanes (C_7–C_{30}, 1 μL was injected by liquid injection alone). Quantification was performed according to a previously reported method (Zhao et al., 2022; Liu et al., 2019, 2021). Compounds were quantified using the internal standard method using six-point linear least squares calibration curves, each of which was constructed using external standards. At the same time, the compound concentration is calibrated using internal standards to eliminate errors caused by experimental operations and instrument status. Information on quantitative method performance parameters is shown in Table S1.

2.8. *Odor activity value (OAV) analysis*

The contribution of VOCs to aroma was evaluated by calculating OAV. The OAV was calculated by dividing the concentration of the compound in Huangjiu by its odor threshold in ethanol or aqueous solution. Aroma descriptions and odor thresholds (ethanol solution or water as matrix) were taken from references (Chen et al., 2013, 2019; van Gemert, 2011; Wang et al., 2022; Flavornet and Human Odor Space, 2004).

2.9. *Statistical analysis*

All experiments were repeated thrice and results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) and Duncan's multiple comparison test ($p < 0.05$) were used to analyze the significance of differences between samples using the SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA). PCA, hierarchical cluster analysis (HCA) and PLS-DA were performed using SIMCA software (version 14.1; Umetrics, Umea, Sweden). MetaboAnalyst 5.0 was used for K-means clustering algorithm analysis (KMCA). Plotting was performed using the Origin software (version 2022; OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. *Effect of caramel colors addition on color and turbidity of Huangjiu*

First, the color indices of the three caramel colors were evaluated. The basic color parameters of the nine caramel colors in aqueous solutions are listed in Table 1. Class I caramel colors had a lower color intensity but higher red and yellow indices, while Class IV had the opposite properties of Class I. The color parameters of Class III caramel colors were between Class I and Class IV. Color intensity reflects the coloring ability of caramel colors (Sengar and Sharma, 2014), and the coloring ability of the three classes of caramel colors followed the order Class IV > Class III > Class I, among which the color intensity of Class IV caramel colors were about twice as high as the other two classes, indicating that it had the strongest coloring ability in aqueous solution (Table 1).

Nine caramel colors were added to Huangjiu at the proportion of 0.20% (w/v), and changes in the color of Huangjiu were evaluated. The addition of all three classes of caramel colors decreased the L* and h* values and increased the a* , b* , and C* values of Huangjiu, significantly ($p < 0.05$) (Fig. 1A & S1A). This indicated that caramel colors were able darkens the brightness of Huangjiu and made it reddish and yellow. The order of ΔE and BI values was Class III > Class I > Class IV. Among them, the ΔE and BI values of Class III caramel colors were about more than twice that of Class I, and the values of Class I is about twice as much as that of Class IV, indicating that the coloring ability of Class III caramel colors in Huangjiu were much stronger than that of the other two classes. Notably, Class IV, which had the highest color intensity in

Table 1

Color intensity, red index, and yellow index of the nine caramel colors.

Caramel color classes	Caramel color products	Chroma/EBC	Red index	Yellow index
Class I	I-A	15723.27 ± 217.87 ^{h}	6.71 ± 0.03^{b}	10.62 ± 0.05^{b}
	I-B	43270.44 ± 576.42^{d}	6.18 ± 0.05^{c}	9.26 ± 0.06^{c}
	I-C	4905.66 ± 377.36^{i}	7.79 ± 0.22^{a}	12.27 ± 0.46^{a}
Class III	III-D	32982.46 ± 547.81 ^{f}	5.30 ± 0.08^{d}	7.82 ± 0.08^{d}
	III-E	34824.56 ± 662.27^{e}	5.29 ± 0.06^{d}	7.87 ± 0.07^{d}
	III-F	30789.47 ± 696.25 ^{g}	5.28 ± 0.07^{d}	7.90 ± 0.07^{d}
Class IV	IV-G	64627.45 ± 359.42^{b}	4.00 ± 0.02^{e}	6.01 ± 0.02^{e}
	IV-H	62666.67 ± 271.69^{c}	4.07 ± 0.01^{e}	6.15 ± 0.02^{e}
	IV-I	68862.75 ± 543.39^{a}	4.04 ± 0.02^{e}	6.07 ± 0.02^{e}

$^{a\text{-}f}$ Different letters within the same column indicate a significant difference ($p < 0.05$).

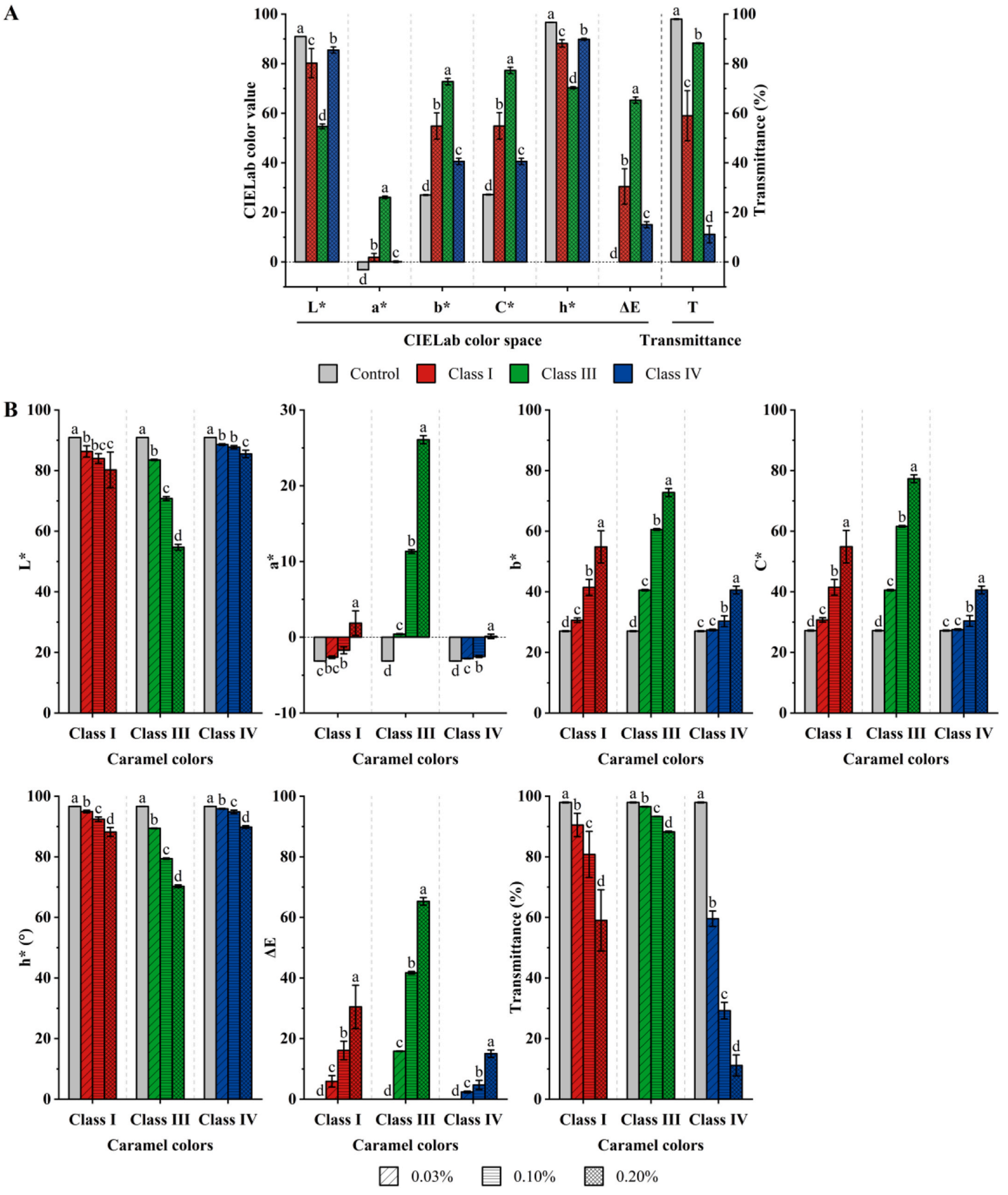

Fig. 1. Effect of different classes (A) and amounts (B) of caramel colors on CIELab color parameters and transmittance of Huangjiu. Different a-d letters in the same group indicate significant differences ($p < 0.05$).

water among the three classes of caramel colors, however had the weakest coloring ability on Huangjiu. This indicated that the coloring ability of the Class IV caramel colors was substantially affected by the Huangjiu matrix.

Transmittance can be used to characterize the clarity of alcoholic beverages. The decrease in transmittance indicated that the clarity of Huangjiu could have been reduced due to turbidity (Hou et al., 2020; Shih et al., 2020; Su et al., 2017). All three classes of caramel colors significantly reduced ($p < 0.05$) the transmittance of Huangjiu, indicating that the addition of caramel colors caused turbidity (Fig. 1A). The degree of change in transmittance was in the order Class IV > Class I > Class III. The transmittance of Huangjiu only decreased by about 10% after adding Class III caramel colors, but it dropped considerably by about 80% with Class IV caramel colors. This may be because Class III caramel colors have the same charge as most colloidal substances in Huangjiu, whereas Class IV caramel colors, in contrast, have positive and negative charges that combine to form neutral macromolecular solids, leading to flocculation, turbidity, and precipitation (Myers and Howell, 1992; Wang and Wang, 2006). The difference in charge between Class III and Class IV caramel colors could be related to the different catalysts used in their production. The effects of different Class I caramel color products on the transmittance of Huangjiu varied greatly within the group, which may be due to the fact that Class I caramel colors were produced without the use of specific catalysts, resulting in differences in the charge carried by different Class I caramel color products depending on the process.

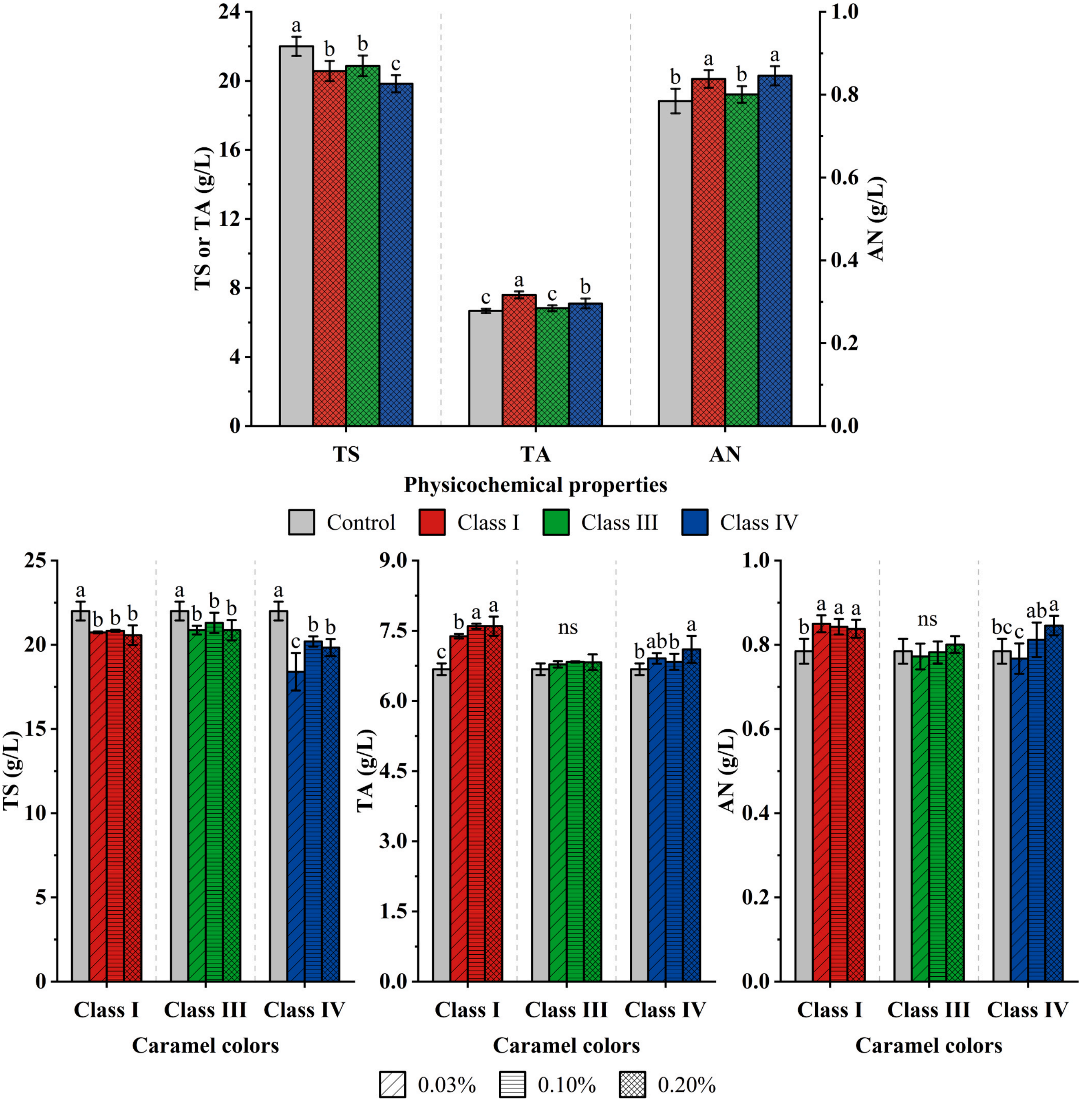

Fig. 2. Effect of different classes (A) and amounts (B) of caramel colors on the physicochemical properties of Huangjiu, including total sugar (TS), titratable acidity (TA), and amino nitrogen (AN). Different a-d letters in the same group indicate significant differences ($p < 0.05$).

The transmittance results appear to explain the variation in the coloring capacity of the caramel colors. Class III caramel colors interacted with Huangjiu, producing less precipitation, and most of the pigmented material of the caramel colors was retained to the maximum extent in the wine. Therefore, Class III caramel colors had the highest coloring capacity in Huangjiu. In contrast, although Class IV caramel colors had a high color intensity in aqueous solutions, their coloring ability in Huangjiu was significantly reduced due to a large amount of precipitation produced in Huangjiu, which may lead to the precipitation of pigment substances in the caramel colors.

Simultaneously, the effects of caramel color addition amounts (0.03%, 0.10%, and 0.20% m/V) on the color and transmittance of Huangjiu were also studied (Fig. 1B & S2B). With increasing addition amounts, the three classes of caramel colors showed consistency but different efficiencies in the changes in Huangjiu color and transmittance. At the unit addition amount, Class III caramel colors changed the color of Huangjiu substantially more than Classes I and IV, and had the highest coloring efficiency. In addition, Class III caramel colors produced the least amount of precipitation in Huangjiu under unit addition amount.

3.2. *Effect of caramel colors addition on physicochemical properties of Huangjiu*

Due to precipitation, caramel color is likely to affect the physicochemical properties of Huangjiu. As shown in Fig. 2A, the addition of the three classes of caramel colors significantly decreased ($p < 0.05$) the TS in Huangjiu. Classes I and IV caramel colors significantly increased ($p < 0.05$) the TA and AN of the Huangjiu. Class III caramel colors had no significant effect ($p \geq 0.05$) on the TA and AN in Huangjiu.

With increasing addition amounts of caramel color, the TA increased slightly for Huangjius with Class I caramel colors, and the TS, TA and AN increased slightly for Huangjius with Class IV caramel colors (Fig. 2B). However, for Class III caramel colors, there were no significant differences in the physicochemical properties of Huangjiu at different addition amounts, and the changes in the physicochemical properties of Huangjiu were small.

3.3. *Effect of caramel colors addition on sensory characteristics of Huangjiu*

The addition of caramel colors may also affect the sensory characteristics of Huangjiu. First, the sensory characteristics of the 0.20% caramel-colored aqueous solutions were evaluated. Among the 11 aromas and 5 taste attributes, the caramel-colored aqueous solutions only had sour, sweet, bitter, umami and astringent taste, and did not show obvious aroma (Fig. 3A). Sourness was stronger in Class I, and astringency was stronger in Class IV.

Sensory evaluation of Huangjiu with caramel color added was performed. A total of three taste and seven aroma attributes of Huangjiu were significantly affected ($p < 0.05$) by the three classes of caramel colors (Fig. 3B). All three classes of caramel colors significantly enhanced ($p < 0.05$) the bitterness of Huangjiu, and Class I caramel colors significantly enhanced ($p < 0.05$) the sourness of Huangjiu. Class IV caramel colors had the strongest astringency in aqueous solution, but they reduced the astringency of Huangjiu, which could be due to the

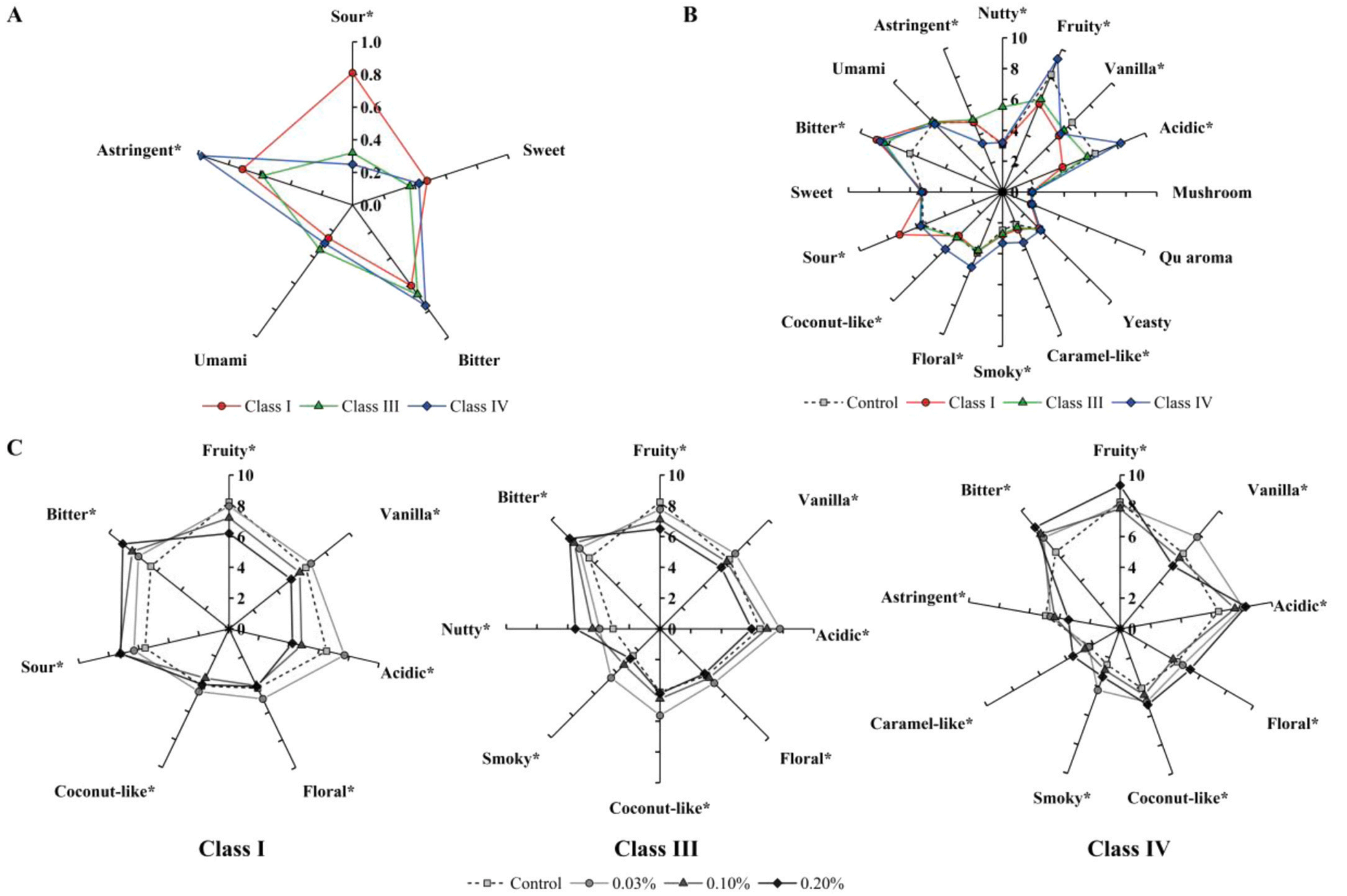

Fig. 3. Sensory attributes of different classes of caramel-colored aqueous solutions (A), and the effect of different classes (B) and amounts (C) of caramel colors on the sensory characteristics of Huangjiu. * Indicates significant differences between different samples ($p < 0.05$).

precipitation of some astringent substances. Among the aroma attributes, all three caramel colors significantly attenuated ($p < 0.05$) the vanilla aroma of Huangjiu. Both Class I and Class III caramel colors significantly attenuated ($p < 0.05$) fruity and acidic aromas, whereas the opposite was true for Class IV caramel colors. Class III caramel colors significantly enhanced ($p < 0.05$) the nutty aroma of Huangjiu, and Class IV caramel colors significantly enhanced ($p < 0.05$) caramel-like, floral, and coconut-like aromas. Caramel colors did not have aroma characteristics in water but altered the aroma of Huangjiu, which implied that caramel colors interacted with the aroma compounds in Huangjiu and affected their release.

Likewise, the addition amounts of caramel colors influenced the sensory characteristics of Huangjiu (Fig. 3C). The bitterness of all three classes of caramel colors significantly increased ($p < 0.05$) with increasing amounts of caramel colors. This may be caused by the bitter substances contained in the caramel color (Li et al., 2021). Meanwhile, the sourness of Class I caramel colors in Huangjiu significantly increased ($p < 0.05$) with the increase in the addition amount, whereas the sourness of Classes III and IV caramel colors did not change significantly ($p \geq 0.05$). The overall aroma of Huangjiu tended to be enhanced and then weakened with increasing amounts of Classes I and III. The overall aroma was stronger with the 0.03% addition and weaker with the 0.20% addition. However, Class IV caramel colors showed a different pattern. With an increase in the addition amount of Class IV caramel colors, the overall aroma of Huangjiu tended to be enhanced. This shows that the effect of caramel color on the aroma of Huangjiu is related to the classes and addition amount, and that there may be complex interactions between caramel color and Huangjiu.

3.4. *Effect of caramel colors addition on volatile organic compounds (VOCs) of Huangjiu*

To further investigate the effects of caramel color on the aroma of Huangjiu, VOCs were analyzed using GC-MS. A total of 49 VOCs were identified in Huangjiu, including 8 alcohols, 15 esters, 8 aldehydes, 2 furans, 5 acids, 2 ketones, 5 phenols, 2 pyrazines, and 2 other compounds. The quantitative results are shown in Table S1. The effects of the three classes of caramel colors on the concentrations of different VOCs in the headspace were different (Fig. 4A). Class I caramel colors significantly increased ($p < 0.05$) the concentrations of aldehydes and furans, Class III caramel colors significantly increased ($p < 0.05$) the concentrations of aldehydes and pyrazines, and Class IV caramel colors significantly increased ($p < 0.05$) the concentrations of compounds other than pyrazines.

ANOVA showed significant changes ($p < 0.05$) in the headspace concentrations of 40 VOCs, including 22 VOCs with OAVs > 1 (Fig. 4B). The three classes of caramel colors showed consistent effects on 13 VOCs, all of which significantly decreased ($p < 0.05$) the concentrations of ethyl 3-phenylpropanoate, 4-vinylguaiacol, ethyl benzoate, and ethyl octanoate, and significantly increased ($p < 0.05$) the concentrations of 2-phenylacetaldehyde, 2,3-butanedione, γ-nonanolactone, octanoic acid, furfural, acetal, acetaldehyde, ethyl 2-hydroxy-4-methylpentanoate, and hexanoic acid. Class I caramel colors significantly increased ($p < 0.05$) the concentration of 5-methylfurfural. Class III caramel colors significantly increased ($p < 0.05$) the concentration of pyrazines in Huangjiu, which could be responsible for its enhanced nutty aroma. Class IV caramel colors significantly increased ($p < 0.05$) the concentrations of phenol, 2-phenylethyl alcohol, 3-methylbutanoic acid, ethyl lactate, ethyl acetate, butanoic acid, 2-phenylethyl acetate, diethyl succinate, 1-butanol, ethyl propanoate, benzyl alcohol, ethyl butanoate, 1-hexanol, 3-methylbutanal, benzaldehyde, ethyl 2-methylpropanoate, and 1-propanol. The results of the OAV analysis are presented in Table S2. The weakening fruity aroma of Huangjius with Classes I and III caramel colors could be related to the lower concentrations of ethyl hexanoate (OAV > 1) and ethyl octanoate (OAV > 1). The enhanced floral and coconut-like aromas of Huangjius with Class IV caramel colors may be related to the increased concentrations of 2-phenylacetaldehyde (OAV > 1), 2-phenylethyl alcohol (OAV > 1), and γ-nonanolactone (OAV > 1). The same proportion of caramel color (0.20% w/v) was added to the Huangjiu-simulated solution and analyzed by GC-MS. However, only two furans and two pyrazines were detected among the 49 VOCs, indicating that the effect of caramel color on the headspace concentrations of most VOCs was due to changes in the release of VOCs.

The PCA, HCA, and KMCA are unsupervised multivariate statistical analysis methods that classify samples based on their similarity in a multidimensional space. Forty significantly affected ($p < 0.05$) VOCs screened using ANOVA were subjected to PCA, HCA, and KMCA. In the PCA score plot, there was a clear separation between the control and sample groups with different caramel color classes, indicating that the addition of caramel colors changed the characteristics of the VOCs in Huangjiu, and there were differences among the different classes of caramel colors (Fig. 4C). HCA further showed that the Huangjiu samples were clustered into three categories: control group, sample group with Classes I and III caramel colors, and sample group with Class IV caramel colors. Simultaneously, the KMCA (K=3) was used to verify the HCA results. The KMCA clustering results were represented by different colors and ellipse ranges in Fig. 4C. It was found that the clustering of KMCA was the same as that of HCA. The above results indicated that there was indeed an interaction between caramel color and VOCs, which changed the concentration of VOCs released into the headspace of Huangjiu. The effects of Classes I and III caramel colors on VOCs in Huangjiu were similar, whereas those of Class IV caramel colors were different.

PLS-DA is a supervised multivariate statistical analysis method used to screen variables that make a prominent contribution to sample classification. A PLS-DA model was developed using the screened 40 VOCs. The K-fold cross-validation (K = 7) showed $R^2X = 0.702$, $R^2Y = 0.965$, and $Q^2 = 0.879$, indicating that the model had good robustness and predictive ability. The intercepts of R^2Y and Q^2 in the permutation test (n = 200) were 0.388 and − 0.499, respectively, which satisfied the recommended values (R^2Y intercept = ~0.3; Q^2 intercept < 0.05), indicating that the PLS-DA model did not overfit (Fig. 5C). The score plot of the PLS-DA model showed that different sample groups were successfully separated (Fig. 5A). A loading plot was used to assess the specific VOCs that contributed to differences between the sample groups (Fig. 5B). Moreover, 5-methylfurfural was closely associated with Class I caramel-colored Huangjius, and two pyrazines were closely associated with Class III caramel-colored Huangjius. These compounds may originate from the caramel color itself.

Furthermore, the value of variable importance in projection (VIP) was calculated, the VOCs that contributed more to distinguishing the sample groups in the PLS-DA model were explored, and the reasons for the changes in the concentrations of key compounds were analyzed according to their content in the caramel colors. The nine compounds with VIP values > 1 were 5-methylfurfural, 2,5-dimethylpyrazine, 2-ethylpyrazine, 2-phenylacetaldehyde, ethyl benzoate, ethyl octanoate, ethyl 3-phenylpropanoate, 2,3-butanedione, and octanoic acid (Fig. 5D). These nine compounds were quantified in the Huangjiu-simulated solutions with the same proportion of caramel colors (Table S3). 5-Methylfurfural was detected in Class I caramel colors, but its content in the caramel color solution only accounted for approximately 1/3 of the increase in the concentration of 5-methylfurfural in Huangjius with Class I caramel colors. This indicated that Class I caramel colors not only provided a small amount of 5-methylfurfural to Huangjiu but also enhanced the release of 5-methylfurfural, which could be used as a marker for the addition of Huangjius with Class I caramel colors. 2,5-Dimethylpyrazine was detected in Classes III and IV caramel color solutions, and its content was higher than the increase in the concentration of 2,5-dimethylpyrazine in the corresponding Huangjiu. This indicated that Classes III and IV caramel colors weakened the release of 2,5-dimethylpyrazine from Huangjiu. 2-Ethylpyrazine was detected in Class III caramel color solutions and its content was approximately equal to the increase in the

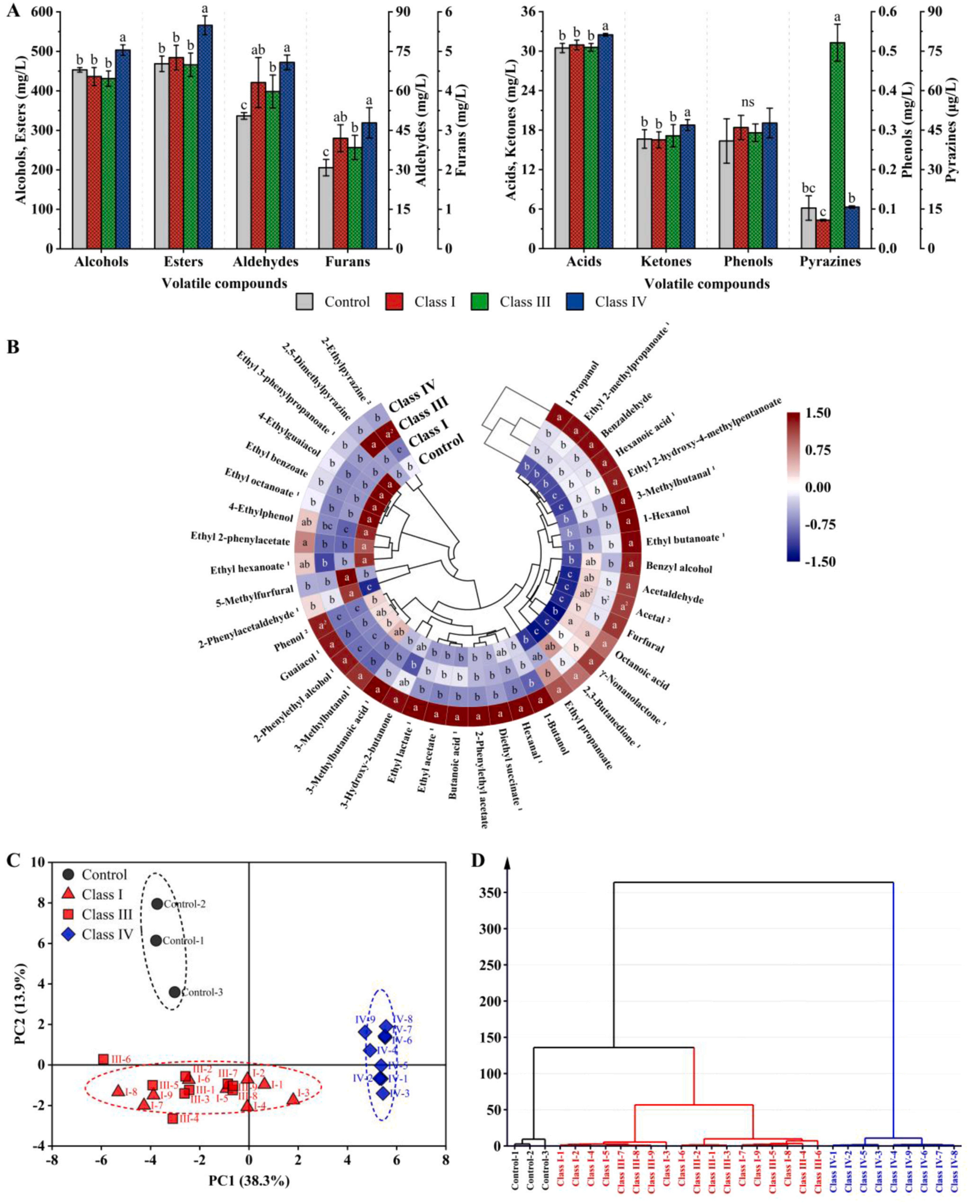

Fig. 4. Effect of different classes of caramel colors on the headspace concentration of different VOCs in Huangjiu (A), and the heat map (B), combination diagram of PCA and KMCA (C), and HCA dendrogram (D) of VOCs with significant differences ($p < 0.05$) in one-way ANOVA. Different a-d letters in (A) in the same VOCs group indicate significant differences ($p < 0.05$), and ns indicates no significant differences ($p \geq 0.05$). Different a-d letters in the same compound group in (B) indicate significant differences, ¹ indicates that the compound had an OAV> 1 in all samples, and ² indicates that the compound had an OAV> 1 only in some specific samples.

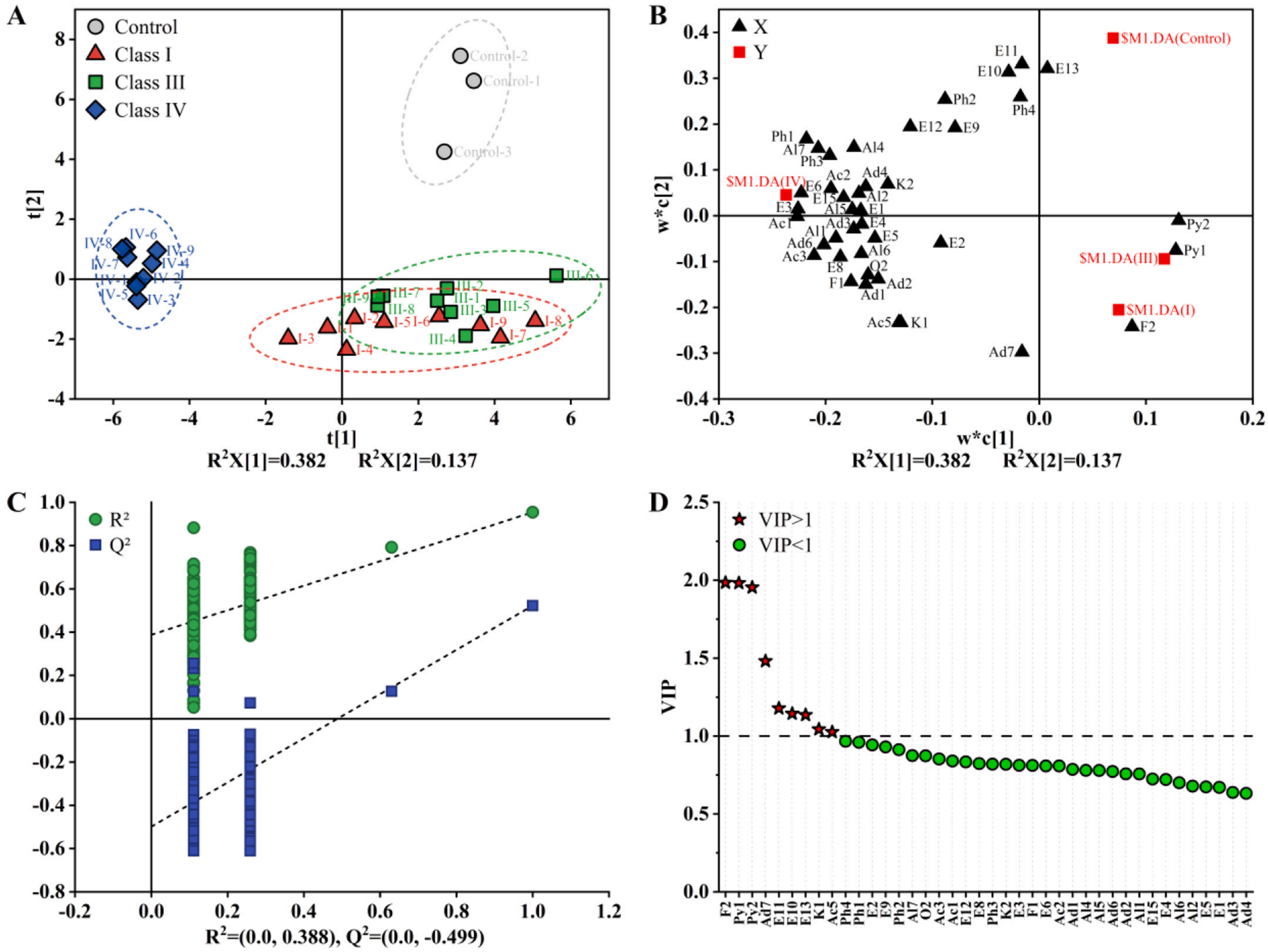

Fig. 5. PLS-DA of VOCs in Huangjius with different classes of caramel colors. (A) PLS-DA score plot. (B) PLS-DA loading plot, with black letters and triangles together indicating VOCs (see Table S1 for coding meanings), and \$M1.DA(Control), (I), (III), and (IV) represent the Huangjiu sample groups for control, Class I, Class III, and Class IV caramel colors, respectively. (C) Permutation test of the PLS-DA model. (D) VIP values of VOCs in the PLS-DA model.

concentration of 2-ethylpyrazine in Huangjius with Class III caramel colors. This indicated that Class III caramel colors had little effect on the release of 2-ethylpyrazine from Huangjiu, and the increase in its concentration was mainly due to the addition of Class III caramel colors. Class I caramel colors did not contain 2-ethylpyrazine, but its addition led to a decrease in the headspace concentration of 2-ethylpyrazine in Huangjiu, indicating that it may weaken the release of 2-ethylpyrazine. The remaining six compounds were not detected in the three classes of caramel colors, indicating that they interacted with the caramel colors, resulting in changes in their release abilities.

A possible mechanism for the interaction of caramel colors with VOCs is that the polar molecules in the caramel colors may interact with water, altering the ratio of free to associated water. This may lead to a decrease in the solubility of polar VOCs and an increase in the gas/liquid partition coefficient, thereby increasing their release (Piccone et al., 2012). This could explain why all three classes of caramel colors enhanced the release of 2,3-butanedione from Huangjiu. Simultaneously, during this process, solute interactions may occur and create an intramolecular hydrophobic environment, thereby reducing the release of nonpolar VOCs (Piccone et al., 2012). In contrast, the addition of high-molecular-weight polymers such as melanoidins in caramel colors may interact with pyrazines through π-π non-covalent bonding (Gigl et al., 2021). This could explain why the caramel colors weakened the release of 2,5-dimethylpyrazine and 2-ethylpyrazine in Huangjiu. High-molecular-weight polymers can also undergo hydrophobic interactions with esters (Andriot et al., 2004), which may lead to a weakened release of esters, such as ethyl octanoate, ethyl benzoate, and ethyl 3-phenylpropionate. The transmittance of Huangjiu was considerably reduced after the addition of Class IV caramel colors. The appearance of turbid substances could have an effect similar to that of salting on the VOCs of Huangjiu, resulting in an enhanced release of a large number of VOCs. However, there are limited reports on the composition and structure of caramel colors, making it unfeasible to establish a specific interaction mechanism.

The effect of caramel color addition amounts on the headspace concentrations of different types of VOCs in Huangjiu is shown in Fig. S1. With an increase in the amount, the addition of the three classes of caramel colors had similar effects on acids and phenols, whereas the effects on aldehydes and furans were different. Alcohols and esters first increased and then decreased with the addition of Classes I and III caramel colors, whereas Class IV caramel colors continuously increased. With an increase in Class III caramel colors, the headspace concentration of pyrazines in Huangjiu gradually increased, which confirmed the conclusion that pyrazines were introduced by the addition of Class III caramel colors. With the addition of Classes I and III caramel colors, the release of total VOCs in Huangjiu first increased and then decreased. This two classes of caramel colors promoted aroma release at low concentrations and suppressed it at high concentrations (Paravisini et al., 2017). In contrast, with the addition of Class IV caramel colors, the release of total VOCs in Huangjiu showed an enhanced trend (Fig. S2A). This was similar to the pattern obtained by the sensory evaluation, suggesting that changes in compound release could have been the main factor affecting the aroma of Huangjiu. The ANOVA results showed that the releasability of 30, 36, and 39 VOCs in Huangjiu changed significantly ($p < 0.05$) with the addition of Classes I, III, and IV caramel colors, respectively (Fig. S2B–D). More than half of the VOCs with OAVs > 1 may have contributed to the aroma changes in Huangjiu. In terms of the overall aroma and VOCs release results, Classes I and III caramel colors were more suitable for use in Huangjiu at low added amounts (0.03%), whereas Class IV was more suitable for higher amounts (0.20%).

4. Conclusion

The three classes of caramel colors significantly altered the quality of Huangjiu to varying degrees. The three classes of caramel colors interacted with Huangjiu to produce varying degrees of turbidity owing to their different net charges. The coloring ability of caramel colors in Huangjiu is not only related to its own color intensity, but also to its degree of turbidity in Huangjiu, as the pigmented material precipitates. Caramel colors had a more pronounced effect on the flavor quality of Huangjiu than on its physicochemical properties. Caramel colors affected the aroma characteristics of Huangjiu by altering the release of VOCs, rather than by contributing its own aroma. The change in the release of VOCs was mainly due to the interaction between caramel colors and VOCs. However, the composition of caramel colors is currently unclear, and the specific interaction mechanism still needs to be further investigated.

CRediT authorship contribution statement

Zhilei Zhou: Project administration, Funding acquisition, Conceptualization, Methodology, Resources, Data curation, Visualization, Writing – review & editing. **Shaopu Liu**: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft. **Yingzhu**: Investigation, Data curation, Writing – review & editing. **Shuangping Liu**: Resources, Software, Methodology. **Zhongwei Ji**: Validation, Investigation. **Qingxi Ren**: Methodology, Writing – review & editing. **Xingxiang Pan**: Supervision, Resources. **Jian Mao**: Funding acquisition, Supervision, Resources, Writing – review & editing.

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

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105932.

References

Andriot, I., Le Quéré, J.-L., Guichard, E., 2004. Interactions between coffee melanoidins and flavour compounds: Impact of freeze-drying (method and time) and roasting degree of coffee on melanoidins retention capacity. Food Chem 85 (2), 289–294. https://doi.org/10.1016/j.foodchem.2003.07.007.

Chen, H.X., Gu, Z.B., 2014. Effect of ascorbic acid on the properties of ammonia caramel colorant additives and acrylamide formation. J. Food Sci. 79 (9), C1678–C1682. https://doi.org/10.1111/1750-3841.12560.

Chen, S., Xu, Y., Qian, M.C., 2013. Aroma characterization of Chinese rice wine by gas chromatography–olfactometry, chemical quantitative analysis, and aroma reconstitution. J. Agric. Food Chem. 61 (47), 11295–11302. https://doi.org/10.1021/jf4030536.

Chen, S., Wang, C.C., Qian, M., Li, Z., Xu, Y., 2019. Characterization of the key aroma compounds in aged Chinese rice wine by comparative aroma extract dilution analysis, quantitative measurements, aroma recombination, and omission studies. J. Agric. Food Chem. 67 (17), 4876–4884. https://doi.org/10.1021/acs.jafc.9b01420.

Flavornet and Human Odor Space (2004). Odorants. Retrieved October 18, 2022 from: 〈http://www.flavornet.org/flavornet.html〉.

Gigl, M., Hofmann, T., Frank, O., 2021. NMR-based studies on odorant–melanoidin interactions in coffee beverages. J. Agric. Food Chem. 69 (50), 15334–15344. https://doi.org/10.1021/acs.jafc.1c06163.

Gigl, M., Frank, O., Gabler, A., Koch, T., Briesen, H., Hofmann, T., 2022. Key odorant melanoidin interactions in aroma staling of coffee beverages. Food Chem 392, 133291. https://doi.org/10.1016/j.foodchem.2022.133291.

Gu, Z.X., Jin, Z., Schwarz, P., Rao, J.J., Chen, B.C., 2023. Unraveling the role of germination days on the aroma variations of roasted barley malts via gas chromatography-mass spectrometry based untargeted and targeted flavoromics. Food Chem 426, 136563. https://doi.org/10.1016/j.foodchem.2023.136563.

Han, G.M., Dai, L.M., Sun, Y.H., Li, C., Ruan, S.L., Li, J.M., Xu, Y., 2021. Determination of the age of dry red wine by multivariate techniques using color parameters and pigments. Food Control 129, 108253. https://doi.org/10.1016/j.foodcont.2021.108253.

Hofmann, T., Czerny, M., Calligaris, S., Schieberle, P., 2001. Model studies on the influence of coffee melanoidins on flavor volatiles of coffee beverages. J. Agric. Food Chem. 49 (5), 2382–2386. https://doi.org/10.1021/jf0012042.

Hou, C.Y., Hou, Z.T., Lin, C.M., Shih, M.K., Chen, Y.W., Lai, Y.H., 2020. Adding α-pinene as a novel application for sulfur dioxide-free in red wine. Int. J. Food Prop. 23 (1), 167–177. https://doi.org/10.1080/10942912.2020.1716798.

Kreutzmann, S., Thybo, A.K., Bredie, W.L.P., 2007. Training of a sensory panel and profiling of winter hardy and coloured carrot genotypes. Food Qual. Prefer. 18 (3), 482–489. https://doi.org/10.1016/j.foodqual.2006.05.009.

Li, H., Zhang, W.C., Tang, X.Y., Wu, C.J., Yu, S.J., Zhao, Z.Q., 2021. Identification of bitter-taste compounds in class-III caramel colours. Flavour Fragr. J. 36 (3), 404–411. https://doi.org/10.1002/ffj.3652.

Liang, J., Cao, P., Wang, X.D., Gao, P., Xu, H.B., Ma, N., 2019. Dietary intake assessment of caramel colours and their processing by-products 4-methylimidazole and 2-acetyl-4-tetrahydroxy-butylimidazole for the Chinese population. Food Addit. Contam.: Part A 36 (7), 1009–1019. https://doi.org/10.1080/19440049.2019.1615137.

Liu, S.P., Chen, Q.L., Zou, H.J., Yu, Y.J., Zhou, Z.L., Mao, J., Zhang, S., 2019. A metagenomic analysis of the relationship between microorganisms and flavor development in Shaoxing mechanized huangjiu fermentation mashes. Int. J. Food Microbiol. 303, 9–18. https://doi.org/10.1016/j.ijfoodmicro.2019.05.001.

Liu, S.P., Ma, D.L., Li, Z.H., Sun, H.L., Mao, J.Q., Shi, Y., Han, X., Zhou, Z.L., Mao, J., 2021. Assimilable nitrogen reduces the higher alcohols content of huangjiu. Food Control 121, 107660. https://doi.org/10.1016/j.foodcont.2020.107660.

Liu, S.P., Sun, H.L., Liu, C.X., Zhou, Z.L., Mao, J.Q., Hu, Z.M., Xu, X.B., Han, X., Zhang, S. J., Mao, J., 2021. Reducing biogenic amine in seriflux and huangjiu by recycling of seriflux inoculated with Lactobacillus plantarum JN01. Food Research International 150, 110793. https://doi.org/10.1016/j.foodres.2021.110793.

Liu, Y.C., Zhou, Z.L., Mao, J., 2020. Effect of caramel color on the stability of rice wine. Farm Prod. Process. 22, 21–28. https://doi.org/10.16693/j.cnki.1671-9646(X).2020.11.047.

Myers, D.V., Howell, J.C., 1992. Characterization and specifications of caramel colours: an overview. Food Chem. Toxicol. 30 (5), 359–363. https://doi.org/10.1016/0278-6915(92)90061-O.

Ortega-Heras, M., González-Sanjosé, M.L., 2009. Binding capacity of brown pigments present in special Spanish sweet wines. LWT - Food Sci. Technol. 42 (10), 1729–1737. https://doi.org/10.1016/j.lwt.2009.04.001.

Paravisini, L., Moretton, C., Gouttefangeas, C., Nigay, H., Dacremont, C., Guichard, E., 2017. Caramel flavour perception: impact of the non-volatile compounds on sensory properties and in-vitro aroma release. Food Res. Int. 100, 209–215. https://doi.org/10.1016/j.foodres.2017.07.003.

Piccone, P., Lonzarich, V., Navarini, L., Fusella, G., Pittia, P., 2012. Effect of sugars on liquid–vapour partition of volatile compounds in ready-to-drink coffee beverages. J. Mass Spectrom. 47 (9), 1120–1131. https://doi.org/10.1002/jms.3073.

Scotter, M.J., 2011. Methods for the determination of European Union-permitted added natural colours in foods: a review. Food Addit. Contam.: Part A 28 (5), 527–596. https://doi.org/10.1080/19440049.2011.555844.

Sengar, G., Sharma, H.K., 2014. Food caramels: a review. J. Food Sci. Technol. 51 (9), 1686–1696. https://doi.org/10.1007/s13197-012-0633-z.

Shih, M.K., Lai, Y.H., Lin, C.M., Chen, Y.W., Hou, Z.T., Hou, C.Y., 2020. A novel application of terpene compound α-pinene for alternative use of sulfur dioxide-free white wine. Int. J. Food Prop. 23 (1), 520–532. https://doi.org/10.1080/10942912.2020.1742735.

Su, L.T., Mao, J., Zhou, Z.L., 2017. Precipitation analysis and control of ginger seasoning wine. J. Food Sci. Biotechnol. 36 (10), 1054–1058. https://doi.org/10.3969/j.issn.1673-1689.2017.10.008.

van Gemert, L.J., 2011. Odour Thresholds: Compilations of Odour Threshold Values in Air, Water and Other Media (Edition 2011). Oliemans Punter & Partners BV, Utrecht, the Netherlands.

Vollmuth, T.A., 2018. Caramel color safety – an update. Food Chem. Toxicol. 111, 578–596. https://doi.org/10.1016/j.fct.2017.12.004.

Wang, J., Zhang, B., Wu, Q., Jiang, X.Y., Liu, H.J., Wang, C.Z., Huang, M.Q., Wu, J.H., Zhang, J.L., Yu, Y.G., 2022. Sensomics-assisted flavor decoding of coarse cereal Huangjiu. Food Chem 381, 132296. https://doi.org/10.1016/j.foodchem.2022.132296.

Wang, J.G., 2007. Changes of color, flavor and taste of rice wine during the storage. China Brewing 10, 48–52. https://doi.org/10.3969/j.issn.0254-5071.2007.10.014.

Wang, J.G., Wang, Q., 2006. Characteristics of caramel color and its application in Chinese rice wine. China Brew 11, 62–64. https://doi.org/10.3969/j.issn.0254-5071.2006.11.019.

Wang, N., Zhang, L.L., Ren, X.J., Chen, S., Zhang, Z., 2023. Metabolomic fingerprinting based on network analysis of volatile aroma compounds during the forced aging of Huangjiu: Effects of dissolved oxygen and temperature. Frontiers in Nutrition 10, 1114880. https://doi.org/10.3389/fnut.2023.1114880.

Zhang, Z.W., Li, J.Y., Fan, L.P., 2019. Evaluation of the composition of Chinese bayberry wine and its effects on the color changes during storage. Food Chem 276, 451–457. https://doi.org/10.1016/j.foodchem.2018.10.054.

Zhang, Z.W., Yu, Q., Li, J.W., Fan, L.P., 2020. Effect of package oxygen on color, color-related compounds, and volatile composition of Chinese bayberry wine after bottling. LWT 128, 109430. https://doi.org/10.1016/j.lwt.2020.109430.

Zhao, Y.Z., Liu, S.P., Yang, Q.L., Han, X., Zhou, Z.L., Mao, J., 2022. Saccharomyces cerevisiae strains with low-yield higher alcohols and high-yield acetate esters improve the quality, drinking comfort and safety of Huangjiu. Food Res. Int. 161, 111763. https://doi.org/10.1016/j.foodres.2022.111763.