Elucidating oxidation-based flavour formation mechanism in the aging process of Chinese distilled spirits by electrochemistry and UPLC-Q-Orbitrap-MS/MS

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A B S T R A C T

The oxygen-based oxidation process is leading toward the understanding of the flavour formation mechanism of aged Baijiu. However, the oxygen-based oxidation process in Baijiu aging is difficult to explore because of (1) the composition of Baijiu produced in different batches varies a lot and (2) the spontaneous oxidation needs several years or even decades to occur. Hence, the flavour formation mechanism of Baijiu aging was investigated using electrochemistry and UPLC-Q-Orbitrap-MS/MS, which enabled us to solve the two difficulties above. The oxidation reaction on gold surface could accelerate Baijiu aging by catalyzing the transformation from alcohols to acids. Although natural aging contributed more diverse compound changes to Baijiu, the oxygen-based oxidation process during natural aging was similar to that in the electrochemical oxidation as a whole. This study provides new insights to flavour formation mechanism of aged liquors and provides a new way for liquor-related enterprises to optimise the aging process.

1. Introduction

According to the group standard issued by the China Alcoholic Drinks Association, aged Baijiu (also known as Chinese distilled spirits, or Chinese liquor) is brewed by the traditional production technology (solid-state fermentation) and 'aged' once it reaches the age of 3 years or more (He, Yangming, Górska-Horczyczak, Wierzbicka, & Jeleń, 2021; Jia et al., 2021; Liu & Sun, 2018). The main components of liquors include water and alcohol, accounting for 98%~99% of the total volume (Bendig, Lehnert, & Vetter, 2014; Kew, Goodall, Clarke, & Uhrín, 2017; Zhao et al., 2018). Moreover, various organic compounds, including phenols, aldehydes, carboxylic acids, and esters, are the key ingredients of aged Baijiu and play a vital role in Baijiu flavour (Song, Zhu, et al., 2020; Zheng, Sun et al., 2016). After the aging period, the liquor becomes aged Baijiu with a unique flavour (Song, Jing, et al., 2020; Zhu et al., 2020). However, due to the remarkable complexity of liquor composition, the understanding of the flavour formation mechanism of aged Baijiu is still superficial and will need to step up the pace to reach the molecular level (Jia et al., 2021; Jiang, Xie, Wan, Chen, & Zheng, 2019). Since the brewing technology of Chinese Baijiu was established, the major purpose of aging is considered to improve the stability of

liquor flavour, which is achieved mainly through (1) the volatilisation of substances with a lower boiling point than ethanol and (2) the spontaneous transformation of chemically active substances into chemically inert substances (Liu and Sun, 2018; What's that stuff? Baijiu, 2018). Because of the extremely slow reaction rate of the chemical reactions, such as esterification, and their imbalance in freshly produced liquor (named as *Xinjiu*), long-term aging is necessary to increase the content of flavour compounds in Baijiu (Li, Fan, & Xu, 2021). During natural aging, high-quality pottery jars with micropores around are used to ensure gas exchange between Baijiu and air (Fang, Du, Jia, & Xu, 2019; Jin, Zhu, & Xu, 2017). Thus the understanding of the spontaneous maturation of Baijiu in modern chemistry is centred on the oxidation process with alcohols, phenols and aldehydes as substrates (Wei, Li, He, Yu, Tian, & Wu, 2020; Xiong et al., 2020). Oxygen is considered the initial oxide, which is key to the formation of flavour compounds (del Alamo-Sanza, Pando, & Nevares, 2014). Therefore, the oxygen-based oxidation process is highly correlated with the flavour formation mechanism of aged Baijiu.

However, the oxygen-based oxidation process in Baijiu aging is difficult to explore because of two reasons. Firstly, the composition of *Xinjiu* produced in different batches varies a lot, which has a significant

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influence on the investigation of material changes during oxidation (Jia, Fan, et al., 2020). Fan et al. discussed the differences in flavour components between *Xinjiu* and aged liquors (Fan & Qian, 2005). They analyzed the flavour dilution (FD) value, which is the ratio of the concentration of the odorant in the initial extract to its concentration in the most dilute extract in which the odour is still detectable (Grosch, 2001), and determined that the aroma compounds in the aged liquors had higher FD values than that in *Xinjiu*. Zhang's team (Ma et al., 2014) investigated the flavour component transformation of Fen-flavour liquor (Fen is an 'aroma' as well as the name of a producer, which is a light aroma liquor and characterised by the mixed flavour of grain, sweet, and vinegar) (Qiao, Zhang, Wang, & Ma, 2012). They confirmed the physicochemical property change of aged Fen-flavour liquor, which resulted from the synergistic effect of volatilisation of low boiling-point substances, oxidation of dissolved oxygen (DO), esterification, and hydrolysis. Jia's team (Jia, Li, et al., 2020) investigated the aging process of liquors using foodomics and discovered 15 organic acids, 8 esters, and some carbonyl compounds, which were differential compounds of typical aged liquors. Many research results have confirmed the componential differences of liquors from different aging times (Chen, Wang, Qian, Li, & Xu, 2019). However, as the semi-mechanized processes are widely used in Baijiu production, the differences between processing batches may be the main source of the substance differences in liquors, even though the raw material looks the same in every batch (Liu and Sun, 2018; Zhu et al., 2020). There are even contradictory reports on the quantitative analysis of volatile chemicals (Zhu et al., 2020). Secondly, the aging process has a low reaction rate and spontaneous oxidation needs several years or even decades to occur (Wang et al., 2021). To the best of our knowledge, the analyzing method as aging and maturation indicators is still insufficient in the distilled spirits industry.

Therefore, the flavour formation mechanism of liquors during aging was investigated using electrochemistry in this study. The characteristics of this method enable it to solve the two difficulties previously mentioned. Firstly, electrochemical oxidation was continuously conducted in situ to explore the composition differences of the same batch of liquors at different extents of reaction, which circumvented the uncertainty of the research results caused by the composition differences of different batches of *Xinjiu* and ensured the reliability of the research results (Yang et al., 2020; Zhao et al., 2019). Secondly, electrochemical oxidation was accelerated and the oxidation reaction could be finished within dozens of minutes (Fang et al., 2014), which ensured the feasibility of the research method. The results confirmed the catalytic effect of oxygen in the aging of liquors. Importantly, this study provides a new idea to understand the flavour formation mechanism of aged liquors and provides a new way for liquor-related enterprises to optimise the aging process.

2. Experimental section

2.1. Materials

The freshly produced Baijiu (named as *Xinjiu*) and 5-year aged Baijiu were collected from the manufacturer Xiangjiao Group Ltd., Shaoyang, China. All the Baijiu samples studied in this research were directly collected from storage containers without any additive. Mention of brand or firm names does not mean the research contact with the manufacturer, nor is the mention for advertising. KCl and agarose were purchased from Aladdin (Shanghai, China), used without further purification. Electrolyte solutions were freshly prepared by ultra-pure water with a resistivity of 18.2 MΩ·cm^{-1}.

2.2. Electrochemical oxidation

Add Baijiu (3 mL) to the first beaker and label this beaker "working beaker". Add 0.1 mol L^{-1} KCl solution (3 mL) to the next beaker and label this beaker "counter beaker". A gold electrode with a working area of 2.0 cm^2 was employed as the working electrode, an Ag/AgCl electrode as the reference electrode. The working electrode and reference electrode were inserted into the "working beaker". A platinum wire was employed as counter electrode. The counter electrode was inserted into the "counter beaker". A salt bridge was used to connect the two beakers thus the three-electrode system was connected. The electrochemical workstation (CHI instrument, Austin, TX, USA) adopted chronoamperometry with the constant voltages (0.20 V, 0.51 V, and 0.78 V) to record current–time curve (I-T curve).

2.3. UPLC-MS analysis

Sample pretreatment method: The sample in the volume of 1 mL was extracted by methanol–acetonitrile solution (1:1, V/V) in the same volume, under vortex movement for 60 s and ultrasonic oscillation for 60 min. The solution was placed at −20 °C for 1 h, then centrifuged at 14,000 RCF (reactive centrifugal force) and 4 °C for 20 min, and the supernatant was used for chromatographic analysis. Chromatographic analysis was performed by an ultra-high performance liquid chromatography (Thermo Scientific Vanquish UPLC system, Massachusetts, USA), using a Waters HSS T3 (100 mm × 2.1 mm, 1.9 um) LC column. The binary mobile phases consisted of solution A (0.1% formic acid–water solution) and solution B (0.1% formic acid-acetonitrile-isopropanol solution). The total flow rate was set at 0.3 mL min^{-1} and the liner gradient program was as follows: 0–6 min, 90% B; 6–15 min, 40% B; 15–17 min, 90% B, with an injection volume of 2 µL and a column temperature of 40 °C.

The mass spectrometry system adopts a Thermo Scientific Q Exactive hybrid quadrupole-Orbitrap mass spectrometer, equipped with electrospray (ESI) ion source and Xcalibur software. The spray voltage was 3.0 kV. The capillary temperature was 350 °C, and the auxiliary gas heating temperature was 320 °C. Raw data files acquired from UPLC-Q Exactive Orbitrap analysis were collected by Xcalibur software, and then imported into Progenesis QI (Waters Corporation, Milford, USA) for processing metabolic profiling data. The resulting data was imported to SIMCA 13.0 for untargeted analysis (PCA), targeted analysis (PLS-DA), and VIP variable analysis.

2.4. GC analysis

The constituent analysis of Baijiu samples was performed by Agilent 7890B gas chromatography (Agilent Technologies Co. Ltd.). GC separation was achieved on an Agilent CP-Wax 57 CB capillary column (0.25 mm × 50 m × 0.2 µm) with a flow rate of 1.0 mL/min. 4.9 mL Baijiu sample was mixed with 0.1 mL internal standard (2-ethylbutyric acid and *n*-pentyl acetate). The initial oven temperature was maintained at 40 °C and held for 5 min, raised to 50 °C at 3 °C min^{-1} and held for 6.5 min, then to 90 °C at 6 °C min^{-1} and held for 5 min, then to 130 °C at 10 °C min^{-1} and held for 2 min, then to 190 °C at 5 °C/min and maintained for 1.4 min, finally raised to 195 °C at 10 °C/min and maintained for 20 min. The qualitative identification was performed by comparing the retention times between the targeted compounds and the reference standards.

2.5. Total dissolved solids and dissolved oxygen analysis

Total dissolved solids (TDS) was measured by a Xiaomi TDS meter (Xiaomi Technology Co., Ltd, Beijing, China). Dissolved oxygen (DO) was measured by a DO meter (AZ-Instrument, Beijing, China).

2.6. Statistical analysis and data treatment

The data are presented as the mean value of triplicate measurements (n = 3), error bars indicate standard deviations (±S.E.M), and the asterisk indicates the P value. *P < 0.05 and **P < 0.01 compared with the control, as estimated by one simple *t* test. Statistical analysis was

performed by Origin 2020 (OriginLab Co., Northampton, USA).

3. Results and discussion

3.1. Electrochemical system for Baijiu oxidation

As shown in the diagram (Fig. 1a), a conventional three-electrode electrochemical system was used in this study. To avoid the interference of the products on the counter electrode to the products on the working electrode, the working and counter electrodes were placed in different electrolytic cells and connected by a salt bridge (Engel, Holtmann, Ulber, & Tippkötter, 2019). The reference and counter electrodes were placed in the same cell. The redox properties of the liquor samples in a wide electrochemical voltage range were investigated using cyclic voltammetry (CV). To eliminate the interference of electromigration on redox current, 0.1 M KCl was added to the liquor samples. As shown in Fig. 1b, the CV curve scanned by the gold electrode in potassium chloride solution shows two oxidation peaks with peak potentials of 0.58 and 0.81 V. Similar to typical CV with a gold electrode, the two oxidation peaks indicated the adsorption of oxygen to two different crystal surfaces (Zheng, Yang et al., 2016). After adding the liquor samples, the CV curve displays two oxidation peaks produced by oxygen adsorption, and the peak potentials of these oxidation peaks decrease to 0.51 and 0.78 V. It has been reported that the mechanism for the oxygen adsorption on gold was shown to proceed via a three-step reaction sequence (Bruckenstein & Shay, 1985), as shown in the insert in Fig. 1b. No new oxidation or reduction peak was detected in the CV curve of the liquor samples. However, the CV peak potential of the oxygen adsorption or desorption on gold is related to the molecular interaction between oxygen and foreign material in the electrolyte solution (Masuda, Ikeda, & Uosaki, 2013). The peak potential of the oxygen adsorption peak decreased, indicating the interactions between compounds contained in liquors and active oxygen on the gold surface.

3.2. Effect of electrochemical oxidation on the mutual transformation of compounds in Baijiu

On the basis of the peak potentials of oxygen adsorption, two voltages (i.e. 0.51 and 0.78 V) were selected for the electrochemical oxidation of the liquor samples. Moreover, a lower potential (i.e. 0.20 V) was selected as the control. These three voltages were set, and the current–time (I-T) curve was tested with the original liquor samples as electrolytes. To avoid the material interference caused by additional electrolyte, no electrolyte was added in the I-T curve test. The liquor samples contained some ionisable organic acids, which ensured the smooth current loop during electrolysis. As shown in Fig. 2a, the I-T curve exhibits an 'L' shape, indicating that the charging process of the electric double layer between the gold electrode and the electrolyte was completed instantaneously after the circuit was connected and presented a relatively stable change trend of current. The dissolved oxygen (DO) concentration in the electrolyte reflects the consumption rate of active oxygen. Thus, three different voltages (i.e. 0.20, 0.51, and 0.78 V) were set. And DO concentrations in the electrolyte were determined after the electrochemical oxidation of liquors in the same time, 20 min. As shown in Fig. 2b (blank boxes), compared with that of the original liquor samples (*Xinjiu*), the DO concentrations of the electrochemically treated liquor samples all decreased significantly under the three voltages. The higher the voltage was, the more obvious the decrease trend of the DO concentration. Under the same voltage (0.78 V), the longer the oxidation time was (20, 60, and 120 min), the greater the reduction of the DO concentration. DO concentration in saturated water at room temperature and 1 atm pressure is ~7.0 ppm (Saravia et al., 2016). As seen, DO concentration in original liquor sample is 6.28 ppm, indicating that the presence of dissolved alcohols, organics, and esters can alter the saturation value of DO. At 0.78 V, the consumption rate of DO was calculated as 3.85×10^{-2} ppm min^{-1}, the DO concentration was reduced from 6.28 to 5.51 ppm in 20 min. The oxidation–esterification equilibrium in liquors (Deng et al., 2020), where the first step is the oxidation of alcohols, which is a rate control step, and the second step is the formation of corresponding esters from acids and ethanol, the extent of which is related to the equilibrium constant. When the active oxygen in the electrochemical oxidation process catalyses the transformation of alcohols, the content of corresponding esters increases. Different compounds contained in the liquor samples have different solubility. Generally, the solubility of esters is lower than that of corresponding acids; hence, the total dissolved solids (TDS) of liquors will increase. Therefore, the TDS of the electrolyte (liquor) is an important indicator of the oxidation ability of active oxygen after electrochemical oxidation. As shown in Fig. 2b (filled boxes), compared with that of the original liquor samples, the TDS of the electrochemically treated liquor samples all increased significantly under the three voltages. The higher the voltage was, the more obvious the increase trend of the TDS. Under the same voltage, the longer the oxidation time was, the greater the increase of the TDS. The formation rate of soluble solids was calculated as 0.26 ppm min^{-1} at 0.78 V. The results demonstrate a negative correlation between the variation trend of DO and TDS. As seen, both DO and TDS showed significant changes and reflected a negative correlation, which confirmed the catalytic effect of electrochemical oxidation on the mutual transformation of compounds in liquors.
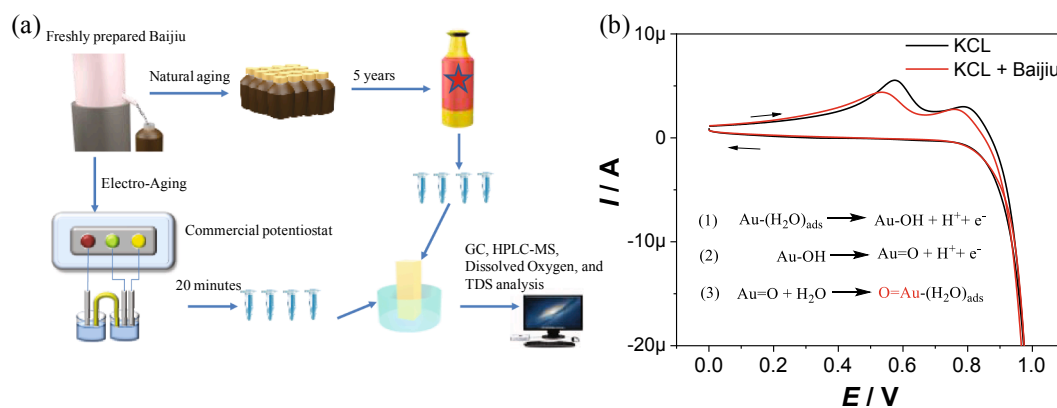

Fig. 1. (a) Schematic representation of the three-electrode electrochemical system used in this study. (b) CV curves scanned by the gold electrode in 0.1 M KCL (in black) and in 0.1 M KCL containing 10 mM Baijiu (in red), the insert shows the mechanism for the oxygen adsorption on gold. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
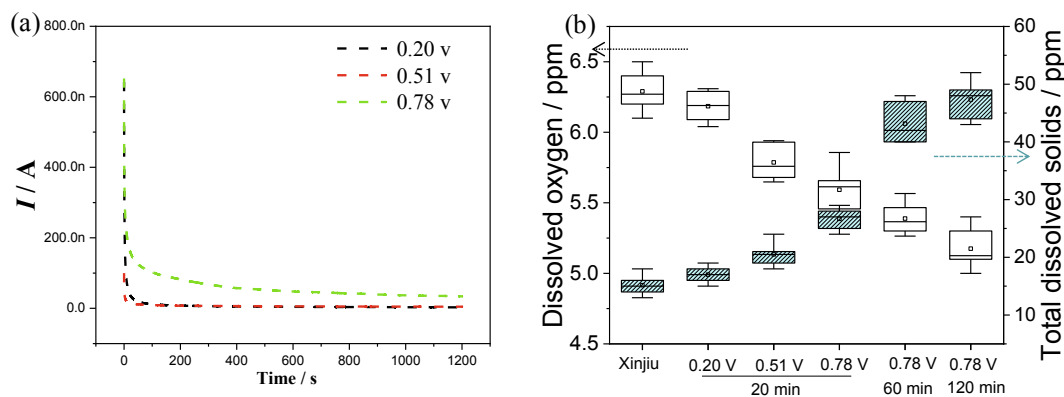
Fig. 2. (a) Chronoamperometry (I-T curve) of the gold electrode in liquors at different voltages (0.20 V, 0.51 V, and 0.78 V). (b) DO (blank boxes) and TDS (filled boxes) concentrations plotted vs. different liquors (*Xinjiu*, 0.20 V-20 min, 0.51 V-20 min, 0.78 V-20 min, 0.78 V-60 min, and 0.78 V-120 min). The data are presented as the mean value of triplicate measurements (n = 3).

3.3. Comparison between the electrochemical oxidation and natural aging

The inter-group and intra-group variabilities reflected by the original UPLC-Q-Orbitrap-MS/MS data of the samples were analysed using principal component analysis (PCA). After dimensionality reduction analysis, the relative coordinate points for each principal component were determined and the PCA score chart was obtained. The distance of each coordinate point represents the degree of aggregation and dispersion between samples. Generally, a short distance indicates a high similarity between samples, whereas a long distance indicates a significant difference between samples. To compare the similarities and differences between natural aging and electrochemical oxidation, liquor naturally aged for 5 years in a pottery jar was selected as the control group. As shown in Fig. 3a, naturally aged and electrochemically oxidized liquors were different from *Xinjiu*. Moreover, the higher the oxidation voltage was, the greater the difference between electrochemically oxidized liquor and *Xinjiu*. Under the same voltage, the longer the oxidation time was, the greater the difference between electrochemically oxidized liquor and *Xinjiu*.

PCA is an unsupervised analysis method. On the basis of the results of PCA, the supervised analysis method partial least squares discriminant analysis (PLS-DA) was used to further analyse the inter-group and intragroup variabilities of the liquor samples. As shown in Fig. 3b, the PLS-DA score chart also indicated obvious differences between naturally aged and electrochemically oxidized liquors and *Xinjiu*. Moreover, permutation testing was used to evaluate the reliability of the PLS-DA model. Generally, a low intercept value of Q_2 (i.e. < 0) indicates good model fitting. In the permutation testing chart, shown in Fig. 3c, the intercept value of Q_2 was -0.533, which proved the reliability of the fitting effect of the PLS-DA model. Then, the influence of electrochemical oxidation on compound changes during liquor aging was evaluated by variable importance (VIP) analysis. The experimental group was subjected to oxidation at 0.78 V for 20 min. Meanwhile, *Xinjiu* was selected as the control group. The compounds with VIP > 1 in the PLS-DA model were the markers with significant differences. Student's t test combined with multivariate analysis was used to screen the different substances between the groups (VIP > 1, $P < 0.05$). As shown in Fig. 3d, after electrochemical oxidation, 24 marker compounds (VIP values are shown in Tab. S1) exhibited significant differences, which further confirmed the catalytic effect of electrochemical oxidation on the mutual transformation of liquor compounds. Three of the marker compounds are the important flavour compounds, including nonanoic acid, phenethylamine, and isobutyric acid (Liu and Sun, 2018).

The change in the concentration of marker compounds not only indicates the interconversion of these substances, but also indicates the cluster similarity and evolutionary relationships between liquors from different groups. Therefore, a phylogenetic tree was obtained based on

the constituent analysis of the 24 marker compounds. As shown in Fig. 4, cluster analysis of the heat map shows that the liquors can be divided into 3 groups: *Xinjiu*, 0.20 V-20 min (cluster A); 5 year, 0.78 V-20 min (cluster B); 0.51 V-20 min, 0.78 V-120 min (cluster C). The results confirmed that the samples obtained under low-potential treatment are similar to *Xinjiu* (cluster A, 0.20 V-20 min) and the samples obtained under high-potential treatment have high similarity with the samples naturally aged for 5 years (cluster C, 0.78 V-20 min). On the one hand, a higher voltage can accelerate the rate of interfacial electron transfer, thus accelerating the rate of oxidation reaction. On the other hand, partial oxidation reactions have higher energy barrier and can only occur at higher voltages. The above results also imply the diversity of oxidizable substances in liquor samples. In the natural aging process, the reaction rate is very slow due to the fact that the natural aging process relies only on dissolved oxygen.

VIP analysis between naturally aged Baijiu (5 years) and *Xinjiu* was conducted. As shown in Fig. S1 in supplementary information, after natural aging for 5 years, 40 marker compounds (VIP values are shown in Tab. S2) exhibited significant differences (VIP > 1, $P < 0.05$). Eight of the marker compounds are the important flavour compounds, including ethyl vanillate, butyl butyrate, ethyl lactate, 2-phenylethyl acetate, benzyl alcohol, 2-butanol, octanoic acid, and heptanoic acid (Liu and Sun, 2018). The similarities between natural aging and electrochemical oxidation were compared. As shown in Table 1, the comparison of these marker compounds showed that 16 compounds were common differential substances after the samples were subjected to the two treatment methods (naturally aging and electrochemical oxidation). That is, the material changes in liquors caused by electrochemical oxidation and natural aging were similar overall. The results indicated that, although natural aging presented more diverse material changes, the oxygen-based oxidation process during natural aging was similar to that in the electrochemical oxidation as a whole. This result confirmed the importance of oxygen in promoting the flavour formation mechanism of aged Baijiu.

3.4. Elucidating the oxidation-based flavour formation mechanism by electrochemical oxidation and natural aging

3 alcohols, 3 acids, and 3 esters were selected as the indicators for quantitative change and these compounds were selected for these reasons: (1) the compounds that contributed to the main aroma and flavour of the liquor, and (2) the compounds related to the safety indicators according to the national standards of Chinese liquor. The content of the alcohols (methyl alcohol, *n*-butyl alcohol, *n*-hexyl alcohol) decreases after electrochemical oxidation (shown in Fig. 5a), and the content of acids (acetic acid, *n*-butyric acid, *n*-hexylic acid) increases after electrochemical oxidation (shown in Fig. 5b), which confirms the
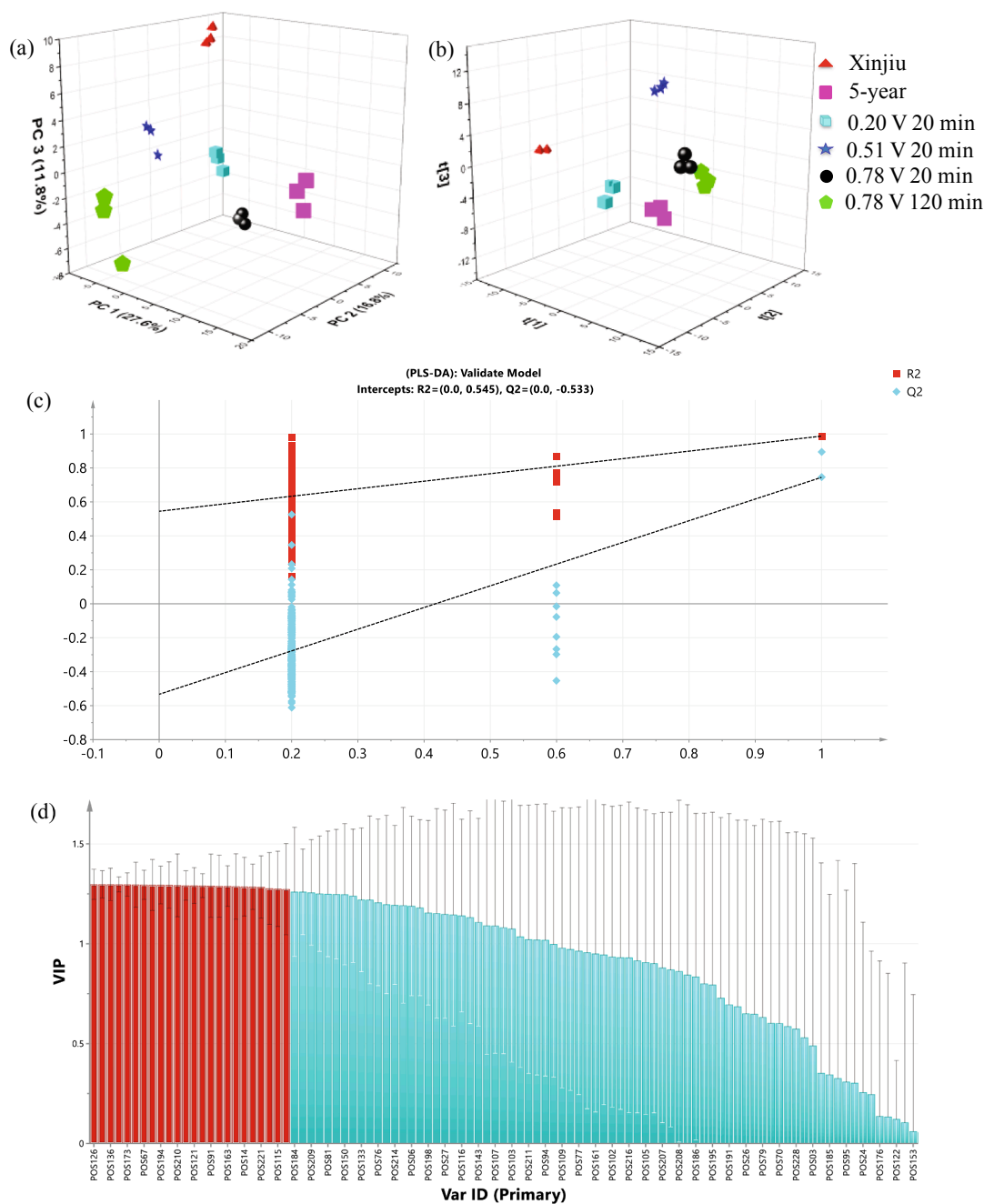
Fig. 3. PCA (a), PLS-DA (b) score plot of the naturally aged and electrochemically oxidized liquors (*Xinjiu*, 5-year aged, 0.20 V-20 min, 0.51 V-20 min, 0.78 V-20 min, and 0.78 V-120 min). (c) Permutation testing for PLS-DA model. (d) Variable importance (VIP) analysis.

mechanism that electrochemical oxidation accelerates the transformation from alcohols to acids. Acids are also the basic flavour compounds in liquors. Their main contribution is to enhance liquor flavour, reduce flavour impurities (such as hydrogen sulphide), and promote the formation of ester compounds to accelerate liquor aging. As shown in Fig. 5c, the content of corresponding esters increases along with the increase of acids, which confirms the transformation from the initial reactant (alcohols) to the final product (esters). During natural aging, the containers used to store liquors are usually pottery jar made of natural clay. The walls of the pottery jar have micropores, which contribute to slight air permeability. Therefore, during the long-term natural aging, the oxygen from the outside can continuously enter the pottery jar to make up for the oxygen consumed by the oxidation

reaction of alcohols to ensure that the DO concentration in liquors is maintained in a saturated state. After electrochemical oxidation, the content of various acids increases, which is consistent with the change trend of the DO concentration in the electrolyte (Eq. (1)).

$$RCH_2OH + Au = O_2 \rightarrow Au + RCOOH + H_2O \text{ (rate control step)} \quad (1)$$

The first step of oxidation–esterification equilibrium is the rate control step (i.e. oxidation of alcohols). Electrochemical oxidation accelerates the oxidation of alcohols thus increases of the concentration of corresponding esters (Eq. (2)).

$$RCOOH + C_2H_5OH \rightleftharpoons RCOOC_2H_5 + H_2O \quad (2)$$
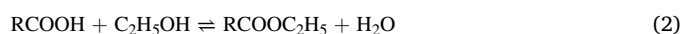
In the process of natural aging, multiple chemical reactions occurred.
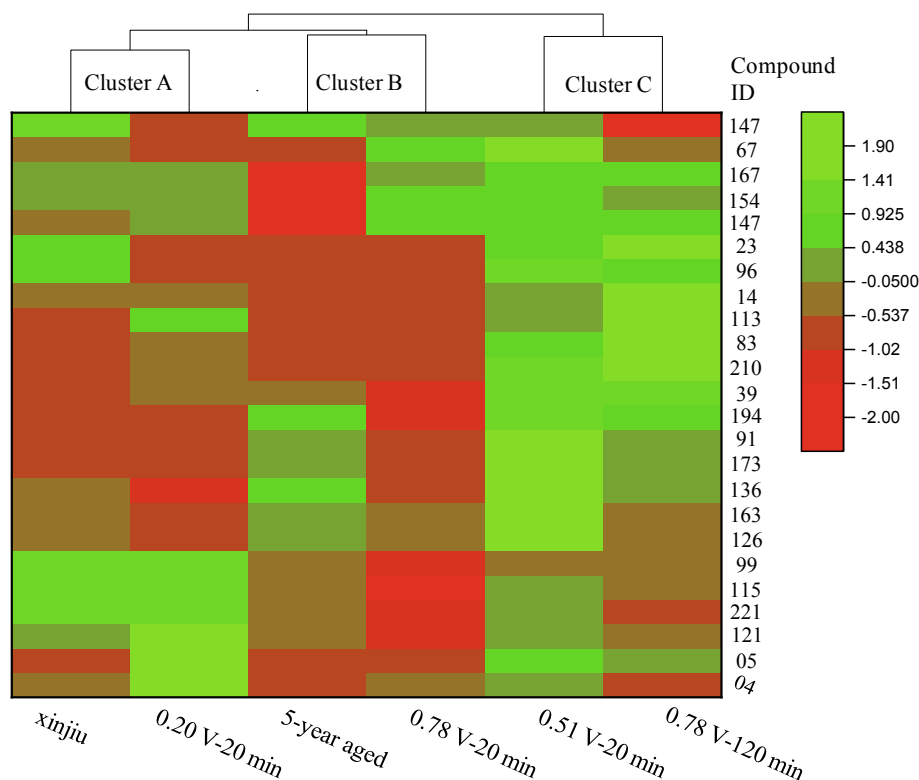
Fig. 4. Heat map of 24 marker compounds between naturally aged and electrochemically oxidized liquors. Blue and red patches indicate high and low concentrations of the marker compounds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Comparison of the marker compounds caused by electrochemical oxidation and natural aging.

ID NO.	Name	CAS NO	Formula	M W	RT(min)	VIP Value *Xinjiu* vs. 5 Y	VIP Value *Xinjiu* vs. 0.78 V
04	Urocanic acid	104–98-3	$C_6H_6N_2O_2$	138.0431	0.660	1.257	1.297
154	Arachidonic acid	506–32-1	$C_{20}H_{32}O_2$	304.2404	9.272	1.329	1.286
173	3-Oxopalmitic acid	928–19-8	$C_{16}H_{30}O_3$	270.2196	9.555	1.310	1.296
05	Triethanolamine	102–71-6	$C_6H_{15}NO_3$	149.1053	0.557	1.308	1.293
113	Eremantholide A	58030–93-6	$C_{19}H_{24}O_6$	348.1576	5.292	1.317	1.288
115	Docosanamide	3061–75-4	$C_{22}H_{45}NO$	339.3503	10.751	1.270	1.274
136	Cetaben	55986–43-1	$C_{23}H_{39}NO_2$	361.2978	9.564	1.220	1.297
14	Tegaserod	145158–71-0	$C_{16}H_{23}N_5O$	301.1891	2.063	1.269	1.285
147	Bis(4-ethylbenzylidene) sorbitol	9072–96-1	$C_{24}H_{30}O_6$	414.2046	7.155	1.311	1.291
194	2-(14,15-Epoxyeicosatrienoyl) glycerol	848667–56-1	$C_{23}H_{38}O_5$	394.2699	8.350	1.286	1.294
23	Sphinganine	764–22-7	$C_{18}H_{39}NO_2$	301.2982	7.355	1.323	1.290
39	Phenethylamine	64–04-0	$C_8H_{11}N$	121.0895	0.729	1.277	1.296
67	*N*-Methylpyrrolidone	872–50-4	C_5H_9NO	99.0689	1.587	1.277	1.295
83	Methyl palmitate	112–39-0	$C_{17}H_{34}O_2$	287.2826	7.033	1.283	1.276
96	Hexadecasphinganine	28457–97-8	$C_{16}H_{35}NO_2$	273.2668	6.688	1.327	1.294
99	Hexadecanamide	629–54-9	$C_{16}H_{33}NO$	255.2563	9.373	1.262	1.273

Alcohols were oxidized into aldehydes, which in turn oxidized into acids. Thus the alcohol content in liquor decreased and the aldehyde and acid content increased and reached new equilibrium after storage. Meanwhile, liquor contains a variety of organic acids, which react with alcohols to produce esters. Due to the particular aroma of the newly formed esters, so the liquor becomes aged Baijiu with distinct aroma and flavour. In the process of electrochemical oxidation of freshly produced liquor, oxygen speeds up the oxidation of alcohols. Among electrochemical oxidation, gold electrode acts as catalysts, the unpaired *d*-orbital electron of gold ensured its catalytic activity. The efficiency of its catalytic reaction depends on the adsorption step and its crystal lattice orientation will significantly affect its catalytic efficiency. Therefore, the influence of crystal lattice orientation density on its efficiency in catalytic conversion of liquor alcohol substances should be discussed in the

future of work.

4. Conclusions

Combining electrochemistry and UPLC-Q-Orbitrap-MS/MS, we report the oxidation-based flavour formation mechanism in Baijiu aging. Both DO and TDS showed significant changes and reflected a negative correlation, which confirmed the catalytic effect of electrochemical oxidation on the mutual transformation of liquor compounds. Although natural aging presented more diverse compound changes, the oxygen-based oxidation process during natural aging was similar to that in the electrochemical oxidation as a whole, which confirmed the importance of oxygen in promoting the flavour formation mechanism of aged Baijiu. The first step of oxidation–esterification equilibrium is the rate control
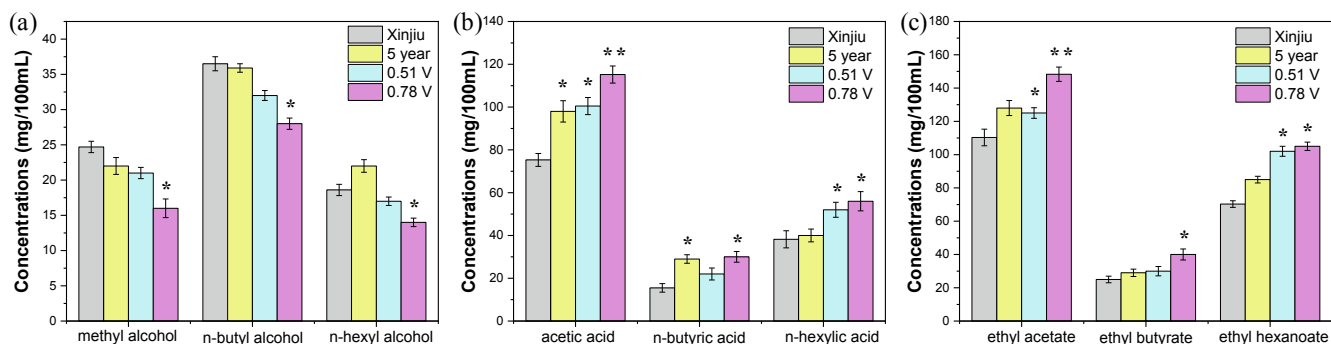
Fig. 5. The content of alcohols (a), acids (b), and esters (c) in naturally aged and electrochemically oxidized liquors (*Xinjiu*, 5-year aged, 0.51 V-20 min, and 0.78 V-20 min), alcohols: methyl alcohol, *n*-butyl alcohol, and *n*-hexyl alcohol; acids: acetic acid, *n*-butyric acid, and *n*-hexylic acid; esters: ethyl acetate, ethyl butyrate, and ethyl hexanoate. The data are presented as the mean value of triplicate measurements (n = 3).

step (i.e. oxidation of alcohols). Electrochemical oxidation accelerates the transformation from alcohols to acids and increase of the concentration of corresponding esters, thus contribute to the distinct aroma and flavour of aged Baijiu. This study also demonstrate that the combination of electrochemistry and UPLC-Q-Orbitrap-MS/MS can be a valid tactic to analyze liquor sample that with remarkable complexity in composition.

CRediT authorship contribution statement

Qing Zheng: Conceptualization, Funding acquisition, Supervision. **Zihao Wang:** Investigation, Methodology, Data curation, Formal analysis. **Ayuan Xiong:** Investigation, Methodology. **Yaru Hu:** Investigation, Methodology. **Ying Su:** Investigation, Methodology, Validation. **Kun Zhao:** Investigation, Methodology, Validation. **Yougui Yu:** Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.129596.

References

Bendig, P., Lehnert, K., & Vetter, W. (2014). Quantification of Bromophenols in Islay Whiskies. *Journal of Agricultural and Food Chemistry, 62*(13), 2767–2771. https://doi.org/10.1021/jf405006e.

Bruckenstein, S., & Shay, M. (1985). An in situ weighing study of the mechanism for the formation of the adsorbed oxygen monolayer at a gold electrode. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry, 188*(1), 131–136. https://doi.org/10.1016/S0022-0728(85)80057-7.

Chen, S., Wang, 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. *Journal of Agricultural and Food Chemistry, 67*(17), 4876–4884. https://doi.org/10.1021/acs.jafc.9b01420.

del Alamo-Sanza, M., Pando, V., & Nevares, I. (2014). Investigation and correction of the interference of ethanol, sugar and phenols on dissolved oxygen measurement in wine. *Analytica Chimica Acta, 809*, 162–173. https://doi.org/10.1016/j.aca.2013.11.053.

Deng, Y., Xiong, A., Zhao, K., Hu, Y., Kuang, B., Xiong, X., ... Zheng, Q. (2020). Mechanisms of the regulation of ester balance between oxidation and esterification in aged Baijiu. *Scientific Reports, 10*(1). https://doi.org/10.1038/s41598-020-74423-z.

Engel, M., Holtmann, D., Ulber, R., & Tippkötter, N. (2019). Increased biobutanol production by mediator-less electro-fermentation. *Biotechnology Journal, 14*(4), 1800514. https://doi.org/10.1002/biot.201800514.

Fan, W., & Qian, M. C. (2005). Headspace solid phase microextraction and gas chromatography−olfactometry dilution analysis of young and aged Chinese "Yanghe Daqu" liquors. *Journal of Agricultural and Food Chemistry, 53*(20), 7931–7938. https://doi.org/10.1021/jf051011k.

Fang, C., Du, H., Jia, W., & Xu, Y. (2019). Compositional differences and similarities between typical Chinese Baijiu and Western liquor as revealed by mass spectrometry-based. *Metabolomics., 9*(1), 2.

Fang, Y., Wang, W., Wo, X., Luo, Y., Yin, S., Wang, Y., ... Tao, N. (2014). Plasmonic imaging of electrochemical oxidation of single nanoparticles. *Journal of the American Chemical Society, 136*(36), 12584–12587. https://doi.org/10.1021/ja507097y.

Grosch, W. (2001). Evaluation of the key odorants of foods by dilution experiments aroma models and omission. *Chemical Senses, 26*(5), 533–545. https://doi.org/10.1093/chemse/26.5.533 %J Chemical Senses.

He, X., Yangming, H., Górska-Horczyczak, E., Wierzbicka, A., & Jeleń, H. H. (2021). Rapid analysis of Baijiu volatile compounds fingerprint for their aroma and regional origin authenticity assessment. *Food Chemistry, 337*, Article 128002. https://doi.org/10.1016/j.foodchem.2020.128002.

Jia, W., Du, A.n., Fan, Z., Zhang, R., Li, Y., Shi, Q., ... Chu, X. (2021). Molecular mechanism of the role of Mare Nectaris in the Feng-Flavor Baijiu aging. *LWT, 135*, 110254. https://doi.org/10.1016/j.lwt.2020.110254.

Jia, W., Fan, Z., Du, A.n., Li, Y., Zhang, R., Shi, Q., ... Chu, X. (2020). Recent advances in Baijiu analysis by chromatography based technology–A review. *Food Chemistry, 324*, 126899. https://doi.org/10.1016/j.foodchem.2020.126899.

Jia, W., Li, Y., Du, A.n., Fan, Z., Zhang, R., Shi, L., ... Chu, X. (2020). Foodomics analysis of natural aging and gamma irradiation maturation in Chinese distilled Baijiu by UPLC-Orbitrap-MS/MS. *Food Chemistry, 315*, 126308. https://doi.org/10.1016/j.foodchem.2020.126308.

Jiang, X., Xie, Y., Wan, D., Chen, M., & Zheng, F. (2019). GUITAR-enhanced facile discrimination of aged Chinese Baijiu using electrochemical impedance spectroscopy. *Analytica Chimica Acta, 1059*, 36–41. https://doi.org/10.1016/j.aca.2019.01.050.

Jin, G., Zhu, Y., & Xu, Y. (2017). Mystery behind Chinese liquor fermentation. *Trends in Food Science & Technology, 63*, 18–28. https://doi.org/10.1016/j.tifs.2017.02.016.

Kew, W., Goodall, I., Clarke, D., & Uhrín, D. (2017). Chemical diversity and complexity of scotch whisky as revealed by high-resolution mass spectrometry. *Journal of the American Society for Mass Spectrometry, 28*(1), 200–213. https://doi.org/10.1021/jasms.8b05374.

Li, M., Fan, W., & Xu, Y. (2021). Volatile compounds sorption during the aging of Chinese Liquor (Baijiu) using Pottery Powder. *Food Chemistry, 345*, Article 128705. https://doi.org/10.1016/j.foodchem.2020.128705.

Liu, H., & Sun, B. (2018). Effect of fermentation processing on the flavor of Baijiu. *Journal of Agricultural and Food Chemistry, 66*(22), 5425–5432. https://doi.org/10.1021/acs.jafc.8b00692.

Ma, Y., Qiao, H., Wang, W., Chen, T., Du, X., Zhai, X., & Zhang, S. (2014). Variations in physicochemical properties of Chinese Fenjiu during storage and high-gravity technology of liquor aging. *International Journal of Food Properties, 17*(4), 923–936. https://doi.org/10.1080/10942912.2012.678536.

Masuda, T., Ikeda, K., & Uosaki, K. (2013). Potential-dependent adsorption/desorption behavior of perfluorosulfonated ionomer on a gold electrode surface studied by cyclic voltammetry, electrochemical quartz microbalance, and electrochemical atomic force microscopy. *Langmuir, 29*(7), 2420–2426. https://doi.org/10.1021/la304705k.

Qiao, H., Zhang, S., Wang, W., & Ma, Y. (2012). Effects of high-gravity field on Fenjiu maturation. *Innovative Food Science & Emerging Technologies, 16*, 198–204. https://doi.org/10.1016/j.ifset.2012.05.013.

Saravia, L. P. H., Sukeri, A., Gonçalves, J. M., Aguirre-Araque, J. S., Brandão, B. B. N. S., Matias, T. A., … Bertotti, M. (2016). CoTRP/Graphene oxide composite as efficient electrode material for dissolved oxygen sensors. *Electrochimica Acta, 222*, 1682–1690.

Song, X., Jing, S.i., Zhu, L., Ma, C., Song, T., Wu, J., … Chen, F. (2020). Untargeted and targeted metabolomics strategy for the classification of strong aroma-type baijiu (liquor) according to geographical origin using comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. *Food Chemistry, 314*, 126098. https://doi.org/10.1016/j.foodchem.2019.126098.

Song, X., Zhu, L., Jing, S.i., Li, Q., Ji, J., Zheng, F., … Sun, B. (2020). Insights into the role of 2-methyl-3-furanthiol and 2-furfurylthiol as markers for the differentiation of chinese light, strong, and Soy Sauce Aroma Types of Baijiu. *Journal of Agricultural and Food Chemistry, 68*(30), 7946–7954. https://doi.org/10.1021/acs.jafc.0c04170.s002.

Wang, C., Wan, Q., Cheng, J., Lin, S., Savateev, A., Antonietti, M., & Wang, X. (2021). Efficient aerobic oxidation of alcohols to esters by acidified carbon nitride photocatalysts. *Journal of Catalysis, 393*, 116–125. https://doi.org/10.1016/j.jcat.2020.11.021.

Wei, D., Li, L., He, S., Yu, J., Tian, X., & Wu, Z. (2020). Improving the lipid oxidation in pork fat processing for Chi-aroma Baijiu through pretreatments and segmented soaking with liquor. *LWT, 130*, Article 109624. https://doi.org/10.1016/j.lwt.2020.109624.

What's that stuff? Baijiu. (2018). C&EN Global Enterprise, 96(33), 30-31. DOI:10.1021/cen-09633-feature4.

Xiong, A., Zhao, K., Hu, Y., Yang, G., Kuang, B., Xiong, X., … Zheng, Q. (2020). Influence of electrochemical oxidation on the maturation process of the distilled spirit. *ACS Omega, 5*(29), 18349–18355. https://doi.org/10.1021/acsomega.0c02090.

Yang, J., Chen, Y., Zhen, X.-T., Dong, X., Ye, L.-H., Zheng, H., & Cao, J. (2020). Oxidative metabolism of typical phenolic compounds of Danshen by electrochemistry coupled to quadrupole time-of-flight tandem mass spectrometry. *Food Chemistry, 315*, Article 126270. https://doi.org/10.1016/j.foodchem.2020.126270.

Zhao, D., Shi, D., Sun, J., Li, A., Sun, B., Zhao, M., … Zheng, F. (2018). Characterization of key aroma compounds in Gujinggong Chinese Baijiu by gas chromatography–olfactometry, quantitative measurements, and sensory evaluation. *Food Research International, 105*, 616–627.

Zhao, K., Liu, L., Zheng, Q., Gao, F., Chen, X., Yang, Z., … Yu, Y. (2019). Differentiating between ageing times of typical Chinese liquors by steady-state microelectrode voltammetry. *Microchemical Journal, 151*, 104244. https://doi.org/10.1016/j.microc.2019.104244.

Zheng, Q., Yang, Y., Yan, Y., Yu, Y., Liu, Y., Gao, W., … Shao, H. (2016). The long-range effect induced by untying hydrogen bonds for single cell test using SECM. *Electrochimica Acta, 207*, 135–142. https://doi.org/10.1016/j.electacta.2016.04.174.

Zheng, Y., Sun, B., Zhao, M., Zheng, F., Huang, M., Sun, J., … Li, H. (2016). Characterization of the Key Odorants in Chinese Zhima Aroma-Type Baijiu by gas chromatography-olfactometry, quantitative measurements, aroma recombination, and omission studies. *Journal of Agricultural and Food Chemistry, 64*(26), 5367–5374. https://doi.org/10.1021/acs.jafc.6b01390.

Zhu, L., Wang, X., Song, X., Zheng, F., Li, H., Chen, F., … Zhang, F. (2020). Evolution of the key odorants and aroma profiles in traditional Laowuzeng baijiu during its one-year ageing. *Food Chemistry, 310*, 125898. https://doi.org/10.1016/j.foodchem.2019.125898.