1\textsuperscript{H}-NMR and viscosity studies of hydrogen bonding of Chinese rice wine

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\textbf{ABSTRACT}

The viscosity of Chinese rice wine gradually increases with the maturing process, resulting in a better taste due to longer contact with the tongue. Herein, the structure of water–ethanol was detected by hydrogen nuclear magnetic resonance (\textsuperscript{1}H-NMR) and viscosity measurement to define the strength of hydrogen bonding. Through single-factor experiments, it can be seen that most of the flavors have a promoting effect on alcohol–water hydrogen bonding, with the exception of aldehydes. And the factors influencing hydrogen bonding in decreasing order were determined by Plackett–Burman (PB) test. The relationship between hydrogen bonding strength and flavor in Chinese rice wine with 18% (v/v) alcohol concentration was investigated through multiple regression analysis, with an adjusted \(R^2\) of 0.925. The results of study provide important insight for the quality control and molecular mechanism of viscosity increasing during Chinese rice wine aging.

\textbf{1\textsuperscript{H}-NMR y estudios de viscosidad del enlace de hidrógeno del vino de arroz chino}

\textbf{RESUMEN}

La viscosidad del vino de arroz chino aumenta gradualmente durante su añejamiento, haciendo que su sabor sea más agradable debido a su contacto más prolongado con la lengua. El presente estudio detectó la estructura de agua-etanol empleando la resonancia magnética nuclear de hidrógeno (\textsuperscript{1}H-NMR) y la medición de la viscosidad para definir la fuerza de adhesión del hidrógeno. A través de experimentos de factor único se comprobó que la mayoría de los sabores promueve la adhesión del hidrógeno al alcohol-agua, excepción hecha de los aldehídos. La prueba Plackett-Burman (PB) determinó los factores que, en orden decreciente, inciden en la adhesión del hidrógeno. Utilizando un análisis de regresión múltiple, se investigó la relación existente entre la fuerza de adhesión del hidrógeno y el sabor del vino de arroz chino mediante la concentración de alcohol 18% v/v, con una \(R^2\) ajustada de 0.925. Los resultados del estudio proporcionan importantes conocimientos para el control de calidad y el mecanismo molecular que provoca la creciente viscosidad del vino de arroz chino durante su añejamiento.

\textbf{1. Introduction}

Chinese rice wine is a water and ethanol solution with a distinct flavor and taste. A traditional Chinese fermented alcoholic beverage, this wine is one of the most popular alcoholic beverages in China with an annual consumption of millions of liters (Chen & Xu, 2010; Yu, Ying, Fu, & Lu, 2006). Water (\(\text{H}_2\text{O}\)) and ethanol (\(\text{EtOH}\)) account for approximately 95% of Chinese rice wine (Sato & Buchner, 2004). In water–ethanol solutions, hydrogen bonding occurs with water–water, water–ethanol, and ethanol–ethanol molecules. Because many free ethanol molecules are found in the solution, newly brewed Chinese rice wine has a relatively poor taste. In the manufacturing process, rice wine is usually matured in pottery jars for at least one year. After the maturation, the organoleptical stimulation of ethanol is reduced. Due to the strong polarity of water and ethanol, the change of the hydrogen bonding structure of water–ethanol molecules has been thought to reduce the irritating taste caused by the free ethanol (Mo, Fan, & Xu, 2009). However, few researchers have scientifically demonstrated the relationship between the water–ethanol structure and the viscosity. Viscosity increasing suggested the mellowing of the wine taste. Several studies investigated the interaction between the water and ethanol molecules and the hydrogen bonding structure in aged alcoholic beverages (Burikov, Dolenko, Patsaeva, Starokurov, & Yuzhakov, 2010).

In the field of solution chemistry, the study of water–alcohol solutions is one of the most attractive subjects. However, the study of the hydrogen bonding between water and ethanol in wines has not been pursued as much as in solution chemistry. Franks and Ives (1966) reviewed the physicochemical and thermodynamic properties of the alcohol–water mixtures. Coccia, Indovina, Podo, and Viti (1975) analyzed the hydrogen nuclear magnetic resonance (\textsuperscript{1}H-NMR) signals in ethanol–water mixtures throughout an entire range of concentrations. Nishi et al. (1988) examined the molecular association in ethanol–water mixtures using mass spectroscopic analysis. Hydrogen bonding under high temperature and pressure in methanol and methanol/water mixtures was studied by Dougan et al. (2004). Schwerdtfeger, Köhler, Potte, and Kaatze (2001) examined the dielectric relaxation of hydrogen bonded liquids in mixtures of monohydric alcohols with n-alkanes. Sato and Buchner (2003) discussed the cooperative dynamics of the H-bond system in 2-propanol and water.
mixtures. The molecular structure of a concentrated alcohol-water mixture was reported by Dixit, Crain, Poon, Finney, and Soper (2002). In some alcohol–water mixtures, the existence of clathrate structures was reported by Murthy (1999). Dimitroulis, Kainourgikakis, Raptis, and Samios (2015) studied the diffusion of water in simulated water and n-alcohol systems. Moreover, the hydrogen bond network is characterized by rather stable topology. Campen and Kubicki (2010) calculated ethanol (OH) frequencies and interaction energy on hydrogen bonding for the H₂O–H₂O, CH₃OH–H₂O, and H₂O–CH₃OH dimers.

In previous studies, most scholars exclusively used water hydroxyl proton peaks to determine hydrogen bonding strength using high-resolution ¹H-NMR spectroscopy (Mizuno et al., 2000). Most salts, acids, and phenols contained in Japanese shochu had been studied (Nose, Masasjo, & Ueda, 2004). However, ¹H-NMR only offered abstract results confirming the degree of hydrogen bonding in beverages (Nose et al., 2005). In this work, we investigated the ¹H-NMR spectra of water and ethanol hydroxyl proton peaks in addition to measuring viscosity in model solutions. Chinese rice wine is composed of nutrients like sugars, alcoholic, aldehydes, as well as acids and salts (Mo et al., 2010). The effects of flavor constitutions on hydrogen bonding were analyzed by the chemical shift of two OH peaks in ¹H-NMR and by measuring viscosity. The relationship between the hydrogen bonding of water–ethanol in Chinese rice wine and flavor constitutions has been examined.

2. Materials and methods

2.1. Materials

Water was deionized and distilled twice under the presence of potassium permanganate (KMnO₄) and sodium hydroxide (NaOH). Regents including 99.7% ethanol, sugars, acids, alcohols, aldehydes, esters, and alcohols were of analytical reagent (AR) grade. All reagents were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. The ubbelodhe viscometer and other glass columns were purchased from Hefei Meifeng Chemical Instrument Co., Ltd. The constant temperature water baths were produced by Shanghai Pingxuan Scientific Instruments Co., Ltd. The superconducting nuclear magnetic resonance (NMR) spectral instrument was produced by Agilent from the United States. The Chinese rice wine sample was provided by Anhui Gu Nan Feng Industrial Co., Ltd.

2.2. ¹H-NMR spectroscopy

¹H-NMR spectra were measured with a JEOL EX-400 spectrometer operating at 400 MHz at 20°C. The deuterated reagent was COCD₃₂, containing 0.03% (volume fraction) tetramethylsilane (TMS) external reference, produced by CIL, U.S. The TMS solution was sealed at the center of a 5 mm capillary tube.

2.3. Viscosity

The viscosity was measured by ubbelodhe viscometer (Agoston, Ötsc, & Jakoby, 2005; Thompson, Kaiser, & Jorgenson, 2006). Capillary diameter was 0.6–0.7 mm. The test temperature was 30°C. The temperature of the sample was maintained constant within ±0.05°C by a circulating and thermostatic cistern and the measurement error within 30 s.

2.4. Chinese rice wine sample

Chinese rice wine production process requires three times filtration, so finished product is a clear and transparent liquid. For further experiments, the samples of Chinese rice wine were treated with 3000 r/min for 30 min.

2.5. Effect of flavor chemicals in Chinese rice wine on hydrogen bonding in alcohol water

The aroma of aged Chinese rice wine Qu is one of the most important factors that influences the flavor of Chinese rice wine (Mo, Fan, & Xu, 2012). It was shown that the concentrations of most flavor chemicals increased progressively from the first day to the fourth day of fermentation, and then decreased gradually (Mo et al., 2010).

Most of the flavor components in Chinese rice wine contain polar groups. So we design an experimental scheme to investigate the effect of flavor chemicals on hydrogen bonding. Blending of 18% (v/v) ethanol aqueous solution with glucose concentration of 1 × 10⁻⁵, 1 × 10⁻⁴, 1 × 10⁻³, 1 × 10⁻², 1 × 10⁻¹, and 1 mol/L, we determine hydrogen bonding strength. The X axis is log value of concentration. Y axis is hydrogen bonding strength (Δδ, η). The research method for other flavor chemicals is the same as glucose.

2.6. Statistical analysis

All data were an average of three experiments. Nuclear magnetic data were processed using MestreNova software (Funasaki, Nomura, Ishikawa, & Neya, 2001), and chemical formulas were plotted using Chemdraw software.

The multivariate linear stepwise regression analysis can comprehensively analyze the explanation degree of each factor on the dependent variable, that is, the ability of each flavor compounds to interpret the hydrogen bonding of Chinese rice wine. Therefore, by using this method, the quantitative relationship between the various factors and the alcohol–water hydrogen bonding strength in Chinese rice wine can be obtained.

3. Results and discussion

3.1. Determination of hydrogen bonding strength index

¹H-NMR measures the relative number of protons and determines the type of association. More importantly, ¹H-NMR details the association of intermolecular hydrogen bonding (Oliveira & Vasconcellos, 2006). ¹H-NMR chemical shifts reflect the average properties or conditions of the hydrogen bonding structure of the three types of hydrogen bonding interactions in ethanol-water (EtOH-H₂O) solutions: water–ethanol, water–water, and ethanol–ethanol (Matsugami et al., 2016; Nishi et al., 1988). The various ethanol mole concentrations (x_เอ = 0.0–1.0) in water and ethanol mixtures were studied using ¹H-NMR spectra. The results are shown in Table 1. Figure 1 shows the ¹H-NMR spectra of non-ethanol, 5%, 18%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% (v/v) and pure ethanol solutions without H⁺ or OH⁻ impurity (Gereben
ethanol concentration. Therefore, the methyl and methylene peaks were used as the internal reference peaks.

Within $0 < \chi_e < 1.000$, ethanol was dissolved in water (Figure 2(b)). The chemical shifts were much higher for ethanol hydroxyl protons in liquid than in pure ethanol ($\delta_{\text{OH}} = 4.994; \chi_e = 1$). The water molecule’s proton chemical shift of system was much smaller than that of pure water ($\delta_{\text{HOH}} = 4.685; \chi_e = 0$). This could be explained by the associated hydrogen bond affecting the ethanol-aqueous solution system. As the proton external environment changes, its chemical shift changes; the original alcohol–ethanol and water–water structure were destroyed. The microscopic association presents a new state at different ethanol mole concentrations. This conclusion was consistent with that of who studied the ethanol–water system by analyzing Raman and infrared (IR) spectroscopy (Nauta & Miller, 2000).

Dixit et al. (2002) argued that the thermodynamic change after mixing ethanol and water was due to incomplete mixing of hydrogen bonds between molecules within the system. Hence, to determine the hydrogen bonding strength, we read two proton peaks in different ethanol concentrations of ethanol–water solution. $\delta(x)$ is the solution of ethanol and water hydroxyl proton weighted average chemical shift:

$$\delta(x) = \delta w(x) - \delta w(1-\chi)(1-x)$$

where $\delta e(x)$, $\delta w(x)$ are the proton chemical shifts of water and ethanol in the mixtures at molar fraction $x$ of ethanol, respectively.

We assumed that an ideal solutions means that no association exists in the mixed system. $\delta^x(w)$ was considered as the ideal mixing of pure water and pure ethanol hydroxyl proton weighted average chemical shift:

| Mole Fraction of H$_2$O |
|------------------------|
| 0.0000 | 4.685 | 4.685 | 4.685 | 1 |
| 0.0161 | 4.673 | 4.6988 | 4.673 | 0.9839 |
| 0.0326 | 4.664 | 4.7050 | 4.6850 | 0.9664 |
| 0.0492 | 4.649 | 4.7142 | 4.6885 | 0.9358 |
| 0.0681 | 4.623 | 4.7030 | 4.6971 | 0.8819 |
| 0.0892 | 4.601 | 4.7466 | 4.7103 | 0.8276 |
| 0.1123 | 4.556 | 4.7662 | 4.7125 | 0.7619 |
| 0.1374 | 4.509 | 4.7904 | 4.7267 | 0.6808 |
| 0.1645 | 4.439 | 4.8211 | 4.7363 | 0.5783 |
| 0.1936 | 4.327 | 4.8611 | 4.7248 | 0.4444 |
| 0.2247 | 4.329 | 4.9156 | 4.8986 | 0.2623 |
| 0.2567 | 4.994 | 4.994 | 4.994 | 0 |

Over the ethanol mole fraction range $x_e$, the water hydroxyl proton and the alcoholic hydroxyl proton were independently separated and were easy to distinguish. In the pure aqueous solution, only the water hydroxyl proton peak was observed; the hydroxyl proton chemical shift of water was 4.685. In the pure ethanol solution, the hydroxyl proton chemical shift of ethanol was 4.994. According to the national standard of Chinese rice wine, the wine alcohol content is about 18% (v/v). In this work, 18% ethanol concentration (v/v) in the ethanol–water solutions. (b) The $^{1}$H-NMR spectra showed water and ethanol hydroxyl proton peaks.
\[ \delta^O(\chi) = \delta e(0)\chi + \delta w(1)(1 - \chi) \]  

(2)

where \(\delta w(1)\), \(\delta e(0)\) are the hydroxyl proton chemical shift of pure water and pure ethanol, respectively. Equation (3) explains the difference between the ethanol–water solution and the ideal mixture:

\[ \Delta\delta = \delta^O(\chi) - \delta(\chi) \]  

(3)

The greater the \(\Delta\delta\), the greater the mixed state of ethanol and water molecules from the ideal mix, indicating a stronger intermolecular H-bond association.

Figure 2(c) shows the specificity of the change in the molar fraction of \(\delta^O(\chi)\), \(\delta(\chi)\) with various concentrations of ethanol. When the ethanol concentration \(x\) was 0.550, \(\Delta\delta\) reached a maximum, which was consistent with the viscosity measurement (Figure 2(d)). The reason for this observation may be the stable structure of the hydrogen bond formed at this time between ethanol and water molecules. The molar ratio of ethanol to water in the ethanol–water solution was close to 1:2, causing the formation of a stable cyclic trimer association structure, under strong hydrogen bonding (Nauta & Miller, 2000).

3.2. Effects of flavor constitutes on the hydrogen bonding of model Chinese rice wine

Chinese rice wine is a complex colloidal solution containing many substances, such as sugars, acids, alcohols, aldehydes, esters that affect the taste, as well as water and ethanol (Cao, Xie, Wu, & Lu, 2010; Shen, Ying, Li, Zheng, & Hu, 2011).

Different flavors can affect the chemical shift due to their different concentrations in Chinese rice wine. The factors affecting hydrogen bonding should be integrated into Chinese rice wine. Sugars, acids, alcohols, and esters are prone to forming hydrogen bonding because of their strong polar groups.

The proton exchange between water and ethanol molecules is promoted by the combined effect of the flavor substances. So, the two peaks coalesced to a single peak in the \(^1\)H-NMR spectrum. The \(\delta\) value of the merged proton peak in the alcoholic simulation system was smaller than in the Chinese rice wine system with the same alcohol concentration, with a deviation between 0.006 and 0.015 ppm. This indicates that flavor constitutes dissolved in Chinese rice wine strengthened the alcohol–water hydrogen bond association, as shown in Figure 3.

3.3. Effects of sugars and acids

For the first time, changes were displayed in the two OH proton signals in the \(^1\)H-NMR spectra and the viscosity value with increasing sugar concentration (Figure 4). Maltose is a disaccharide, and glucose is a monosaccharide. A maltose molecule consists of two molecules of glucose. As shown in Figure 4, the \(\Delta\delta\) value and viscosity of maltose are greater than glucose at the same concentration. We assumed that the dimer has a greater effect on hydrogen bonds than the monomer. From the data in Figure 4, the \(\Delta\delta\) value based on a hydroxyl group (-OH) was defined as 0.011–0.018. The
viscosity of maltose and glucose reached a maximum at 1 mol/dm$^3$ and maltose had a higher viscosity.

The effects of citric acid were examined at the same low concentrations on the OH proton chemical shifts in both the Chinese rice wine model and the Chinese rice wine (Figure 5). At $1 \times 10^{-3}$ mol/dm$^3$, citric acid caused a low-field chemical shift in the Chinese rice wine model (Figure 5(b)). Notably, in the 18% (v/v) EtOH-H$_2$O solution without solutes, both the OH peaks of water and ethanol were distinct, as mentioned above. Even with $1 \times 10^{-4}$ mol/L citric acid, the two peaks were still observed separately (Figure 5(c)). However, the two OH peaks of ethanol and water in $1 \times 10^{-1}$ mol/dm$^3$ citric acid were not shown in Figure 5(a); The ethanol peak was located at a much lower field of $\delta = 5.11$). The chemical shift of the water proton peak might represent the hydrogen bond strength in this system. So, the $\delta$ value of water proton peak increased with increasing citric acid concentration (Figure 5(d)).

In 18% (v/v) EtOH-H$_2$O solutions with $1.0 \times 10^{-1}$ mol/dm$^3$ citric acid, the peaks of water and ethanol merged into a single peak, indicating that the proton exchange reaction between water and ethanol molecules was promoted by the dissolved citric acid (Nose & Hojo, 2006). The hydrogen bond lifetime is extremely short, measured in picoseconds. The unification of the two peaks indicates that the proton exchange had accelerated. As citric acid concentration increased, additional low-field chemical shift changes were simultaneously observed. The results were consistent with those of Mizuno, Miyashita, and Shindo (1995). They examined the rate constants of the exchange reactions of the ethanol-water mixtures with $^1$H-NMR spectra, showing that the specific rate of exchange between water and ethanol was proportional to the concentration of the added acid or base.

The promotion of proton exchange between water and ethanol molecules accompanied by the strengthened hydrogen bonding was observed for other acids in the Chinese rice wine model. The effects of malic acid, pyruvic acid, acetic acid, and lactic acid in Chinese rice wine were also investigated (Figure 6). All acids examined caused $\Delta \delta$ and viscosity to increase with increasing ethanol concentration. The effects of an acid on hydrogen bonding may be divided into two aspects: the undissociated acid molecule (HA) and H$^+$ from the acid. We concluded that acids caused the tight association between H$_2$O and EtOH molecules. From the $\Delta \delta$ graph, the effect of acids on hydrogen bond association may be related to the numbers of –OH, –COOH, and –C = O groups. The acids investigated were arranged based on hydrogen bonding: malic acid, lactic acid, pyruvic acid, and acetic acid. Lactic acid has one carboxyl group (–COOH) and one hydroxyl group (–OH), and malic acid has two carboxyl groups. The value of the $\Delta \delta$ based on a carboxyl group was estimated to be 0.123 from comparing the value of $\Delta \delta$ caused by $1.0 \text{ mol/dm}^3$ malic acid with lactic acid.

In the Chinese rice wine model, sugars and acids strengthen the ethanol-water structure according two detection indicators. Merged proton peaks suggested stronger hydrogen bonding than the single peak of water and ethanol in $^1$H-NMR.

3.4. Effects of alcohols and esters

The alcohols have an oxygen atom with strong electronegativity (Lilly, Lambrechts, & Pretorius, 2000; Moreira, Mendes, Hogg, & Vasconcelos, 2005) which caused a more stable association structure among alcoholic and water molecules (Figure 7).
Three situations have resulted in the hydrogen bond association changing: (1) The alcoholic hydroxyl group forms an intermolecular hydrogen bond with the oxygen atom at position 1. (2) An alcohol is directly used to form a stable 12-membered ring hydrogen bond association structure. The number of 12-membered rings containing alcohol determines the strength of the hydrogen bond association in the system. (3) The number of 12-membered rings in the ethanol–water structure determines the intensity of the hydrogen bond association in the system.

At the same alcohol concentration, the dependent $\Delta\delta$ value of phenylethyl alcohol was less than that of the isoamyl alcohol (Figure 8). The reason for this may be that the benzene ring, as a side chain group of the alcohol, has a larger spatial structure than the long carbon chain, which is not conducive to creating associations with the original alcohol group water molecules (Lalanne, Andanson, Tassaing, Danten, & Besnard, 2004). Hence, a more stable association structure is formed. From the linearity between viscosity value and alcohol concentration, we determined that the dependent viscosity value of phenylethyl alcohol is greater than that of isoamyl alcohol, perhaps because the relatively strong hydrophobic interaction of the benzene ring caused the viscosity of the system to increase (Nose & Hojo, 2006; Nose et al., 2005).

As ethyl acetate and ethyl lactate concentration increased, the $\Delta\delta$ value increased, accompanied by enhanced hydrogen bond association (Figure 9). Additionally, the $\Delta\delta$ value of ethyl lactate was greater than that of ethyl acetate. Hydrolysis equilibrium was attained in the system with added esters. Ethyl lactate and ethyl acetate were separated from lactic acid and ethanol, acetic acid and ethanol, respectively. The effect of lactic acid was stronger than that of acetic acid on hydrogen bond association, aligning with the findings of
Figure 7. A model for the intimate association among water and ethanol molecules with the assistance of stronger hydrogen-bonding donors (alcohols).

Figura 7. Modelo para la asociación íntima entre moléculas de agua y etanol con la asistencia de donantes más fuertes para la adhesión de hidrógeno (alcoholes).

Figure 8. (a) Change in $\Delta \delta$ value in the Chinese rice wine model containing isoamyl alcohol or phenethyalcohol. (b) Effects of isoamyl alcohol or phenethyalcohol on the viscosity of the Chinese wine model.

Figura 8. (a) Cambio en el valor $\Delta \delta$ en el modelo de vino de arroz chino con contenido de alcohol isoamílico o alcohol fenetílico. (b) Efecto del alcohol isoamílico o el alcohol fenetílico en la viscosidad del modelo de vino chino.

Figure 9. (a) Change in $\Delta \delta$ value in the Chinese rice wine model containing ethyl acetate or ethyl lactate. (b) Effects of ethyl acetate or ethyl lactate on the viscosity of the Chinese wine model.

Figura 9. (a) Cambio en el valor $\Delta \delta$ en el modelo de vino de arroz chino con contenido de acetato de etilo o lactato de etilo. (b) Efectos de acetato de etilo o lactato de etilo en la viscosidad del modelo de vino chino.
previous research. The effect of ethyl acetate on the viscosity of the Chinese rice wine model first increased and then decreased with increasing ester concentration in the Chinese rice wine model. When the concentration of ethyl acetate reached $1 \times 10^{-7}$ mol/L, the viscosity of the system reached its maximum. When the concentration of ethyl acetate was more than $1 \times 10^{-2}$ mol/L, the interaction between the molecules of ethyl acetate was enhanced (Withers, John, Gwenn, John, & Alistair, 1994). The contribution of ethyl acetate to the system was greater than to hydrogen bonding. Therefore the viscosity of the system started to decrease due to the high viscosity of ethyl acetate.

### 3.5. Effects of aldehydes

The effects of aldehydes were also investigated (Figure 10). Surprisingly, we noticed that $\Delta \delta$ and the viscosity value decreased with increasing aldehyde concentration in the model Chinese rice wine. The carbonyl (aldehyde) group was selective for the intermolecular association of either ethanol or water. Therefore, the structure of the water and ethanol association was weakened by the presence of small amounts of aldehydes. Benzaldehyde has a benzene ring that is a larger structure than a straight chain, so affecting the association between ethanol and water was more difficult for benzaldehyde. Thus the $\Delta \delta$ value of benzaldehyde was greater than that of acetaldehyde at the same concentration.

### 4. Flavor constitutes on hydrogen bonding by applied multiple regression analysis

#### 4.1. Multiple regression analysis (MRA)

We also analyzed the relationship between the hydrogen bonding that was affected by flavor chemicals or the chemical group. However, the primary and secondary sequences of flavor chemicals that affected the hydrogen bonding were unknown. The Plackett–Burman experiment design (Ma, Wang, Tang, & Yang, 2016) involves a two-part partial factor design method. Based on the principle of non-fully balanced block, the factors can be estimated using the fewest number of tests. The most important factors are selected from many factors. For our study, we used the single factor, and the Plackett–Burman experiment was performed in combination with the content range for the Chinese rice wine. The test design and response values are shown in Table 2. We observed that the unification of the two peaks was due to the combined influence of flavor matters, just like was observed for citric acid. So the chemical shift of the merged peak was considered a response value. The results are shown in Table 3. The credibility of the first two factors was about 80%.

The multiple nonlinear regression analysis method is based on a set of data to find the relationship between

![Figure 10](image1.png)

**Figure 10.** (a) Change in $\Delta \delta$ value in model Chinese rice wine containing benzaldehyde or acetaldehyde. (b) Effects of benzaldehyde or acetaldehyde on the viscosity of model Chinese wine.

**Table 2.** Experimental design and response value of Plackett–Burman for flavor constituents.

| Number | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $\Delta \delta$ |
|--------|------|------|------|------|------|------|------|------|------|--------|-------------|
| 1      | 1    | -1   | 1    | -1   | -1   | 1    | 1    | 1    | 1    | -1     | 4.456       |
| 2      | 1    | 1    | -1   | -1   | -1   | 1    | 1    | 1    | 1    | 1      | 4.494       |
| 3      | -1   | 1    | -1   | -1   | -1   | -1   | 1    | 1    | 1    | 1      | 4.492       |
| 4      | 1    | -1   | 1    | 1    | -1   | -1   | 1    | 1    | 1    | 1      | 4.462       |
| 5      | 1    | 1    | -1   | 1    | 1    | -1   | -1   | -1   | 1    | 1      | 4.483       |
| 6      | 1    | 1    | 1    | -1   | 1    | 1    | -1   | -1   | -1   | 1      | 4.474       |
| 7      | -1   | -1   | 1    | 1    | 1    | -1   | -1   | -1   | -1   | 1      | 4.463       |
| 8      | -1   | -1   | -1   | 1    | 1    | -1   | -1   | -1   | -1   | 1      | 4.457       |
| 9      | -1   | -1   | -1   | -1   | 1    | 1    | -1   | -1   | -1   | 1      | 4.489       |
| 10     | 1    | -1   | -1   | -1   | -1   | 1    | 1    | -1   | -1   | 1      | 4.475       |
| 11     | 1    | -1   | -1   | -1   | -1   | -1   | 1    | 1    | -1   | 1      | 4.452       |
| 12     | -1   | -1   | -1   | -1   | -1   | -1   | -1   | 1    | 1    | -1     | 4.443       |

**Table 3.** Factors, levels, and major response-effect analysis of Plackett–Burman experiments for flavor constituents.

| Mass Concentration (mg/L) | Variable | Low Level | High Level | Coefficients | P-Value | Importance Order |
|---------------------------|----------|-----------|------------|--------------|---------|-----------------|
| Intercept                 |          | -         | -          | 4.47         | 0.00033 | -               |
| $X_1$                     |          | 100       | 150        | 0.0040       | 0.33618 | 6               |
| $X_2$                     |          | 5000      | 7500       | 0.0063       | 0.22472 | 3               |
| $X_3$                     |          | 100       | 150        | -0.0027      | 0.45762 | 7               |
| $X_4$                     |          | 300       | 450        | 0.0083       | 0.17380 | 1               |
| $X_5$                     |          | -         | -          | 0.00083      | 0.78162 | 9               |
| $X_6$                     |          | 50        | 75         | 0.0057       | 0.24866 | 4               |
| $X_7$                     |          | -         | -          | -0.00033     | 0.90966 | 10              |
| $X_8$                     |          | 150       | 225        | 0.0082       | 0.17717 | 2               |
| $X_{10}$                  |          | 15        | 20         | 0.002       | 0.54887 | 8               |
the possible factors. The statistical methods applied to the expression of variables result in the best nonlinear mathematical expression. When applied multiple nonlinear regression analysis, setting the dependent variable and selecting the significant factor as independent variables are necessary. Multivariate nonlinear regression analysis was performed using alcohol content, citric acid content and malic acid content as independent variables and the chemical shift of hydrogen bonding as the dependent variable.

Multivariate nonlinear regression analysis is a holistic analysis of the degree of influence of each explanatory variable that we used to explain the relationship between comprehensive hydrogen bonding of Chinese rice wine and ingredients. Therefore, the multi-nonlinear stepwise regression method was applied. A mathematical model was selected to explain the quantitative relationship between hydrogen bonding and factors. The coefficient $R^2$ was 0.925. The variance analysis of the equation yielded an $F$-value of 370.599 and a $p$-value of 0.000. The equation is extremely significant. Then it is possible that the developed model can predict the age of Chinese rice wine. Because there is a strong correlation between the strength of hydrogen bonding and Vintage wine. And the overall hydrogen bonding strength of Chinese rice wine can be calculated from this developed model, then the wine age can be inferred.

The regression model can be stated as:

$$Y = 3.969 + 0.39[J] - 0.346[N] + 0.768[P] + 0.150[N^2]$$
$$-1.179E - 5[J^2] - 0.055[N^3] + 1.568[P^2] + 0.001[J^2N]$$
$$-0.005[J^2P] - 0.022[JNP]$$

(5)

In the formula: [$J$] is alcohol content, [$N$] is citric acid content, [$P$] is malic acid content.

### 4.2. Validated experiment

Ten different typical Chinese rice wines were purchased from the market as a group of new samples. Then we measured the alcohol content, critical acid content, and malic acid content in these new samples and put these data to the equation model that has been built. The hydrogen bonding strength of Chinese rice wine should been calculated. The results are shown in Table 4.

The error range was between $-0.02\%$ and $0.18\%$, proving this developed model fitted the strength of hydrogen bonding in Chinese rice wine.

### 5. Conclusions

This study of hydrogen bonding in Chinese rice wine to control mouthfeel is a promising strategy. The index of hydrogen bonding strength was $\Delta \delta$ and $\eta$. Most of flavors, such as sugars and acids, caused the association between water and ethanol molecules, whereas aldehydes did not. This is also consistent with the results of fluorescence spectroscopy and it identifies more flavors than it determines the order of influence (Qiao, Zhang, & Wang, 2013). The value of the $\Delta \delta$ based on a carboxyl (-COOH) group was estimated to be 0.123, and that of a hydroxyl (-OH) group was about 0.011–0.018. The two peaks of water and ethanol combined into single peak when adding citric acid. Citric acid promoted proton exchange between water and ethanol. A more stable association among alcoholic and water molecules was built when alcohols were present. Through PB text, the influencing factors from strong to weak on the strength of the hydrogen bonding were citric acid, malic acid, maltose, benzaldehyde, ethyl-lactate, isoamyl alcohol, 2-phenylethyl, and ethyl acetate. The regression equation can fit the hydrogen bonding strength of Chinese rice wine. Validation experiment results show that determined regression equation can fit the hydrogen bonding strength in Chinese rice wine ($R^2 = 0.925$).

### Disclosure statement

No potential conflict of interest was reported by the authors.

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