Research Article

Preliminary Study on Ultrasonic Ageing Zhenjiang Vinegar Mechanism Based on Maillard Simulation System

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In this study, ultrasonic technology was used to treat Zhenjiang vinegar, and the effects on the physicochemical characteristics of Zhenjiang vinegar were investigated. The influences of ultrasound time and power on the number of induced hydroxyl radicals and superoxide radicals were also investigated. Besides, the novel simulation system of the Maillard reaction was built to research the effects of different ultrasonic times and power treatment on Zhenjiang vinegar. The results show that, under the conditions of ultrasonic treatment, the changes of Zhenjiang vinegar physiochemical index, such as color, reducing sugar, and amino acid, are consistent with those of natural ageing. In addition, ultrasound can produce a cavitation effect and cracking water molecules to produce hydroxyl radicals and superoxide radicals, so as to achieve the ageing effect of vinegar.

1. Introduction

Fermented vinegar has widely been used in China and other Asian countries as a food condiment for hundreds of years [1, 2]. Zhenjiang Vinegar, which is one of the most famous traditional fermented vinegar types in China, is world-famous for its special elegant and complex aroma [3]. It is widely accepted like wine because of the harsh taste, pungent smell, and some possible harmful side-effects; fresh vinegar needs a specific process known as ageing in which a specific time period was used to produce high-quality vinegar [4]. Traditionally, ageing vinegar in barrel ageing systems is a common practice used for improving vinegar quality and stability. However, considering the disadvantages of natural ageing such as high production cost and long time period, it is critical and essential to study innovative ageing technology.

Recently, scientists have put great attempts into promoting brand new ageing techniques and enhancing present techniques. Present studies showed that there are diverse physical methods displaying considerable potential for accelerating the ageing process, including ultrasonic wave, electric fields, ultra-high pressure, and gamma irradiation [5–10]. It was found that the ultrasound (16–60 kHz) was adept to expedite oxidation, condensation, and polymerization of ethanol, aldehydes, esters, and olefins in wines [11]. Zheng et al. [10] found an optimum treatment for speeding up fresh red wines ageing, which enhanced wines with excellent taste and flavor. Reference [12] found that the high-pressure intervention in the scope from 80 MPa to 120 MPa not beyond 2 hours was shown to considerably improve the wine taste. Better apprehension of each divisor that impacts the quality of vinegar ageing process combined the economic and operational reasons; ultrasonic is a potential physical method to accelerate the vinegar ageing process [13].

Ultrasound, especially with high power and low frequency, has the advantage of pollution-free and being speedy. Over the last decades, abundant works have
2.1. Chemicals. Chemicals used in this study were furfural, n-Octane, fructose, glycine, methylene blue, vanillin, anhydrous ethanol, and aniline which were purchased from Sinopharm Group Chemical Co., Ltd. In this study, all chemicals and solvents used were of analytical grade.

2.2. Samples. The simulated Maillard reaction system was constructed to be 100 mL of 0.1 mol/L fructose and glycine aqueous solution. All the vinegar samples of different storing times used in this study were collected from Jiangsu Hengshun Vinegar Co., Ltd. Samples of vinegar were divided into 2 kinds of different treatments. One is the naturally aged vinegar samples, which were aged traditionally for different periods such as 12, 18, 32, and 44 months and kept in glasses. The samples of fresh and aged vinegar were stored at 4°C in a refrigerator. Samples with ultrasonic were treated according to the method of Wang et al. [9].

2.3. Color Evaluation. Color is one significant sensory characteristic of vinegar, which affects the consumers’ overall acceptability. The change of color can well reflect the process of Maillard reaction. Color measurement is measured by Hunterlab Spectrophotometer. The $L^*$, $a^*$, and $b^*$ values (CIELAB parameters) were monitored by using the software CromaLab [26], which better test the color of wines and permit better differentiation [27–30]. In this study, the CIE Lab system was used to define the color of vinegar. Record the color difference value of the vinegar $L^*$, $a^*$, and $b^*$, measured three times, and take the average. Value of $L^*$ indicates the brightness, the value of 0–100; the greater the value the greater the brightness; $a^*$ represents the values of red/green, positive partial red, and negative partial green; $b^*$ represents the yellow/blue value, positive yellow, and negative blue. The value $\Delta E^*$ is measured as the Euclidean distance between two points in the three-dimensional space defined by $L^*$, $a^*$, and $b^*$ and tells from color differences:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}. \quad (1)$$

2.4. Determination of Reducing Sugar. The content of reducing sugar in the samples was analyzed as stated in the GB/T 5009.7–2016 method including different ages of traditional aged vinegar and optimum ultrasonic experimental conditions treated vinegar (ultrasonic power 50 W/100 mL, time 75 min, and ethanol addition 0.75% (V/V)). All vinegar samples were determined in triplicate.

2.5. Determination of Free Amino Acid. Automatic amino acid analyzer was exploited to measure the content of free amino acids in the vinegar samples. The vinegar sample to be tested was diluted 20 times with 1% sulfosalicylic acid solution, centrifuged at 10000 rpm for 15 min, and the supernatant was clarified through 0.22 μm microporous membrane filter for the determination of the use of the machine.

Amino acid analysis chromatographic conditions are fluorescence detector, 4.0 × 125 mm C18 column; column temperature, 40°C; flow rate, 1.0 mL/min; and detection wavelength, Ex 440 nm, Em 570 nm.

2.6. Determination of Hydroxyl Radicals. The hydroxyl radical evoked by the cavitation of ultrasound was assessed indirectly by using methylene blue as the radical scavenger and ultraviolet-visible spectrophotometer as the testing method. [31, 32] The methylene blue was handled with
dissimilar ultrasonic treatments and the maximum absorption wavelength was measured by UV-visible spectrophotometer full-wavelength scanning. The hydroxyl radical produced by ultrasound was also measured by the decrease of absorbance at the maximum absorption wavelength.

2.7. Determination of Superoxide Radicals. Vanillin aniline fluorescent reagent synthesis method: 1.5 g vanillin was dissolved in 20 mL of anhydrous ethanol; 0.9 g of aniline was added dropwise, refluxed at 80°C, for 5 h; most of the solvent was distilled off; and a pale yellow solid was obtained after cooling. Recrystallize from anhydrous ethanol and vacuum dry. $2 \times 10^{-4}$ mol/L vanillin aniline ethanol solution was configured.

In a 10 mL colorimetric tube, add 2 mL of vanillin aniline ethanol solution, 2 mL of sonicated aqueous solution, and 1.5 mL of Tris-HCL buffer solution and bring the volume to the mark. The fluorescence intensity was measured under the excitation wavelength of 282 nm, and the emission wavelength of 340 nm. Then dilute to the final stages of the reactions [34, 35]. The fluorescence intensity of each substance under ultrasonic induction; therefore, the absorbance at 420 nm and 294 nm ($\Delta \text{Abs}$) of each solution was recorded on a UV software (Beijing Beifen-Ruili) spectrophotometer with a 1 cm path length quartz cell. All samples were done three times in parallel.

2.8. Determination of Maillard Reaction Process. In view of a large variety of components in vinegar, it is difficult or even impossible to find out the specific changes and mechanisms of each substance under ultrasonic induction; therefore, the content of vinegar should be higher when studying the ultrasonic accelerated vinegar ageing mechanism. The representative substances that have an important influence on the quality of vinegar were studied as objects. The results of this study are also easy to compare with the relatively clear mechanism of change in the natural maturation process of these substances. The Maillard reaction is one of the most important reactions in the vinegar ageing process. Therefore, it is necessary to construct the Maillard simulation system to study the mechanism of ultrasound on its action.

Maillard reaction model system is the 100 mL of 0.1 mol/L fructose and glycine solution. The degree of browning and the amount of intermediate product of the Maillard reaction were measured according to the way of Gu et al. [33]. According to the literature, absorbance at 294 nm was served to examine the formation of the intermediate compounds of the Maillard reaction and the size of the UV-visible absorption at 420 nm in the Maillard reaction system is related to the final stages of the reactions [34, 35]. The browning indices were evaluated by spectrophotometry, recording the absorbance at 420 nm and 294 nm ($A_{420 \text{ nm}}, A_{294 \text{ nm}}$) of each model system against distilled water. The results were recorded on a UV software (Beijing Beifen-Ruili) spectrophotometer using a 1 cm path length quartz cell. All samples were done three times in parallel.

2.9. Data Analysis. All model systems were prepared in duplicate and the analysis was performed in triplicate. Excel 2010, Origin 8.0, and SPSS Statistics 17.0 were applied to complete the data analysis. The significant differences between the different samples were obtained through a one-way ANOVA analysis with a level of $P < 0.05$.

3. Results and Discussion

3.1. Color Evaluation. As Table 1 shows, the color of the vinegar was significantly affected by the ageing time and different storage times have a significant effect on vinegar $L^*$, $a^*$, $b^*$. For the traditionally natural aged vinegar samples with the extension of storage time, the values showed a downward trend. Lightness is the attribute of a visual sensation according to which a given visual stimulus appears to be more or less light, ranging from “light” to “dark” [26]. Table 1 shows that all the $L^*$ values of vinegar samples were investigated varying from 2.07 to 0.49 units; $a^*$ values were between 5.54 and 1.12 units. Besides, $b^*$ values were between 4.21 and 0.95. According to Oszmianski [36, 37], these minute dissimilarities between the vinegar samples were by reason to the oxidation instead of the enzymatic treatment. However, the previous literature [38, 39] reported that the ultrasound can accelerate oxidation, esterification, and other reactions. The values of the ultrasonic treated vinegar ($L^* = 2.41, a^* = 6.37, b^* = 5.25$) were higher as compared to nonultrasonic treated vinegar. The value of $\Delta E^*$ was found between the ultrasonic treated vinegar and fresh vinegar ($\Delta E^* = 0.94$). These colorimetric data present that the ultrasonic treated vinegar samples exhibited mainly red and yellow tint, which is also a consumer favor. The result shows that a tester can distinguish the color of these vinegar samples. In addition, the reducing sugar in vinegar declining with the extension of ageing time, combined with the later experimental verification Maillard reaction products, increased after ultrasonic treatment, which is consistent with the results of the color change of vinegar treatment.

3.2. Effect of Ultrasound on Hydroxyl Radicals. Figures 1 and 2 show the methylene blue solution UV-visible absorbance curve along with the change of ultrasonic time and power. Besides, these results showed that methylene blue solution has the maximum absorption peak at 664 nm and gradually decreases with the increase of ultrasonic time and power. These results show that the concentration of methylene blue solution gradually decreased to prove that the ultrasonic treatment of hydroxyl radicals generated and hydroxyl radical concentration gradually increased over ultrasonic time and power. As shown in Figure 3, it is not hard to see that in the initial period of time, the production of hydroxyl radical increased rapidly. As the time extended to 75 min later, the rate of increase slowed down. The reason here might be that the increased time for ultrasonic treatment increases the vinegar temperature too high. The vapor pressure in bubbles increases and consequently bubbles closed to enhance the buffer effect and the cavitation role becomes weakened. Therefore, in this study, the ultrasonic time is 75 min.

As shown in Figure 4, with the increase of ultrasonic power, the intensity of the ultrasonic wave generated in unit
time increases, and the cavitation effect produced is stronger [40]. Therefore, when the cavitation bubbles generated by the aqueous solution are crushed, high temperature and high-pressure environment occurs; the number of free radicals generated will be increased sufficiently.

Hydroxyl radical is the most active oxygen free radical [41]. Ultrasound-induced hydroxyl radicals can cause oxidation of sugars, amino acids, proteins, and esters in vinegar [42]. The production of free radicals can speed up the breakage and formation of various substances in vinegar, accelerate the ripening of vinegar, and shorten the ageing time [43].

3.3. Effect of Ultrasound on Superoxide Radica. From Figures 5 and 6, it can be seen that superoxide radicals in the solution that has not been sonicated are not detected; however, a large number of free radicals are generated in the aqueous solution after sonication, which proves that the ultrasonic treatment produces superoxide radicals in the solution and has a significant effect. According to Figure 5, the number of superoxide radicals generated increases with the increase of the ultrasound time and reaches the maximum level at 40 min after sonication. Figure 6 shows that the number of superoxide radicals produced by ultrasound gradually decreases with the increase of ultrasonic power of 10 W/100 mL. The generation of superoxide radicals is due to the action of ultrasound on the oxygen molecules in the aqueous solution. The reason for the above research results is that, on the one hand, it is finally converted into hydroxyl radical through a series of reactions which is consistent with the results of the previous studies on hydroxyl radicals, and, on the other hand, superoxide anions can dismutate to produce hydrogen peroxide and oxygen [44].

Superoxide radicals in water can be considered as a base, which can accept H+ from a protonated superoxide radical HOO−. In one way, the mechanism is largely to convert it into other more active oxygen ions. The base achieves the promotion of chemical reaction.

3.4. Effect of Ultrasound on Browning Degree of Maillard Reaction. Table 1 shows that after the ultrasonic treatment, the color of the vinegar becomes lighter. The content of

| Age (month) | L*           | a*           | b*           | ΔE*        | Reducing sugar (g/100 mL) |
|-------------|--------------|--------------|--------------|------------|--------------------------|
| 0           | 2.07 ± 0.38c | 5.54 ± 0.17d | 4.21 ± 0.23bc| 0.00       | 4.06 ± 0.15d             |
| 12          | 1.24 ± 0.24b | 5.31 ± 0.20d | 3.23 ± 0.10bc| 0.85       | 3.39 ± 0.14c             |
| 18          | 0.89 ± 0.02c | 4.56 ± 0.13c | 2.05 ± 0.11b | 1.03       | 2.51 ± 0.16ab            |
| 32          | 0.64 ± 0.04c | 2.22 ± 0.11c | 1.83 ± 0.09bc| 2.79       | 2.92 ± 0.18b             |
| 44          | 0.49 ± 0.02d | 1.12 ± 0.02c | 0.95 ± 0.02c | 1.00       | 2.24 ± 0.14b             |
| Ultrasonic  | 2.41 ± 0.01d | 6.37 ± 0.02d | 5.25 ± 0.13d | 0.94       | 3.03 ± 0.45c             |

Table 1: Effect of color change and reducing sugar in the natural ageing process of vinegar and ultrasound treated vinegar.

![Figure 1: UV-visible spectra of methylene blue solution at different ultrasonic times.](image)
reducing sugar decreased by nearly 50% from 4.06 g/100 mL to 2.24 g/100 mL. Besides, during the traditionally natural ageing process, the changes of vinegar free amino acids are shown in Table 2. It is revealed that as the ageing time becomes longer, the content of free amino acids decreased by 1041.35 mg/100 mL down to 757.87 mg/100 mL. Moreover, after ultrasonic treatment, the content of amino acids in vinegar decreased, consistent with the trend of natural ageing. And ultrasound can produce lots of hydroxyl radicals. Combined with the above experimental results analysis, we speculate that the changes are related to the progress of the Maillard reaction. The monosaccharides in Zhenjiang vinegar is mainly glucose and fructose. And glycine in the vinegar free amino acid mass fraction is relatively high. In particular, fructose-glycine can form a kind of Amadori compounds [45, 46]. Therefore, in this experiment, the fructose-glycine simulation system was used to verify that ultrasound may speed up the Maillard reaction. As far as we know, the melanoidin produced during the Maillard reaction can make the color of the vinegar darker, so that the degree of browning can be directed to the extent of the Maillard reaction.

As can be seen from Figure 7, the degree of browning of the fructose-glycine simulation system gradually increased with the ultrasonic time rise. And the results showed that...
Table 2: Changes in the free amino acids in the vinegar during the ageing process.

| Amino acids | Fresh | 12 months | 18 months | 32 months | 44 months | Ultrasound treated | Taste       |
|-------------|-------|-----------|-----------|-----------|-----------|--------------------|-------------|
| Asp         | 82.43 | 67.24     | 51.90     | 48.03     | 46.76     | 52.22              | Sour        |
| Glu         | 115.64| 114.61    | 120.12    | 127.85    | 134.72    | 128.96             | Fresh       |
| Val<sup>E</sup> | 77.15 | 66.66     | 63.48     | 58.54     | 48.86     | 65.65              | Astringent  |
| Leu<sup>E</sup> | 107.87| 92.73     | 85.18     | 82.42     | 78.73     | 89.50              | Astringent  |
| Gly         | 91.65 | 70.70     | 66.49     | 63.85     | 61.84     | 64.73              | Strong sweet|
| Ala         | 118.64| 103.56    | 94.81     | 90.06     | 83.65     | 94.46              | Strong sweet|
| Thr<sup>E</sup> | 42.13 | 41.30     | 38.39     | 35.48     | 32.73     | 38.57              | Sweet       |
| Ser         | 52.49 | 45.15     | 44.46     | 41.94     | 40.89     | 42.27              | Sweet       |
| Met<sup>E</sup> | 13.36 | 14.39     | 13.65     | 12.57     | 9.29      | 12.06              | Sweet       |
| Phe<sup>E</sup> | 34.18 | 31.83     | 45.29     | 39.79     | 45.45     | 39.56              | Sweet       |
| Pro         | 61.46 | 38.97     | 43.16     | 32.81     | 35.68     | 36.89              | Sweet       |
| Lys<sup>E</sup> | 45.65 | 36.55     | 37.30     | 27.95     | 21.19     | 27.21              | Slight sweet|
| Ile<sup>E</sup> | 47.59 | 51.91     | 45.73     | 40.61     | 37.45     | 46.39              | Bitter      |
| Arg         | 62.87 | 50.75     | 32.20     | 34.40     | 29.21     | 33.86              | Bitter      |
| Tyr         | 49.81 | 35.48     | 30.72     | 31.44     | 27.78     | 31.29              | Slight bitter|
| His         | 27.77 | 15.00     | 16.18     | 11.57     | 10.12     | 16.44              | Slight bitter|
| Trp<sup>E</sup> | 10.48 | 14.32     | 17.63     | 17.54     | 13.52     | 14.88              | Slight bitter|
| **Total**   | 1041.35 | 891.18   | 836.69    | 796.85    | 757.87    | 843.94             |             |

<sup>E</sup> represents the essential amino acids, <sup>T</sup> represents the total amino acids, and <sup>N</sup> represents the nonessential amino acids.
the degree of browning increased slowly after 60 min of sonication. In Figure 8, with the ultrasonic power rise, the degree of browning of the simulated system showed a tendency to increase first and then decrease and reached the maximum when the ultrasonic power density was 50 W/100 mL. This result is consistent with our previous experimental conclusions [9]. The apparent increase in the absorbance indicates that the midterm product of the Maillard reaction is accumulating. This difference could be related to the acoustic cavitation. Since this cavitation activity can be viewed as a dramatic concentration of acoustic energy resulting in localized high stresses, temperatures, and/or fluid velocities, its biological consequences should be understood by those who are trying to either optimize or minimize its effects [47]. Additionally, the violent collapse of bubbles can generate exceedingly steep heat and pressure, which can produce free radicals and so forth [48], and then small molecules can rearrange [4]. The results show that the ultrasonic wave can strengthen the fructose-glycine Maillard reaction, promote the system browning, and induce the middle stage and the formation of advanced stage products.

3.5. Effect of Ultrasound on the Amount of the Intermediate Products in Maillard Reaction. In the Maillard reaction process, there are some noncolor intermediates, such as small molecules of ketones and aldehydes, which are also important indicators of the Maillard reaction [34]. As can be seen from Figures 9 and 10, without ultrasonic processing, the intermediate product of the Maillard reaction has not been detected in the simulation system solution. Applying the ultrasonic vinegar sample, however, the intermediate product of the reaction significantly altered. Results (Figures 9 and 10) showed that accompanied with the ultrasonic time and ultrasonic power rise, the median product of the Maillard reaction increased gradually, reaching the maximum at 75 min and 50 W/100 mL, respectively. The change of Maillard intermediates may largely be due to the fact that ultrasound can induce some oxygen ions and hydroxyl radicals in the aqueous solution [49], and the production and rearrangement of these oxygen ions and hydroxyl radicals can promote the Maillard reaction of the fructose-glycine simulation system, leading to the production of intermediates. The degree of browning and the number of intermediate products in the ultrasonic treatment
simulation system can be used to prove the ultrasonic catalytic Maillard reaction.

4. Conclusion

In this study, under the conditions of ultrasonic processing, the changes of Zhenjiang vinegar physiochemical index, such as color, reducing sugar, and amino acid, are consistent with those of natural ageing. In addition, the sonicated vinegar produces hydroxyl radicals, which generally appear to increase with increasing ultrasound time and ultrasound power. The present study reveals that the mechanism of ultrasonic ageing lies in the cavitation of ultrasound to induce the generation of radicals, such as hydroxyl radicals and superoxide radicals, catalyze the oxidation reaction in vinegar, and verify it by the fructose-glycine Maillard simulation system. The results showed that ultrasonic treatment had positive impacts on the vinegar ageing process. For the future, much research is needed to determine the exact role of ultrasound in vinegar ageing.

Data Availability

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

All authors declare no conflicts of interest.

Authors’ Contributions

Xiaoke Ma and Tingting Li contributed equally to this study.

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