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Effect of ultrasound-assisted fermentation on physicochemical properties and volatile flavor compounds of Chinese rice wine

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ABSTRACT

The traditional fermentation method for Chinese rice wine (RW) production has some limitations such as difficulty in controlling quality, long brewing time, and thin taste. In this study, ultrasonic technology was adopted by adding enzyme with the aim of improving the fermentation efficiency and volatile flavor quality of RW. Results showed that the ethanol yield was increased by 23.53 % and the fermentation time was reduced by nearly 2 days by ultrasonic treatment of (one-time, 28 kHz, 1 h, 35 W/L) on the first day. Flavor analysis (following the ultrasonic treatment) showed that the flavors, especially the esters and alcohols in RW, also enhanced most significantly. The contents of isobutyl acetate, ethyl butyrate, ethyl hexanoate, and phenethyl acetate increased by 58.03 %, 107.70 %, 31.84 %, and 18.71 %, respectively. The results showed that the brewing process could be simplified, brewing time could be reduced and volatile flavor of RW could be improved by ultrasound.

1. Introduction

Traditional Chinese rice wine (RW) has been popular in China for about thousands years. RW brewing refers to a process of simultaneous saccharification and solid-state fermentation by mixed saccharifying starters, such as wheat starter and distiller's yeast [1]. However, a wide variety of microorganisms are present in the medium during brewing, and the quality of starter varies from region to region, which leads to an uncontrollable fermentation process and inconsistent product quality of RW [2]. In addition, traditionally fermented RW also has some limitations including thin taste, long brewing time, slow product conversion, and limited application of high-technology [3,4]. Traditional research methods mainly focused on the screening of bacteria or process optimization [5,6]. Although some work has been done to help address the limitations of traditional brewing, there are still lots to be done.

Ultrasound, as a new non-thermal physical processing technique, with its excellent equipment and relatively advanced theoretical research [7,8], as well as its low energy consumption, high efficiency, and low thermal effects, has extensively been utilized to overcome the drawbacks of conventional fermentation [9–12]. Some studies have showed that the application of ultrasonic technology in brewing can enhance the fermentation efficiency and quality [13]. Schläfer et al. noticed that the ethanol yield after

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ultrasound treatment increased by 2.7 times compared to the control. They also realized that ultrasound-treated samples yielded about 29 % more ethanol than heat-treated samples [14]. Zheng et al. found that the total ester content of steeped greengage wine after ultrasonic treatment (45 kHz, 360 W, 15 °C for 30 min) increased by 24.59 % compared with the control [15].

In addition, studies have shown that compared with yellow wine, the flavor of RW is relatively simple and does not require the typical aroma and taste given by wheat koji [16]. Therefore, on the basis of liquefaction method, it is theoretically feasible to further use enzyme preparations and pure yeast to completely replace koji in brewing RW, and it can simplify the brewing system, facilitate microbial control and quality control of RW products, and make it easier for mechanized and automated production [17].

At present, research on ultrasound-assisted fermentation is mainly focused on the pure culture fermentation process in the laboratory. Therefore, this study established an enzymatic brewing process for RW by simulating actual production. By adding α-amylase to liquefy the starch, it is hydrolyzed from long chain starch to short chain dextrin and oligosaccharides, to assist saccharifying enzyme in converting starch into fermentable sugars, and analyzed the impact of ultrasound on the physicochemical properties and volatile compounds during the fermentation process.

2. Materials and methods

2.1. Materials

Polished rice was supplied by Danyang Jiahe Rice Industry Co., Ltd (Jiangsu, China). α-Amylase and saccharifying enzyme were bought from Shanghai Yuanye Biotechnology Co., Ltd (China). Rose Bengal Agar was acquired from Beijing Landbridge Technology Co., Ltd (China). 2-Octanol was bought from Shanghai Macklin Biochemical Co., Ltd (China). All other reagents were of analytical grade and obtained from Sinopharm Chemical Co. Ltd (Shanghai, China).

2.2. Yeast strains and culture conditions

The *S. cerevisiae* 1048 used in the research was bought from China Center of Industrial Culture Collection (Beijing, China). The strain was inoculated onto YPD medium and cultured at 30 °C with shaking (160 r/min).

2.3. Ultrasonic equipment

The multi-frequency scanning ultrasonic equipment used in this research (developed by our research group, Jiangsu University) is shown in Fig. 1. This instrument has two ultrasonic emission modes: fixed frequency and sweeping frequency.

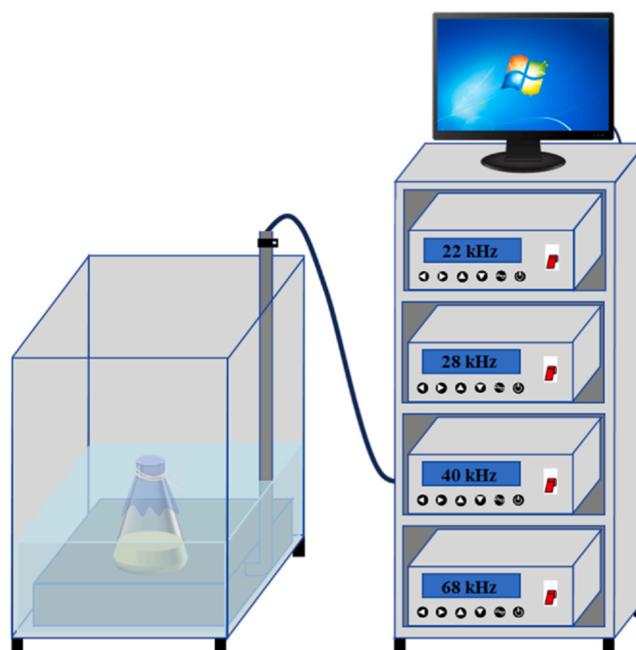

Fig. 1. Pulsed multi-frequency scanning ultrasonic equipment. 1: computer control system; 2: ultrasonic generator; 3: ultrasonic pool; 4: sample treatment vessel; 5: water; 6: ultrasonic transducer.

Fig. 2. Enzymatic brewing process of rice wine in laboratory.

2.4. Enzymatic brewing process and key points

2.4.1. Enzymatic brewing of RW in the laboratory

The soaked rice was cooked in a pressure cooker. After cooling, liquefied and saccharified. Finally, proceed with fermentation. The enzymatic brewing process of RW in the laboratory is displayed in Fig. 2.

2.4.2. Key points

(1) Raw material: polished rice without impurities or obvious broken rice;
(2) Soaking: Add water to soak raw materials after washing. Soak the rice until there is no white heart in the center, which will be easily crushed;
(3) Cooking: Thoroughly wash the soaked ingredients and place them in a high pressure cooker. Steam until the rice is fragrant, loose, soft, transparent, and non-sticking;
(4) Liquefaction: After cooling, α-amylase is added for liquefaction;
(5) Saccharification: After liquefaction is completed, saccharifying enzymes are added for saccharification;
(6) Fermentation: Control the appropriate ratio of material water ratio, the inoculum ratio, fermentation temperature, and fermentation time for fermentation.
(7) Sample collection: The specimen was collected after centrifugation at 8000 r/min for 10 min, and refrigerated at − 80 °C before oenological analysis.

2.5. Establishment of enzymatic brewing process

2.5.1. Determination of liquefaction conditions

The effect of liquefaction temperature (50, 60, 70, 80, 90, and 100 °C), liquefaction time (40, 60, 80, 100, 120, and 140 min) and different amount of α-amylase (20, 40, 60, 80, 100, and 120 U/g) on the dextrose equivalent (DE) value of rice liquid mash were studied under the control conditions of liquefaction temperature of 80 °C, liquefaction time of 60 min and α-amylase addition amount of 60 U/g.

2.5.2. Determination of saccharification conditions

The effect of saccharification temperature (50, 55, 60, 65, 70, and 75 °C), saccharification time (1, 2, 3, 4, 5, and 6 h) and varied amount of saccharifying enzyme (50, 100, 150, 200, 250, and 300 U/g) on the reducing sugar content of rice liquid mash were studied under the control conditions of saccharification temperature of 65 °C, saccharification time of 4 h and saccharifying enzyme addition amount of 200 U/g.

2.5.3. Determination of fermentation conditions

The effect of material to water ratio (1:0.5, 1:1, 1:1.5, 1:2, 1:2.5, and 1:3 g/mL), the inoculum ratio (2.5 %, 5 %, 7.5 %, 10 %, 12.5 %, and 15 % v/v), fermentation temperature (16, 20, 24, 28, 32, and 36 °C), and fermentation time (2, 3, 4, 5, 6, and 7 d) on the alcohol content of RW under the control conditions of the ratio of material to water at 1:1 g/mL, the inoculum ratio of 10 % (v/v), fermentation temperature of 28 °C, and fermentation time of 4 d.

2.6. Optimization of ultrasonic conditions

Under the control conditions of ultrasonic mode of 1 time, ultrasonic phase of the 1st day, ultrasonic frequency of 28 kHz, ultrasonic time of 1 h, ultrasonic power of 30 W/L, the effects of ultrasonic mode (1, 2, 3, 4, and 5 times), ultrasonic phase (0, 1st, 2nd, 3rd, 4th, and 5th days), and ultrasonic frequency (22, 28, 40, and 68 kHz), ultrasonic time (0.5, 1, 2, 3, 4, and 5 h) and ultrasonic power (25, 30, 35, 40, and 45 W/L) on the alcohol content of RW were studied.

2.7. Determination of DE value

2.7.1. Determination of reducing sugar content

The 3, 5-dinitrosalicylic acid (DNS) method was used to determine the reducing sugar [18]. 1 mL sample was mixed with 2.0 mL DNS reagent and placed in boiling water for 5 min. After, it was cooled quickly under running water, and distilled water added to 25 mL, and vortexed. Absorbance was measured at 540 nm. The reducing sugar content was calculated by using the formula (1):

$$Y_1 = c \times v/m \times 100\% \tag{1}$$

where Y_1 is the reducing sugar content (%); c is the reducing sugar concentration (g/mL); v is the sample volume (mL); m is the sample mass (g).

2.7.2. Determination of solid content

The solid content was measured by constant weight method at 105 °C. Appropriate amount of samples were put into a constant mass drying beaker and baked in an oven at 105 °C until constant weight. The solid content was calculated by the formula (2):

$$Y_2 = m_2/m_1 \times 100\% \tag{2}$$

#where Y_2 is the solid content (%); m_1 is the mass before constant weight (g); m_2 is the mass after constant weight (g).

2.7.3. Determination of DE value

The DE value is expressed as the glucose equivalent of the reducing sugar in the sample, that is, the percentage of the reducing sugar to the dry matter of the syrup, and the DE value was calculated by the following formula (3):

$$Y = Y_1/Y_2 \times 100\% \tag{3}$$

where Y is the DE value (%); Y_1 is the reducing sugar content (%); Y_2 is the solid content (%).

2.8. Determination of physiochemical properties

The alcoholic strength, reducing sugar, total acid, and pH of the RW were determined by the official methods outlined in the Chinese National Standard (GB 13662-2018).

2.9. Determination of volatile flavor components

The profile of the volatile flavor components was quantified by using head-space solid-phase microextraction (HS-SPME) combined with GC-MS instrument. The injection port was operated in a split less model. Each sample (8 mL) was put in a 20 mL SPME glass vial together with 1 g of sodium chloride and 10 μL of the internal standard 2-octanol. The samples were equilibrated at 50 °C for 5 min and extracted for 25 min to adsorb volatile compounds. The GC-MS equipped with Rtx-WAX capillary column (30 m long × 0.25 mm internal diameter, 0.25 μm film thickness, Agilent Technology, USA) was used to measure the volatile compounds of every sample. The oven temperature program was as follows: 40 °C (5 min), 5 °C/min to 150 °C (2 min), 10 °C/min to 230 °C (10 min) and finally 20 °C/min to 240 °C (5 min). The flow rate of carrier gas (helium) was 1 mL/min. The ion energy for the electron impact (EI) was kept at 70 eV. The ion source temperature was set at 230 °C. The chromatograms were recorded by monitoring the total ion currents in the 30–550 mass range. The compounds were identified by matching the mass spectra with the NIST17 mass spectral database. Semi-quantification of the volatile compounds was calculated according to the formula [19]:

$$C_i = C_s \times A_i/A_s$$

where C_i is the relative concentration of analyzed sample (mg/L), *Cs* is the final concentration of internal standard in sample (mg/L), *Ai* is the peak area of analyzed sample, and *As* is the peak area of internal standard.

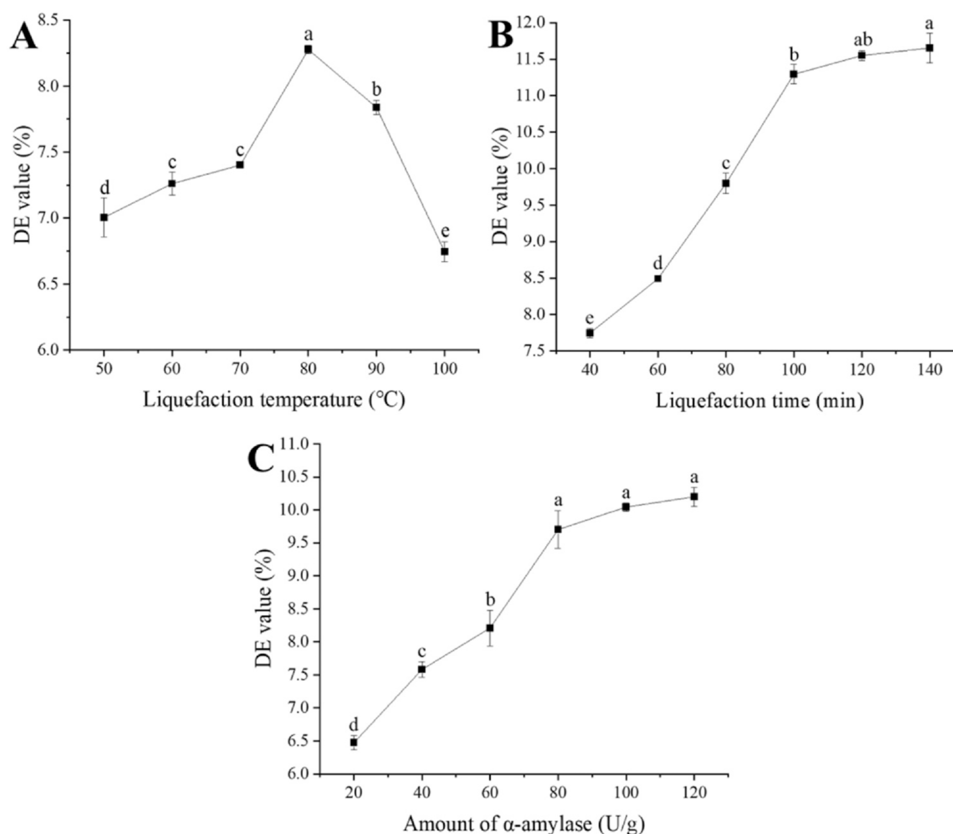

Fig. 3. Effects of different liquefaction conditions on DE value. A: liquefaction temperature; B: liquefaction time; C: amount of α-amylase. Different letters suggested significant difference within the same line ($p < 0.05$).

2.10. Statistical analysis

Statistical analysis was conducted using SPSS 26.0 (SPSS Inc., Chicago, Ill, U.S.A.). The data obtained was analyzed for variance (ANOVA), and the significance level was considered at $p < 0.05$. All experiments were done in triplicates and data were expressed as means ± standard deviations.

3. Results and discussion

3.1. Determination of liquefaction conditions

For the degree of liquefaction, it is commonly measured by the dextrose equivalent value (DE value) of the liquefied starch, which indicates the degree of starch hydrolysis and the degree of saccharification, and generally refers to the percentage of glucose (including all reducing sugars measured) in the measured syrup dry matter.

The activity of α-amylase, which plays a key role in the liquefaction of rice, is affected by temperature (Fig. 3A). With the increase of liquefaction temperature, the binding efficiency of α-amylase and substrate was improved. Therefore, DE value of rice liquefying mash increased with the increase of liquefaction temperature, reaching the maximum value (8.28 %) at the liquefaction temperature of 80 °C, which rapidly decreased when the temperature exceeded 80 °C. This is because too high temperature denatures the protein gradually, and the activity of amylase is reduced, thus affecting the hydrolysis efficiency of starch.

With the increase of liquefaction time, DE value showed an upward trend reaching 11.55 % at 100 min at first, and then tended to be stable (Fig. 3B). At the early stage of liquefaction, the substrate starch concentration was high, and α-amylase was fully combined with the substrate, and the starch molecules were decomposed into small oligosaccharides and glucose, and the DE value in the liquefaction mash increased rapidly. However, with the continuous hydrolysis of starch molecules, the concentration of substrate decreased, and the hydrolysis products also inhibited and passivated the activity of α-amylase, resulting in no further increase in the liquefaction DE value.

As shown in Fig. 3C, the DE value of rice liquefying mash rapidly enhanced with the increase of α-amylase addition when the amount of α-amylase was 20–80 U/g. When α-amylase added was 80 U/g, the DE value was 9.70 %, and then the DE value of rice liquid mash increased slightly and tended to be stable. When the amount of α-amylase is less, the substrate concentration is higher, so the enzyme can fully bind to the substrate. However, when the added amount of α-amylase exceeds a certain amount, since the

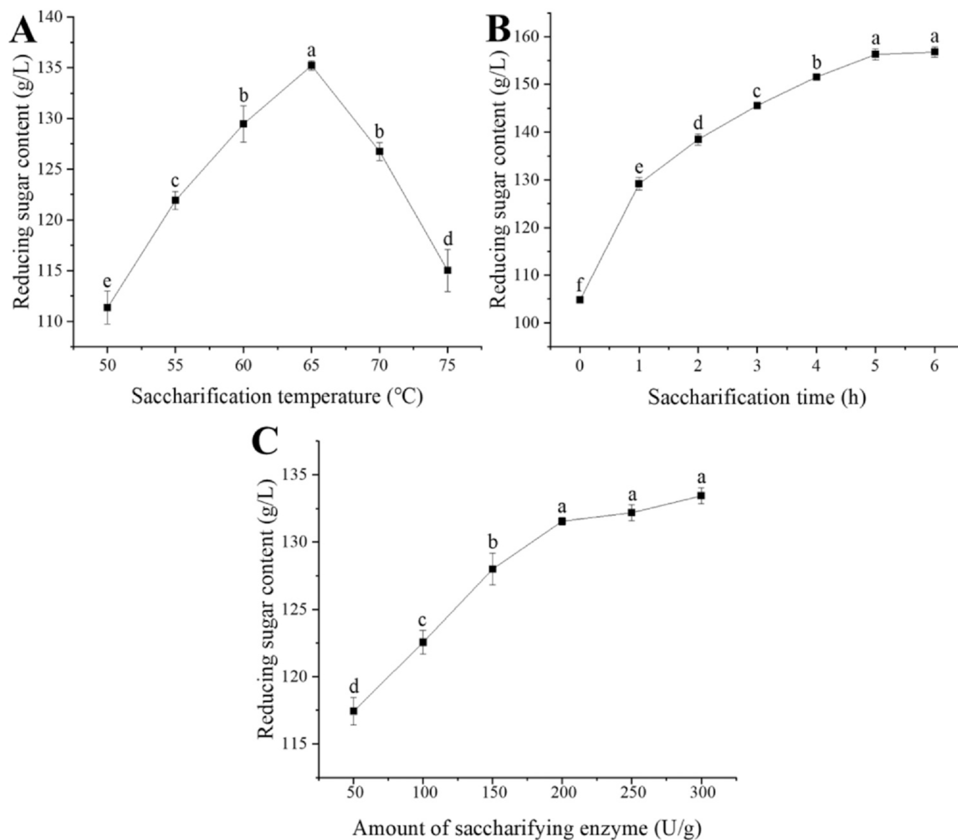

Fig. 4. Effects of different saccharification conditions on reducing sugar content. A: saccharification temperature; B: saccharification time; C: amount of saccharifying enzyme. Different letters suggested significant difference within the same line ($p < 0.05$).

substrate has fully combined with the enzyme and there is no excess substrate to react with the enzyme, the continued increase of α-amylase has little effect on the DE value.

In summary, the liquefaction temperature of 80 °C, the liquefaction time of 100 min and the addition amount of α-amylase of 80 U/g were selected as the liquefaction conditions.

3.2. Determination of saccharification conditions

On the basis of liquefaction, the effect of saccharification temperature, saccharification time and saccharifying enzyme on reducing sugar content were studied to determine the saccharification conditions.

The reducing sugar content in saccharification mash first enhanced and then decreased with the increase in saccharification temperature (Fig. 4A). The reducing sugar content reached its maximum value at 65 °C (135.22 g/L), but rapidly decreased when the temperature exceeded this temperature (65 °C). This is because the temperature affects the activity of saccharifying enzyme. When the temperature reached about 65 °C, the activity of saccharifying enzyme was the strongest, and the full contact between the enzyme and the substrate accelerated the reaction rate. When the temperature exceeded 65 °C, the protein began to denature, and the activity of the enzyme decreased accordingly, thus affecting the saccharifying enzyme rate and resulting in the reduction of the reducing sugar content in the saccharifying mash.

Fig. 4B showed the two stages of the saccharification process. At the first stage, the saccharification time was 0–5 h. As the saccharification time increased, the reducing sugar content showed a rapid increasing trend, and the reducing sugar content was 156.17 g/L at the 5th h. After the second stage, saccharification time was 5 h, and the reducing sugar content was almost unchanged with the extension of saccharification time. This is because in the first stage, the substrate concentration was higher, the substrate fully combined with the enzyme, and the reaction rate was faster, resulting in a rapid increase in the reducing sugar content. As saccharification progressed, the substrate in the mash gradually decreased, the saccharification rate also decreased, and the reducing sugar content slowly increased and tend to stabilize.

As shown in Fig. 4C, by increasing the amount of saccharifying enzyme, the reducing sugar content showed a rapid rise at first, and then stabilized. When the added saccharifying enzyme was between 50 and 200 U/g, the reducing sugar content increased rapidly as the amount of saccharifying enzyme increased. The reason for this phenomenon is that, when the amount of saccharifying enzyme added is small, the substrate concentration is high, and the enzyme can fully bind with the substrate. At this time, adding

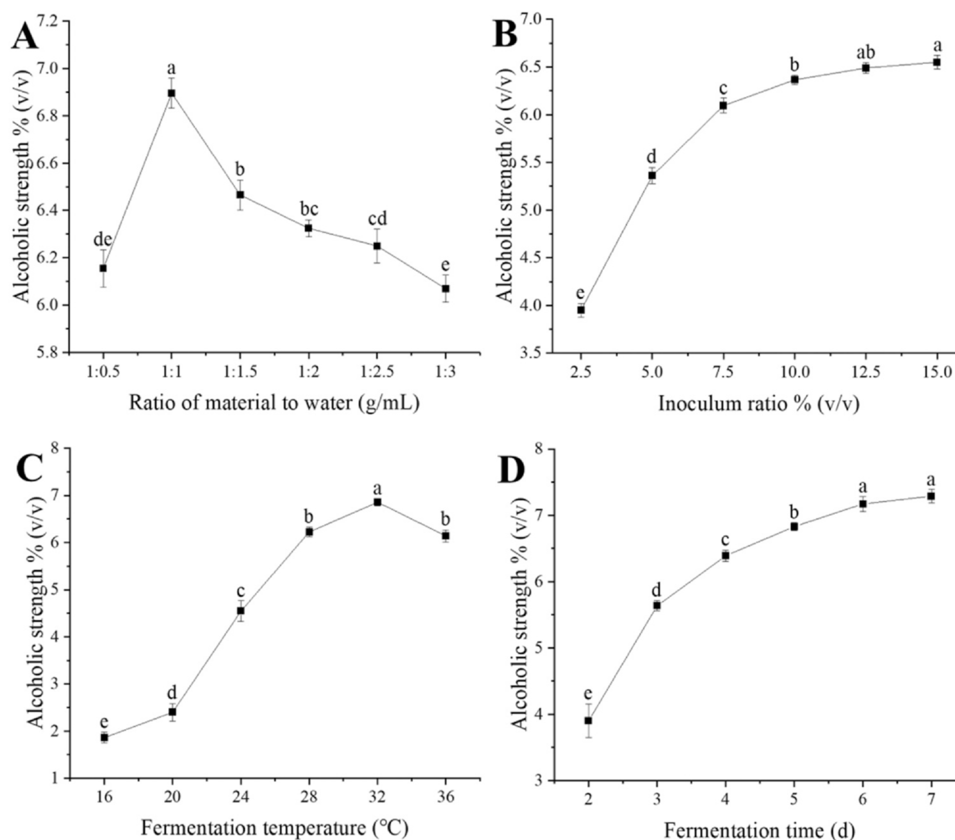

Fig. 5. Effects of different fermentation conditions on alcoholic strength of rice wine. A: ratio of material to water; B: inoculum ratio; C: fermentation temperature; D: fermentation time. Different letters suggested significant difference within the same line ($p < 0.05$).

saccharifying enzyme will accelerate the rate of saccharification reaction, and the reducing sugar content of saccharifying mash will also rapidly increase. When the amount of saccharifying enzyme added exceeded 200 U/g, the reducing sugar content remained basically unchanged. This is because, the substrate had fully combined with the enzyme, and further addition of saccharifying enzyme had no significant effect on the reducing sugar content.

In summary, the temperature of 65 °C, time of 5 h, and enzyme amount of 200 U/g were selected as the saccharification conditions for subsequent research.

3.3. Determination of fermentation conditions

Fig. 5A showed the effect of material water ratio on the alcoholic strength of RW. As added water increased, the alcohol content first increased, and then decreased. When the ratio of material to water was 1:1 g/mL, the alcoholic strength reached the maximum value, which was 6.90 % (v/v). This is because when the amount of water added is at a relatively low level, the substrate concentration in the fermentation mash increases, and the yeast is in a high osmotic pressure environment, which inhibits the growth and metabolism of the yeast, and thus reducing its fermentation activity, and consequently resulting in lower alcohol content [20]. When the amount of water added was at a relatively high level, it led to a decrease in the concentration of fermentable sugars in the mash, causing the fermentation to end prematurely and eventually resulting in a lower alcohol content and relatively low flavor substances in the RW.

With the increase in the amount of *S. cerevisiae* (inoculant) in RW fermentation, the alcoholic strength initially increased, and reached 6.37 % (v/v) when the inoculated amount was 10 % (v/v), and then stabilized (Fig. 5B). This is because when the amount of yeast (inoculant) is small, its reproduction/activity is slow, and so the sugar in the fermentation liquid cannot be fully utilized, resulting in low alcohol content. When the amount, however, is too high, because the yeast itself needs a lot of sugar for growth and reproduction, the sugar is not converted into ethanol, thus the alcohol content of RW is not increased.

Fig. 5C showed the influence of fermentation temperature on the alcoholic strength of RW. With the increase in fermentation temperature, the alcoholic strength of RW increased first, and then decreased. When the fermentation temperature was 32 °C, the alcoholic strength of RW was the highest, 6.86 % (v/v). This is because as temperature increases, the metabolism of yeast is accelerated, and the ability to produce ethanol is enhanced, leading to the increase in alcohol content of RW. However, when the fermentation temperature is too high, the *S. cerevisiae*, *Aspergillus* and other microorganisms in the fermentation liquid metabolize and propagate too quickly, which causes not only premature aging of bacteria, but also affect the microbial enzyme production

process and enzyme activity in the fermentation liquid; and thus, the alcohol content in the fermentation liquid is low, and also lead to sour and astringent taste of RW, thereby affecting the quality.

As fermentation time increased, the alcoholic strength of RW increased rapidly initially, then reached 7.17 % (v/v) on the 6th day, and then tended to stabilize (Fig. 5D). At the early stage of fermentation, *S. cerevisiae* may have converted sugars into ethanol through its own metabolism, leading to a rapid increase in the alcohol content of RW. Due to the lack of nutrition and the effect of alcohol, brewing yeast gradually dies, and thus the alcohol content remains basically unchanged.

The fermentation conditions of 1:1 g/mL (material to water), 10 % (v/v) inoculum, 32 °C (temperature) and 7 d (i.e. fermentation time) were used as the basis for further investigation on the influence of ultrasound in the brewing of RW.

3.4. Optimization of ultrasonic conditions

The influence of different ultrasonic modes on the alcoholic strength of RW is shown in Fig. 6A. Compared with the control, the alcohol content of RW was significantly increased by 11.89 %− 14.81 % ($p < 0.05$) following ultrasound, and there was no

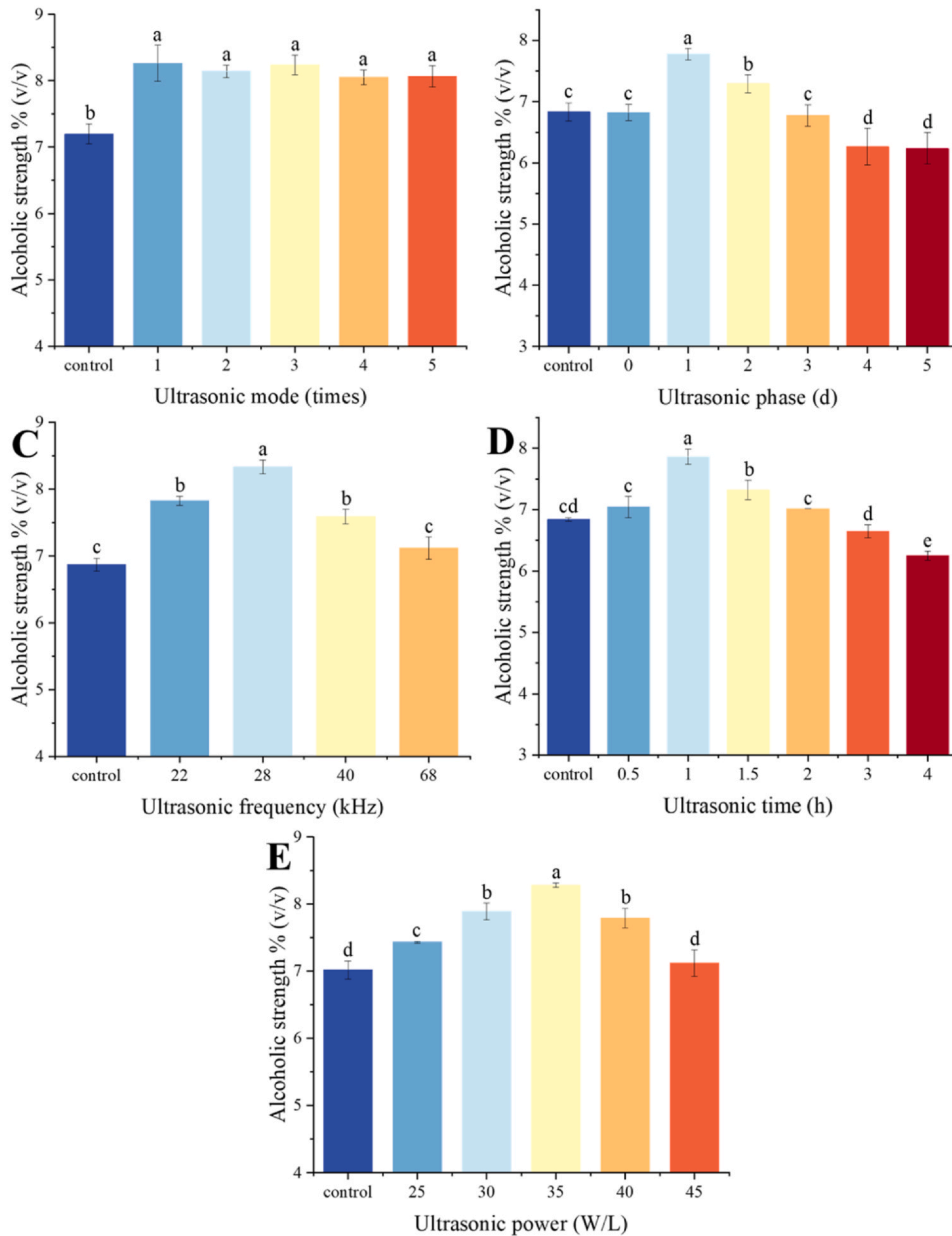

Fig. 6. Optimization of different ultrasonic conditions on alcoholic strength of rice wine. A: ultrasonic mode; B: ultrasonic phase; C: ultrasonic frequency; D: ultrasonic time; E: ultrasonic power. "Control" means no ultrasonic treatment. Different letters suggested significant difference within the same line ($p < 0.05$).

significant difference between different ultrasound modes. This phenomenon may be linked to the fact that after one-time ultrasonic stimulation, the permeability of microbial cells was changed, making it conducive to the secretion of enzymes [21], and so the cells could repair the damage on time to resist the stimulation [22]. Short-term ultrasound loading every day can also effectively stimulate microbial cells to promote RW fermentation [23].

Fig. 6B shows the influence of different ultrasonic treatment phases on the alcoholic strength of RW. On the 1st day, the alcohol content of RW was the highest, reaching 7.776 % (v/v), which was significantly increased by 13.79 % compared with the control ($p < 0.05$). Ultrasonic treatment on the 2nd day also promoted the improvement of alcohol content of RW to a certain extent, reaching 7.294 % (v/v), which was significantly increased by 6.73 % compared with control ($p < 0.05$). Ultrasonic treatment on day 0 and 3 had no significant effect in improving/increasing the alcohol content of RW, while ultrasonic treatment on the 4th and 5th day had inhibitory effect on the alcohol content of RW. The reason for this phenomenon may be that, on the 1st day, the fermentation substrate was rich in nutrients, the microorganisms grew rapidly, the enzyme production was rich, and the fermentation substrate was sensitive to external stimuli [24]. However, in the stable stage, growth and metabolism slowed down, and cell resistance was better [1].

With the increase in ultrasonic frequency, the alcoholic strength of RW increased first, and then decreased (Fig. 6C). When the ultrasonic frequency was 28 kHz, the alcohol content of RW was the highest, reaching 8.332 % (v/v), which was significantly increased by 21.32 % compared with the control ($p < 0.05$). Compared with the control group, the alcohol content of RW in the 22 kHz and 40 kHz groups significantly increased by 13.95 % and 10.51 %, respectively ($p < 0.05$). However, 68 kHz ultrasonic treatment of *S. cerevisiae* had no significant effect on the improvement of alcohol content of RW. This observation may be illustrated by the fact that, by increasing frequency, the frequency of cavitation also increases, and likewise the sonochemical effect, and consequently increasing the activation effect on the microorganisms [25]. However, too high a frequency could easily cause the generation and collapse of bubbles, which will in turn cause greater shear stress to microbial cells, thereby causing cell rupture, and eventually killing the microorganisms, and thereby reducing metabolites [26,27].

As shown in Fig. 6D, by increasing ultrasonic time, the alcoholic strength of RW first increased and then decreased. After a 1 and 1.5 h ultrasonic treatment on the 1st day, the alcoholic strength of RW was significantly increased compared with the control group ($p < 0.05$), the alcohol content of RW treated by ultrasound for 1 h was the highest, reaching 7.864 % vol, which was significantly increased by 14.97 % compared with the control group ($p < 0.05$). Ultrasonic treatment for 0.5, 2 and 3 h did not promote an increase in the alcohol content of RW, but ultrasonic treatment for 4 h had an inhibitory effect on the alcohol content of RW. The reason for this observation could be that, too short an ultrasonic time may not effectively stimulate the cell membrane; and with the increase in the ultrasonic time, it will overstimulate *S. cerevisiae*, resulting in permanent cell damage that cannot be repaired, and thus reducing the metabolic activity of microorganisms [28].

As shown in Fig. 6E, increasing the ultrasonic power resulted in the alcoholic strength of RW increasing (initially) and then decreasing. After 25, 30, 35, and 40 W/L ultrasonic treatment, the alcoholic strength of RW was significantly increased compared with the control ($p < 0.05$), 35 W/L sample had the best improvement effect, reaching 8.28 %vol, which was significantly increased by 18.03 % compared with control ($p < 0.05$); 45 W/L ultrasonic treatment of *S. cerevisiae* did not promote increase in the alcohol content of RW, and this could possibly be associated with the power (as low power cannot promote the flow of bacterial cell material). However, when it is too high, it will reduce the stability of the microbial surface cell wall, resulting in the thinning of the cell wall, which will cause cell rupture, cause microbial death, and affect cell multiplication [29,30].

From the study results, the physiochemical properties during RW fermentation and volatile flavor components were thus studied under the condition of one-time, 28 kHz, 1 h, 35 W/L on the 1st day.

3.5. Physiochemical properties of RW

3.5.1. Alcoholic strength

The alcoholic strength of RW in both the control and the ultrasonic treated samples first rapidly increased and then stabilized (Fig. 7A). In the early stage of fermentation, *S. cerevisiae* utilizes the reducing sugars produced during the saccharification stage to produce alcohol, resulting in a rapid increase in alcohol intensity [31]. The slow increase in alcohol during the later stage of fermentation is due to the stable decline in yeast growth, which is inhibited by metabolic products such as carbon dioxide and organic acids, resulting in a gradual stabilization of alcohol production [32]. The fermentation time of RW after ultrasonic treatment was shortened by nearly 2 days, and the ethanol yield was comparable to that of the sample without ultrasonic treatment. Ultrasonic treatment not only shortened the fermentation time, but also increased alcohol yield. On the 7th day of fermentation, the alcoholic strength of RW in the ultrasonic treatment was 23.53 % higher than that in the control group ($p < 0.05$). This discovery is due to the fact that ultrasound can improve the permeability of cell membrane and enzyme activity, thereby promoting material transport, accelerating proliferation, increasing the number of metabolites, and thereby increasing ethanol production [24,33].

3.5.2. Reducing sugar content

As shown in Fig. 7B, the reducing sugar content of RW in the control group first decreased, showed an upward trend on the 4th day, and then began to decrease. This suggests that enzymes may have further broken down the starch in the substrate, leading to an increase in reducing sugar content [34]. The reducing sugar is then utilized and consumed, causing its content to decline as *S. cerevisiae* grows. The reducing sugar content of RW in the ultrasonic treated sample increased first and then decreased rapidly. The increase in reducing sugar (at the early stage) after ultrasonic treatment may be due to the self-repair of *S. cerevisiae* or adaptation to the fermentation environment [35]. It requires less reducing sugar for its own growth and fermentation, and saccharifying enzymes continue to play a role. Therefore, there was a tendency to increase the reducing sugar content of RW in the ultrasonic group, and

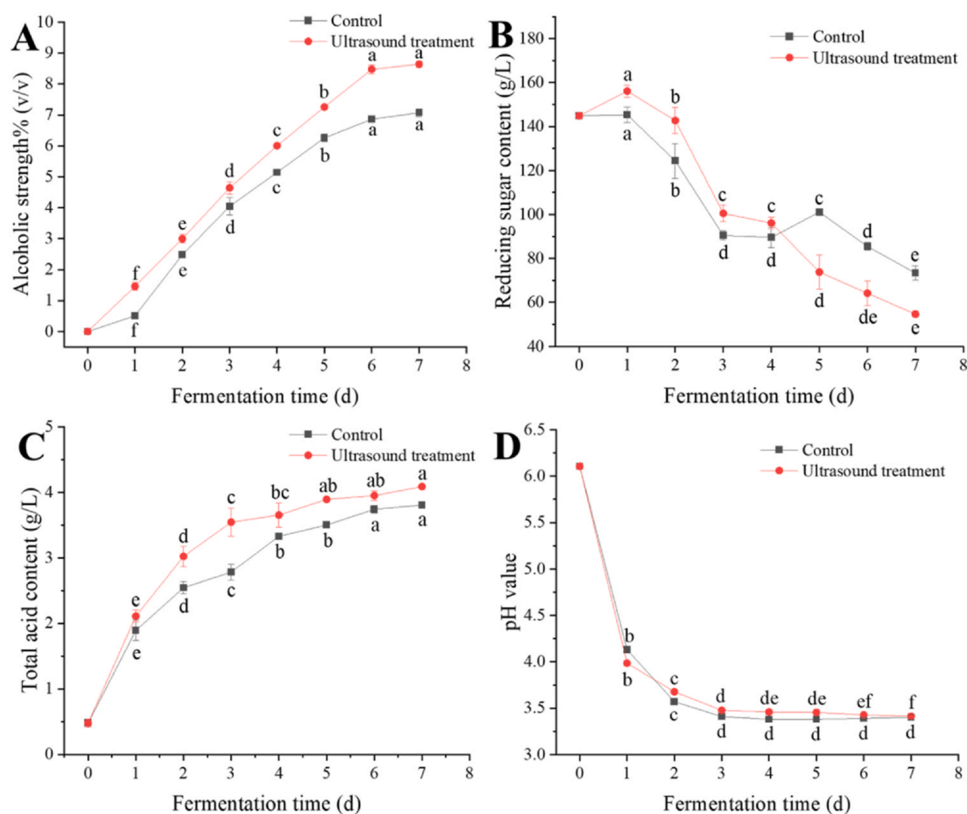

Fig. 7. Changes in physiochemical properties during fermentation of rice wine with or without ultrasonic treatment. A: alcoholic strength; B: reducing sugar content; C: total acid content; D: pH value. "Control" means no ultrasonic treatment. Ultrasonic treatment was carried out by one-time, 28 kHz, 1 h, 35 W/L on the 1st day. Different letters suggested significant difference within the same line ($p < 0.05$).

these reducing sugars were then used by *S. cerevisiae* to produce ethanol. On the 7th day of fermentation, reducing sugar content of RW in ultrasonic group was 54.65 g/L, which was significantly decreased by 25.47 % compared with control group ($p < 0.05$). This may be due to the effect of cavitation and microfluidics during ultrasound, which increase the metabolism of *S. cerevisiae*, leading to the consumption of more sugars to produce alcohol and acids.

3.5.3. Total acid content

Total acid content as fermentation progresses is affected by lactic or yeast fermentation to produce organic acids, as well as protein hydrolysis into smaller fragments (such as peptides) and amino acids [36]. As shown in Fig. 7C, the total acid content of RW in the control and the ultrasonic sample initially increased and then stabilizing. On the 7th day of fermentation, the total acid content of RW under ultrasonic treatment was 4.09 g/L, which was significantly increased by 7.43 % ($p < 0.05$). This was mainly due to the improvement of the growth and reproduction of *S. cerevisiae* after ultrasonic treatment, the increase of biomass and the further increase of total acid content. The late stabilization of total acid content may be due to the inhibition of yeast growth and organic acid production by metabolites such as alcohol and organic acids [37].

3.5.4. pH value

The pH value of the fermented RW in both the control and ultrasonic samples decreased rapidly (initially) and then stabilized ($p < 0.05$) (Fig. 7D). This phenomenon is related to the rapid growth of bacteria at the early stages of fermentation, such that the fermentation liquid produces a certain amount of lactic acid, and the pH value in the mash is rapidly reduced [38]. In the subsequent fermentation process, the yeast rapidly consumes glucose and produces a large amount of metabolites such as acids and alcohols, thereby reducing the pH value of the fermentation liquid, inhibiting the growth of other harmful microorganisms and their own acid production [39].

3.6. Analysis of volatile flavor components

As shown in Table 1, 57 volatile flavor components were detected during RW fermentation, including 20 alcohols, 11 esters, 8 acids, 4 alkanes, 4 aldehydes, 3 alkenes, 3 ketones, 2 phenols, and 2 other flavor substances.

Esters are the largest flavor components in RW, typically presenting fruity and flower aromas that provide a pleasant smell to RW. Esters are formed by the esterification reaction of alcohols and acids, or by the biosynthesis of alcohols acetyltransferase on substrates higher alcohols and acetyl-coA [40]. Small changes in the concentration of these secondary metabolites can have significant effect on

Table 1
Volatile flavor compounds in rice wine with or without ultrasonic treatment.

Number	RT (min)	Compound	Relative content (mg/L)	
			Control	Ultrasound
		Esters		
Z1	5.765	Isobutyl acetate	0.13	0.20
Z2	6.441	Ethyl butyrate	0.46	0.95
Z3	7.403	Ethyl isovalerate	—	0.03
Z4	9.051	Isoamyl acetate	—	11.42
Z5	12.816	Ethyl hexanoate	0.77	1.02
Z6	16.200	Ethyl lactate	0.24	0.20
Z7	20.510	Ethyl octanoate	—	19.25
Z8	23.816	Butyrolactone	0.39	0.35
Z9	24.561	Ethyl decanoate	—	0.65
Z10	28.684	Phenethyl acetate	11.13	13.22
Z11	36.387	Ethyl palmitate	1.49	1.08
		Alcohols		
C1	8.681	Isobutyl alcohol	104.18	117.66
C2	10.243	1-Butanol	0.47	0.29
C3	12.297	Isoamyl alcohol	488.24	563.80
C4	13.755	1-Undecanol	0.25	—
C5	13.865	1-Dodecanol	0.43	0.81
C6	18.827	6-Methyl-1-octanol	238.75	294.95
C7	19.991	7-Tetradecanol	273.42	303.20
C8	20.117	2,4-Dimethyl-1-heptanol	187.85	297.57
C9	20.362	2,7-Dimethyl-1-octanol	—	261.60
C10	20.620	1-Heptanol	89.95	82.68
C11	20.874	3-Methyl-2-octanol	0.15	0.39
C12	21.115	2-Ethylhexanol	18.68	19.54
C13	21.703	2-Nonanol	—	1.33
C14	22.415	1-Tetradecanol	0.37	—
C15	22.457	2-Ethylcyclohexanol	1.05	0.82
C16	22.847	2,3-Butanediol	2.61	3.29
C17	29.583	Geraniol	—	1.03
C18	30.177	Benzyl alcohol	0.22	0.17
C19	30.990	Phenethyl alcohol	131.98	144.98
C20	49.675	4-Hydroxybenzyl alcohol	—	0.14
		Aldehydes		
Q1	6.577	Acetaldehyde	—	1.68
Q2	7.693	Hexanal	0.13	0.17
Q3	11.111	Heptanal	—	0.04
Q4	21.924	2,5-Dihydroxyacetophenone	7.53	—
		Acids		
S1	20.445	5-Ethyl-2-nonanol	159.40	306.38
S2	22.670	2-Methylpropanoic acid	30.57	31.86
S3	24.071	Butanoic acid	0.91	0.98
S4	25.091	3-Methylpentanoic acid	18.19	19.54
S5	29.409	Hexanoic acid	—	11.55
S6	33.622	Caprylic acid	7.84	12.59
S7	36.452	Decanoic acid	2.44	3.47
S8	38.339	Benzoic acid	6.13	4.21
		Alkanes		
W1	13.541	Nonyl-cyclopropane	1.47	1.13
W2	13.855	7-Hexadecene	—	0.28
W3	18.021	Tetradecane	0.48	1.26
W4	36.910	Dotriacontane	1.12	—
		Phenols		
F1	32.706	Phenol	8.38	3.90
F2	35.531	2-Methoxy-4-vinylphenol	3.25	4.45
		Alkenes		
X1	10.122	4-Methyl-1-pentene	—	0.44
X2	12.309	2-Pentene	—	285.76
X3	13.616	2-Dodecene	—	0.57
		Ketone		
T1	14.387	2-Octanone	258.07	244.18
T2	16.562	3-Nonanone	0.15	0.07
T3	17.515	2-Nonanone	0.58	0.48
		Other		
O1	13.391	Sec-Butylbenzene	1.21	2.55
O2	18.204	Pentane	0.88	0.89

Note: “—” indicates that the substance was not detected.

the sensory quality of final products. After the ultrasonic treatment, isobutyl acetate, ethyl butyrate, ethyl hexanoate, and phenethyl acetate increased by 58.03 %, 107.70 %, 31.84 %, and 18.71 %, respectively compared with the control. At the same time, ethyl isovalerate, isoamyl acetate, ethyl octanoate, and ethyl decanoate were produced with contents of 0.03, 11.42, 19.25, and 0.65 mg/L, respectively. The contents of ethyl lactate, ethyl palmitate, and butyrolactone decreased.

Alcohols are key components of RW fermentation and can be synthesized through amino acid metabolism, glycolysis pathway, methyl ketone reduction, and degradation of linoleic acid and linolenic acid [41]. After ultrasonic treatment, the total alcohol content and types increased, among which isobutyl alcohol, isoamyl alcohol, 2,4-dimethyl-1-heptanol, 3-methyl-2-octanol, 2-ethylhexanol, 2,3-butanediol, and phenethyl alcohol increased by 12.95 %, 15.48 %, 58.41 %, 153.87 %, 4.60 %, 26.13 %, and 9.86 %, respectively, compared with the control.

Aldehydes have a lower threshold, but they are important flavor compounds in wine, able to integrate and coordinate the flavor of wine. Although 2,5-dihydroxyacetophenone disappeared, the content of hexanal increased and acetaldehyde and heptanal were produced, which give RW more grassy and fruity aroma.

Acids can be obtained by lipid oxidation or by the conversion of aldehydes or ketones. In addition, acids can react with alcohols to form esters and provide wine aromas [42]. Alkene is also an important volatile flavor substance in RW. After ultrasonic treatment, the types and contents of acids and alkenes increased.

4. Conclusions

This study established an enzymatic brewing process for RW in the laboratory and simplified the brewing system by simulating actual production, facilitating the quality control of RW. On this basis, the effects of ultrasonic mode, ultrasonic phase, ultrasonic frequency, ultrasonic time, and ultrasonic power on the alcoholic strength of RW were studied. The results showed that ultrasound can increase the yield of ethanol, shorten fermentation time, and increase the type and content of esters. This study proposed a green physical technology (ultrasound) applied to traditional fermentation, which can provide a theoretical and/or technical basis for the research and development of enzymatic RW brewing.

CRediT authorship contribution statement

Xu Haining: Validation, Conceptualization. **Yan Pengfei:** Validation, Conceptualization. **Yang Mengyuan:** Validation, Conceptualization. **Mintah Benjamin Kumah:** Writing – review & editing, Methodology. **Gao Xianli:** Methodology, Investigation. **Zhang Rong:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Hao Jing:** Writing – original draft, Methodology, Investigation, Conceptualization.

Declaration of Competing Interest

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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