Research Article

Quality Comparison of Hawthorn Wines Fermented by *Saccharomyces cerevisiae* with and without Pulp Contact and Pectase Treatment

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This study evaluated the chemical and volatile composition of hawthorn wines fermented with *Saccharomyces cerevisiae* A3.12 with and without pulp contact and pectase treatment during fermentation. The results indicated that the addition of pectase was in favor of pH decrease and clarification. The pectase-treated wines had significantly higher concentrations of acetic acid, ethyl octanoate, and isoamyl acetate than the non-enzyme-treated wines. Furthermore, the nonextracted wines had significantly higher concentrations of isoamyl alcohol, phenylethyl alcohol, ethyllactate, and isoamyl acetate than the extracted wines. The first principal component separated the wine from the nonextracted juice with pectase from other samples based on the higher concentrations of isoamyl acetate (0.14 µg/g), diethyl butylenedioate (0.07 µg/g), 2-phenylethyl acetate (0.23 µg/g), and acetoin (1.47 µg/g). The wine from nonextracted pulpy juice with pectase was significantly more aromatic than the others and 26.2% of the consumers were found to be willing to purchase this product.

1. Introduction

Chinese hawthorn (*Crataegus pinnatifida*) is a member of family Rosaceae. It is an edible fruit used in traditional Chinese medicine to improve digestion and increase appetite [1]. Hawthorn is an important plant in traditional Chinese medicine and is recommended for the treatment of some diseases such as tumors and cardiovascular disease related to the production of radical species resulting from oxidative stress [2, 3].

Compared with hawthorn fruits, studies on hawthorn wine fermentation and volatiles in hawthorn wines are limited. Hawthorn wine can be produced by fermentation with *Saccharomyces cerevisiae*. He et al. investigated the chemical characteristics and antioxidant capacities of hawthorn wines by the fermentation of different yeast strains [4]. It was found that the wine compositions and antioxidant activities showed expected variations. In spite of the studies described above, it still needs strive to optimize the fermentation processing to improve the quality of hawthorn wine.

In order to improve fruit wine quality, many different enzymes were applied to solve the processing problems [5–7]. For example, Apolinar-Valiente et al. reported β-galactosidase was used to affect oligosaccharide composition of Monastrell red wines [8]. González-Neves et al. found that maceration enzymes affect the anthocyanin profile and the stability of the color of grape wines [9]. In our previous study, that pulp contact and protease were proved to be able to improve the intensity and complexity of jujube wine aroma due to the increase of the assimilable nitrogen [10]. Pectases have been used in wine production since the 1960s. The pectase can help break down the fruit material and extract flavors from the mash, and also the presence of pectin can improve the clarity in finished wine [11, 12]. In spite of the studies described above, the effects of pectase on the chemical and volatile profiles of hawthorn wines remain unclear. Thus, the aim of this study was to evaluate the chemical and volatile composition of the resultant hawthorn wines fermented by *S. cerevisiae* A3.12 with and without pulp contact. The effectiveness of pectase application in hawthorn wine flavor...
modification was investigated as well. The information gained would be useful for the processing in order to produce hawthorn wines with differential characteristics.

2. Materials and Methods

In this study, we followed the methods of Zhang et al. (2016) [10] with slight modification. The detailed procedure was as follows.

2.1. Yeast Strains and Culture Media. S. cerevisiae A3.12 commercial wine yeast (Angel Yeast Co., Ltd., China) was used in the hawthorn fermentation. 2.4 gram freeze-dried yeast powder was propagated in the 150 mL broth of fresh juice (adjusted to Brix of 4.0% described below) at 38 °C for 1 h under static conditions and yeasts grew to 10^7 CFU/mL for use immediately.

2.2. Pretreatment of Hawthorn Juice. The variety of hawthorn sample used in this study was Yubeihong, an improved breed cultivated in north part of Henan province, China. The dried hawthorn (950g) was rinsed in 2850 mL distilled water (1:3w/v) overnight. Then Xinzhen, Henan, China. The hawthorn was cultivated in north part of Henan Province, China. The dried sample used in this study was Yubeihong, an improved breed cultivated in north part of Henan province, China. The dried hawthorn (950g) was rinsed in 2850 mL distilled water (1:3w/v) overnight. Then the hawthorn was cooked at 90 °C for 5 h and continually at 100 °C for 1 h. The cooked hawthorn was divided into two lots. The first lot was extracted manually by 4 layer cotton-gauze filter to remove the pulp (Brix 13.7%). The second lot was not extracted and Brix was detected as 14.3%. The sugar concentrations of extracted and unextracted juice were 135.4 and 143.9 g/L, respectively. All experiments were performed at least in triplicate to collect enough samples.

2.3. Fermentation and Pectase Treatment. The hawthorn juice fermentation was carried out in 1000-mL sterile Erlenmeyer conical flasks (plugged with cotton wool and then wrapped with aluminium foil), and each flask contained 800 mL of extracted hawthorn juice or unextracted pulpy hawthorn juice. Before inoculation, the samples were divided into two portions. One portion was subjected to pectase treatment (Imperial Jade biotechnology Co., Ltd, China; Enzymatic activity ≥3 × 10^5 μg; enzyme dosage of 0.3 g/L) at 45 °C for 1 h and the other portion was kept at 45 °C for 1 h as control. All four different samples were inoculated with 1.25% (v/v) yeast broth culture. The fermentation was conducted at 22 °C statically for 24 days. All pectase treatments and controls were carried out in triplicate. Samples collected at the end of the fermentation were centrifuged at 3,000g for 15 min, and the resulting supernatant was stored at 4 °C for the other ten days before further analysis.

2.4. Measurement of pH, Brix, and Clarity. The total soluble solids (Brix) and pH were measured at the indicated time points by using a refractometer (PAL-1, ATAGO, Japan) and a pH meter (Mettler-Toledo, Switzerland), respectively. The clarity of wine was recorded at 680 nm using a Leng Guang 722 spectrophotometer [13] and distilled water is used as a control. Samples were analyzed in triplicate for each wine.

2.5. Analysis of Total Sugars, Reducing Sugars, and Methanol. The contents of total sugar, reducing sugars, and methanol were quantified according to the National Standard of the People’s Republic of China [14].

2.6. Extraction and Analysis of Volatile Compounds. Simultaneous distillation extraction was developed to extract and concentrate volatile compounds from hawthorn wine and performed with a Likens-Nickerson apparatus [15]. For distillation, 150 mL hawthorn wine mixed with 50 distilled water and 60 mL dichloromethane were placed in a round-bottom flask in a water-bath (around 50 °C) for 2 h. When the extraction was performed, chilled water was circulated through the cold finger condenser. 1 mL phenylethyl acetate dichloromethane solution (0.62 mg/mL) was added in the extract as internal standard. The samples were concentrated to 1.0 mL for further GC-MS analysis.

GC-MS analysis of volatile aromatic compounds was carried out on an Agilent 5973 mass select detector (Agilent Corporation of America) directly coupled to a HP-5 gas chromatograph (60 m × 250 μm × 0.25 μm). The temperature of the GC-MS transfer line was 280 °C in the electron impact (EI) mode (70 eV), scanning from m/z 35-550 in one scan. The column oven was programmed from 50 °C (after 2 min) to 280 °C at 4 °C/min and the final temperature was held for 15 min. The voltage of the electronic multiplier tube (EMT) was 230 V above tuning.

The mass spectral identification of aromatic compounds was carried out by comparing to the Nist11.L (USA Agilent Corporation). Qualitative analysis (mass spectral data) was verified by comparing the retention indices and mass spectra of identified compounds. The relative quantity of each compound was determined using acetic acid phenyl ester dichloromethane as the internal standards, without considering recovery of aroma compounds and response factors [16]; all sorts of aroma components were analyzed quantitatively as follows:

$$\text{Aroma components extraction (mg/g)} = \frac{(\text{peak area of aroma components} \times \text{quality of internal standard})}{(\text{internal standard peak area} \times \text{quality of sample})}.$$  \hspace{2cm} (1)

2.7. Sensory Analysis. Four wine samples (140 mL per sample) were assessed by a panel of seven trained flavorists from our department [17]. The four wine samples were assessed in the following order: (i) wine from the extracted juice with pectase; (ii) wine from the extracted juice without pectase; (iii) wine from nonextracted pulpy juice with pectase; (iv) wine from nonextracted pulpy juice without pectase. A set of descriptive terms for nine attributes were rated on a five-point
scale for the intensity perceived, where zero indicated that the descriptor was not perceived and twenty indicated a very high intensity.

2.8. Consumer Acceptability Test. The consumer acceptability tests were made by 150 students in our department and four wine samples (6 L per sample) were assessed. A set of descriptive terms, overall, taste and flavor acceptance, were rated on a nine-point scale for the intensity of liking, where zero indicated the least and nine indicated the best [18]. Besides, a questionnaire survey of puncher intention was presented as well.

2.9. Statistical Analysis. The statistical differences of the effect of yeast treatment on the volatiles of hawthorn wine fermented with and without pulp (or pectase treatment) were evaluated using analysis of variance (ANOVA) in SPSS (version 12.0). All tests of significance were conducted at a probability level of \( P < 0.05 \). Means and standard deviations were obtained from triplicate fermentation samples. The volatile and aroma profiles for pectase-treated hawthorn wines and control were further analyzed using principal component analysis (PCA) to characterize the multidimensional data.

3. Results and Discussion

3.1. Physicochemical Properties of Hawthorn Wine with and without Extraction and Enzyme Treatment. As shown in Table 1, after fermentation, the pH value was slightly lower (Brix value and clarity were higher) for pectase-treated wines than that without enzyme treatment for both extracted and nonextracted wines. The results indicated that the addition of pectase was in favor of pH decrease and clarification, which was consistent with the results reported by Cheirisilp and Umsakul [19]. However, the cell number was much higher for nonextracted wine compared with extracted wine for both enzyme and nonenzyme treatments, which indicated that nonextracted wines might be better for cell growth due to more complicated nutrition. Four wines have similar characteristics in terms of total sugars change and total reducing sugars changes after fermentation. The total soluble solids were 5.87–6.80 °Brix, while the total sugars and total reducing sugars are 7.4–8.64 g/L and 5.50–6.75 g/L, respectively (Table 1). The pectin was degraded completely by pectase treatment. The methanol content was higher for pectase-treated wine than nonenzyme-treated wines, which indicated that pectase treatment would lead to more methanol production [20]. According to Agricultural Industry Standard of the People’s Republic of China [21], the methanol contents in all wine samples are strictly regulated at <400 mg/L.

3.2. Volatiles. Thirty-eight volatiles were identified and quantified in the hawthorn wines with thirty-four volatiles being present in all wines (Table 2). The results showed pulp contact with pectase could improve the complexity of wine aroma. The alcohols accounted for the highest amount in hawthorn wines. Isoamyl alcohol was likely derived from leucine via the Ehrlich pathway [22]. Phenylethyl alcohol was second most abundant, which was likely derived from phenylalanine via the Ehrlich pathway [23]. The nonextracted wines had significantly higher concentrations of isoamyl alcohol and phenylethyl alcohol than the extracted wines. There was statistically significant difference between the concentrations of alcohols after extraction, including 1-propanol, isobutyl alcohol, isoamyl alcohol, 3-hexen-1-ol, 2,3-butanediol, alphaterpineol, phenylethyl alcohol, and 3-ethyl-1-butanol, which could be related to the higher availability of relative amino acids in pulp juice [24].

The second most abundant volatile group in the hawthorn wines was the acid group (Table 2). The major organic acids in hawthorn wine were acetic acid and isobutyric acid, which represented above 59% of the total organic acids. The pectase-treated wines had significantly higher concentrations of acetic acid than the nonenzyme-treated wines. The results indicated that pectase treatment seemed to promote the production of acetic acid.

| Table 1: Physicochemical properties and total reducing sugar concentration of extracted and nonextracted wine, with and without pectase treatment. |
|-----------------|-----------------|-----------------|-----------------|
|                  | Extracted        | Control          | Nonextracted    | Control          |
| pH               | 2.82 ± 0.01\(^a\) | 2.89 ± 0.02\(^b\) | 2.79 ± 0.02\(^c\) | 2.90 ± 0.03\(^d\) |
| Total soluble solids (°Brix) | 6.38 ± 0.10\(^e\) | 5.93 ± 0.23\(^f\) | 6.80 ± 0.65\(^g\) | 5.87 ± 0.32\(^h\) |
| Clarity (%)      | 91.17 ± 3.78\(^i\) | 79.91 ± 1.66\(^j\) | 61.98 ± 0.67\(^k\) | 85.84 ± 1.22\(^l\) |
| Cell count (10⁶ cfu/mL) | 9.30 ± 0.71\(^m\) | 11.5 ± 1.65\(^n\) | 69.40 ± 10.56\(^o\) | 35.00 ± 18.38\(^p\) |
| Total sugars (g/L) | 8.22 ± 0.68\(^q\) | 7.40 ± 1.48\(^r\) | 8.64 ± 0.87\(^s\) | 7.85 ± 0.78\(^t\) |
| Total reducing sugars (g/L) | 6.75 ± 0.64\(^u\) | 5.80 ± 0.94\(^v\) | 6.40 ± 2.78\(^w\) | 5.50 ± 1.11\(^x\) |
| Ethanol (% v/v)  | 7.53 ± 0.21\(^y\) | 6.50 ± 0.20\(^z\) | 7.13 ± 0.21\(^{ab}\) | 7.67 ± 0.21\(^c\) |
| Total acid (g/L)  | 10.68 ± 0.25\(^d\) | 9.83 ± 0.16\(^e\) | 12.28 ± 1.87\(^f\) | 10.24 ± 0.15\(^g\) |
| Pectin (g/L)      | n.d. \(^h\)      | 2.67 ± 0.14\(^i\) | n.d. \(^j\)      | 1.99 ± 0.05\(^k\) |
| Methanol (mg/L)   | 349.68 ± 10.32\(^l\) | 65.70 ± 6.86\(^m\) | 362.36 ± 14.41\(^n\) | 117.30 ± 6.34\(^o\) |

\(^{a,b,c,d}\) Statistical analysis at 95% confidence level with same letters indicating no significant difference.
The third most abundant volatile group in the hawthorn wines was the ester group (Table 2). Most of the esters in the hawthorn wines were produced during fermentation. The nonextracted wines had significantly higher concentrations of ethyl lactate and isoamyl acetate than the extracted wines. Furthermore, the enzyme-treated wines had higher concentrations of both ethyl octanoate and isoamyl acetate than the nonenzyme-treated wines, which is consistent with the quantities of octanoic acid and \(n\)-decanoic acid between the two kinds of wines. The results indicated that the

<table>
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<tr>
<th>Group</th>
<th>Compound</th>
<th>Extracted</th>
<th>Control</th>
<th>Nonextracted</th>
<th>Control</th>
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<tr>
<td>Alcohol</td>
<td>Ethyl 3-hydroxybutyrate</td>
<td>0.06 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Ester</td>
<td>Butyro lactone</td>
<td>0.21 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.30 ± 0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.41 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diethyl butanedioate</td>
<td>0.06 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.07 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aldehyde</td>
<td>Furfural</td>
<td>1.77 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.88 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.51 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.20 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Ketone</td>
<td>Acetoin</td>
<td>0.58 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.47 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.70 ± 0.11&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Others</td>
<td>Eugenol</td>
<td>0.38 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
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n.d.: not detected; <sup>a,b,c,d</sup> Statistical analysis at 95% confidence level with same letters indicating no significant difference.
production of fatty acids has the positive correlation with the production of ethyl esters, which is in agreement with other report by Li et al. [24].

In Table 2, it could be found that pectase treatment could enhance eugenol, which is main wine flavor. Furthermore, pulp contact can promote production of isoamyl alcohol, phenylethyl alcohol, isoamyl acetate, and ethyl lactate. Hence, the application of pulp contact was effective in enhancing the intensity of hawthorn wine aroma. Furthermore, the addition of pectase to nonextracted juice could accelerate the release of odor-active volatiles such as 3-ethoxy-1-propanol, acetic acid, octanoic acid, n-decanoic acid, isoamyl acetate, ethyl octanoate, ethyl octadecanoate, and eugenol.

3.3. Sensory Characteristics of Hawthorn Wine. The sensory profiles of the hawthorn wines were represented in a spider web plot as shown in Figure 1. The nonextracted wine with pectase treatment showed auburn color and was significantly more aromatic than the rest. These results were in agreement with the results of Table 2. The nonextracted wine with pectase treatment was more acidic than the rest, probably due to higher level of total acid (Table 1).

3.4. Principal Component Analysis of Volatiles in Hawthorn Wine. Principal component analysis (PCA) was applied to discriminate the volatile profiles of the hawthorn wines (Figures 2(a) and 2(b)). The first principal component (PC1) accounted for 50.4% of the total variance in the data set, while the second principal component (PC2) accounted for 31.8% of the total variance. The PCA biplot separated the thirty-eight different volatile compounds and the four different samples.

The first principal component (PC1) separated the wine from the extracted wine with pectase from other samples based on the higher concentrations of medium-chain fatty acids (octanoic and n-hexadecanoic acid) and medium-chain ethyl esters (ethyl octanoate, ethyl palmitate, and ethyl octadecanoate). PC1 also separated the wine from the nonextracted wine without pectase, based on alcohols (butanol and 3-methylthiopropanol), acids (isobutyric and butanoic acid), and esters (ethyl 3-hydroxybutyrate and butyro lactone). PC2 separated the nonextracted wine with enzyme addition and extracted wine without enzyme based on higher concentrations of isoamyl acetate (0.14 μg/g), diethyl butanedioate (0.07 μg/g), 2-phenylethyl acetate (0.23 μg/g), and acetoin (1.47 μg/g) in the former and higher acetic acid in the latter.

3.5. Consumer Acceptability Test of Hawthorn Wine. The results of consumer acceptability test were shown in Figure 3. The wine from nonextracted pulpy juice with pectase (B) showed favorable acceptability compared to the others. It was also found that 26.2% of the consumers were willing to purchase this product. These results were in agreement with the results of sensory characteristics.

4. Conclusion

The chemical and volatile composition of hawthorn wines fermented with S. cerevisiae with and without pectase was evaluated. The methanol content was much higher for pectase-treated wine than nonenzyme-treated wines. Although the addition of pectase was in favor of clarification and filtration of wine, the nonextracted wine without pectase treatment was significantly more aromatic than the rest. The results suggested that pulp contact could intensify and diversify the
Figure 2: Biplot of principal component analysis of hawthorn wines. (a) Wine from the extracted juice with pectase (●); wine from the extracted juice without pectase (⧫); wine from nonextracted pulpy juice with pectase (○); wine from nonextracted pulpy juice without pectase (▲); (b) aroma compositions: (1) 1-propanol; (2) isobutyl alcohol; (3) 1-butanol; (4) isoamyl alcohol; (5) 3-ethoxy-1-propanol; (6) 3-hexen-1-ol; (7) 2,3-butanediol; (8) alpha-terpineol; (9) 3-methylthiopropanol; (10) benzyl alcohol; (11) phenylethyl alcohol; (12) 3-ethyl-1-butanol; (13) acetic acid; (14) isobutyric acid; (15) butanoic acid; (16) 2-methyl butyric acid; (17) hexanoic acid; (18) octanoic acid; (19) n-decanoic acid; (20) 9-decenoic acid; (21) n-hexadecanoic acid; (22) isoamyl acetate; (23) ethyl hexanoate; (24) ethyl pyruvate; (25) ethyl octanoate; (26) ethyl 3-hydroxybutyrate; (28) butyrolactone; (29) diethyl butanedioate; (30) 2-phenylethyl acetate; (31) ethyl palmitate; (32) Ethyl 9-hexadecenoate; (33) ethyloctadecanoate; (34) ethyloleate; (35) furfural; (36) 5-methyl furfural; (37) acetoin; (38) eugenol.

Figure 3: Consumer acceptability test of hawthorn wine. Left ordinate represents score of acceptance and right ordinate represents percentage of purchase intention. (A) Wine from the extracted juice with pectase, (B) wine from the extracted juice without pectase, (C) wine from nonextracted pulpy juice with pectase, and (D) wine from nonextracted pulpy juice without pectase.

Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References


