

Chemical and volatile composition of jujube wines fermented by *Saccharomyces cerevisiae* with and without pulp contact and protease treatment

Wenye ZHANG¹, Lei ZHANG¹, Chunping XU^{1*}

Abstract

This study evaluated the chemical and volatile composition of jujube wines fermented with *Saccharomyces cerevisiae* A1.25 with and without pulp contact and protease treatment during fermentation. Yeast cell population, total reducing sugar and methanol contents had significant differences between nonextracted and extracted wine. The nonextracted wines had significantly higher concentrations of ethyl 9-hexadecenoate, ethyl palmitate and ethyl oleate than the extracted wines. Pulp contact also could enhance phenylethyl alcohol, furfuryl alcohol, ethyl palmitat and ethyl oleate. Furthermore, protease treatment can accelerate the release of fusel oils. The first principal component separated the wine from the extracted juice without protease from other samples based on the higher concentrations of medium-chain fatty acids and medium-chain ethyl esters. Sensory evaluation showed pulp contact and protease could improve the intensity and complexity of wine aroma due to the increase of the assimilable nitrogen.

Keywords: fermentation; flavor; jujube wine; volatiles; protease.

Practical Application: Control of jujube wine fermentation with pulp contact and protease treatment.

1 Introduction

Chinese jujube (*Ziziphus jujuba* Mill), belongs to the Rhamnaceae, Order Rhamnales, is a native fruit of China (Li et al., 2007a). It is a deciduous fruit tree that blooms in early summer, ripens in autumn and grows in the temperate and subtropical areas of the Northern Hemisphere, especially the drier parts of north China. Jujube 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.

Compared with jujube fruits, studies on jujube wine fermentation and volatiles in jujube wines are limited. Jujube wine can be produced by fermentation with *Saccharomyces cerevisiae*. Liu & Zhao (2011) investigated the effect of initial sugar content, inoculation concentration of yeast, fermentation temperature, and pH on jujube wine quality. Li et al. (2013b) found that the jujube peel is the main factor of bitter taste for jujube wine. Chun et al. (2012) investigated the free amino acids and flavors in fermented jujube wine by the methods of HPLC and GC/MS. Park et al. (2009) investigated the effect of different hydrostatic pressure and freezing treatment on microbial counts, physicochemical properties, and sensory characteristics of jujube wine.

Fusel oils, or the higher alcohols produced during fermentation, play an important part in the bouquet of wine and the flavour of beer (Stevens, 1960). It has been reported that fusel oils are produced from the degradation of amino-acids by the Ehrlich pathway during wine fermentation (Sentheshanmuganathan

& Elsdén, 1958). In spite of the studies described above, it still need strive to optimize the fermentation processing to improve the quality of wine by the increase of fusel oil concentration. Piddocke et al. (2011) was found to specifically liberate branched-chain amino acids and enhance the synthesis of fusel oils during fermentation. The aim of this study was to evaluate the chemical and volatile composition of the resultant jujube wines fermented by *S. cerevisiae* A1.25 with and without pulp contact. The effectiveness of protease application in jujube wine flavor modification was investigated as well. The information gained would be useful for the processing in order to produce jujube wines with differential characteristics.

2 Materials and methods

2.1 Yeast strains and culture media

S. cerevisiae A1.25 commercial wine yeast (Angel Yeast Co., Ltd., China) was used in the jujube fermentations. One gram freeze-dried yeast powder were propagated in the 200 mL broth of fresh juice (adjusted to °Brix of 4.7% described below) at 38 °C for 15 min and then 28 °C for 1 h under static conditions and yeasts grew to 10⁷ CFU/mL for use immediately.

2.2 Pretreatment of jujube juice

Dry jujube was purchased from a local wholesale centre in Xinzheng, China. Two kilogram dry jujube fruits were washed and then rinsed in 8 L distilled water overnight. Then the jujube was cooked at 90 °C for 5 h and continually at 100 °C for 1 h.

Received 18 Sept., 2015

Accepted 07 Feb., 2016

¹College of Food and Biology Engineering, Zhengzhou University of Light Industry, Zhengzhou, China

*Corresponding author: c.p.xu@zzuli.edu.cn

The cooked jujube was divided into two lots. The first lot was extracted manually by 4 layer cotton-gauze filter to remove the pulp (°Brix 16.3%). The second lot was not extracted and °Brix was detected as 15.7%. Both lots were acidified to pH 3.6 with 50% w/v food grade DL-malic acid (Tianjin Deen Chemical Co., Ltd, China).

2.3 Fermentation and protease treatment

The jujube juice fermentations were carried out in 1000-mL sterile Erlenmeyer conical flasks (plugged with cotton wool and then wrapped with aluminium foil), and each flask contained 500 mL of extracted jujube juice or unextracted pulpy jujube juice. Before inoculation, the samples were divided into two portions. One portion was subjected to protease treatment (Imperial Jade biotechnology Co., Ltd, China; Enzymatic activity $\geq 2 \times 10^7$ $\mu\text{g/g}$; enzyme dosage of 0.15 g/L) at 45 °C for 1 h and the other portion was kept as control. All four different samples were inoculated with 10% (v/v) yeast broth culture. The fermentation was conducted at 20 °C statically for 9 days. All protease treatments and controls were carried out in triplicate. Samples collected at the end of the fermentation were centrifuged at 3,000 g for 15 min, and the resulting supernatant was stored at 4 °C for other ten days before further analysis.

2.4 Measurement of pH and °Brix

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 (Daudt & Fogaça, 2013). Samples were analyzed in triplicate for each wine.

2.5 Analysis of reducing sugars and methanol

A segmented continuous flow analysis system is used for the routine determination of total reducing sugars based on methods developed by Lever (1973) utilizing the reaction of reducing sugars with p-hydroxybenzoic acid hydrazide. The methanol content was quantified according to the National Standard of the People's Republic of China (2006).

2.6 Analysis of free amino acids

Ion chromatography with integrated pulsed amperometric method is used for determination of free amino acids by a Dionex ICS-5000 SP (Thermo Fisher Scientific Inc., Dionex, USA) (Yu et al., 2002). Standard reference material amino acids were purchased from Waters, MA, USA.

2.7 Analysis of fusel oil

The contents of *n*-propanol, isobutanol and isoamylol were determined by Agilent 7820A Gas Chromatograph (19091N-133, 60 m \times 250 μm \times 0.25 μm). The standard references (Sigma) were used to prepare the standard solution. The wine samples were filtered through a membrane filter (0.45 μm , Millipore). The temperature of the GC 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 45 °C

(after 2 min) to 200 °C at 10 °C/min and the final temperature was held for 10 min. The voltage of the electronic multiplier tube (EMT) was 230 V above tuning.

2.8 Extraction and analysis of volatile compounds

Simultaneous distillation extraction was developed to extract and concentrate volatile compounds from jujube wine and performed with a Likens-Nickerson apparatus (Schultz et al., 1977). For distillation, 150 mL jujube wine mixed with 50 distilled water and 60 mL dichloromethane were placed in a round-bottom flask in a water-bath (60 °C) for 2 h. When the extraction was performed, chilled water was circulated through the cold finger condenser. One mL acetic acid phenyl ester 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 \times 250 μm \times 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 (Cai et al., 2002), all sorts of aroma components were analyzed quantitatively as follow:

$$\text{Aroma components extraction } (\mu\text{g/g}) = (\text{peak area of aroma components} \times \text{quality of internal standard}) / (\text{internal standard peak area} \times \text{quality of sample})$$

2.9 Sensory analysis

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

2.10 Statistical analysis

The statistical differences of the effect of different yeast treatment on the volatiles of jujube wine fermented with and without pulp (or protease treatment) were evaluated using analysis of variance (ANOVA). 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 protease-treated jujube wines and control were further analysed using principal component analysis (PCA) to characterize the multidimensional data.

3 Results and discussion

3.1 °Brix, pH, yeast growth, total reducing sugar, alcohol and methanol concentrations

As showed in Table 1, the four wines have similar characteristics in terms of °Brix change and pH changes after fermentation. The pH values fluctuated from 3.56 to 3.63 and Brix values were reduced to 5.5-5.9° for all four jujube wines. However, the cell number was higher for nonextracted wine compared with extracted wine for both enzyme and nonenzyme treatments. The total reducing sugar was also higher for nonextracted wines compared with extracted wines (Table 1). In addition, the final ethanol reached 6.05-6.55% (v/v) for four wines and was slightly higher in the enzyme-treated wines; however, this difference was not significant. The methanol content was lower for nonextracted wine than extracted wines, which indicated

that nonextracted wines remained at more acceptable level to the consumer's health than extracted wines.

3.2 Free amino acids

As showed in Table 2, the contents of free amino acids in the protease-treated wines were higher than those in the nonenzyme-treated wines except arginine and histidine. The amount of total free amino acids in the wine from nonextracted pulpy juice was higher than that in the wine from extracted juice. The results indicated that protease treatment could enhance the concentration of assimilable nitrogen, which was one of the important nutrients for yeast in wine fermentation (Perestrelo et al., 2006).

3.3 Fusel oil

The contents of *n*-propanol, isobutanol and isoamylol were quantified in the four wines (Table 3). The amount of total three fusel oils was in the order of wine from nonextracted pulpy juice with protease > wine from the extracted juice with protease > wine from nonextracted pulpy juice without protease > wine

Table 1. Physico-chemical properties and total reducing sugar concentration of extracted and nonextracted wine, with and without protease treatment.

	Extracted		Nonextracted	
	Protease	Control	Protease	Control
pH	3.56 ± 0.02 ^a	3.56 ± 0.03 ^a	3.61 ± 0.04 ^a	3.63 ± 0.04 ^a
Total soluble solids (°Brix)	5.90 ± 0.14 ^a	6.05 ± 0.07 ^a	5.50 ± 0.28 ^a	5.90 ± 0.28 ^a
Cell count (10 ⁶ cfu/ mL)	12.50 ± 3.11 ^a	10.80 ± 1.84 ^a	80.35 ± 24.15 ^b	42.65 ± 22.03 ^b
Ethanol (% v/v)	6.50 ± 0.55 ^a	6.25 ± 0.33 ^a	6.55 ± 0.35 ^a	6.05 ± 0.92 ^a
Total Reducing sugars (g /L)	3.72 ± 0.21 ^a	3.79 ± 0.26 ^a	4.32 ± 0.32 ^a	4.02 ± 0.35 ^a
Methanol	42.80 ± 5.05 ^a	21.87 ± 2.16 ^b	17.27 ± 0.30 ^c	17.02 ± 0.65 ^c

Table 2. Free amino acid contents of extracted and nonextracted wine, with and without protease treatment.

Free amino acid (mg/L)	Extracted		Nonextracted	
	Protease	Control	Protease	Control
Arginine	486.39 ± 8.92 ^a	485.95 ± 11.21 ^a	488.49 ± 4.15 ^a	481.09 ± 3.68 ^a
Alanine	88.82 ± 2.01 ^b	28.20 ± 0.98 ^a	101.37 ± 3.02 ^d	36.89 ± 1.06 ^c
Theronine	106.47 ± 9.46 ^b	70.78 ± 5.76 ^a	111.99 ± 9.60 ^b	87.44 ± 4.36 ^a
Valine	1495.49 ± 18.42 ^b	1401.81 ± 17.83 ^a	1827.45 ± 20.99 ^d	1616.42 ± 13.52 ^c
Proline	200.43 ± 1.01 ^b	214.80 ± 1.54 ^a	195.85 ± 2.01 ^c	215.16 ± 1.87 ^a
Histidine	25.77 ± 0.63 ^a	24.29 ± 1.05 ^a	18.48 ± 0.37 ^b	19.75 ± 1.43 ^b
Phenylalanine	387.80 ± 11.50 ^b	366.91 ± 5.35 ^a	410.24 ± 6.29 ^c	385.13 ± 5.49 ^b
Glutamate	12.75 ± 0.56 ^a	n.d.	14.12 ± 1.06 ^a	n.d.
Aspartate	72.72 ± 4.14 ^b	26.53 ± 1.33 ^a	84.56 ± 3.98 ^d	48.94 ± 2.64 ^c
Total	2876.64	2619.27	3252.55	2890.82

n.d.: not detected.

Table 3. Fusel oil contents of extracted and nonextracted wine, with and without protease treatment.

Free amino acid (mg/L)	Extracted		Nonextracted	
	Protease	Control	Protease	Control
<i>n</i> -propanol	20.61 ± 0.47 ^a	14.27 ± 0.45 ^b	22.52 ± 0.09 ^c	14.73 ± 0.58 ^b
isobutanol	29.23 ± 1.72 ^a	22.99 ± 0.09 ^b	36.21 ± 0.55 ^c	25.66 ± 0.55 ^d
isoamylol	160.75 ± 1.53 ^a	114.64 ± 1.74 ^b	167.89 ± 3.88 ^c	121.05 ± 2.87 ^d
Total	210.59	151.92	226.62	161.44

from the extracted juice without protease. After treated by protease, the total amount of three fusel oils was increased by 38.62% and 40.37% in the wine from nonextracted and extracted juice, respectively. This indicated that protease treatment could enhance assimilable nitrogen yield and promote the formation of fusel oil production, which also accelerate the formation of aromatic compounds (Jiménez-Martí et al., 2007).

3.4 Volatiles

Thirty four volatiles were identified and quantified in the jujube wines with twenty one volatiles being present in all wines (Table 4). The alcohols accounted for the highest amount in jujube wines. Phenylethyl alcohol was most abundant, which made up about 92% of the total amount of the alcohols in jujube wines. Phenylethyl alcohol was likely derived from phenylalanine via

Table 4. Major volatiles ($\mu\text{g/g}$) for extracted and nonextracted wine, with and without protease treatment.

Group	Compound	Extracted		Nonextracted	
		Protease	Control	Protease	Control
Alcohol					
	2,3-Butanediol	0.43 \pm 0.19 ^a	0.45 \pm 0.26 ^a	n.d.	0.32 \pm 0.16 ^a
	3-Ethoxy-1-propanol	0.61 \pm 0.32 ^a	0.31 \pm 0.16 ^a	0.86 \pm 0.23 ^a	0.69 \pm 0.04 ^a
	Furfuryl alcohol	0.88 \pm 0.30 ^a	0.86 \pm 0.32 ^a	1.16 \pm 0.34 ^a	0.83 \pm 0.22 ^a
	(5-Methyl-2-furyl)methanol	0.07 \pm 0.02 ^a	0.08 \pm 0.03 ^a	0.08 \pm 0.02 ^a	0.06 \pm 0.03 ^a
	3-Methylthiopropanol	0.49 \pm 0.02 ^a	0.62 \pm 0.25 ^a	1.09 \pm 0.09 ^b	0.72 \pm 0.13 ^a
	Benzyl alcohol	0.17 \pm 0.09 ^a	0.14 \pm 0.09 ^a	0.25 \pm 0.11 ^a	0.23 \pm 0.10 ^a
	Phenylethyl alcohol	27.16 \pm 3.63 ^a	26.91 \pm 2.45 ^a	41.10 \pm 3.98 ^b	38.71 \pm 3.16 ^b
Total		29.81	29.37	44.54	41.56
Acid					
	Octanoic acid	0.78 \pm 0.10 ^a	0.78 \pm 0.11 ^a	0.10 \pm 0.02 ^b	0.11 \pm 0.03 ^b
	<i>n</i> -Decanoic acid	0.10 \pm 0.03 ^a	0.18 \pm 0.04 ^b	0.04 \pm 0.003 ^c	0.03 \pm 0.006 ^d
	Lauric acid	n.d.	n.d.	0.03 \pm 0.002 ^a	0.02 \pm 0.003 ^b
Total		0.88	0.96	0.17	0.16
Ethyl ester					
	Ethyl 3-hydroxybutyrate	0.03 \pm 0.006 ^a	0.02 \pm 0.005 ^a	0.05 \pm 0.003 ^b	0.06 \pm 0.019 ^b
	Ethyl octanoate	0.09 \pm 0.001 ^a	0.11 \pm 0.02 ^a	n.d.	n.d.
	Ethyl 3-phenylpropionate	0.02 \pm 0.006 ^a	0.02 \pm 0.003 ^a	n.d.	n.d.
	Ethyl decanoate	0.03 \pm 0.008 ^a	0.04 \pm 0.01 ^b	0.01 \pm 0.006 ^c	0.01 \pm 0.005 ^c
	Ethyl laurate	n.d.	0.01 \pm 0.005 ^a	0.04 \pm 0.004 ^b	0.06 \pm 0.008 ^c
	Ethyl myristate	n.d.	n.d.	0.04 \pm 0.006 ^a	0.04 \pm 0.004 ^a
	Ethyl 9-hexadecenoate	0.02 \pm 0.006 ^a	0.01 \pm 0.005 ^a	0.14 \pm 0.02 ^b	0.20 \pm 0.046 ^b
	Ethyl palmitate	0.03 \pm 0.01 ^a	0.03 \pm 0.01 ^a	0.15 \pm 0.08 ^b	0.18 \pm 0.04 ^b
	Ethyl linoleate	n.d.	n.d.	0.11 \pm 0.04 ^a	0.14 \pm 0.02 ^a
	Ethyl oleate	0.04 \pm 0.007 ^a	0.03 \pm 0.003 ^a	0.14 \pm 0.07 ^b	0.20 \pm 0.05 ^b
	Ethyl stearate	0.07 \pm 0.004 ^a	0.06 \pm 0.006 ^a	0.04 \pm 0.01 ^b	0.05 \pm 0.01 ^c
Total		0.33	0.34	0.72	0.94
Aldehyde					
	Furfural	2.52 \pm 0.99 ^a	1.63 \pm 0.51 ^a	2.61 \pm 0.47 ^a	1.61 \pm 0.66 ^a
	5-Methyl furfural	1.35 \pm 0.64 ^a	1.03 \pm 0.48 ^a	1.78 \pm 0.75 ^a	0.83 \pm 0.46 ^a
	Benzeneacetaldehyde	0.15 \pm 0.07 ^a	0.12 \pm 0.05 ^a	0.23 \pm 0.09 ^a	0.15 \pm 0.01 ^a
	5-Hydroxymethylfurfural	0.28 \pm 0.16 ^a	n.d.	0.29 \pm 0.13 ^a	0.14 \pm 0.02 ^a
Ketone					
	4-Cyclopentene-1,3-dione	0.15 \pm 0.04 ^a	0.12 \pm 0.03 ^a	0.24 \pm 0.05 ^a	0.07 \pm 0.04 ^a
	3-methyl-1,2-Cyclopentanedione	0.13 \pm 0.03 ^a	0.04 \pm 0.01 ^b	0.16 \pm 0.08 ^a	n.d.
	beta-Damascenone	0.01 \pm 0.001 ^a			
Others					
	gamma-Nonanolactone	n.d.	n.d.	n.d.	0.03 \pm 0.006 ^a
	(2,6,6-Trimethyl-2-hydroxycyclohexylidene) acetic acid lactone	n.d.	n.d.	0.02 \pm 0.005 ^a	0.01 \pm 0.007 ^a
	Methyl hexadecanoate	0.01 \pm 0.001 ^a	n.d.	0.01 \pm 0.001 ^a	0.01 \pm 0.001 ^a
	2-Acetylfuran	0.14 \pm 0.03 ^a	0.13 \pm 0.04 ^a	0.17 \pm 0.05 ^a	0.13 \pm 0.005 ^a
	2-Acetyl pyrrole	n.d.	n.d.	0.17 \pm 0.03 ^a	0.01 \pm 0.006 ^b
	2-Methoxy-4-vinylphenol	0.06 \pm 0.02 ^a	0.05 \pm 0.01 ^a	0.05 \pm 0.01 ^a	0.03 \pm 0.006 ^b

n.d.: not detected.

the Ehrlich pathway (Bell & Henschke, 2005). There was no statistically significant difference between the concentrations of other alcohols after protease treatment, except phenylethyl alcohol, 3-ethoxy-1-propanol and 3-methylthiopropanol, which could be related to the higher availability of relative amino acids in pulpy juice (Li et al., 2013c).

The second most abundant volatile group in the jujube wines was the ester group (Table 4). Most of the esters in the jujube wines were produced during fermentation. The nonextracted wines had significantly higher concentrations of ethyl 9-hexadecenoate, ethyl palmitate and ethyl oleate than the extracted wines. However, the extracted wines had higher concentrations of both ethyl octanoate and ethyl decanoate than the nonextracted wines, which is consistent with the quantities of octanoic acid and *n*-decanoic acid between the two kinds of wines. The results indicated that pulp contact seemed to the production of fatty acids has the positive correction with the production of ethyl esters, which is in agreement with other report by Li et al. (2013c).

Some quantitatively minor volatile compounds were also identified, such as β -damascenone in all wines and (2,6,6-Trimethyl-2-hydroxycyclohexylidene) acetic acid lactone in the nonextracted wines.

In Table 4, it could be found that pulp contact could enhance phenylethyl alcohol, ethyl palmitat and ethyl oleate, which are main wine flavors. Furthermore, pulp contact can promote to produce ethyl myristate, ethyl linoleate, lauric acid and gamma-nonanolactone, (2,6,6-Trimethyl-2-hydroxycyclohexylidene) acetic acid lactone. Hence, the application of pulp contact was effective to enhance the intensity of jujube wine aroma.

3.5 Sensory characteristics of jujube wine

The sensory profiles of the jujube wines were represented in a spider web plot as shown in Figure 1. From the analysis of variance (ANOVA), no significant differences were found for all sensory attributes except for 'aroma' attribute. This was likely due to high standard deviations, which were probably caused by variations of panellists sensitivity to different aroma attributes. The nonextracted wine with protease treatment was significantly more aroma than the rest. It was probably ascribed to the higher level of ethyl ester (such as ethyl 9-hexadecenoate, ethyl palmitate, ethyl linoleate and ethyl oleate) and fusel oils (Tables 3 and 4). The extracted wine without protease treatment had significantly less scores for acidic attributes than the rest (Figure 1).

3.6 Principal component analysis of volatiles in jujube wine

Principal component analysis (PCA) was applied to discriminate the volatile profiles of the jujube wines (Figures 2A and 2B). The first principal component (PC1) accounted for 69% of the total variance in the data set, while the second principal component (PC2) accounted for 25% of the total variance. The PCA biplot separated the twenty-nine different volatile compounds and the four different samples.

The first principal component (PC1) separated the wine from the extracted juice without protease from other samples based on the higher concentrations of medium-chain fatty acids (octanoic and decanoic acids) and medium-chain ethyl esters (ethyl 3-phenylpropionate, ethyl octanoate, ethyl decanoate and ethyl stearate). PC1 also separated the wine from the extracted juice with protease, but there was no significant difference

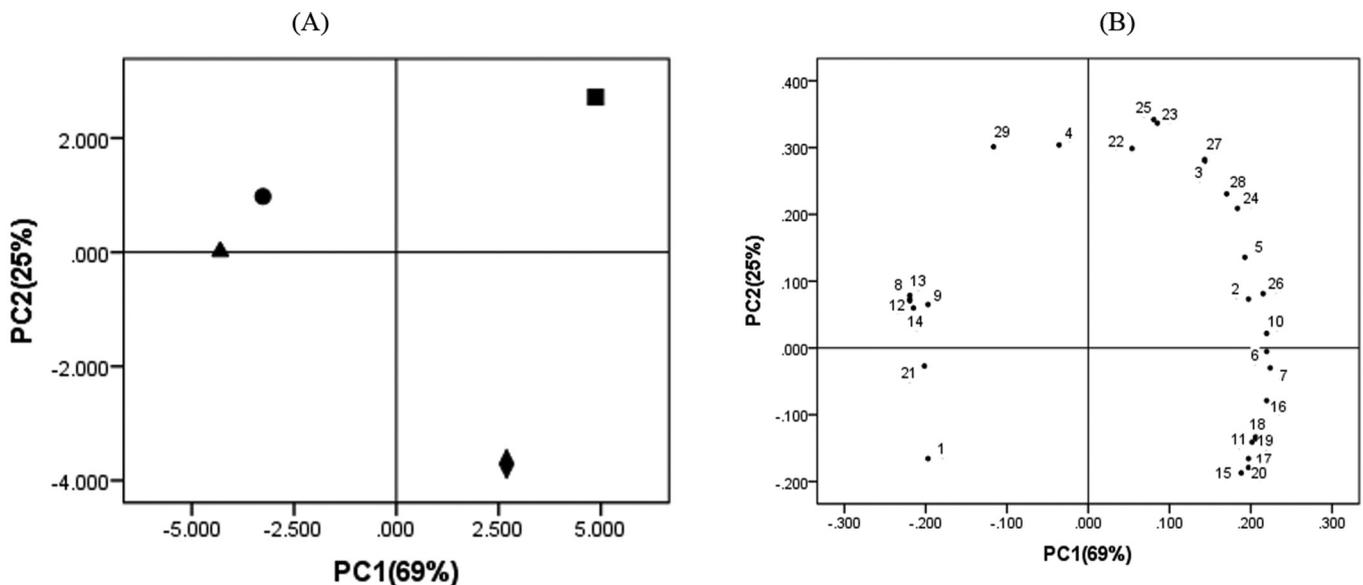


Figure 1. Biplot of principal component analysis of jujube wines: (A) wine from the extracted juice with protease (•), wine from the extracted juice without protease (▲), wine from nonextracted pulpy juice with protease (■), wine from nonextracted pulpy juice without protease (◆); (B) (1) 2,3-Butanediol; (2) 3-Ethoxy-1-propanol; (3) Furfuryl alcohol; (4) (5-Methyl-2-furyl)methanol; (5) 3-Methylthiopropanol; (6) Benzyl alcohol; (7) Phenylethyl Alcohol; (8) Octanoic acid; (9) *n*-Decanoic; (10) Lauric acid; (11) Ethyl 3-hydroxybutyrate; (12) Ethyl Octanoate; (13) Ethyl 3-phenylpropionate; (14) Ethyl Decanoate; (15) Ethyl laurate; (16) Ethyl myristate; (17) Ethyl 9-hexadecenoate; (18) Ethyl Palmitate; (19) Ethyl Linoleate; (20) Ethyl Oleate; (21) Ethyl Stearate; (22) Furfural; (23) 5-Methyl furfural; (24) Benzeneacetaldehyde; (25) 4-Cyclopentene-1,3-dione; (26) (2,6,6-Trimethyl-2-hydroxycyclohexylidene)acetic acid lactone; (27) 2-Acetylfuran; (28) 2-Acetyl pyrrole; (29) 2-Methoxy-4-vinylphenol.

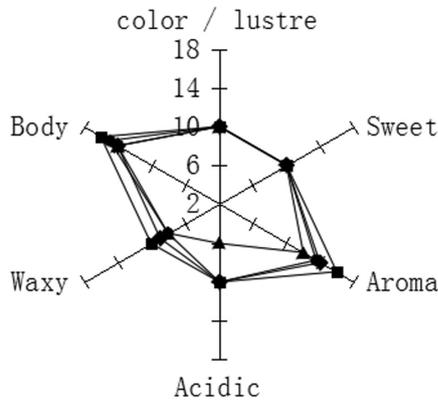


Figure 2. Aroma profile of jujube wines: wine from the extracted juice with protease (●); wine from the extracted juice without protease (▲), wine from nonextracted pulpy juice with protease (■), wine from nonextracted pulpy juice without protease (◆).

between both wines from the extracted juice with and without protease. PC1 separated the nonextracted wine for both enzyme and nonenzyme addition based on higher concentrations of benzeneacetaldehyde, 2-acetylfuran and 2-acetyl pyrrole in the former and higher ethyl palmitate, ethyl linoleate, ethyl 9-hexadecenoate and ethyl oleate in the latter.

4 Conclusion

In this study, the chemical and volatile composition of jujube wines fermented with *S. cerevisiae* with and without protease was evaluated. The chemical composition of the jujube wine from the extracted juice was different from the nonextracted juice. Alcohol and Ethyl esters were important volatile compounds produced during jujube fermentation. The results suggested that pulp contact and protease could improve the intensity and complexity of wine aroma due to the increase of the assimilable nitrogen.

References

- Bell, S. J., & Henschke, P. A. (2005). Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian Journal of Grape and Wine Research*, 11(3), 242-295. <http://dx.doi.org/10.1111/j.1755-0238.2005.tb00028.x>.
- Cai, J. B., Liu, B. Z., Ling, P., & Su, Q. D. (2002). Analysis of free and bound volatiles by gas chromatography and gas chromatography-mass spectrometry in uncased and cased tobaccos. *Journal of Chromatography. A*, 947(2), 267-275. [http://dx.doi.org/10.1016/S0021-9673\(02\)00015-8](http://dx.doi.org/10.1016/S0021-9673(02)00015-8). PMID:11883660.
- Chun, M. S., Kim, S. J., & Noh, B. S. (2012). Analysis of free amino acids and flavors in fermented jujube wine by HPLC and GC/MS. *Korean Journal of Food Science and Technology*, 44(6), 779-784. <http://dx.doi.org/10.9721/KJFST.2012.44.6.779>.
- Daudt, C. E., & Fogaça, A. de O. (2013). Phenolic compounds in Merlot wines from two wine regions of Rio Grande do Sul, Brazil. *Food*

Science and Technology, 33(2), 355-361. <http://dx.doi.org/10.1590/S0101-20612013005000045>.

- Jiménez-Martí, E., Aranda, A., Mendes-Ferreira, A., Mendes-Faia, A., & Olmo, M. I. (2007). The nature of the nitrogen source added to nitrogen depleted vinifications conducted by a *Saccharomyces cerevisiae* strain in synthetic must affects gene expression and the levels of several volatile compounds. *Antonie van Leeuwenhoek*, 92(1), 61-75. <http://dx.doi.org/10.1007/s10482-006-9135-1>. PMID:17252314.
- Lever, M. (1973). Colorimetric and fluorometric carbohydrate determination with p-hydroxybenzoic acid hydrazide. *Biochemical Medicine*, 7(2), 274-281. [http://dx.doi.org/10.1016/0006-2944\(73\)90083-5](http://dx.doi.org/10.1016/0006-2944(73)90083-5). PMID:4634162.
- Li, J. W., Fan, L. P., Ding, S. D., & Ding, X. L. (2007a). Nutritional composition of five cultivars of Chinese jujube. *Food Chemistry*, 103(2), 454-460. <http://dx.doi.org/10.1016/j.foodchem.2006.08.016>.
- Li, A., Ding, Y., Chen, J., & Zeng, J. (2013b). The bitter substances origin and composition analysis in jujube wine. *Journal of Chinese Institute Of Food Science and Technology*, 7, 236-241.
- Li, X., Lim, S. L., Yu, B., Curran, P., & Liu, S. Q. (2013c). Mango wine aroma enhancement by pulp contact and b-glucosidase. *International Journal of Food Science & Technology*, 48, 2258-2266.
- Liu, Y. H., & Zhao, Q. X. (2011). Studies on the fermentation technics of dry wild jujube wine. *Advanced Materials Research*, 236-238, 2951-2956. <http://dx.doi.org/10.4028/www.scientific.net/AMR.236-238.2951>.
- National Standard of the People's Republic of China. (2006). *GB/T 15038-2006: analytical methods of wine and fruit wine*. Beijing: China Standard Press.
- Park, H. J., Kim, K. Y., & Jeong, H. S. (2009). Quality changes of jujube wine by hydrostatic pressure and freezing treatment during storage. *Journal of the Korean Society of Food Science and Nutrition*, 38(1), 89-97. <http://dx.doi.org/10.3746/jkfn.2009.38.1.089>.
- Perestrello, R., Fernandes, A., Albuquerque, F. F., Marques, J. C., & Camara, J. S. (2006). Analytical characterization of the aroma of *Tiata Negra* Mole red wine: Identification of the main odorants compounds. *Analytica Chimica Acta*, 563(1-2), 154-164. <http://dx.doi.org/10.1016/j.aca.2005.10.023>.
- Piddocke, M. P., Fazio, A., Vongsangnak, W., Wong, M. L., Heldt-Hansen, H. P., Workman, C., Nielsen, J., & Olsson, L. (2011). Revealing the beneficial effect of protease supplementation to high gravity beer fermentations using '-omics' techniques. *Microbial Cell Factories*, 10(1), 27. <http://dx.doi.org/10.1186/1475-2859-10-27>. PMID:21513553.
- Schultz, H. T., Flath, R. A., Mon, T. R., Eggling, S. B., & Teranishi, R. (1977). Isolation of volatile components from a model system. *Journal of Agricultural and Food Chemistry*, 25(3), 446-449. <http://dx.doi.org/10.1021/jf60211a038>.
- Sentheshanmuganathan, S., & Elsdén, S. R. (1958). Formation of tyrosol by *Saccharomyces cerevisiae*. *The Biochemical Journal*, 69(2), 210-218. <http://dx.doi.org/10.1042/bj0690210>. PMID:13546168.
- Stevens, R. (1960). Beer flavor. I. Volatile products of fermentation: a review. *Journal of the Institute of Brewing*, 66(6), 453-471. <http://dx.doi.org/10.1002/j.2050-0416.1960.tb01740.x>.
- Yu, H., Ding, Y. S., Mou, S. F., Jandik, P., & Cheng, J. (2002). Simultaneous determination of amino acids and carbohydrates by anion-exchange chromatography with integrated pulsed amperometric detection. *Journal of Chromatography. A*, 966(1-2), 1-2. [http://dx.doi.org/10.1016/S0021-9673\(02\)00739-2](http://dx.doi.org/10.1016/S0021-9673(02)00739-2). PMID:12214708.