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Evaluation of different *Saccharomyces cerevisiae* strains on the profile of volatile compounds and polyphenols in cherry wines

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ABSTRACT

Tart cherries of 'Early Richmond', widely grown in Shandong (China), were fermented with six different *Saccharomyces cerevisiae* strains (BM4×4, RA17, RC212, D254, D21 and GRE) to elucidate their influence on the production of volatiles and polyphenols. Acetic acid and 3-methylbutanol were found in the highest concentrations among all identified volatiles with all six yeast strains, followed by 2-methylpropanol and ethyl lactate. RA17 and GRE cherry wines were characterised by a higher amount of esters and acids. D254 wine contained a higher concentration of alcohols. With respect to polyphenols, five phenolic acids and four anthocyanins were identified among all tested samples, with chlorogenic and neochlorogenic acids, cyanidin 3-glucosylrutinoside and cyanidin 3-rutinoside being the major compounds. When using principal component analysis to classify the cherry wines according to the volatiles and polyphenols, they were divided into three groups: (1) RA17 and GRE, (2) RC212 and D254 and (3) BM4×4 and D21.

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1. Introduction

Shandong (a province situated in eastern China) is the main region of cherry production in the country. The cherry crop occupies more than 8000 ha in this province, with an estimated annual harvest of 60,000 tons, creating a sales revenue of over 2 billion Chinese yuan (Han et al., 2008). However, cherry is relatively perishable, and is often not availably served as fresh table fruit after transportation; thus, it is often processed to juice and wine, especially with the sour varieties (*Prunus cerasus* L.). In Shandong, for many years, the cherry wine has been an important segment of the fruit and vegetable industry. As Shandong cherry wine became more popular in China over the past decade, its characteristic and distinct flavour began to receive more scrutiny from the consumers.

Aroma is a most important and distinguishing characteristic of cherry wine. It is derived from hundreds of volatile compounds from cherry berry, and from wine-making by yeasts and aging process. Considering the fact that (1) most of the odorous compounds are produced during fermentation, and (2) yeasts have a significant effect on the sensory characteristics of wines, the selection of a proper yeast strain is critical for the development of the desired cherry wine style (Patel & Shibamoto, 2002). During fermentation, the function of yeasts in the production of flavour is to release varietal volatile compounds from cherry precursors, and in the meantime, to synthesise *de novo* yeast-derived volatile compounds (Molina, Swiegers, Varela, Pretorius, & Agosin, 2007; Molina et al., 2009). Today, a wide range of wine yeast strains are commercially available, which offers us the opportunity to explore one or more suitable yeasts to assure a rapid and reliable fermentation process, and give wines a consistent and predictable quality (Rodriguez, Lopes, Barbagelata, Barda, & Caballero, 2010). However, to the best of our knowledge, there have been limited studies performed to evaluate the effect of *Saccharomyces cerevisiae* strains on the flavour profiles of cherry wines. Furthermore, cherry wines are still under-researched among fruit wines. Hence, the first objective of this study is to employ the headspace solid phase microextraction with gas chromatography–mass spectrometry (HS-SPME-GC–MS) technique for the identification and quantification of volatiles of cherry wines inoculated with six commercial yeast strains.

Phenolic compounds are also important group of cherry wine constituents, and they greatly contribute to the sensory properties by affecting the colour and taste (Czyzowska & Pogorzelski, 2002). These compounds are natural antioxidants, and possessing neuroprotective and potent cancer-preventive properties, which are considered as beneficial and proved (Lee, Hur, Lee, & Lee, 2005; Yoo, Al-Farsi, Lee, Yoon, & Lee, 2010). Wine, in comparison with other sources, contains relatively high amounts of highly diversified polyphenols. Most of the phenolic compounds pass from berry to the wine during extraction and fermentation, and a few are newly formed, such as some free phenolic acids and flavonic isomers (Wulf & Nagel, 1980). One of the possibilities of the occurrence of new polyphenols in wine may be transformation (e.g.

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enzymatic) of substances contained in fruit. Enzymes of fruit origin and microorganisms responsible for fermentation may lead to oxidation or hydrolysis of native components (Nagel & Wulf, 1979), therefore, when fermenting the same must, the employment of whatever yeast strains are straightly associated with the composition and content of polyphenols in the final product. Here, in the current investigation, the second purpose was to elucidate the effect of different yeast species on the profiles of polyphenols in cherry wines, with special attention paid to the phenolic acids and anthocyanins.

2. Materials and methods

2.1. Chemicals

Folin–Ciocalteu's phenol reagent, chlorogenic acid, *p*-coumaric acid, neochlorogenic acid, *p*-hydroxybenzoic acid, caffeic acid, cyanidin 3-glucoside, gallic acid, anhydrous sodium sulphate, ethanol (chromatographic grade) and methanol (99%) were purchased from Sigma Chemical Co. (St. Louis, MO). Cyanidin 3-rutinoside, cyanidin 3-glucosylrutinoside and peonidin 3-rutinoside were obtained from Polyphenols Laboratories (Sandnes, Norway). All volatile standards were purchased from Aldrich (Milwaukee, Wis., USA) and Fluka (Buchs, Switzerland). All other chemicals used were of analytical or high performance liquid chromatography (HPLC) grade.

2.2. Yeast strains

Six *S. cerevisiae* commercial strains including Lalvin BM4×4, RA17, RC212, D254, D21 and GRE, purchased from Lallemand (France), were used in this work.

2.3. Preparation of cherry wine

Fruits of Early Richmond picked at commercial maturity during the 2009 harvest season from the 'menlou' cherry orchard (Yantai, China) were used in this work. Cherries were crushed, manually deseeded and poured into 20 L bottles where they were treated pectinolytic preparation Lallzyme EX-V pectinase (20 mg/L) for 10 h at 20 °C. Then, sucrose was added up to 210 g/L; sulphur dioxide additions were made as needed to maintain approximately 30 mg/L of free sulphur dioxide. Initial must analysis results were as follows: total sugars 165 g/L, titratable acidity 7.9 g/L, pH 4.03. Fermentation occurs under control temperature (20 °C) with an association of *S. cerevisiae* strains (250 mg/L) as recommended by the producer. Fermentation progress was monitored by measuring the total reducing sugars (TRS) using a glucose oxidase test strip (Chemstrip, Boehringer Mannheim Corp., Indianapolis, IN) until the sugar content was reduced below 1 g/L. The finished young wine was filtered through four layers of fine cheesecloth into 750 mL wine bottles that were subsequently corked and stored in an 18 °C storage chamber until the analysis. All samples were done in triplicate.

2.4. Analytical method

Total reducing sugars (TRS), titratable acidity (expressed as g/L of malic acid), pH, total soluble solids (TSS), ethanol, volatile acidity (VA), and free SO_2 were determined according to the official methods (OIV, 2005).

2.5. Total phenolics

The total content of phenolics was measured according to the Folin–Ciocalteu colorimetric method (Rakitzis, 1975). Briefly, 1 mL of appropriately diluted sample or a standard solution was added to 9 mL of distilled water, 1 mL of Folin–Ciocalteu phenol reagent and 10 mL of 7% Na_2CO_3 solution. The mixture was then immediately diluted to a volume of 25 mL in a volumetric flask with distilled water and left to stand for 90 min at 23 °C. The absorbance was read at 750 nm, and results are expressed as milligrams of gallic acid equivalents (GAE) per litre of cherry wine.

2.6. Total anthocyanins

Total anthocyanins were determined by the pH differential method (Kim, Heo, Kim, Yang, & Lee, 2005). Cherry wine dissolved in 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) were measured simultaneously at 510 and 700 nm after 15 min of incubation at 23 °C. The content of total anthocyanins was expressed as milligrams of cyanidin 3-glucoside equivalents (CGE) per litre of cherry wine. A molar absorptivity of 26,900 L/mol cm was used for cyanidin 3-glucoside, which has the molecular mass of 449.2 g/mol.

2.7. Fractionation of polyphenols

For the easier separation of anthocyanins from nonanthocyanin phenolics during HPLC analysis, a simple fractionation was performed using two connected and preconditioned C18 Sep-Pak cartridges (Waters, Milford, MA, USA), as described by Kim et al. (2005). Briefly, a load of 200 μL phenolic extract was applied onto the cartridges, and washed with 6 mL of 0.01 N aqueous HCl to get rid of sugars, acids, and other water-soluble compounds. Nonanthocyanins were obtained by rinsing cartridges with 20 mL of ethyl acetate and followed by elution. Finally, the adsorbed anthocyanins were eluted with 10 mL of methanol with 0.1% (v/v) HCl, collected, dissolved in 50% aqueous methanol and distilled water, and stored at −4 °C under nitrogen gas to prevent oxidation until HPLC analysis.

2.8. HPLC analysis of polyphenols

A reversed-phase HPLC system (Hewlett–Packard model 1100; Palo Alto, CA) consisting of a 20 μL sample loop, a photodiode array detector, a quaternary pump, and a vacuum degasser was employed for the analysis of polyphenols as reported previously (Kim et al., 2005). Separation was performed on a C18 reversed-phase Symmetry Analytical column (5 μm × 250 mm × 4.6 mm; Waters Corp., Milford, MA) thermostated at 30 °C. The mobile phase was composed of a solvent A (0.1% H_3PO_4 in HPLC grade water) and solvent B (0.1% H_3PO_4 in HPLC grade acetonitrile). Linear solvent gradient was applied as follows (total 60 min): 92% A/8% B at 0 min, 89% A/11% B at 4 min, 87% A/13% B at 25 min, 80% A/20% B at 27.5 min, 40% A/60% B at 50 min, 92% A/8% B at 55 min, and 92% A/8% B at 60 min. The flow rate was at 1.0 mL/min, and the runs were monitored at the following wavelengths: phenolic acids at 320 nm, and anthocyanidins at 520 nm. UV–visible spectra were simultaneously recorded from 200 to 600 nm during sample running. For quantification analysis, the standard curves of individual phenolics that relate various concentrations of authentic standard solutions to the areas of their corresponding peaks were obtained.

2.9. Qualitative analysis of volatile compounds

A portion of 5 mL cherry wine, 5 μL of 3-octanol (internal standard, 100 mg/L standard solution in ethanol) and 1.5 g of NaCl were added into 15 mL headspace vial. Vials were capped with a Teflon septum and an aluminium cap (Chromacol Ltd., Herts, England). Volatile compounds were extracted from the headspace using a fibre of 50/30 μm DVB/CAR/PDMS (Supelco, Bellefonte

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