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Dynamic changes in phenolic compounds, colour and antioxidant activity of mulberry wine during alcoholic fermentation

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ABSTRACT

Dynamic changes in total phenolics (TP), total flavonoids (TF), total anthocyanins (TA), two main monomer anthocyanins, cyanidin-3-*O*-glucoside (C3G) and cyanidin-3-*O*-rutinoside (C3R), colour and antioxidant activities as well as correlations among these factors were investigated in mulberry wine during alcoholic fermentation. TP and TF increased rapidly from day 0 to 3 and showed unobvious changes from day 3 to 10, whereas TA, C3G and C3R increased first and then decreased. C3G and C3R reached their maximum at days 1 and 2, respectively; thereafter, C3G decreased rapidly, whereas C3R was more stable. Five colour parameters, L*, a*, b*, C* and H*, changed significantly from day 0 to 2 and showed unobvious changes from day 2 to 10. For antioxidant activity, changes of the scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH·) and reducing power were similar to changes of colour parameters. TP, TF, TA and C3R exhibited significant correlations ($P < 0.01$) with antioxidant activity, whereas C3G exhibited weaker correlations.

1. Introduction

Morus alba L., which belongs to the *Morus* genus and the *Moraceae* family, is a type of perennial woody plant (Ercisli & Orhan, 2007). This genus has 24 species with one subspecies, and 100 varieties are known to date (Ercisli & Orhan, 2007). It has the characteristics of wide adaptability and easy cultivation because it adapts to a wide area of tropical, subtropical, and temperate zones in the northern hemisphere and to the tropics of the southern hemisphere (Ercisli & Orhan, 2007; Natić et al., 2015). It is also widely distributed in most areas of China. Ripe mulberry, which belongs to the fruit of *Moraceae* family, is oval purple-black or white jade. Mulberry belongs to the group of berries and has the characteristics of thin skin, succulence and rich nutrition. Furthermore, mulberry has a strong seasonal characteristic and a short harvest season; the harvesting period is between May and June in Beijing, China. It is unfavourable for storage and transportation because it is susceptible to spoilage at room temperature. To prolong its shelf

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life, postharvest mulberry should be processed quickly. Mulberry can be consumed either fresh or processed, and it can be processed into many forms, including syrup, jam, pulp, ice cream, vinegar, concentrate, and fruit wine (Gundogdu, Muradoglu, Sensoy, & Yilmaz, 2011).

Mulberries have been reported to exhibit several biological activities, including antioxidant (Kamiloglu, Serali, Unal, & Capanoglu, 2013; Yang, Yang, & Zheng, 2010), anti-inflammatory (Liu & Lin, 2013, 2014), hypolipidaemic (Yang et al., 2010) and neuroprotective effects (Kang, Hur, Kim, Ryu, & Kim, 2006), which are linked to the presence of phenolics in mulberry. Black mulberries have cyanidin-based anthocyanins, particularly cyanidin-3-O-glucoside (C3G) and cyanidin-3-O-rutinoside (C3R), and numerous flavonoids, including rutin, quercetin and isoquercitin (Ercisli & Orhan, 2007; Özgen, Serçe, & Kaya, 2009), as well as chlorogenic acid, gallic acid and caffeic acid (Kamiloglu et al., 2013).

Mulberry wine is one product of mulberries. It has the advantages of fruit wine and is consistent with the shift of Chinese alcoholic beverage industry's policies of low consumption grain and low alcohol content. Furthermore, mulberry wine can be more adaptable to changes in the alcohol consumption market and exhibits a huge development space and market potential. Therefore, processing mulberry into fruit wine not only makes full use of sericulture resources but also greatly improves the economic benefits of sericulture and can enrich fruit wine varieties and boom the fruit wine market.

Current research into mulberry wine brewing has primarily focused on analysing the volatile aromatic compounds (Butkhup et al., 2011), evaluating the colour parameters and antioxidant activity (Kalkan Yildirim, 2006) and optimising the fermentation process for preparation (Wang et al., 2013). However, research into dynamic changes in phenolic compounds, colour, and antioxidant activity of mulberry wine during alcoholic fermentation is not as common. Furthermore, because processing mulberry into fruit wine exhibits many of the advantages mentioned above, it is necessary to study the changes in phenolic compounds, colour, and antioxidant activity that occur when processing mulberry into mulberry wine. Therefore, dynamic changes in the phenolic compounds, colour and antioxidant activity of mulberry wine during alcoholic fermentation were investigated in this study. Dynamic changes in the content of total phenolics (TP), total flavonoids (TF), total anthocyanins (TA), two main monomer anthocyanins, i.e., C3G and C3R, colour, and antioxidant activity as well as the correlations between them were analysed in mulberry wine during alcoholic fermentation of four mulberry varieties, which may provide a theoretical basis for processing mulberry into mulberry wine.

2. Materials and methods

2.1. Materials

C3G (Purity ≥ 98.0%, HPLC) was purchased from Chengdu Must Bio-technology Co., Ltd. (Chengdu, Sichuan, China). C3R (Purity ≥ 98.0%, HPLC) was purchased from Sigma (St. Louis, MO, USA). The other reagents used were of analytical grade.

Completely ripe mulberries were picked from Daxing, Beijing, China. *Saccharomyces cerevisiae* was LALVIN CY3079 (LALLEMAND, Birkerød, Denmark). Pectinase was purchased from ENARTIS (Novara, Italy).

2.2. Vinification process

The vinification process of mulberry wine was performed according to a mulberry wine patent by Sheng and Huang (2011). Completely ripe mulberries were picked out and then crushed (added SO_2 60 mg/l, pectinase 30 μl/l). Then, sucrose was added to adjust the sugar content, and the mixture was inoculated with active dry yeast at 0.25 g/l. The fermentation temperature was controlled at 20–25 °C. The cap was punched twice a day during the first seven days of fermentation. The skins were removed on day 7.

Samples of 30 ml were taken every day from day 0 to day 10 during fermentation to determine the changes in the related indicators. Fermentation was done with three parallel sets for each mulberry variety. Samples were placed in a –20 °C refrigerator for subsequent experiments after centrifugation (5000 × *g*, 10 min).

2.3. Determination of TP, TF and TA content

The Folin–Ciocalteu method with some modification was used for the determination of the TP content (Li et al., 2014; Ma et al., 2013, 2014). Briefly, 1 ml of sample, 60 ml distilled water, and 5 ml Folin–Ciocalteu reagent were added in a 100 ml volumetric flask successively. After reaction for 5 min, 15 ml of 20% Na_2CO_3 was added. The solution was diluted with water to 100 ml and mixed well. The mixture was allowed to react at room temperature in the dark for 2 h, and absorbance was measured at 765 nm. The results were expressed as gallic acid equivalents (GAE) (mg/l of GAE). The TF content was determined according to a previously described protocol (Peinado, de Lerma, Moreno, & Peinado, 2009) with some modification. Briefly, in a 10 ml centrifuge tube, 100 μl of sample was mixed with 1 ml distilled water and 100 μl $NaNO_2$ (0.5 mol/L), and the mixture was allowed to react for 5 min. At the end of the reaction, 200 μl $AlCl_3$ (0.3 mol/L) was added and the mixture was allowed to stand for 6 min. Finally, 1 ml NaOH (1 mol/L) and 5 ml distilled water were added to the reaction mixture, and absorbance was read at 510 nm. The results were expressed as catechin equivalents (mg/l of CTE). The amount of TA content was determined using the pH differential method (Lee, Durst, & Wrolstad, 2005) with some modification. Briefly, the absorbance of sample in 0.025 mol/L potassium chloride solution (pH 1.0 buffer) and 0.4 mol/L sodium acetate buffer (pH 4.5 buffer) was measured simultaneously at 510 and 700 nm by a UV-vis spectrophotometer and calculated using the equation $A = (A_{510} - A_{700})\ pH1.0 - (A_{510} - A_{700})\ pH4.5$. The TA content was calculated using the following formula: TA content = $(A \times MW \times DF \times 1000)/(\varepsilon \times 1)$, where A is the absorbance, MW is the molecular weight of cyanidin-3-glucoside (449 g mol–1), DF is the dilution factor, and ε is the molar extinction coefficient of cyanidin-3-glucoside (29600). The results were expressed as C3G equivalents (mg/l of C3GE).