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Evaluation of antioxidant capacity and flavor profile change of pomegranate wine during fermentation and aging process

Yongli Lan [a], Jin Wu [a], Xuejiao Wang [a], Xuchun Sun [a], Robert M. Hackman [b], Zhixi Li [a], Xianchao Feng [a,*]

[a] College of Food Science and Engineering, Northwest A&F University, No. 22 Xinong Road, Yangling, Shaanxi 712100, China
[b] College of Agricultural and Environmental Sciences, University of California, Davis One Shields Avenue, Davis, CA 95616, USA

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ABSTRACT

Antioxidant properties and flavor characteristic profile of pomegranate wine during winemaking were investigated. The total phenol content and radical scavenging activity exhibited a slightly decrease in the end edge. Punicalagins and gallic acid were revealed to be the most abundant phenolic compounds, followed by ellagic acid and vanillic acid. These constituents were mainly responsible for the effective antioxidant capacity of pomegranate wine. The major changes of flavor qualities occurred in the initial stage, particularly 0–4 day of fermentation. Fermentation significantly reduced the relative content of aldehydes, ketones, heterocyclic and aromatic compounds, but promoted the generation of esters and alcohols. This is the first time of using E-nose and E-tongue to monitor odour and taste changes in the brewing process of pomegranate wine. The study may provide a promising instruction for improving functional features and quality control of the pomegranate wine.

1. Introduction

Pomegranate (*Punica granatum* L.) is mainly grown in occidental Asia and Mediterranean Europe, also in warm climate areas of the Americas and other parts of the world (Pagliarulo et al., 2016). Nowadays, pomegranate is regarded as a diet source of bioactive compounds due to its health promoting effects, such as maintenance of redox balance, protection from cardiovascular diseases, diabetes, Alzheimer's and cancer et al. (Viuda-Martos, Fernández-López, & Pérez-Álvarez, 2010; Xuan et al., 2014). However, the consumption of the fresh pomegranate fruit is somewhat inconvenient due to the difficulty of taking the edible arils out. Products such as juices, fruit vinegar, jellies, grenadine syrup et al. are commercially booming and well appreciated throughout the world because of their desirable taste and aroma, flavor and interesting nutritional features (Ferrara, Cavoski, Pacifico, Tedone, & Mondelli, 2011; Viuda-Martos et al., 2010). Nevertheless, huge waste of pomegranate fruits particularly secondary quality and over-ripe fruits still exists and the quality of processed products needs to be further improved. Therefore, with the aim of minimizing production losses and improve the quality of wine, new uses and methods for pomegranate processing should be developed. The elaboration of pomegranate wine has been recently put forward as a novel approach of exploitation of pomegranates (Lantzouraki, Sinanoglou, Tsiaka, Proestos, & Zoumpoulakis, 2015).

Wine fermentation can not only increase the number and total concentration of the volatiles but health benefits (Lim, Jeong, & Shin, 2012). For the foregoing reasons, pomegranate wines have already attracted extensive attention and have been investigated thoroughly. Significant volatile compositional alterations and the characteristic flavor formation of pomegranate wines are happening during the fermentation, leading to an alluring, unique and fragrant pomegranate product (Lantzouraki et al., 2015).

Only a few studies have sporadically reported the specific bioactive compounds of pomegranate wine to show that fermentation could promote the production of some novel functional ingredients (Berenguer et al., 2016; Ordoudi et al., 2014). However, the detailed real time change of antioxidant properties still needs to be further studied during the whole fermentation and aging process. Volatile compounds are important factors that affect the flavor quality of pomegranate wine. Nevertheless, to the best of our knowledge, the real-time changes of flavor characteristics and particular compounds in pomegranate wine during winemaking process have not been well investigated. Therefore, the effective real-time monitoring of wine fermentation during the whole process is necessary to reduce stuck and sluggish fermentation. The understanding of the changes of antioxidant capacity, taste and aroma characteristics as well as particular compounds during

* Corresponding author.
E-mail address: fengxianchao1@hotmail.com (X. Feng).

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winemaking will also enable, in turn, the improvement of the quality of pomegranate wine.

Electronic nose (E-nose) and electronic tongue (E-tongue) could objectively detect and analyze the slight variation in taste and aroma of different samples. GC–MS technology has the advantages of requiring less sample, rapid analysis, and high sensitivity and could identify what the specific flavor compounds are (Yang et al., 2016). However, a separation and enrichment process is necessary before volatile analysis since pomegranate wine has complex constituents with a high level of volatile substances, notoriously. Solid phase micro-extraction (SPME) technology is widely used for this purpose owing to its outstanding advantages.

The main objectives of the present study were to investigate the antioxidant properties and flavor profile changes of functional pomegranate wine during fermentation and aging process. Total phenols content (TPC), total anthocyanin content (TAC), DPPH free radical, super oxide anion and hydroxyl radicals scavenging activity as well as phenolic profiles were monitored throughout the whole winemaking process. Overall tastes and aromas quality and dynamic changes of flavor ingredients in pomegranate wine were also monitored during winemaking process using the aforementioned technologies. This is the first time to use E-tongue and E-nose systems to monitor ferments of pomegranate wine. Additionally, the quality parameters including pH, titratable acidity (TA), total soluble solid (TSS), alcohol content and organic acid profiles were also monitored throughout the whole winemaking process.

2. Materials and methods

2.1. Chemicals and reagents

ALCOHOL ACTIVE DRY YEAST was purchased from Angel Yeast Co., Ltd (Hubei, China). 2-octanol was GC grade and from Sigma-Aldrich (Steinhem, Germany). All chemicals and reagents used were HPLC grade and obtained from Tianjin Chemical Company, Ltd. (Tianjin, China).

2.2. Sample preparation

The *Bright red sweet* pomegranate fruits were donated by a local industry (Xian Danruoer Pomegranate Brewery Co., Ltd, Xian, China). Arils were manually separated from the pith of pomegranate fruits. Pomegranate juice was collected by pressing the arils with a laboratory beating apparatus (HausElec, Qingdao, China) and then filtrated through gauze. The obtained pomegranate juice (TSS content, 16 °Brix; TA, 0.546 g citric acid/100 mL; pH value, 3.79) of approximately 10 L was stored at −20 °C for use.

TSS of pomegranate juice was adjusted to 20 °Brix in a 2500 mL glass fermentation cylinder. After addition of the activated yeast at rates of 1.0 g/L, fermentation was started and temperature was kept at 28 ± 1 °C throughout the fermentation process. After day 3, the wine was conducted a secondary fermentation by adding to pectinase (3 g/L), sulphurous acid (1 mL/L) and yeast (1 g/L). The wine was transferred to a new glass vessel and placed in darkness and at room temperature for 10 days of aging. Wine samples were prepared on 0, 2, 4, 6, 8, 10, 18 and 26 days and then sealed and stored at −20 °C until analysis.

2.3. Determination of quality parameters

The pH was measured using a MP220 portable pH-meter (Mettler-Toledo, Switzerland). TA was detected by titrating with 0.01 M NaOH and expressed as g citric acid. TSS (°Brix) of the wine samples was analyzed using an A.Krüss Optronic GmbH (Hamburg, Germany) refractometer. Alcohol content (%) was determined using a pycnometer.

2.4. Organic acids analysis

Organic acids were measured using a Shimadzu LC-15C system equipped with degasser, quaternary pump, autosampler, ZORBAX SB-C18 chromatographic column (4.6 × 250 mm; Agilent, USA) and a SPD-15C UV-detector (Shimadzu, Japan) (Berenguer et al., 2016). Briefly, all samples were centrifuged for 5 min at 15000*g* (model H2050R-1, Hettich Zentrifugen, Chengdu, China) and then the supernatant was filtered through a 0.45 μm PVDF membrane. The mobile phase for isocratic elution consisted of 0.1% phosphoric acid solution with a flow rate of 0.7 mL/min, loading volume of 10 μL and ultraviolet detector's wavelength of 210 nm. The qualitative and quantitative characterization of citric, malic, tartaric, oxalic and lactic acids were based on the standards and the related chromatographic peaks area.

2.5. Determination of polyphenol and antioxidant activities

2.5.1. Determination of total phenolic content

Total phenols content (TPC) was determined by the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965). Samples (0.5 mL of) were diluted 10 times and mixed with 1 mL of the Folin-Ciocalteu reagent and 2 mL 7.5% sodium carbonate. The mixture was vortexed and allowed to stand for 5 min at 50 °C. The absorbance was measured with a spectrophotometer at 760 nm. Gallic acid was used to construct a calibration curve and expressed as gallic acid equivalent (GAE).

2.5.2. Determination of total anthocyanin content

The total anthocyanin content (TAC) was determined by the previous method with some modifications (Castro-Vázquez, Díaz-Maroto, González-Viñas, & Pérez-Coello, 2009). Briefly, the pH of wine samples was brought to 1.0 with potassium chloride and 4.5 with sodium acetate buffers. All absorbance were measured at room temperature using distilled water as the blank. The absorption of solutions was measured at 510 nm and 700 nm. The results were expressed as milligrams of cyanidin-3-glucoside (cy-3-glu) equivalents per liter.

2.5.3. Dpph·antioxidant capacity assay

DPPH radical scavenging activity was determined as described by (Yamaguchi, Takamura, Matoba, & Terao, 1998). The diluted sample (2 mL) was added to 0.1 mM DPPH in ethanol (2 mL) and then vortexed. The absorbance was measured at 517 nm after 30 min of incubation in dark. Percent DPPH scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging } (\%) = [(\text{Control absorbance-Sample absorbance})/(\text{Control absorbance})] \times 100\% \quad (1)$$

2.5.4. Super oxide anion and hydroxyl radicals scavenging activity

Generation of superoxide radicals (O^{2-}) and hydroxyl radicals ($OH^{\cdot}$) were carried out according to literature (Liu, Ooi, & Chang, 1997). The radical inhibition was calculated by the following equation:

$$\text{Inhibition } (\%) = [(Ao - As)/Ao] \times 100 \quad (2)$$

where Ao represents the absorbance of control and As represents the absorbance of sample.