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Research Article
Phenolic Compounds Content and Antioxidant Activity of Mulberry Wine During Fermentation and Aging

[1]XiaoHua Xie, [2]Lingli Zhang and [2]Xueling Gao

[1]Chuzhou Vocational and Technical College, 2188 Fengle Road, Chuzhou, Anhui, China
[2]School of Tea and Food Science, Anhui Agricultural University, 130 West Changjiang Road, Hefei, Anhui, China

Abstract

Background and Objective: Mulberry is a delicious yet delicate fruit with a high nutritional value. However, nutritional values are changed when mulberry is processed into wine. This study aims to reveal the changes in phenolic compounds and antioxidant activity during fermentation and aging of wines made from mulberry fruit. **Materials and Methods:** Total anthocyanins (TAC), total phenolics (TPC), total tannins (TTC) and total flavonoids (TFC) as well as antioxidant activities were measured in mulberry wine during alcoholic fermentation and aging. Data were analyzed by one-way ANOVA followed by Duncan's range test using Prism™. **Results:** Overall, fermentation increased the TPC, TTC and TFC. TAC reached it maximum (911.73 mg L^{-1}) at day 1 of fermentation and then reached it minimum (158.80 mg L^{-1}) value aged to 90 days ($p<0.05$). Changes in the free radical scavenging activity and reducing power were similar to changes in the ferric reducing antioxidant power. During the wine-making process, antioxidant capacity increased during fermentation and decreased during aging. **Conclusion:** Mulberry wine has a higher TAC, TPC, TTC, TFC and antioxidant activity and the changes in TAC, TPC, TTC and TFC were consistent with the changes in antioxidant activity during fermentation and aging.

Key words: Mulberry, mulberry wine, aging, polyphenols, antioxidant

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Corresponding Author: Xueling Gao, School of Tea and Food Science, Anhui Agricultural University, 130 West Changjiang Road, Hefei, Anhui, China
Tel: 86-551-65786342

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mulberry (*Morus* spp.) is widely distributed across the world. Mulberry fruits have a sweet flavor and are abundant in phytochemicals such as ascorbic acid, phenolic compounds, anthocyanins and other flavonoid compounds[1,2]. Due to their abundant bioactive compounds, mulberries offer a number of health benefits to consumers, including antioxidant, anticancer, neuroprotective, hypolipidemic and anti-atherosclerosis functionalities[3-5]. However, mulberries have a short harvesting season and have sensitivity to storage and transport, for instance fresh mulberries can only be kept for several days in a refrigerator. As a result, mulberry fruit is popularly made into jam, pies, wines and liquor[6,7].

In addition to taste, consumers also care about the health benefits of eating fruits, especially the bioactive compounds and their bioavailability. Mulberry wine is a popular alcoholic drink consumed in Asia, due in part to the potential health benefits related to its bioactive composition[6,8]. Alcoholic fermentation of mulberry results in more abundant phenolic compounds and a purple-black color of the final wine[9]. Wine properties are affected by the technology used to produce the wine or during aging[10,11]. The aging process also enhances the organoleptic properties of wine, thus making it more pleasant[12]. These studies showed that it is essential to further evaluate the constituents of mulberry wine.

The objective of this study was to investigate the changes in total anthocyanin, total phenolic, total tannin and total flavonoid contents during alcoholic fermentation and aging of mulberry wine. Antioxidant activities were also tracked. The results of this study provide a better understanding of the types and levels of the bioactive compounds and the overall antioxidant activity in mulberry wine.

MATERIALS AND METHODS

Chemicals: Folin-Ciocalteu reagent, gallic acid, lutin, gallotannic acid and ascorbic acid were obtained from Sinopharm Chemical Reagent (Shanghai, China). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and 2, 4, 6-Tris (2-pyridyl)-1 and 3, 5-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other general chemicals were of analytical grade and were obtained from local suppliers.

Mulberry samples and winemaking: Mulberry fruits (*Morus* spp.) were collected in May of 2016 in the Central-Eastern of China. The fruits in this study had a °Brix of 18±0.26, a titratable acidity of 7.79±0.01 g L^{-1} as citric acid and a pH of 3.69±0.22. Mulberries were crushed into mash and then treated at 16°C for 24 h with pectinase (0.05 g kg^{-1}, Laffort, Sydney, Austrilia) and potassium metabisulphite (70 mg kg^{-1}). The mashes were then adjusted to 20°Brix with food-grade pure sucrose and inoculated with *S. cerevisiae* (Zymaflore F15, Laffort, Sydney, Austrilia) at 0.2 g kg^{-1}. Alcoholic fermentation was carried out at 25°C and ended when the residual sugar content was below about 4.0 g L^{-1}. Separation of the wine pomace was performed at the end of alcoholic fermentation. Potassium metabisulphite (40 mg kg^{-1}) was added and then the wine samples were aged at 16°C for 3 months.

Total phenolic content (TPC): The TPC was determined by the Folin-Ciocalteu method[13]. The absorbance of each sample was determined at 765 nm. The results were expressed as mg gallic acid equivalents (GAE) per one liter of wine (mg GAE L^{-1}).

Total anthocyanin content (TAC): The TAC was estimated using the pH differential method[14]. Aliquots of each sample were diluted with pH 1.0 or 4.5 buffers to the same dilution. The absorbance was measured at 510 and 700 nm in both pH 1.0 and 4.5 buffers. The TAC was calculated using Eq. 1:

$$TAC = \frac{A \times MW \times DF \times V_e \times 1000}{\varepsilon \times 1 \times M} \quad (1)$$

Where:

A = Difference in absorbance between pH 1.0 and 4.5
MW = Molecular weight of cyanidin-3-glucoside (449 g mol^{-1})
DF = Dilution factor
V_e = Extract volume
g = Molar extinction coefficient of cyanidin-3-glucoside (29,600)
M = Mass of the sample extracted

The results were expressed as mg cyanidin-3-glucoside (C3G) equivalents per one liter of wine (mg C3G L^{-1}).

Total flavonoid content (TFC): The TFC was measured using a colorimetric assay adapted from Mahmood *et al.*[15]. The absorbance was measured at 510 nm. The results were expressed as mg rutin equivalents (RE) per one liter of wine (mg RE L^{-1}).

Total tannin content (TTC): The TTC was measured by the Folin-Denis method[16]. The absorbance was measured at 700 nm. The equation obtained for the calibration curve of gallotannic acid (0.2-130 mg L^{-1}) was Y = 0.0236x+0.65 (r = 0.9995). The results were expressed as mg gallotannic acid equivalent per one liter of wine.

Free radical scavenging capacity (DPPH): The DPPH free radical-scavenging capacity was estimated by adding 2.95 μL of 0.1 mM DPPH methanolic solution to 50 μL of the sample extracts. The solution was thoroughly mixed and placed in the dark for 30 min. The absorbance was measured at 517 nm. The results were expressed in mg VC equivalent antioxidative capacity per one liter of wine (mg VC L^{-1}).

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay was adopted from that described by Si *et al.*[17]. The FRAP reagent was prepared fresh daily in acetate buffer (adjusted to pH 3.6 by acetic acid) by mixing TPTZ solution (10 mM in 40 mM HCl) and 20 mM iron chloride solution in a proportion of 10:1:1, respectively. Each sample (90 μL) was mixed with 3.0 mL of the FRAP reagent and incubated for 10 min at 37°C. The absorbance was read at 593 nm. The results were expressed as mmol ferrous ion per one liter of wine (mmol Fe^{2+} L^{-1}).

Reducing power assay (RP): The RP was determined by the methods described by Infante *et al.*[16]. The absorbance was read at 700 nm after standing for 2 min, the final result was expressed as mg RP equivalent per one liter of wine (mg RP L^{-1}).

Statistical analysis: Data were expressed as the means±standard deviation (SD) of triplicate determinations. Mean differences at $p<0.05$ level were determined by one-way ANOVA followed by Duncan's range test using Prism™ v6.0 software.

RESULTS

Bioactive components of mulberry wines during fermentation: The total contents of anthocyanin (TAC), phenolic (TPC), total tannin (TTC) and flavonoid (TFC) were measured in mulberry wine over 4 days of fermentation (Fig. 1). The TAC of mulberry wine

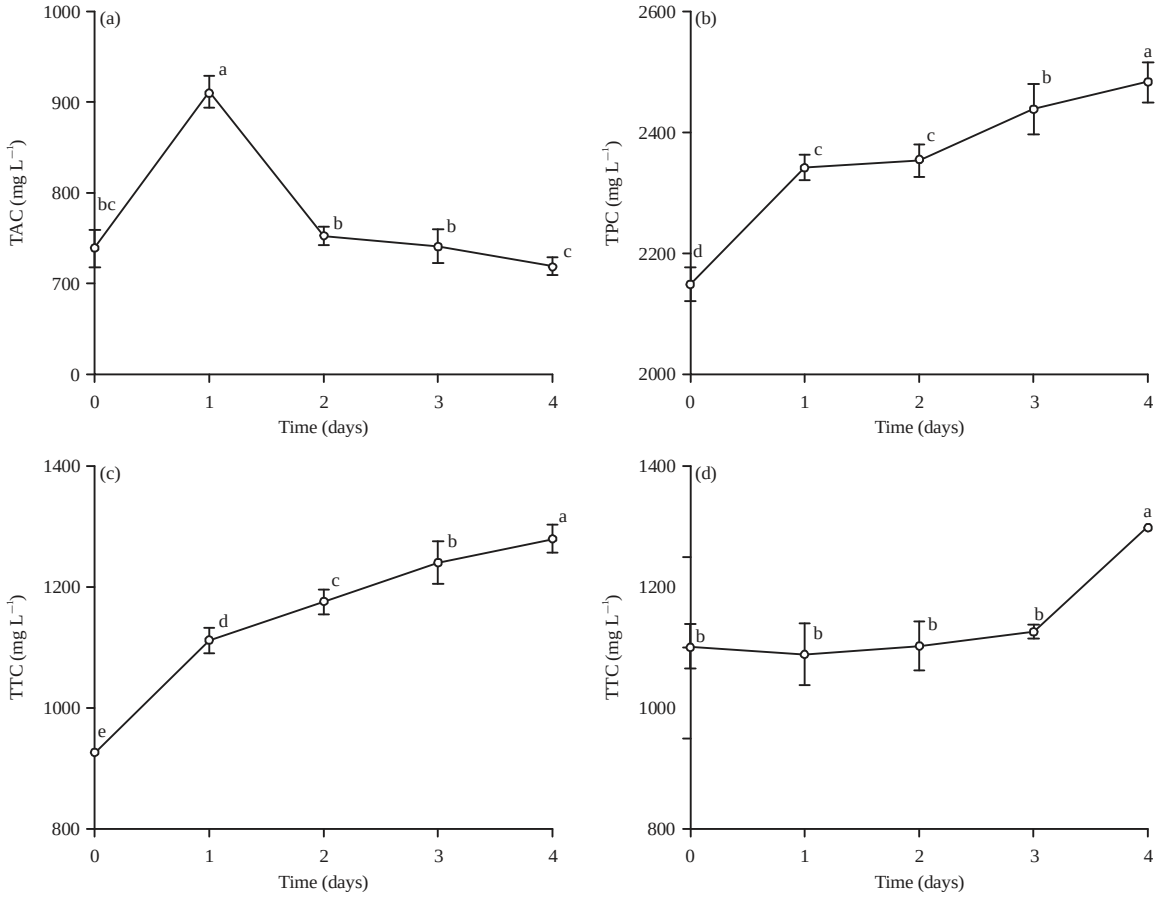

Fig. 1(a-d): Changes in the contents of (a) Total anthocyanins content (TAC), (b) Total phenolics content (TPC), (c) Total tannins content (TTC) and (d) Total flavonoids content (TFC) during fermentation of mulberry wine
Different lower cases represent significant differences ($p<0.05$), data were expressed as the Mean± Standard Deviation (SD)

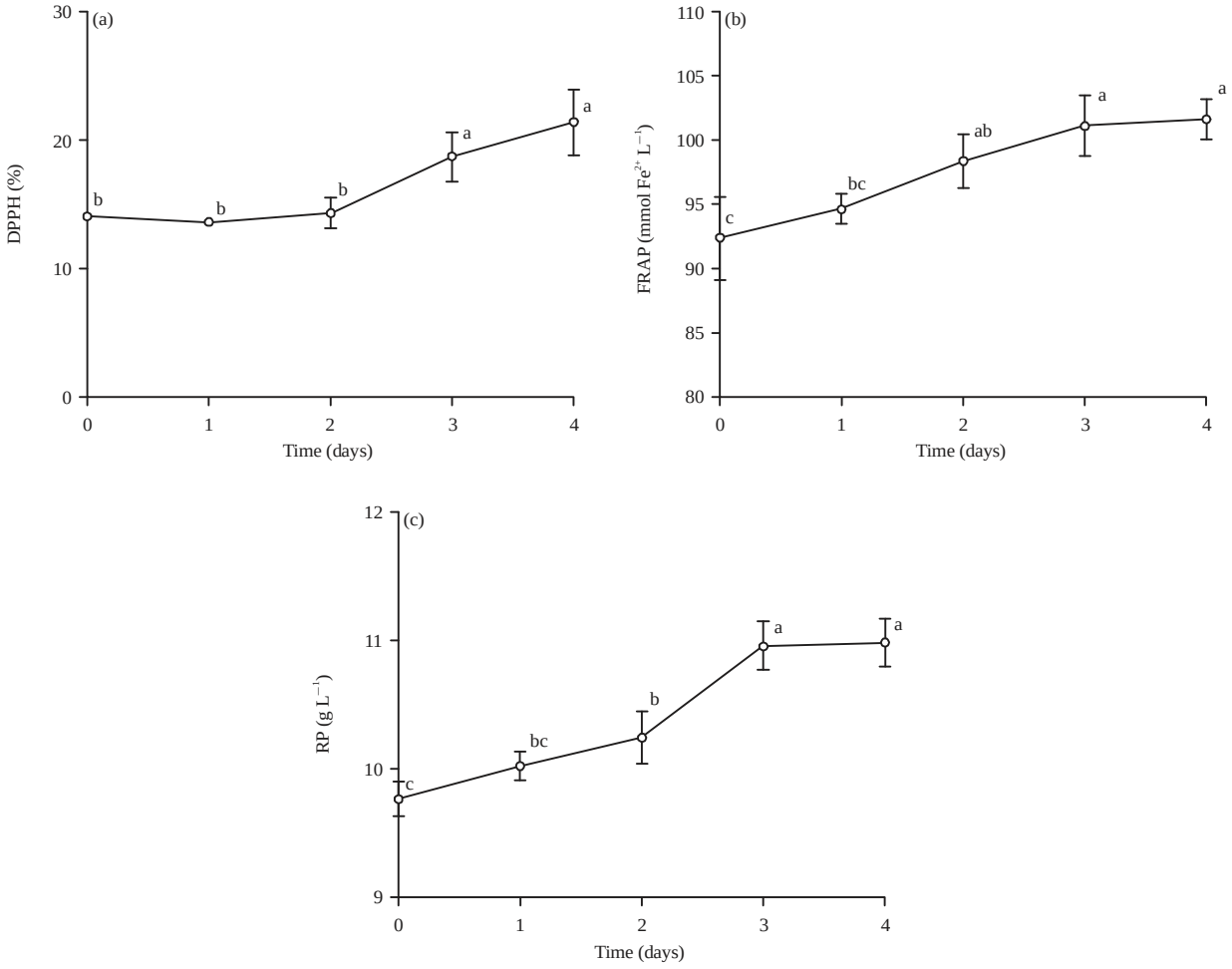

Fig. 2(a-c): Changes in (a) Free radical scavenging capacity (DPPH), (b) Ferric reducing antioxidant power (FRAP) and (c) Reducing power (RP) in mulberry wine during fermentation
Different lower cases represent significant differences ($p<0.05$), data were expressed as the Mean±Standard Deviation (SD)

peaked on day 1 (911.73 mg L^{-1}) and remained slightly decreased from day 2-4 (Fig. 1a).

Increases in TPC were observed on each day of fermentation (Fig. 1b). Changes in the TTC (Fig. 1c) and TFC (Fig. 1d) during fermentation were similar to those observed for the TPC (Fig. 1b). The maximal observed levels of TPC (2482.61 mg L^{-1}), TTC (1279.71 mg L^{-1}) and TFC (664.20 mg L^{-1}) were all on day 4.

Antioxidant activities of mulberry wines during fermentation: The levels of DPPH, FRAP and RP in mulberry wine were assayed during 4 days of alcoholic fermentation (Fig. 2). Each activity increased in mulberry wine during alcoholic fermentation, from day 0-4 and reached their maximum values at day 4.

Bioactive components of mulberry wines during aging: The total contents of anthocyanin (TAC), phenolic (TPC), tannin (TTC) and flavonoids (TFC) were measured in mulberry wine over 90 days of aging (Fig. 3). The TAC, TPC, TTC and TFC decreased during aging. The TAC (Fig. 3a) ranged from 719.39-158.80 mg L^{-1}, while the levels of TPC (Fig. 3b) decreased from 2492.61-1936.52 mg L^{-1}.

Antioxidant activities of mulberry wines during aging: Antioxidant assays (DPPH, FRAP and RP) were performed to evaluate the antioxidant activity of mulberry wine during aging (Fig. 4). The changes in antioxidant activities were consistent with the changes in the TAC, TPC, TTC and TFC in mulberry wines, which decreased during aging.

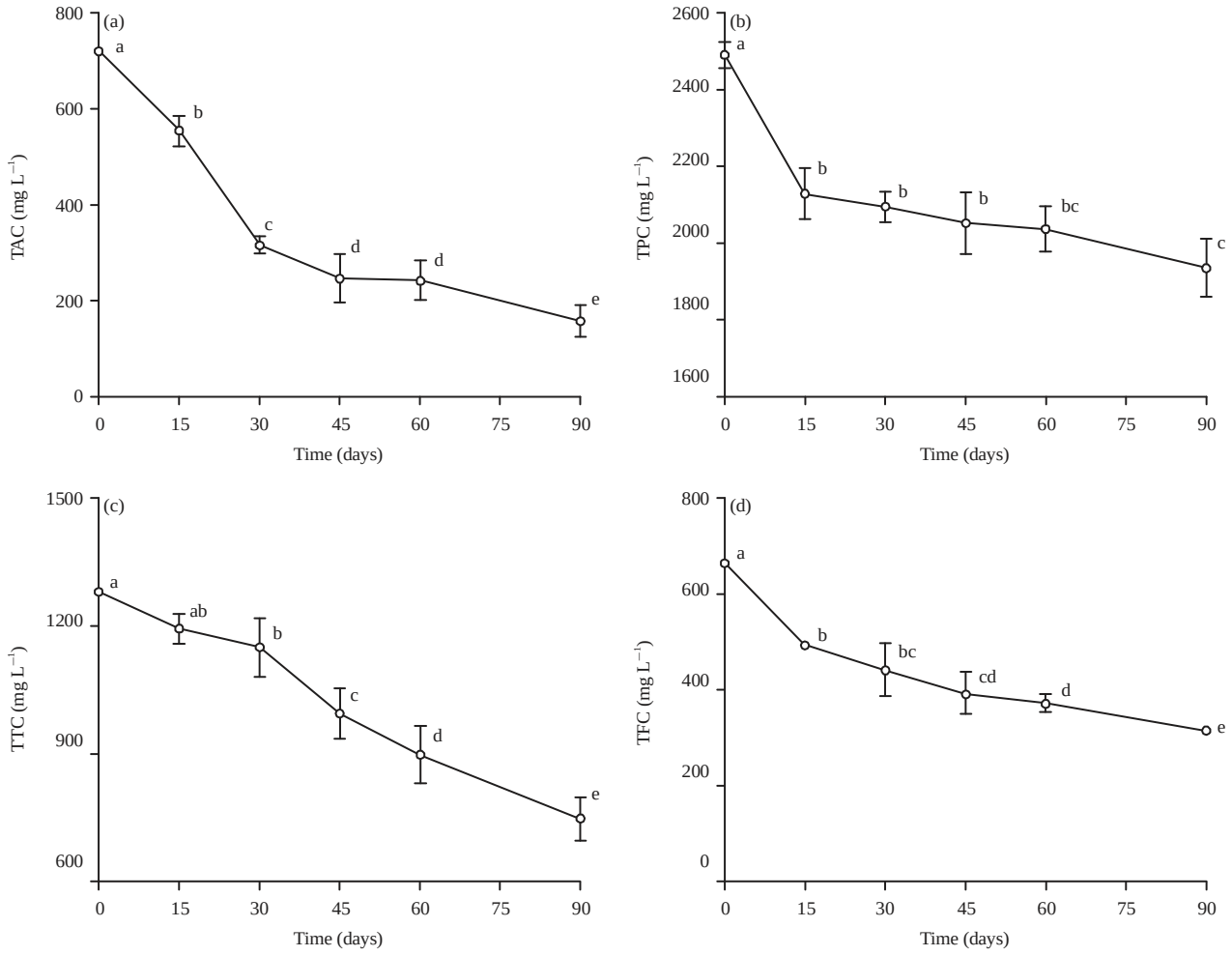

Fig. 3(a-d): Changes in the (a) Total anthocyanins content (TAC), (b) Total phenolics content (TPC), (c) Total flavonoids content (TTC) and (d) Total flavonoids content (TFC) in mulberry wine during aging for 90 days
Different lower cases represent significant differences ($p<0.05$), data were expressed as the Mean±Standard Deviation (SD)

DISCUSSION

Mulberry fruits are rich in phenolic compounds, anthocyanins and other flavonoid compounds[18]. A previous study indicated that anthocyanins were transferred into mulberry juice via maceration prior to wine fermentation[19]. In this study, the levels of anthocyanins in mulberry wine increased from day 0-1 during fermentation, but decreased from day 2-4, indicating that the rate of anthocyanin degradation might be greater than the initial rate of anthocyanin dissolution during the 1st day[20]. The changes in TPC and TFC during alcoholic fermentation of mulberry juice were consistent with red wine of Di Egidio *et al.*[21].

As reported by Lim *et al.*[22] and Zhang *et al.*[23], changes in TPC and antioxidant capacity correlate during the wine making process. In this study, the antioxidant activities (Fig. 2a-c) and phenolics content (Fig. 1b) of mulberry wine continually increased during fermentation.

Anthocyanins have a low stability and their degradation is influenced by pH, light, temperature and oxygen[24]. In this study, the total anthocyanin contents decreased during mulberry wine aging. Since most phenolic substances are not sensitive to light, heat or oxygen[25], the phenolics content decreased more slowly during the aging period. It has been reported that phenolics are responsible for antioxidant capacity[26,27]. Furthermore, Ramful *et al.*[28] reported that the phenolics content of mauritian citrus fruit pulp extracts correlated strongly with the antioxidant activities as determined by the FRAP assays.

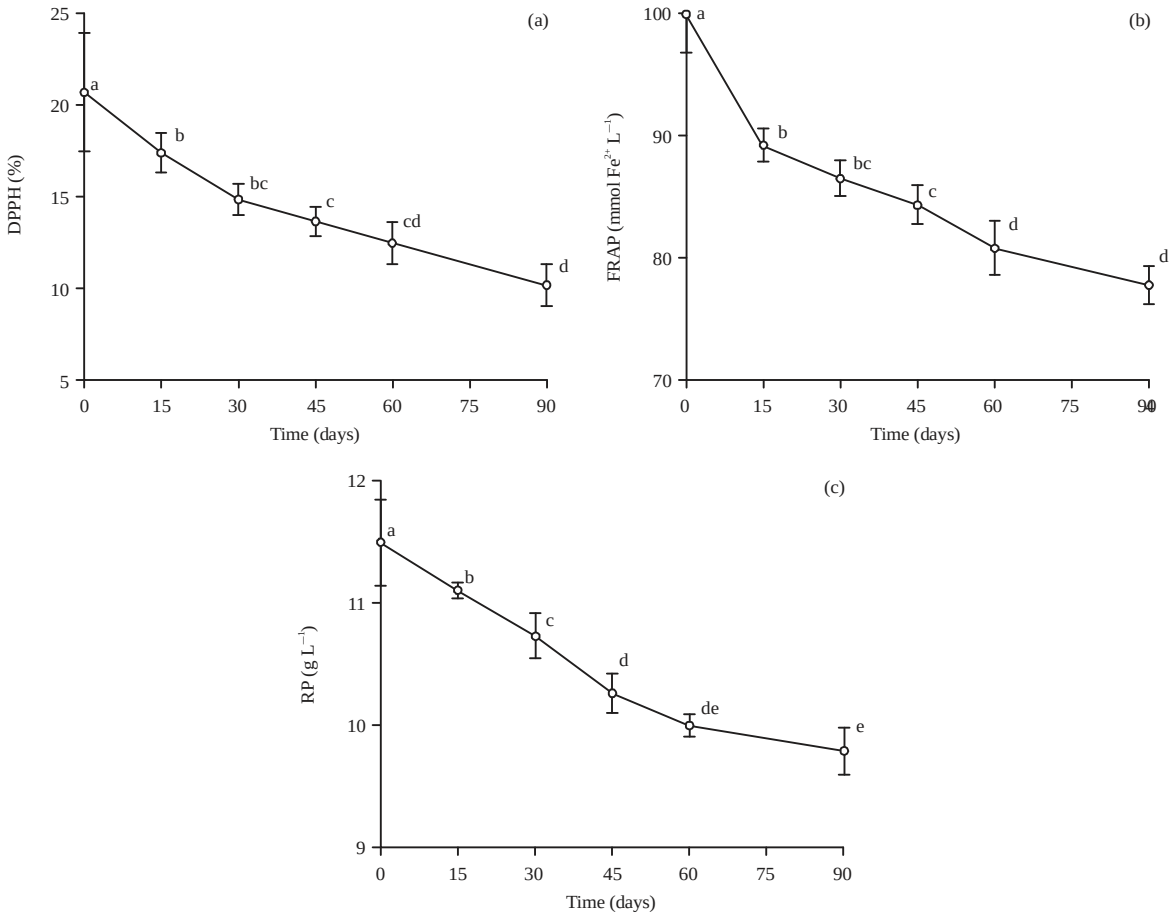

Fig. 4(a-c): Changes in (a) Free radical scavenging capacity (DPPH), (b) Ferric reducing antioxidant power (FRAP) and (c) Reducing power (RP) of mulberry wine during aging over 90 days
Different lower cases represent significant differences ($p<0.05$), data were expressed as the Mean±Standard Deviation (SD)

CONCLUSION

Mulberry juice was fermented and aged so that the total contents of anthocyanins (TAC), phenolics (TPC), flavonoids (TFC), tannins (TTC) as well as the antioxidant activities could be analyzed during the wine-making process. Mulberry wine has high TAC, TPC, TFC and TTC contents and antioxidant activity. The changes in the antioxidant activity were consistent with the changes in the TAC, TPC, TFC and TTC during fermentation and aging. These results indicate that changes in the timing of the mulberry wine-making process may more effectively deliver the antioxidant capacities of the mulberry fruit to the consumer.

SIGNIFICANCE STATEMENT

This study showed that the changes in antioxidant activities of mulberry wines were consistent with the levels of phenolic compounds in mulberry.

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