Kor. J. Microbiol. Biotechnol.
Vol. 36, No. 1, 67–71(2008)

Changes of Physicochemical Properties and Antioxidant Activities of Red Wines during Fermentation and Post-fermentation

No, Jae-Duck, Dae-Hyoung Lee[1], Young-Soo Hwang[2], Sang-Han Lee[3], Dae-Hyung Lee, and Jong-Soo Lee*
Dept. of Life Science and Genetic Engineering, Paichai University, Daejeon 302-735, Korea
[1]Bae Sang Myun Brewery Co,. Ltd., Pocheon 487-840, Korea
[2]Dept. of Horticulture, Chungnam National University, Daejeon 305-764, Korea
[3]Department of Food Science and Technology, Kyungpook National University, Daegu 702-701, Korea

The goal of this study was to vinify four varieties of grapes, namely *Vitis labrusca* L (Gerbong), *Vitis labrusca* B (Campbell Early), *Vitis labrusca* (Muscat Bailey A) and *Vitis hybrid* (Sheridan), and to investigate the changes in the physicochemical properties and antioxidant activity of the red wines during fermentation and post-fermentation. The ethanol content of the four red wines varied only slightly from 11.4%-12.8%, indicating that no significant change occurred during the fermentation and post-fermentation. The total anthocyanin and phenol contents as bioactive compounds were the highest level in the *Vitis labrusca* B red wine. The antioxidant activity was also the highest of 88.9% after 10 days fermentation in the *Vitis labrusca* B red wine and showed from only 36.6% to 61.7% in the other red wines, though the range decreased to 33.1%-64.1% during post-fermentation for 120 days at 4°C. Our results show that the *Vitis labrusca* B red wine has the potential to become a functional red wine because of its high antioxidant activity.

Key words: Antioxidant activity, fermentation, post-fermentation, red wines

Introduction

Various fruits and grapes have recently received attention because they have nutraceuticals with health-stimulating properties. Grapes contain 0.3%-0.5% of various organic acids, such as tartaric acid and malic acid, as well as some free sugars and vitamins. They also contain a large amount of polyphenol compounds, including anthocyanin and proanthocyanidins [23], flavonoids, and phenolic acids [7, 32]. Approximately 75% of grape polyphenols exist in the seeds and skin, and the major polyphenols in grape skin are procyanidins and proanthocyanidins, as well as condensed cyanidine-3-glucosides, malvidin-3-glucosides, and peonidine-3-glucosides [18, 31]. These phenolic compounds have several health benefits such as antioxidant activity [12], scavenging of active harmful oxygen radicals [4, 9, 10, 33, 36], inhibition of oxidation on lipoprotein [17, 30, 32], low density lipoprotein [24, 35], inhibition of platelet aggregation [5], anti-inflammatory action [25], and the lowering of blood cholesterol by grape resveratrol and antimicrobial activity [20, 29].

The health benefits of red wine have been widely reported [7, 8, 11, 16, 27, 28, 34]. These benefits may originate from the abundance of phenolics. Amous et al. reported a close correlation between the 1,1-diphenyl-2-picryhydrazyl (DPPH) radical scavenging activity and the hydroxyl radical scavenging activity of red wine [1]; in addition, some studies on the vinification of various varieties of Korean grapes have acknowledged the quality and acceptability of such wines [21, 22]. Red wines, however, are limited with respect to offering unique characteristics, acceptability, and highly valuable physiological functionality. Aside from several papers on the antioxidant activity of red wines in general, few studies have focused on the antioxidant activity of Korean red wines [6, 19].

In this study, we investigated the optimal fermentation conditions for the making of functional red wines as well as the physicochemical properties and antioxidant activity of red wines during four months of post-fermentation at 4°C.

---

*Corresponding author
Tel: 82-42-520-5388, Fax: 82-42-520-5388
E-mail: biotech8@pcu.ac.kr

Materials and Methods

Grapes, yeast and chemicals

In 2005, we purchased from a commercial grape market four varieties of Korean grapes: *Vitis labrusca* L (Gerbong), *Vitis labrusca* B (Campbell Early), *Vitis labrusca* Muscat Bailey A) and *Vitis hybrid* Sheridan). Furthermore, in preparing the wines, we used *Saccharomyces cerevisiae* K-7 [13-15] from the Laboratory of Food Biotechnology at Paichai University (Daejeon, Republic of Korea). We purchased DPPH from Sigma Chemical Co. (St. Louise, Mo., USA). Unless otherwise specified, all chemicals were of analytical grade.

Vinification

First, we crushed four varieties of grapes and then added sugar to a concentration level of 24°Brix. After adding 150 ppm of $K_2S_2O_5$ and leaving the mixture for 5 h, we inoculated 1% of *S. cerevisiae* K-7, which had been cultured in the must for 24 h. The must was then fermented for 10 days at 25°C. Finally, we filtered the fermented broths, adjusted the SO_2 content to 150 ppm, and then stored the broths at 4°C for 120 days.

General analysis

The pH values were measured with a pH meter (Fisher Scientific, Colorado, USA), and the titratable acidity was estimated after titration with 0.1 N NaOH to a pH of 7.0; the tartaric acid (%) was then calculated from this value. After the distillation of the red wines, we determined the volatile acid in terms of titratable acidity. In addition, we used an alcoholic meter (Ceti Optical Instruments, Belgium) to determine the ethanol content [14]. The color of the red wines, which was determined by a color difference meter (Hunter Associates Laboratories, Reston, USA), is described in terms of lightness (the L value), redness (the a value), and yellowness (the b value). The free sugar content and the organic acid content were analyzed with HPLC, and the proximate analysis of the grapes was determined with the AOAC method [2]. The total anthocyanin content and the total phenol content were determined by the methods of Morris *et al.* [3].

Assay of antioxidant activity

After concentrating 50 mL samples of the red wines to 5 mL samples, we used the Blois method [3] and DPPH to assay the antioxidant activity. We added a 0.8 mL DPPH solution (12.5 mg of DPPH dissolved in 100 mL of ethanol) to 0.2 mL of each sample. The mixture was shaken for 10 s and left for 10 min, after which we determined the absorbance at 525 nm. The antioxidant activity was calculated as [1-(absorbance of the reaction mixture minus absorbance of the sample alone)/absorbance of blank]×100.

Results and Discussion

Grape characteristics

The crude protein content of the four varieties of grapes ranged from 0.12% to 0.16%, and the carbohydrate content ranged from 0.38% to 0.45% (Table 1). There were some differences in the free sugar content of the grapes. However, there was an abundance of glucose and fructose in *Vitis labrusca* L (that is, 20.9 g of glucose and 21.41 g of fructose per 100 g of dried powder). With regard to the organic acids, we found that malic acid was relatively high in *Vitis labrusca* L powder (7.08 g per 100 g of dried powder) and in *Vitis labrusca* powder (6.61 g per 100 g of dried powder). In contrast, tartaric acid was relatively high in *Vitis labrusca* powder (0.07 g per 100 g of dried powder) and in *Vitis labrusca* B powder (0.06 g per 100 g of dried powder). The grapes also had trace elements of other organic acids (data are not shown).

Changes in the physicochemical properties and antioxidant activity of the red wines during fermentation

Table 2 shows the changes in the physicochemical properties of the four kinds of red wines during fermentation at 25°C for 10 days. The ethanol content did not change during 5 days and 10 days of fermentation; furthermore,

Table 1. Proximate components of grape varieties (Unit:%)

Grape variety	Moisture	Crude protein	Crude lipid	Carbohydrate	Ash
Vitis hybrid (Sheridan)	86.93	0.14	0.49	0.38	12.06
Vitis labrusca L (Gerbong)	85.99	0.12	0.34	0.40	13.15
Vitis labrusca (Muscat Bailey A)	86.68	0.13	0.59	0.45	12.15
Vitis labrusca B (Campbell Early)	86.66	0.16	0.57	0.42	12.19

after 10 days of fermentation, there was no significant difference between the red wines with regard to the ethanol content, which ranged from 11.4% to 12.0% (v/v). Choi et al. recently reported that alcohol content of sweet persimmon wine made from sweet persimmon juice with a concentration of 24°Brix was 12.8% after 5 days of fermentation at 25°C [6]. There was also no significant difference in the total acid content of the wines. The total anthocyanin content and the total phenol content, known as bioactive compounds, reached the highest level in the *Vitis labrusca* B red wine.

The antioxidant activity increased significantly from a range of 3.9%-16.4% to 44.7%-86.0% during 5 days of fermentation (Fig. 1). Of the four kinds of red wines, the *Vitis labrusca* B red wine showed the greatest antioxidant activity (88.9% for 10 days of fermentation). The *Vitis labrusca* L red wine also had high antioxidant activity of 61.7% after 10 days of fermentation. Moreover, during fermentation the antioxidant activity increased by a 9.5% in the *Vitis labrusca* L red wine and by 2.9% in the *Vitis labrusca* B red wine. However, the antioxidant activity decreased by 10.8% in the *Vitis hybrid* red wine and by 8.1% in the *Vitis labrusca* red wine. In the case of the *Vitis labrusca* red wine, the decrease in antioxidant activity may be caused by the decrease in the total phenol content and the anthocyanin content, which are known as antioxidants (Table 2). As yet, however, we are unable to explain why the antioxidant activity of the *Vitis hybrid* red wine decreases during fermentation. Further research is needed to isolate, identify and characterize the antioxidants of these red wines.

Choi *et al.* reported that the antioxidant activity of some Korean commercial red wines ranges from 347 mg/L ± 63.2 mg/L to 3659 mg/L ± 555.7 mg/L in terms of ascorbic acid equivalents antioxidant activity as the DPPH radical scavenging activity [6]. Koh et al. reported that Korean red wines have several kinds of phenolic compounds and manifest superoxide radical scavenging activity [19].

Changes in the physicochemical properties and antioxidant activity of the red wines during post-fermentation

Our investigation of changes in the physicochemical properties of four kinds of red wines during 120 days of post-fermentation at 4 revealed that the ethanol content did not significantly change during post-fermentation, except for a decrease of 1.6% in the *Vitis labrusca* red wine (Table 3). However, the total anthocyanin content decreased in all the red wines as the post-fermentation period was prolonged.

With regard to changes in the antioxidant activity of the four kinds of red wines during 120 days of post-fermentation at 4°C, we found, as shown in Fig. 1, that the antioxidant activity decreased from a range of 36.6%-88.9% to 32.2%-51.8%. The decrease in antioxidant activity is likely caused by a decrease in the anthocyanin contents as antioxidant (Table 3).

Our results show that the *Vitis labrusca* B red wine has the potential to become a functional red wine because of its high antioxidant activity and good acceptability [26].

Table 2. Physicochemical properties of various red wines from fermentation of 5 and 10 days at 25

Fermentation periods (days)	Red wines	Ethanol (%)	pH	Total acid (%)	Volatile acid (%)	Total anthocyanin (A_{520})	Color intensity ($A_{520}+A_{420}$)	Browing index (A_{420}/A_{520})	Total phenol (mg/mL)	Color		
										L[1] (lightness)	a[2] (redness)	b[3] (yellowness)
5	Vh. W[4]	11.6	3.7	0.76	0.009	1.224	1.991	0.625	0.71	80.28	+22.72	+0.93
	VlL. W	12.8	3.7	0.58	0.012	0.259	0.574	1.218	0.49	95.99	+4.99	+2.27
	Vl .W	12.0	3.6	0.71	0.011	1.773	2.655	0.497	0.62	75.85	+30.49	-0.89
	VlB. W	12.4	3.6	0.55	0.008	2.142	3.701	0.727	0.98	66.44	+31.38	+5.96
10	Vh. W	11.6	3.8	0.82	0.011	1.373	1.909	0.679	0.70	77.51	+16.14	+1.17
	VlL. W	12.0	3.7	0.64	0.011	0.259	0.522	1.083	0.49	96.39	+3.83	+1.64
	Vl. W	11.4	3.7	0.80	0.012	1.401	2.134	0.522	0.57	84.38	+22.25	-0.39
	VlB. W	11.4	3.7	0.84	0.013	1.770	3.009	0.699	0.93	78.62	+23.96	+4.75

[1] Measures lightness and varies from 100 for perfect white to zero for black.

[2] Measures redness when plus, gray when zero and greenness when minus.

[3] Measures yellowness when plus and blueness when minus.

[4] Vh.W : *Vitis hybrid* (Sheridan) red wine; VlL.W : *Vitis labrusca* L (Gerbong) red wine; Vl.W : *Vitis labrusca* (Muscat Bailey A) red wine; VlB.W : *Vitis labrusca* B (Campbell Early) red wine.

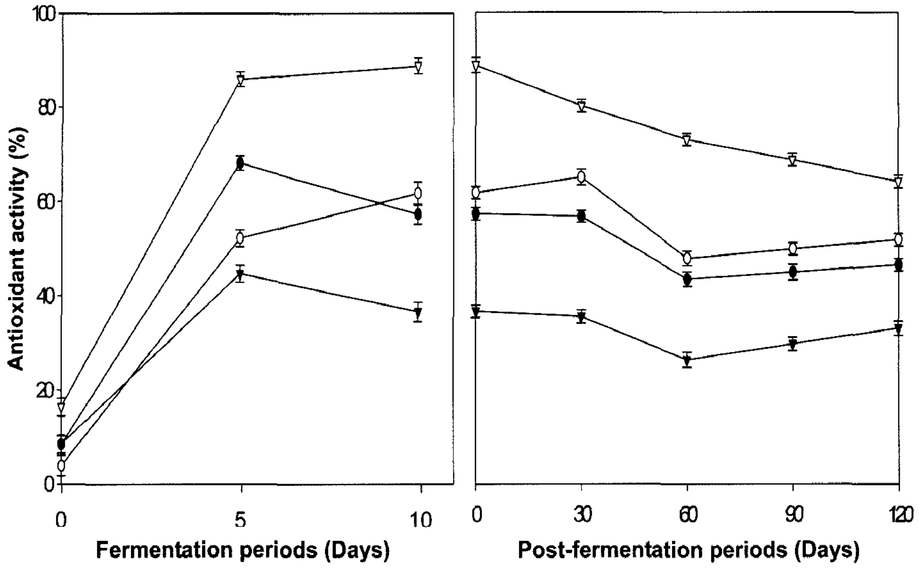

Fig. 1. Changes of antioxidant activity in various red wines during fermentation and post-fermentation. Closed circles : *Vitis hybrid* (Sheridan) red wine; Open circles : *Vitis labrusca* L (Gerbong) red wine; Closed triangles : *Vitis labrusca* (Muscat Bailey A) red wine; Open triangles : *Vitis labrusca* B (Campbell Early) red wine. Values show means±SE from three experiments performed in triplicate.

Acknowledgment

This study was performed as one of project in Grape Research Center, supported by ARPC (Agriculture R&D Promotion Center) technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

REFERENCES

1. Amous, A., D. Makris, and P. Kefalas. 2001. Effect of principal polyphenolic components in relation antioxidant characterics of aged red wines. *J. Agric. Food Chem.* **49**: 5736-5742.
2. AOAC. 1984. *Official Methods Analysis* (14th ed.). AOAC International press, Washington DC.
3. Blois, M. S. 1958. Antioxidant determination by the use of stable free radical. *Nature.* **181**: 1199-1200.
4. Burkitt, M. J. and J. Duncan. 2000. Effects of trans-Resveratrol on Copper-Dependent Hydroxyl-Radical Formation and DNA Damage: Evidence for Hydroxyl-Radical Scavenging and a Novel, Glutathione-Sparing Mechanism of Action, *Arch. Biochem. Biophys.* **381**: 253-263.
5. Chang, S. S., B. Osric-Matijasevic, O. A. H. Hsih, and C. L. Huang. 1977. Natural antioxidants from rosemary and sage. *J. Food Sci.* **42**: 1102-1106.
6. Choi, Y. M., K. W. Yu, N. S. Han, J. H. Koh, and J. S. Lee. 2006. Antioxidant activities and antioxidant compounds of commercial red wines. *J. Food Sci. Nutr.* **35**: 1286-1290.
7. Frankel, E. N., J. Kanner, J. B. German, E. Parks, and J. E. Kinsella. 1993. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet.* **341**: 454-457.
8. Friedman, L. A. and A. W. Kimball. 1986. Coronary heart disease mortality and alcohol consumption in Framingham. *Am. J. Epidemiol.* **124**: 481-489.
9. Husain, S. R., J. Cillard, and P. Cillard. 1987. Hydroxyl radical scavenging activity of Flavonoids. *Phytochemistry.* **26**: 2489-2491.
10. Jayaprakasha, G. K., R. P. Singh, and K. K. Sakariah. 2001. Antioxidant activity of grape seed(*Vitis vinifera*) extracts on peroxidatin models in vitro. *Food Chem.* **73**: 285-290.
11. Kallithraka, S., I. Arvanitoyanins, A. El-zajouli, and P. Kefalas. 2001. The application of an improved method for trans-resveratrol to determine the origin of Greek red wines. *Food Chem.* **75**: 355-363.
12. Kanner, J., E. N. Frankel, R. Granit, B. German, and J. E. Kinsella. 1994. Natural antioxidants in grapes and wines. *J. Agric. Food Chem.* **42**: 64-69.
13. Kim, J. H., D, H. Lee, S. C. Jeong, K. S. Chung, and J. S. Lee. 2004. Characterization of antihypertensive angiotensin I-converting enzyme inhibitor from Saccharomyces cerevisiae. *J. Microbiol. Biotechnol.* **14**: 1318-1323.
14. Kim, J. H., D. H. Lee, S. H. Lee, S. Y. Choi, and J. S. Lee. 2004. Effect of Ganoderma lucidum on the quality and functionality of Korean traditional rice wine, Yakju. *J. Biosci. Bioeng. Jpn.* **97**: 24-28.
15. Kim, J. H., S. H. Lee, N. M. Kim, S. Y. Choi, J. Y. Yoo, and J. S. Lee. 2000. Manufacture and physiological functionality of Korean traditional liquors by using dandelion (Taraxacum platycarpum). *Korean J. Biotechnol. Bioeng.* **28**: 367-371.
16. Kimura, Y., H. Ohminami, H. Okuda, K. Baba, M. Kozawa, and S. Arichi. 1983. Effects of stilbene components of roots of polygonum ssp. on liver injury in peroxidized oil-fed rats.

Planta Med. **49**: 51-54.

17. Kinsella, J. E., E. Frankel, B. German, and J. Kanner. 1993. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol.* **47**: 85-89.
18. Koeppen, B. H. and D. S. Basson. 1966. The anthocyanin pigments of Barlinka grapes. *Phytochemistry.* **5**: 183-187.
19. Koh, K. H., J. H. Lee, K. R. Yoon, S. Y. Choi, and K. L. Seo. 1998. Phenolic compounds of Korean red wine and their superoxide radical scavenging activity. *Food Sci. Biotechnol.* **7**: 131-136.
20. Landolfi, R., R. L. Mower, and M. Steiner. 1984. Modification of platelet function and arachidonic acid metabolism by bioflavonoids. Structure-activity relations. *Biochem. pharmacol.* **33**: 1525-1530.
21. Lee, S. J., J. E. Lee, and S. S. Kim. 2004. Development of Korean red wines using various grape varieties and preference measurement. *Korean J. Food Sci. Technol.* **36**: 911-918.
22. Lee, S. Y., K. H. Lee, K. S. Chang, and S. K. Lee. 2000. The changes of aroma in wine treated with reverse osmosis system. *Korean J. Food Sci. Technol.* **32**: 17-24.
23. Lu, Y. and F. L. Yeap. 1999. The polyphenol contituents of grape pomace. *Food Chem.* **65**: 1-8.
24. Meyer, A., O. Yi, D. Pearson, A. L. Waterhouse, and E. Frankel. 1997. Inhibition of human low density lipoprotein oxidation in relation to phenolic antioxidants in grape(Vitis Vinifera). *J. Agric. Food Chem.* **45**: 1638-1643.
25. Moroney, M. A., M. J. Alcaraz, R. A. Forder, F. Carey, and J. R. Hoult. 1988. Selectivity of neutrophil 5-lipoxygenase and cyclo oxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. *J. Pharm. Pharmacol.* **40**: 787-792.
26. No, J. D., E. N. Lee, D. H. Lee, J. P. Chun, S. H. Lee, and J. S. Lee. 2007. Cardiovascular functionalities and antidementia β-secretase inhibitory activity of red wines. *Food Biotechnology.*(in press).
27. Park, S. Y., J. S. Lee, K. W. Yu, N. S. Han, H. Lee, J. H. Koh, and K. S. Shin. 2006. Anti-complementary polysaccharides in grape wines, *J. Food Sci. Nutr.* **35**: 1232-1236.
28. Renaud, S. and M. de Lorgeril. 1992. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet.* **339**: 1523-1526.
29. Sato, M., N. Ramarathnam, Y. Suzuki, T. Ohkudo, M. Takeuchi, and H. Ochi. 1997. Superoxide radical scavenging activities of wines, and antioxidative properties of fractions recovered from merlot wine pomace. pp. 359. *Food Factors for Cancer Prevention.* Springer, Tokyo.
30. Serafini, M., A. Ghiselli, and A. Ferro-Luzzi. 1994. Red wine, tea, and antioxidants. *Lancet.* **344**: 626.
31. Shim, K. H., K. S. Kang, J. S. Choi, K. I. Seo, and J. S. Moon. 1994. Isolation and stability of anthocyanin pigments in peels. *J. Food Sci. Nutr.* **23**: 279-286.
32. Teissedre, P. L., E. N. Frankel, A. L. Waterhouse, H. Peleg, and J. B. German. 1996. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food and Agric.* **70**: 55-61.
33. Torel, J., J. Cillard, and P. Cillard. 1986. Antioxidant activity of flavonoids and reactivity with peroxy radical. *Phytochemistry.* **25**: 383-385.
34. Tsutomu, T. and K. Yokotsuka. 1999. Angiotensin I -converting enzyme inhibitory peptides from wine. *Am. J. Enol. Vitic.* **50**: 65-68.
35. Williams, R. L. and M. S. Elliot. 1997. Antioxidants in grapes and wine: Chemistry and health effects. pp. 150-173. *In* F. Shaihidi (ed.), *Natural antioxidants: Chemistry, health effects and application.* AOCS Press, Illinois.
36. Yilmaz, Y. and R. T. Toledo. 2006. Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. *J. Food Compost. Anal.* **19**: 41-48.

(Received Oct. 26, 2007/Accepted Dec. 20, 2007)

국문초록

적포도주들의 발효와 후발효 중 물리화학적 성질과 항산화활성의 변화

노재덕 · 이대형[1] · 황용수[2] · 이상한[3] · 이종수*

배재대학교 생명유전공학과, [1](주) 배상면주가 연구소, [2]충남대학교 원예학과, [3]경북대학교 식품공학과

본 연구에서는 고품질의 새로운 적포도주를 개발하기 위하여 먼저 거봉, 켐벨 어리, 마스캇 베리 A와 세레단 포도 품종들을 이용하여 4종류의 적포도주들을 제조한 후 이들의 발효와 후발효중의 물리화학적 성질과 항산화활성의 변화를 조사하였다. 4종류의 적포도주들의 에탄올 함량은 발효기간이나 품종간에 큰 차이없이 11.412.8%를 보였고, 생리활성물질로 알려진 총 안토시아닌과 페놀함량은 켐벨 어리 적포도주에서 가장 높았다. 항산화활성은 켐벨 어리 적포도주에서 발효 10일 후 88.9%로 제일 높았고 다른 포도주에서는 36.661.7%의 항산화활성을 보였다. 그러나, 이들 항산화활성은 후발효 120일 후에는 33.164.1%까지 감소하였다. 이상의 결과들을 요약해볼 때 켐벨 어리 적포도주가 높은 항산화활성과 좋은 기호도를 보여 새로운 가능성 적포도주로 기능성이 있을 것으로 사료된다.