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EFFECTS OF RED GRAPE, WILD GRAPE AND BLACK RASPBERRY WINES ON GROUND PORK DURING REFRIGERATED STORAGE

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The effects of red grape, wild grape and black raspberry wines on the quality of ground pork during a 15 days refrigerated storage period were investigated. The levels of phenolic compounds were the highest in black raspberry wine ($P<0.05$). In contrast, the antioxidant capacities according to ferric reducing antioxidant potential (FRAP) and trolox equivalent antioxidant capacity (TEAC) were not significantly different among the wines ($P>0.05$). The addition of 5% and 10% wine influenced the quality of ground pork by decreasing pH, inhibiting the progression of lipid oxidation and the formation of total volatile basic nitrogen (TVB-N), and stabilizing the red colour of the ground pork compared to control samples to which no wine was added. In ground pork, addition of red grape wine led to lower concentrations of thiobarbituric acid reactive substances (TBARS, 0.19–0.39 mg kg^{-1}) and TVB-N values (69.1–119.9 mg kg^{-1}) than wild grape (0.16–0.43 mg kg^{-1} and 72.0–194.1 mg kg^{-1}, respectively) or black raspberry wine (0.33–0.58 mg kg^{-1} and 81.7–225.4 mg kg^{-1}, respectively) up to 10 days of storage. Results from the present study suggested that the quality of ground pork was affected by wine type and storage period. These effects could be due to phenolic compounds as well as other chemical components of the wines.

Keywords: colour, lipid oxidation, phenolics, pork, wine

Wine is an alcoholic beverage typically made from the fermented juices of grapes, berries and other fruits. Generally, these fruits are rich in natural antioxidants, namely phenolic compounds, including anthocyanins, flavonols, catechins and other flavonoids. During the wine-making process, phenolic compound composition of the fruit undergoes significant changes during the early crushing step, fermentation and aging (Ju et al., 2009). Phenolic compounds play a major role in developing the sensory characteristics of wine, such as colour, astringency and bitterness. Furthermore, most of these compounds have a wide range of biological properties including antioxidant, antimicrobial and anticarcinogenic effects (Liu et al., 2002). Red wine is made from cultivars of *Vitis vinifera,* better known as the European grape, and moderate consumption of this beverage has been associated with reduced risk of cardiovascular disease (Goldfinger, 2003). Korean wines have been traditionally produced with black raspberries (*Rubus coreanus* Miq., called "Bokbunja" in Korea) and wild grapes (*Vitis amurensis,* called "moru"). These wines have been widely used for therapeutic purposes in Korea for centuries (Koh et al., 2003).

Ground meat is highly susceptible to microbial spoilage and chemical deterioration during handling, processing and storage, leading to discoloration and the development of rancid odours. Myoglobin, a globular heme protein, is a major contributor to muscle

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pigmentation, and its oxidation of ferrous oxymyglobin (Fe^{2+}) to ferric metmyoglobin (Fe^{3+}) is responsible for the discoloration of meat during storage. The heme protein and free iron have been regarded as major catalysts for initiating lipid oxidation. This process is the primary reason for quality deterioration in meats as it results in the formation of various aldehyde compounds that produce unpleasant odours, and is also reported to induce myoglobin oxidation (Min & Ahn, 2005). Therefore, the oxidation of lipids and myoglobin in meat are interrelated (Brunton et al., 2000).

Natural antioxidants, such as vegetable extracts, juice concentrates, oil seed products, herbs and spices, are commonly used to reduce oxidative deterioration in meat products (Grün et al., 2006). These natural plant materials contain phenolic compounds. For example, the inhibition of lipid oxidation by rosemary extract and culinary spices (i.e. cloves, nutmeg and curry) has been reported in refrigerated raw ground beef and minced chicken, respectively (El-Alim et al., 1999; Balentine et al., 2006). In addition, rapeseed and pine bark appear to be excellent sources of phenolics, and inhibit protein and lipid oxidation in cooked pork patties (Vuorela et al., 2005).

Considering the reported use of natural antioxidants and antimicrobial agents derived from plant sources, wine could be a probable candidate for preserving red meat products. In the present study, the phenolic compound content and antioxidant capacity of red grape wine and traditional Korean fruit wines (black raspberry and wild grape) were evaluated. These wines were then added to ground pork, and their effects on maintaining the ground pork quality were investigated by assessing lipid oxidation levels, total volatile basic nitrogen content, colour and pH during 15 days of refrigerated storage.

1. Materials and methods

1.1. Materials

Fresh pork (Boston butt) was obtained from a local supermarket (Daejeon, Korea). The pork had a water content of 61±9% and a fat content of 18.4±3.4%. Wines used in this study were purchased from local stores (Daejeon, Korea). The red grape wine was produced in France while the wild grape and black raspberry wines were produced in South Korea.

1.2. Analysis of antioxidant content of the wines

Total phenolic compounds, tartaric esters, and flavonol contents in the wines were determined as previously described by Mazza and co-workers (1999). The wine diluted in 10% ethanol at a 1:10 ratio (0.25 ml) was mixed with 0.1% HCl in 95% ethanol (0.25 ml) and 2% HCl (4.5 ml) in a test tube. After 15 min, the absorbance was measured at 280, 320 and 360 nm to determine the levels of total phenolic compounds, tartaric esters and flavonols, respectively. The contents of these compounds were expressed as mg l^{-1} of gallic acid equivalents (GAE), caffeic acid equivalents (CAE) or quercetin equivalents (QE), respectively. All samples were analysed in triplicate.

1.3. Analysis of the antioxidative capacity of the wines

The ferric reducing antioxidant potential (FRAP) assay was performed according to the method of Wong and co-workers (2006) with a slight modification. FRAP solution was prepared by mixing 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) in 40 mM HCl (10 mM), ferric

chloride solution (20 mM) and acetate buffer (pH 3.6, 1:1:10, v:v:v). Wine (10 µl) was added to the FRAP solution, vortexed, and left for 5 min. Absorbance was then measured at 593 nm. A standard curve was generated using gallic acid standards, and FRAP values were expressed as mg l^{-1} of gallic acid equivalents (GAE).

To determine the trolox equivalent antioxidant capacity (TEAC) of the wines, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals were generated by mixing (1:0.5, v:v) 7 mM ABTS and 2.45 mM potassium persulfate (Shan et al., 2005). The solution was then incubated at room temperature in the dark for 12–16 h. Next, the ABTS radical solution was diluted with ethanol until an absorbance of 0.7±0.02 at 734 nm was achieved. The wine (100 µl, diluted in water at a 1:10 ratio) was mixed with the ABTS radical solution (4 ml) and left for 6 min. Absorbance was then measured at 734 nm. Trolox solution was used as a reference standard, and the results were expressed as µM trolox/l.

The superoxide radical scavenging activity of the wines was measured by the method of Zhang and co-workers (2012) with slight modification. Superoxide anion radicals were generated by phenazine methosulfate-nicotinamide/adenine dinucleotide (PMS/NADH) in the presence of nitro blue tetrazolium (NBT), a compound that turns blue when reduced by superoxide radicals. Wine (100 µl) was mixed with NBT (156 µM, 2 ml) and NADH (468 µM, 2 ml) in sodium phosphate buffer (100 mM, pH 7.4). The reaction was accelerated by adding PMS (60 µM, 100 µl) and incubated at 25 °C for 4 min; absorbance was subsequently measured at 560 nm. The superoxide radical scavenging activity was calculated according to percent inhibition of NBT reduction using the following equation:

$$\text{Radical scavenging capacity (RSC, \%)} = [(Abs_{\text{no wine}} - Abs_{\text{wine}}) / Abs_{\text{no wine}}] \times 100$$

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity of the wine was determined. Wine (30 µl) was mixed with a DPPH radical methanolic solution (100 µM, 2.5 ml), and absorbance was measured at 516 nm. DPPH radical scavenging capacities (RSC) were calculated using the above equation. Ascorbic acid (10 mM) was used as a reference for the assay. All samples for analysis of the antioxidative capacity were analysed in triplicate.

1.4. Preparation of ground pork with wine

The pork was ground with a meat chopper (M-12S, Seoul, South Korea) and the total volume was randomly divided into eight groups. Wine (100 ml) was mixed with the ground pork (1 kg) for the 10% (v/w) red grape, wild grape and black raspberry wine groups. The diluted wines (100 ml) in 12% ethanol (1:1, v:v) were mixed with the ground pork (1 kg) for the 5% (v/w) wine groups. Control I contained only ground pork, and control II (ethanol treatment group) was ground pork (1 kg) mixed with 100 ml of 12% ethanol. The samples were placed in air-permeable polyethylene bags, sealed, and stored at 4 °C until analysed.

1.5. Determination of thiobarbituric acid reactive substances (TBARS) values

Lipid oxidation was measured using the TBARS method. Samples of the meat (5 g each) were transferred to Corning centrifuge tubes, mixed with 20 ml of 10% trichloroacetic acid (TCA) and 100 µl of 7.2% butylated hydroxytoluene (BHT) in ethanol, and homogenized for 1 min with an ultrasonic homogenizer. The mixture was centrifuged at 3500 r.p.m. for 10 min, and the supernatant was filtered through filter paper (Whatman No.1, Whatman International Ltd., Maidstone, UK). A 2-ml aliquot of the filtrate was mixed with 2 ml of a 20 mM 2-thiobarbituric acid (TBA) solution, and placed in a water bath at 90 °C for 20 min. After cooling, absorbance was measured at 532 nm. TBARS values were calculated relative

to a standard curve generated by a solution of 1,1,3,3-tetraethoxypropane (TEP). Results are expressed as TBARS in mg of malondialdehyde (MDA) per kg sample. All samples were analysed in triplicate.

1.6. pH and colour analysis

Four grams of the meat samples were homogenized with 36 ml of distilled water with an ultrasonic homogenizer, and centrifuged at 2400 r.p.m. for 10 min. pH was measured using a digital pH meter. The colour of each sample (5 g) was analysed, and the CIE L* (lightness), a* (red pigmentation), and b* (yellow pigmentation) values were determined (Color Techno System Corp., Tokyo, Japan). All samples were analysed in triplicate.

1.7. Analysis of total volatile basic nitrogen (TVB-N) contents

TVB-N contents of the pork samples were analysed using a Conway microdiffusion assay (Cobb et al., 1973). Each sample (4 g) was homogenized with 36 ml of distilled water, and centrifuged at 2400 r.p.m. for 10 min. The filtrate (1 ml) was pipetted onto the outer chamber of a Conway unit, and 1 ml of 0.01 N H_3BO_3 and 100 µl of indicator (methyl red and bromocresol green) were added to the inner chamber. Next, 1 ml of 50% K_2CO_3 was quickly added to the outer chamber, and the unit was incubated at 37 °C for 120 min. The inner solution was titrated with 0.01 N HCl, and TVB-N values were reported as mg kg^{-1} sample. All samples were analysed in triplicate.

1.8. Statistical analyses

Statistical Analysis System software was used to perform the statistical computations (SAS, 2000). Analysis of variance (ANOVA) with Duncan's multiple range test were performed. P-values <0.05 were considered to be significant.

2. Results and discussion

2.1. Antioxidative properties of the wines

Phenolic contents of the wines are presented in Table 1. The levels of total phenolic compounds (1296 mg GAE/l), tartaric esters (260 mg CAE/l), and flavonols (222 mg QE/l) were the highest in black raspberry wine ($P<0.05$). Red grape wine contained higher concentrations of total phenolic compounds (1017 mg GAE/l) and tartaric esters (254 mg CAE/l), and lower levels of flavonol (113 mg QE/l) than wild grape wine ($P<0.05$).

Antioxidant capacities of the wines were measured with four different methods in this study (Table 1 and Fig. 1). The FRAP and TEAC values of the wines were not significantly different; except wild grape wine had the lowest TEAC level (733 µM TE/l). Black raspberry wine had the greatest DPPH radical scavenging activity but the lowest superoxide anion radical scavenging activity ($P<0.05$). No significant difference in scavenging activity was found between red grape and wild grape wines ($P>0.05$; Fig. 1). The antioxidant activity in wine generally depends on the level and type of phenolic compounds as well as the presence of other components including sulphur dioxide (SO_2) and metals (Rupasinghe & Clegg,

2007). In addition, assessment of antioxidant capacities could be affected by the assay model system since antioxidants may respond in different ways to different oxidant sources in the assay (Prior et al., 2005).

Table 1. The phenolic compound contents and antioxidant capacities of the wines (Values are expressed as the mean ± standard deviation of triplicate analysis.)

	Total phenolics (mg GAE/l)	Tartaric esters (mg CAE/l)	Flavonol (mg QE/l)	TEAC (μM TE/l)	FRAP (mg GAE/l)	pH
Red grape wine	1017±10^{b}	254±0^{b}	113±2^{c}	796±8^{a}	49.5±1.1^{a}	3.36
Wild grape wine	980±14^{c}	202±4^{c}	146.0±4^{b}	733±29^{b}	53.1±1.2^{a}	3.78
Black raspberry wine	1296±10^{a}	260±0^{a}	222±2^{a}	827±28^{a}	52.6±0.1^{a}	3.78

GAE: gallic acid equivalent; CAE: caffeic acid equivalent; QE: quercetin equivalent; TEAC: trolox equivalent antioxidant capacity; FRAP: ferric reducing antioxidant potential; TE: trolox equivalent; values in the same column with a different letter are significantly different (P<0.05)

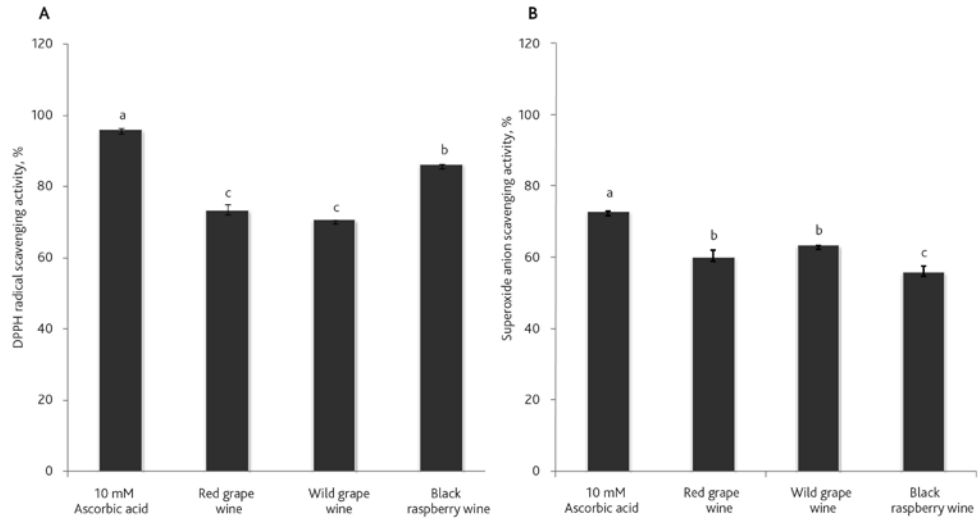

Fig. 1. The DPPH radical (A) and superoxide anion (B) scavenging activities of red grape, wild grape and black raspberry wines. Values with different letters are significantly different (P<0.05)

Wine contains a wide range of flavonoids and other phenolic compounds. According to a study by Ju and co-workers (2009), fermentation can increase the total phenolic content and antioxidant activity of black raspberry wine. In vinification process, the amount of colourless phenolic compounds (i.e. total phenols, tartaric esters and flavonols) increase during alcohol fermentation, and these levels remain stable during further malolactic fermentation and subsequent storage. The wines evaluated in the present study contained higher levels of phenolic compounds than white grape, apple, cherry, plum, raspberry and cranberry wines (Heinonen et al., 1998).

2.2. Effect of the wines on the pH of ground pork

The pH of the control I (6.02–5.98) and control II (5.92–6.22) ground pork samples steadily increased until day 10 during storage. Throughout the experimental period, control I had a significantly higher pH than control II except for day 15 ($P<0.05$; Table 2). Generally, lactic acid accumulation causes muscle pH to decline from approximately 7.1–7.3 to 5.4–5.7 during rigor mortis. The pH of meat begins to slightly increase during storage as proteolytic enzymes degrade myofibrillar proteins. Except for ground pork treated with 10% black raspberry wine, initial (day 1) pH levels of the ground pork treated with 5% and 10% of any wine (5.72–5.85) were lower than those of control I (6.02) and control II (5.92). This was because the wines added to the ground pork were acidic (Table 1) with pH values ranging from 3.36 (red grape wine) to 3.78 (wild grape and black raspberry wines). In most cases, pH levels of ground pork treated with the wines increased up to day 5, and then gradually decreased for the rest of the storage period. Components of wines, such as phenolic compounds, acids and alcohol, might have inhibited microbial growth and proteolytic enzyme activity in the ground pork, resulting in lower pH values compared to the control pork samples. During wine-making, sugars in the fruit juice are converted into alcohol by yeasts. The alcohol is then oxidized into acetic acid by bacteria when the wine is exposed to air. In our study, the ground pork was packaged in air-permeable bags. Therefore, a portion of the alcohol in the wine-treated ground pork could have been converted into acetic acid, thereby decreasing the pH during storage.

Table 2. pH of wine-treated and untreated ground pork during storage at 4 °C

	Day 1	Day 5	Day 10	Day 15
Control I	$^{B}6.02\pm0.04^{a}$	$^{A,B}6.16\pm0.05^{a}$	$^{A}6.57\pm0.51^{a}$	$^{B}5.98\pm0.01^{b}$
Control II	$^{C}5.92\pm0.01^{b,c}$	$^{B,C}5.98\pm0.03^{b}$	$^{B}6.07\pm0.09^{b}$	$^{A}6.22\pm0.03^{a}$
5% red grape wine	$^{A}5.82\pm0.06^{d,e}$	$^{A}5.85\pm0.03^{c,d}$	$^{A}5.80\pm0.03^{b,c}$	$^{B}5.47\pm0.02^{d}$
10% red grape wine	$^{A}5.72\pm0.07^{f}$	$^{A}5.78\pm0.09^{d}$	$^{A}5.81\pm0.03^{b,c}$	$^{B}5.48\pm0.08^{d}$
5% wild grape wine	$^{A}5.85\pm0.03^{c,d}$	$^{A}5.94\pm0.11^{b,c}$	$^{A,B}5.82\pm0.03^{b,c}$	$^{B}5.71\pm0.01^{c}$
10% wild grape wine	$^{B}5.73\pm0.02^{e,f}$	$^{A}5.99\pm0.03^{b}$	$^{B,C}5.66\pm0.16^{c,d}$	$^{C}5.47\pm0.13^{d}$
5% black raspberry wine	$^{B}5.79\pm0.01^{d,e,f}$	$^{AB}6.01\pm0.03^{b}$	$^{A,B}5.88\pm0.12^{b,c}$	$^{C}5.44\pm0.05^{d}$
10% black raspberry wine	$^{A}5.95\pm0.01^{a,b}$	$^{A}6.02\pm0.07^{b}$	$^{B}5.36\pm0.07^{d}$	$^{B}5.30\pm0.08^{e}$

Values are expressed as the mean ±standard deviation of triplicate analysis; Control I: ground pork; control II: ground pork (1 kg) treated with 100 ml of 12% ethanol.
Values in the same column with different lowercase letters are significantly different among treated groups ($P<0.05$); values in the same row with different uppercase letters are significantly different during storage ($P<0.05$)

2.3. Effect of the wines on lipid oxidation in ground pork

Lipid oxidation in the ground pork was measured as TBARS values over 15 days (Table 3). Lipid oxidation was affected by the type of wine used and the storage period. In all groups, TBARS values increased during storage, showing that prolonged storage periods resulted in the progression of lipid oxidation. TBARS values at 15 days for control I (0.90 mg MDA kg^{-1}) and control II (0.66 mg MDA kg^{-1}) markedly increased. Addition of ethanol lowered the TBARS value of control II (0.23–0.66 mg MDA kg^{-1}) compared to that of control I (0.22–

0.90 mg MDA kg^{-1}) during storage. Ethanol may scavenge highly reactive radicals (e.g. hydroxyl radicals) that initiate lipid peroxidation (Min & Ahn, 2005). However, addition of wine to the ground pork more effectively reduced TBARS values compared to ethanol addition.

Table 3. TBARS values (mg MDA/kg) of wine-treated and untreated ground pork during storage at 4 °C

	Day 1	Day 5	Day 10	Day 15
Control I	$^{C}0.22\pm0.00^{b, c, d}$	$^{B}0.47\pm0.10^{a}$	$^{B}0.61\pm0.01^{a}$	$^{A}0.90\pm0.09^{a}$
Control II	$^{C}0.23\pm0.02^{b, c, d}$	$^{B}0.38\pm0.01^{a, b}$	$^{A}0.58\pm0.04^{a}$	$^{A}0.66\pm0.06^{b}$
5% red grape wine	$^{C}0.19\pm0.04^{c, d}$	$^{C}0.22\pm0.02^{c, d}$	$^{B}0.39\pm0.03^{c, d}$	$^{A}0.51\pm0.01^{c, d}$
10% red grape wine	$^{C}0.19\pm0.06^{d}$	$^{C}0.18\pm0.01^{e}$	$^{B}0.28\pm0.01^{e}$	$^{A}0.37\pm0.01^{e}$
5% wild grape wine	$^{A}0.30\pm0.10^{a, b, c}$	$^{A}0.31\pm0.02^{b, c}$	$^{A}0.36\pm0.03^{d}$	$^{A}0.43\pm0.04^{d, e}$
10% wild grape wine	$^{C}0.16\pm0.02^{d}$	$^{B}0.30\pm0.04^{b, c}$	$^{A}0.43\pm0.01^{c}$	$^{A}0.44\pm0.04^{d, e}$
5% black raspberry wine	$^{B}0.33\pm0.01^{a, b}$	$^{C}0.24\pm0.01^{c, d}$	$^{A}0.58\pm0.01^{a}$	$^{A}0.60\pm0.04^{c, b}$
10% black raspberry wine	$^{C}0.41\pm0.01^{a}$	$^{D}0.33\pm0.05^{b, c}$	$^{B}0.51\pm0.01^{b}$	$^{A}0.68\pm0.01^{b}$

Values are expressed as the mean ±standard deviation of triplicate analysis; Control I: ground pork; control II: ground pork (1 kg) treated with 100 ml of 12% ethanol.
Values in the same column with different lowercase letters are significantly different among treated groups ($P<0.05$); values in the same row with different uppercase letters are significantly different during storage ($P<0.05$)

After 5 days, the ground pork treated with any wine at concentrations of 5% and 10% (0.18–0.68 mg MDA/kg) had lower TBARS values than those of control I (0.22–0.90 mg MDA/kg) and control II (0.38–0.66 mg MDA/kg) except for ground pork added with 10% black raspberry wine on day 15 ($P<0.05$). These findings suggest that the considerable protective effect of wines against lipid oxidation was partly related to their phenolic compound contents. During 5–15 days of storage, ground pork treated with 10% red grape wine had significantly lower TBARS values (0.18–0.37 mg MDA/kg; $P<0.05$) than ground pork mixed with 5% red grape wine (0.22–0.51 mg), 5% and 10% wild grape wine (0.30–0.44 mg) and black raspberry wine (0.24–0.68 mg) as well as control groups I and II (0.38–0.90 mg). These results are similar to ones from the study by Youn and co-workers (2007) showing that lipid oxidation is decreased in pork during cold storage by higher concentrations of red wine.

2.4. Effect of the wines on TVB-N levels in ground pork

TVB-N is one of the most widely used compounds for assessing the freshness of pork. TVB-N compounds mainly include ammonia and traces of trimethylamine, and increased TVB-N levels are associated with spoilage due to either bacterial activity or enzymatic degradation. Wine is an alcoholic beverage rich in phenolic compounds. Ethanol acts as an inhibitor of microbial growth and phenolic compounds are also known to have antimicrobial effects. TVB-N values for all groups steadily increased over storage time ($P<0.05$). The TVB-N contents of control I dramatically increased ($P<0.05$) from 82.5 mg kg^{-1} of ground pork to over 200 mg kg^{-1} after 10 days of storage, and finally reached 265.5 mg kg^{-1} after 15 days (Table 4). Addition of ethanol (12%) significantly inhibited TVB-N formation (81.7–203.5 mg kg^{-1}) in control II that had lower values compared to control I (164.3–265.5 mg kg^{-1}) during 5–15 days of storage.

Table 4. TVB-N content (mg/kg) of wine-treated and untreated ground pork during storage at 4 °C

	Day 1	Day 5	Day 10	Day 15
Control I	$^{D}82.5\pm0.1^{b}$	$^{C}164.3\pm9.5^{a, b}$	$^{B}224.9\pm7.2^{a}$	$^{A}265.5\pm10.1^{a}$
Control II	$^{D}67.8\pm3.0^{c}$	$^{C}81.7\pm11.4^{e}$	$^{B}144.7\pm11.3^{c}$	$^{A}203.5\pm15.9^{d}$
5% red grape wine	$^{C}69.1\pm4.8^{c}$	$^{B,C}77.5\pm9.1^{e}$	$^{B}86.8\pm9.2^{e}$	$^{A}142.8\pm4.2^{e}$
10% red grape wine	$^{C}76.2\pm5.0^{b, c}$	$^{C}78.9\pm6.9^{e}$	$^{B}119.9\pm9.1^{d}$	$^{A}215.1\pm11.3^{c,d}$
5% wild grape wine	$^{D}74.0\pm1.9^{b, c}$	$^{C}117.1\pm2.1^{d}$	$^{B}194.1\pm8.1^{b}$	$^{A}241.7\pm9.5^{b}$
10% wild grape wine	$^{D}72.0\pm1.2^{c}$	$^{C}137.2\pm11.1^{c}$	$^{B}184.8\pm9.8^{b}$	$^{A}231.5\pm9.1^{b, c}$
5% black raspberry wine	$^{D}81.7\pm7.0^{b}$	$^{C}148.9\pm13.0^{b, c}$	$^{B}199.7\pm5.7^{b}$	$^{A}241.3\pm12.5^{b}$
10% black raspberry wine	$^{C}128.8\pm4.0^{a}$	$^{B}170.3\pm6.5^{a}$	$^{A}225.4\pm12.0^{a}$	$^{A}226.3\pm8.1^{b, c}$

Values are expressed as the mean±standard deviation of triplicate analysis; control I: ground pork; control II: ground pork (1 kg) treated with 100 ml of 12% ethanol. Values in the same column with different lowercase letters are significantly different among treated groups ($P < 0.05$); values in the same row with different uppercase letters are significantly different during storage ($P<0.05$)

The TVB-N levels in wine groups of ground pork steadily increased, and showed the highest levels on day 15. The 5% and 10% red grape or wild grape wine lowered TVB-N levels in the ground pork compared to control I. For the black raspberry wine groups, TVB-N level was significantly lower than that of control I after 15 days of storage. Addition of 5% red grape wine resulted in the lowest level of TVB-N formation (77.5–142.8 mg kg^{-1}) among the ground pork samples treated with wines after 5 days of storage ($P<0.05$).

2.5. Effect of wines on ground pork colour

The L*, a* and b* values of ground pork during refrigerated storage (Table 5) were determined. L* values (lightness) of all ground pork increased over time. The black raspberry wine groups had significantly lower L* values compared to the other groups. Addition of 10% wine samples decreased lightness of the ground pork more than the 5% wine samples. b* values (yellowness) for most ground pork samples were not significantly affected by storage ($P>0.05$), but the ground pork added with 10% red grape and wild grape wines had significantly decreased yellowness after 10 days of storage ($P<0.05$). At the beginning of the storage period, the degree of yellowness of the ground pork treated with black raspberry wine was significantly lower than that of the other ground pork samples ($P<0.05$). In contrast, ground pork mixed with 10% wild grape wine was more yellow compared to the ground pork treated with 10% red grape and black raspberry wines. The a* value (redness) of control I decreased over the storage period whereas redness of the control II and wine treated groups was not significantly affected by storage up to 15 days ($P>0.05$).

Refrigerated storage increased lightness while decreasing the redness of the untreated ground pork. These findings were similar to the results of previous studies (Juncher et al., 2001). Ground meat is most susceptible to discoloration that results from the oxidation of ferrous-myoglobin (Fe^{2+}) into ferric-metmyoglobin (Fe^{3+}) during storage. This discoloration has also been reported to be caused by lipid oxidation, iron contained in muscle tissue and microbial growth (Vuorela et al., 2005). According to Brunton and co-workers (2000), lipid and myoglobin oxidation in meat are interrelated. Aldehydes as secondary lipid oxidation products can form adducts with protein and alter myoglobin stability, thereby leading to the

Table 5. L*, a* and b* values of wine-treated and untreated ground pork during storage at 4 °C

L* values	Day 1	Day 5	Day 10	Day 15
Control I	$^{B}52.86\pm1.27^{a, b, c}$	$^{B}52.35\pm1.90^{b}$	$^{B}52.80\pm1.67^{b}$	$^{A,B}53.16\pm1.35^{b}$
Control II	$^{B}53.57\pm0.38^{a, b}$	$^{A}55.14\pm0.24^{a}$	$^{A,B}54.58\pm1.06^{a, b}$	$^{A}55.09\pm0.59^{a, b}$
5% red grape wine	$^{B}53.85\pm0.70^{a}$	$^{C}52.76\pm0.59^{b}$	$^{A}56.01\pm0.86^{a}$	$^{A}55.98\pm0.75^{a}$
10% red grape wine	$^{B}52.4\pm0.50^{b, c}$	$^{B}51.85\pm0.39^{b, c}$	$^{A,B}54.00\pm2.04^{a, b}$	$^{A}55.82\pm1.96^{a}$
5% wild rape wine	$^{B}52.13\pm1.15^{c}$	$^{B}51.91\pm1.14^{b, c}$	$^{A,B}53.57\pm0.26^{b}$	$^{A}53.72\pm1.84^{a, b}$
10% wild grape wine	$^{C}49.83\pm0.81^{d}$	$^{C}50.38\pm1.50^{c}$	$^{B}52.42\pm0.56^{b}$	$^{A,B}53.37\pm1.35^{b}$
5% black raspberry wine	$^{B}47.24\pm1.09^{e}$	$^{B}47.36\pm1.61^{d}$	$^{A,B}48.66\pm1.31^{c}$	$^{A}50.85\pm2.04^{c}$
10% black raspberry wine	$^{B,C}43.72\pm0.13^{f}$	$^{C}42.48\pm0.73^{e}$	$^{B}45.76\pm1.97^{d}$	$^{A}48.2\pm1.38^{d}$

b* values	Day 1	Day 5	Day 10	Day 15
Control I	$^{A}11.59\pm0.31^{a, b}$	$^{A}12.05\pm0.18^{b, c}$	$^{A}11.63\pm2.30^{a}$	$^{A}11.67\pm0.33^{a}$
Control II	$^{B}11.41\pm0.46^{b}$	$^{A}13.28\pm0.74^{a}$	$^{B}11.54\pm1.62^{a}$	$^{A,B}12.15\pm0.60^{a}$
5% red grape wine	$^{A}11.46\pm0.49^{b}$	$^{A}11.9\pm0.59^{b, c}$	$^{A}11.44\pm2.07^{a}$	$^{A}11.83\pm0.37^{a}$
10% red grape wine	$^{A,B}11.47\pm0.58^{b}$	$^{A}11.83\pm0.68^{b, c}$	$^{B,C}10.32\pm1.55^{a, b}$	$^{C}9.72\pm0.52^{c}$
5% wild rape wine	$^{A,B}11.81\pm0.18^{a, b}$	$^{A}12.78\pm1.02^{a, b}$	$^{A,B}11.71\pm2.11^{a}$	$^{B}10.21\pm1.17^{b, c}$
10% wild grape wine	$^{A}12.24\pm0.47^{a}$	$^{A}12.61\pm0.85^{a, b, c}$	$^{B}10.38\pm0.93^{a, b}$	$^{B}11.08\pm0.69^{a, b}$
5% black raspberry wine	$^{A,B}10.45\pm0.32^{c}$	$^{A}11.66\pm0.17^{c}$	$^{A,B}10.51\pm1.37^{a, b}$	$^{B}9.30\pm1.18^{c}$
10% black raspberry wine	$^{A,B}8.87\pm0.53^{d}$	$^{A}9.13\pm0.19^{d}$	$^{A,B}8.21\pm1.53^{b}$	$^{B}7.55\pm0.64^{d}$

a* values	Day 1	Day 5	Day 10	Day 15
Control I	$^{A,B}12.55\pm0.72^{a}$	$^{A}13.31\pm1.35^{a}$	$^{B,C}11.40\pm0.26^{a, b}$	$^{C,D}10.39\pm0.68^{b}$
Control II	$^{B}11.03\pm0.69^{b}$	$^{C}9.26\pm1.59^{b, c}$	$^{A,B}11.83\pm0.83^{a}$	$^{A,B}12.63\pm1.32^{a}$
5% red grape wine	$^{B}10.07\pm0.30^{c, d}$	$^{B}9.94\pm0.35^{b, c}$	$^{C}7.81\pm1.05^{d}$	$^{A,B}10.80\pm0.50^{b}$
10% red grape wine	$^{B}9.75\pm0.50^{d}$	$^{B}9.41\pm0.91^{b, c}$	$^{B}9.47\pm1.17^{c}$	$^{B}10.22\pm1.23^{b}$
5% wild rape wine	$^{A}10.9\pm0.65^{b}$	$^{A}10.2\pm1.01^{b, c}$	$^{A}10.20\pm0.41^{b, c}$	$^{B}8.31\pm0.82^{c}$
10% wild grape wine	$^{A}10.59\pm0.40^{b, c}$	$^{B,C}8.98\pm1.07^{c}$	$^{A}10.42\pm0.82^{b, c}$	$^{A,B}10.08\pm0.92^{b}$
5% black raspberry wine	$^{A}10.79\pm0.21^{b, c}$	$^{A}11.0\pm1.17^{b}$	$^{A}10.48\pm1.28^{a, b, c}$	$^{A}10.11\pm0.84^{b}$
10% black raspberry wine	$^{A}11.24\pm0.24^{b}$	$^{A}10.7\pm0.77^{b, c}$	$^{A}11.85\pm0.75^{a}$	$^{A}10.81\pm0.99^{b}$

Values are expressed as the mean±standard deviation of triplicate analysis; control I: ground pork; control II: ground pork (1 kg) treated with 100 ml of 12% ethanol; values in the same column with different lowercase letters are significantly different among treated groups ($P<0.05$); values in the same row with different uppercase letters are significantly different during storage ($P<0.05$)

oxidation of myoglobin and subsequent discoloration. These aldehydes are also associated with TBARS values. Therefore, higher TBARS values observed for control I could have resulted in more oxidation of myoglobin compared to control II, leading to a loss of red colour (Table 3).

Ground pork treated with wines in the present study generally retained red colour during the storage period. The stable red colour of ground pork may be promoted by the antioxidative

capacity of phenolic compounds since natural antioxidants including numerous phenolic compounds have been shown to prevent oxidation, resulting in stable red coloration of ground meat (Brunton et al., 2000). In addition, the red colour of pork treated with wines can be affected by the actual red wine pigmentation. Anthocyanins, the compounds primarily responsible for the red colour of wine, are unstable and highly susceptible to degradation. However, other flavonoids in the wine confer a more stable red colour than that due to anthocyanins alone (Kontoudakis et al., 2001). Furthermore, pigmentation due to anthocyanins is dependent on acidity. Wines with lower pH tend to be redder with more stable colouring, whereas wines with higher pH have more blue pigments that are less stable (Robinson, 2006). Therefore, the decreased pH of ground pork treated wines could have also influenced the red colour of the meat during storage (Table 2).

Wines contain different concentrations of various components including phenolic compounds, transition metals, ethanol, organic acids, SO_2 and biogenic amines (Rupasinghe & Clegg, 2007). SO_2 is a chemical widely used in wine-making due to its antimicrobial and antioxidant activities (Danilewicz, 2007). The concentrations of transition metals in wine, such as iron (0–5 mg l^{-1}) and copper (0.1–0.3 mg l^{-1}), depend on the fruit source, geographical origin of the fruit, and vinification method (Rupasinghe & Clegg, 2007). The levels of metals in wine are important because these metals are known to generate highly reactive hydroxyl or alkoxyl radicals, resulting in oxidative spoilage even at low concentrations. Phenolic compounds can act as antioxidants by scavenging radicals. However, these compounds can also exert prooxidant effects, leading to the formation of highly reactive superoxide radicals. The antioxidant and prooxidant activities of phenolics in wine are affected by phenolic concentration, the presence of transition metal ions and pH levels (Halliwell & Gutteridge, 1984). Therefore, the wines evaluated in the present study might have affected the storage stability of ground pork by the antioxidative capacities of phenolic compounds as well as the interaction of various component contained in the wines.

3. Conclusions

In the present study, black raspberry wine contained more total phenolic compounds than red grape or wild grape wines. The antioxidative activity (FRAP and TEAC) of black raspberry wine was similar to those of the other wines, but superoxide anion radical scavenging capacity was lower than others. The addition of red grape, wild grape and black raspberry wines exerted antioxidative effects on ground pork during 15 days of refrigerated storage by inhibiting lipid oxidation and formation of TVB-N, and stabilizing the red pigmentation of the meat. Among the wines, red grape wine was more desirable on preventing further lipid oxidation or formation of TVB-N. An investigation for the effect of those wines on cooked ground pork regarding consumer acceptability and sensory quality would be needed for further research.

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