[Original Paper]

Effect of Clarification and Stabilization Treatments on *trans*-Resveratrol Content of Delaware Rose Wine Produced from UV-C Irradiated Grapes

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trans-Resveratrol was induced in skins of Delaware grapes, an American variety, which were harvested before full maturity and irradiated with ultraviolet-C (UV-C) light. Rose wine was produced from these grapes. Wine produced by thermovinification yielded approximately 20 mg/L *trans*-resveratrol. The wine was subjected to various clarification treatments. Heating, cooling, gelatin treatment or deacidification with calcium carbonate had no effect on *trans*-resveratrol content, whereas treatment with activated carbon or polyvinylpolypyrrolidone (PVPP) decreased its content. *trans*-Resveratrol was adsorbed from a wine-like model solution [10% ethanol (v/v), pH 3.03] on filtration with cellulose or polyvinylidene fluoride (PVDF), but was not adsorbed on filtration with aluminum oxide or polytetrafluoroethylene (PTFE).

Key words: filtration, fining, resveratrol, stabilization, wine

Introduction

Resveratrol, its glucoside piceid, and their isomers, are stilbene compounds well known for their presence in grapes and wines. These compounds are non-flavonoid phenolics having potential health benefits, such as cardiovascular disease risk reduction (18) and cancer prevention (8). The benefits are thought to be due to their abilities to inhibit low-density lipoprotein (LDL) oxidation (4) and to block platelet aggregation (3, 26) and eicosanoid synthesis (14, 26). *cis*-Resveratrol is either found in very small amounts in grapes or not detected at all, whereas *trans*-resveratrol is usually present in small amounts (9, 10, 12, 17, 35, 37). Resveratrol is mostly bound to glucose before harvest as *cis*- and *trans*-glucosides (9-11, 13, 20, 27, 33, 35, 37, 38). However, larger amounts of both *cis*- and *trans*-resveratrols have been detected in wines than in grapes (1, 2, 5, 7, 16, 19, 21, 23, 24, 28, 30, 36).

trans-Resveratrol is the most commonly studied stilbene compound in relation to temperature in grape-growing sites (5, 37). On the whole, red wines made from grapes grown in places having cool and slightly humid conditions, such as Ontario in Canada, the Rhone Valley, Bordeaux, and Burgundy in France, Oregon in the USA, and Switzerland, tend to have low *trans*-resveratrol contents. Meanwhile, red wines made from grapes grown in the Iberian peninsula (Portugal and Spain), the Mediterranean countries (Italy, Greece, etc.), California, Australia, South America, and South Africa, all of which are relatively warm and dry, have high *trans*-resveratrol contents (2, 5, 30, 31, 32). *trans*-Resveratrol content was low in Japanese red wines made from grapes grown in Japan (25, 28) under hot and very humid conditions.

One of the key factors in producing Japanese red wines with high resveratrol content is considered to be the prevention of their loss during post-fermentation clarification, as grapes grown in Japan have naturally low resveratrol content to begin with (25). Therefore, rose wine was made from UV-C irradiated Delaware grapes for this study, since *trans*-resveratrol can be produced in large

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amounts from the skins of this American variety using the irradiation process from our preliminary experimental work.

Materials and Methods

Preparation of must from Delaware grapes

Delaware grapes were harvested at 14.2–14.6 °Brix and 1.71% titratable acidity (w/v, as tartaric acid) on August 1, 2004.

Grape clusters were arranged in a plastic container lined with aluminum foil and were irradiated for 20 minutes at room temperature, at a distance of 10 cm with UV light (National GL 15, 15 W × 2; power output for sterilization, 4.9 W), with peak output at 254 nm. The clusters were turned over once in the middle of the irradiation process. The treated clusters were kept in the dark at 25 °C for four days.

The irradiated grapes (68 kg) were crushed and potassium metabisulfite (200 mg/L) was added. The crushed and sulfited grapes were heated in a plastic bag at 70 °C for 30 minutes. After cooling in running water, the heated grapes were pressed to obtain 42 L of juice. The pink juice was stored at –20 °C in a plastic bag. The frozen juice was thawed at room temperature immediately before use.

Production of rose wine from irradiated grapes

The juice was ameliorated with sucrose to give 23 °Brix, and then fermented in an air-conditioned room at 20 °C by adding dry wine yeast (*Saccharomyces cerevisiae*, Lalvin EC-1118) to give a content of 300 mg/L. After fermentation, the rose wine was centrifuged at 20,000 × g for 10 minutes and the supernatant obtained was filtered through a 0.45 µm membrane filter (PTFE, Advantec).

Effects of clarification and fining treatment of wine on *trans*-resveratrol content

• Cooling: 500 mL of Delaware rose wine was poured into a glass bottle (720 mL capacity) with a silicone stopper, left to stand at –4 °C for one week, and centrifuged at 9,000 × g for 30 minutes. The supernatant was used for resveratrol analysis.

• Heating: 500 mL of Delaware wine was poured into a glass bottle (720 mL capacity) with a silicone stopper and heated at 60 °C for 30 minutes. The wine was centrifuged at 9,000 × g for 30 minutes, and the supernatant was used for resveratrol analysis.

• Deacidification: Calcium carbonate was added twice to 500 mL of Delaware wine in a beaker to give a concentration of 0.9 g/L. The mixture was stirred at room temperature for one hour with a mechanical stirrer, allowed to stand overnight, then centrifuged at 9,000 × g for 30 minutes; the supernatant was collected and used for resveratrol analysis.

• Treatment with diatomaceous earth: Diatomaceous earth was added to 500 mL of Delaware wine in a beaker to give a concentration of 500 mg/L. The mixture was stirred at room temperature for one hour with a mechanical stirrer, allowed to stand for six hours, then centrifuged at 9,000 x g for 30 minutes; the supernatant was collected and used for resveratrol analysis.

• Treatment with bentonite (Ben-Gel, HoJun Co., Ltd., Mie, Japan), PVPP or activated carbon: Bentonite, PVPP, or activated carbon was added to 500 mL of Delaware wine to give a concentration of 500 mg/L. The mixture was stirred at room temperature for one hour with a mechanical stirrer, allowed to stand for six hours, and then centrifuged at 9,000 × g for 30 minutes. The above fining procedure was repeated and the supernatant obtained was used for resveratrol analysis.

• Treatment with gelatin (Copowrap-G, Otsuka Food Co., Ltd., Osaka), or gelatin plus tannin (Fuji Chemical Industry Co., Ltd., Toyama, Japan): Gelatin (300 mg/L) or gelatin (300 mg/L) plus tannin, was added to Delaware wine. The mixture was stirred at room temperature for one hour, allowed to stand for six hours, and then centrifuged at 9,000 × g for 30 minutes. The above fining procedure was repeated and the supernatant obtained was used for resveratrol analysis.

Effects of wine filtration material on resveratrol content

trans-Resveratrol standard solution (20 mg/L) was prepared by mixing a wine-like model solution with *trans*-resveratrol in ethanol. The wine-like model solution was prepared as follows. Six grams of tartaric acid was dissolved in 2,700 mL of deionized water, and to this was added 300 mL of absolute ethanol. The solution was adjusted to pH 3.03 with 50% NaOH. The wine or the resveratrol standard solution was passed through filters made of cellulose acetate, aluminum oxide, PTFE, or PVDF. Two cellulose acetate membrane filters (Advantec, 0.45 µm pore size, 13 mm diameter; 0.45 µm pore size, 25 mm diameter), two PTFE membrane filters (Advantec, 0.5 µm pore size, 10 mm diameter; Millipore, 0.45 µm pore size, 13 mm diameter), one hydrophilic PVDF membrane filter (Millipore, 0.45 µm pore size, 13 mm diameter), and one aluminum oxide membrane filter (Whatman, 0.2 µm pore size, 10 mm diameter) were used. In addition, industrial filter paper No. 5C, which was made of high-purity cellulose (Advantec, 185 mm diameter), and filter pad NA-16, which was made of natural fiber and diatomaceous earth (Advantec, 200 × 200 mm), were used. *trans*-Resveratrol content was determined before and after filtration.

Determination of *cis*- and *trans*-resveratrols

trans- and *cis*-Resveratrols were determined by high-performance liquid chromatography (HPLC) according to the methods described by Sato et al. (28). Individual extractions were independently conducted by centrifugation in 15-mL conical tubes. *trans*-Resveratrol was analyzed in duplicate and the analytical values were the averages of the results obtained.

Statistical analysis

Statistical analyses (ANOVA and t-test) were carried out for all relevant data using Excel Statistics 2000. LSD analyses based on ANOVA or t-test results were also carried out. The level of significance was established at $p \leq 0.05$.

Results and Discussion

Preparation of rose wine with high *trans*-resveratrol content

Delaware grapes were UV-C irradiated for either 5, 10, 20, or 30 minutes, and stored at 4, 15, 25, or 10 days. The grapes were then analyzed in order to determine grapes that had the highest *trans*-resveratrol content. The maximum content of *trans*-resveratrol was obtained by UV-C irradiation for 20 minutes at room temperature, followed by storage at 25 °C for four days. *trans*-Resveratrol was effectively transferred from grape skins into juice by heating irradiated and crushed grapes at 70 °C for 30 minutes (thermovinification). The juice with the largest amount of *trans*-resveratrol (26.2 mg/L) was used to make the wine.

The Delaware wine, which had a clear pink to light brownish yellow color, had 12.7% alcohol (v/v), extract 2.91 g/100 mL, 0.95% total acidity (w/v, as tartaric acid), 1,604 mg/L total phenols (as gallic acid), 19.9 mg/L *trans*-resveratrol, and a pH of 3.25. In the sensory evaluation of the Delaware wine, the average scores for flavor, aroma, appearance, and overall quality, respectively, were 2.27 [highest score 3.5 - lowest 1; standard deviation (SD) = 1.009), 3.73 (5 - 3; 1.009), 3.73 (5 - 2; 0.905), and 2.64 (4 - 1; 0.924), and the total score was 12.4 (maximum score: 20). The wine had weak aroma, strong bitterness, astringency, and acid taste, as well as pungent taste without oxidized flavor, smoky bacon or smoked odor, and little fruitiness.

Effects of clarification treatment of wine on *trans*-resveratrol content

The same Delaware rose wine as that used in the previous section was heated or chilled, and clarified with activated carbon, bentonite, calcium carbonate, diatomaceous earth, PVPP, or gelatin. The difference in *trans*-resveratrol content was investigated before and after the clarification treatments. The contents of *trans*-resveratrol, total phenols, and flavonoids were high at 19.9, 2,229, and 1,959 mg/L, respectively, owing to heating of the crushed grapes. Treatments with activated carbon, PVPP, and gelatin, respectively, decreased total phenol content by 17.5, 17.9, and 12.5%, and flavonoid content by 18.3, 20.3, and 16.0%, compared with contents before the treatment. On the other hand, compared with the content before treatment, *trans*-resveratrol content decreased by 98.6% on treatment with activated carbon, and by 93.8% on treatment with PVPP. However, little change in *trans*-resveratrol content was observed with gelatin treatment. The wine was deacidified effectively by the addition of calcium carbonate, and there was little change in its *trans*-resveratrol content.

It is speculated that the commercial production of a competitive, resveratrol-rich wine is possible by a combination of thermovinification using grapes with high acidity, resveratrol induction by UV irradiation, and deacidification with a treatment agent (calcium carbonate, for example).

Widely used filter materials for wine were found to remarkably influence resveratrol content, whereas heating

Table 1 Effects of clarification, fining, deacidification, and heating or cooling, on *trans*-resveratrol, total phenol, flavonoid, and non-flavonoid phenol contents and total titratable acidity in Delaware rose wine.

Treatment	Content after treatment (mg/L)				Total titratable acidity
	trans-Resveratrol	Total phenols	Flavonoids	Non-flavonoid phenols	
No addition	19.9	2229	1959	270	1.41
	(0.045)[b]	(53.0)	(11.8)	(11.8)	
Activated carbon (0.1% w/v, ×2)[c]	0.28	1838	1601	237	1.40
	(0.057)	(64.8)	(77.8)	(13.0)	
Bentonite (0.1% w/v, ×2)[c]	19.4	2213	1969	244	1.39
	(0.076)	(5.89)	(2.27)	(5.89)	
Calcium carbonate (5.4 g/L, ×2)[c]	19.2	2342	2034	308	0.49
	(0.210)	(106.1)	(101.4)	(4.71)	
Diatomaceous earth (0.1% w/v, ×2)[c]	19.8	2271	2018	253	1.41
	(0.242)	(5.89)	(17.7)	(11.8)	
PVPP (0.1% w/v, ×2)[c]	1.24	1829	1561	268	1.40
	(0.144)	(29.5)	(23.6)	(5.89)	
Gelatin (0.3 g/L, ×2)[c]	19.9	1950	1645	305	1.39
	(0.151)	(11.8)	(13.0)	(1.19)	
7 days after start of cooling at –4 °C and centrifugation	19.0	2167	1901	266	1.33
	(0.025)	(23.6)	(11.8)	(11.8)	
Heating at 60 °C for 30 minutes and centrifugation	19.7	2221	1961	260	1.40
	(0.038)	(23.6)	(11.8)	(5.89)	
Suction filtration through filter paper[d] (×3)	16.0	2217	1974	243	1.39
	(0.529)	(23.6)	(21.2)	(2.36)	
Ultrafiltration through filter pad[e] (×2)	9.30	—	—	—	1.40
	(0.797)	—	—	—	
Ultrafiltration through filter pad[e] (×2)	9.30	—	—	—	1.40
	(0.797)	—	—	—	

[a] % as tartaric acid.
[b] Values in parentheses show standard deviation.
[c] Addition of clarification or fining substance, followed by centrifugation.
[d] Advantec filter paper No. 5C (185 mm). See Table 2 regarding filter material.
[e] Advantec filter pad NA-16 for wine. See Table 2 regarding filter material.

and cooling of wine hardly had any influence on resveratrol content, as shown in Table 1. Thus, the effects of filter materials were further investigated.

Effects of filter materials on *trans*-resveratrol content in wine

Rose wine produced from UV-irradiated Delaware grapes and three standard *trans*-resveratrol solutions of different contents were used. In our laboratory, two cellulose filters (filter paper No. 5C, Advantec, Tokyo, for filtering small volumes of wine; and filter pad NA-16, Advantec, for large-scale filtration) were used for the small-scale production of wine, and the other five membrane filters were used for analytical purposes (Table 2). The recovery of *trans*-resveratrol was low with cellulose acetate filter for all the samples, and slightly low with PVDF filter. Of the filters used, excellent recovery was achieved using aluminum oxide or PTFE filter. Thus, the filter material appreciably influenced the recovery of resveratrol. In our study, two filters made of aluminum oxide or PTFE were further used. In the case of filtration that was performed twice through filter pad NA-16 or filter paper No. 5C, approximately half of *trans*-resveratrol was lost. The filter manufacturer (Advantec, Tokyo) informed us that the filter pad was made of natural fiber, cellulose (ca. 40%) and diatomaceous earth (ca. 60%). Natural fiber may contain contaminants other than those two components, because filtration with either cellulose filter or diatomaceous earth filter did not lead to any loss of resveratrol. Therefore, attention should be paid to the filter material used to produce resveratrol-containing wine.

There have been several papers published on the effects of fining agents, such as carbon, PVPP, diatomaceous earth, gelatin, and bentonite, on resveratrol content of wine (6, 15, 22, 29, 37, 39). However, there appear to be few on the use of cooling and heating treatments for the stabilization of wine, on the use of calcium carbonate for deacidification, or

Table 2 Effect of filter materials on *trans*-resveratrol content in Delaware rose wine and standard *trans*-resveratrol solutions.

Filter material	*trans*-Resveratrol content (mg/L)		Amount absorbed (mg/L)	Recovery (%)
	Before filtration	After filtration		
Delaware rose wine (produced from grapes harvested on July 21, 2004)				
Aluminum oxide (Whatman, 0.2 μm, 10 mm)[a]	38.9	38.7	0.2	99.5
Cellulose acetate (Advantec, 0.45 μm, 13 mm)		17.3	21.6	44.5
PTFE[b] (Advantec, 0.5 μm, 10 mm)		38.8	0.1	99.7
PVDF[c] (Millipore, 0.45 μm, 13 mm)		34.7	4.24	89.1
Hydrophilic PTFE[a] (Millipore, 0.45 μm, 13 mm)		37.9	1.0	97.4
Delaware rose wine (produced from grapes harvested on August 1, 2004)				
Cellulose (Advantec, filter paper No. 5C, ca. 1 μm, 0.22 mm, 185 mm)	19.9	16.0	3.9	80.5
Cellulose (ca. 40%) & diatomaceous earth (ca. 60%) (Advantec, filter pad NA-16 for wine)		9.30	10.6	46.7
trans-Resveratrol solution A[d]				
Aluminum oxide	0.820	0.718	0.103	87.5
Cellulose acetate		0	0.671	0
PTFE[b]		0.714	0.107	87.0
PVDF[c]		0.621	0.120	83.8
Hydrophilic PTFE[a]		0.572	0.099	85.3
trans-Resveratrol solution B[d]				
Aluminum oxide	4.377	4.320	0.057	98.7
Cellulose acetate		0.568	3.301	14.7
PTFE[b] (Advantec)		4.303	0.074	98.3
PVDF[c]		3.482	0.531	86.8
Hydrophilic PTFE[b]		3.513	0.356	90.8
trans-Resveratrol solution C[d]				
Aluminum oxide	8.569	8.566	0.003	100
Cellulose acetate		1.640	6.437	20.3
PTFE[b]		8.535	0.034	99.6
PVDF[c]		7.375	1.106	87.0
Hydrophilic PTFE[b]		7.542	0.534	93.4

[a] Values in parentheses indicate pore size, thickness, and diameter of each filter in that sequence.
[b] PTFE: Polytetrafluoroethylene.
[c] PVDF: Polyvinylidene fluoride.
[d] Wine-like model solutions (pH 3.1) containing various concentrations of *trans*-resveratrol and 10% ethanol (w/v).

on the effect of filter materials for filtration. With respect to the effects of fining agents, our results are similar to others (30, 34, 36). The experimental results on minimizing the loss of resveratrol during fining and filtration discussed above are considered to be useful for both making wines with high resveratrol contents and analyzing them. The commercial production of wines with high resveratrol contents may be possible with further research aimed at developing a more effective method of inducing resveratrol in grape skins, and less damaging post-fermentation, wine clarification treatments.

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［研　究　報　文］

UV-C 照射デラウェアブドウから製造したロゼワインのリスベラトロール濃度に及ぼす清澄化並びに安定化処理の影響

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要　約

完熟前のデラウェアブドウに UV-C を照射し、果皮にリスベラトロール誘導を行い、このブドウを用いて、ロゼワインの製造を行った。加温醸造法により製造したワインの *trans*-リスベラトロール濃度は約 20 mg/L であった。このワインを用いて種々の清澄化処理を行った。加熱、冷却、ゼラチン処理または炭酸カルシウムによる除酸処理では、*trans*-リスベラトロール濃度に変化は見出せなかったが、活性炭またはポリビニルポリピロリドン（PVPP）処理では減少が認められた。*Trans*-リスベラトロールをモデルワインに溶解した溶液（10%エタノール、pH 3.03）を濾過したとき、*trans*-リスベラトロールはセルロースやポリフッ化ビニリデン（PVDF）膜に吸着されたが、酸化アルミニウムやポリテトラフルオロエチレン（PTFE）膜には吸着しなかった。