［Original Paper］

Light-induced Isomerization of *trans*-Resveratrol to *cis*-Resveratrol in Must and Wine during Fermentation and Storage

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Koshu must containing a large amount of *trans*-resveratrol was put in a clear transparent bottle and fermented under irradiation with a D65 fluorescent lamp whose spectral distribution is similar to that of natural sunlight. The amount of *trans*-resveratrol decreased with fermentation time, while *cis*-resveratrol, the amount of which corresponded to the amount of decrease of *trans*-resveratrol, was formed. A wine-like model solution containing authentic *trans*-resveratrol was stored in each of three transparent glass bottles with different colors (clear transparent, emerald green or brown) and exposed to fluorescent light for 200 days. The amount of *trans*-resveratrol in the emerald green bottle decreased and a certain amount of *cis*-resveratrol was formed. On the other hand, *trans*-resveratrol disappeared completely when stored in the clear transparent bottle. *cis*-Resveratrol may be photochemically formed from *trans*-resveratrol depending on the illumination conditions during vinification and storage of wine.

Key words: isomerization, *trans*-resveratrol, *cis*-resveratrol

Introduction

It is known that resveratrol is present as *trans*- and/or *cis*-resveratrols and/or their glucosides in wine. Romero-Pérez et al. (2001), Jeandet et al. (1991), Versari et al. (2001), Urhovsek et al. (1997), and Sun et al. (2006) reported that most *trans*- and *cis*-resveratrols existed as their glucosides (also called piceids or polydatins) in grape berries. Similarly, Roldán et al. (2003) reported that resveratrol was present mainly in the glycosylated form. Meanwhile, Clare et al. (2004) found that free resveratrol was absent in the must prior to fermentation. That wine contains not only resveratrol glucosides but also free resveratrols is supposed to be due to the transformation of the glucosidic forms of *cis*- and *trans*-resveratrols into the corresponding aglycones.

The possibility of the photochemical isomerization of *trans*-resveratrol to *cis*-resveratrol has been discussed. The photochemical isomerization of stilbene compounds, such as resveratrol, is well known and occurs via the excitation of electrons at double-bonded carbon atoms (Waldeck 1991, Takeuchi et al. 2008). Jeandet et al. (1995) and Mattivi et al. (1995) negated the concept of the light-induced isomerization of *trans*-resveratrol to *cis*-resveratrol and advocated that *cis*-resveratrol glucoside in grape berries is the main source of *cis*-resveratrol in wine. In actual work, the photochemical isomerization of resveratrol may hardly occur because wine fermentation is usually carried out in the dark. On the other hand, fermentation may be performed in rare cases in a room with bright natural light or beneath a fluorescent lamp and wine may be stored in a clear transparent bottle under various light conditions in a wine cellar.

In this paper, we describe the possible presence of *cis*-resveratrol that is formed by the photochemical isomerization of *trans*-resveratrol in wine during vinification and storage.

Materials and Methods

UV irradiation

Grape clusters (50 kg, harvested on September 4, 2004)

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arranged in a plastic container lined with aluminum foil were irradiated for 20 minutes at room temperature at a distance of 10 cm with UV light (Panasonic, National GL 15, 15 W x 2; power output for sterilization, 4.9 W, Tokyo, Japan) with peak output at 254 nm. The clusters were turned over once in the middle of the irradiation. The treated clusters were kept in the dark at 25°C for 4 days in order to induce *trans*-resveratrol formation in skin (Yokotsuka *et al.* 2008).

Fermentation of Koshu wine under fluorescent lamp irradiation

The irradiated grapes 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 27 L of juice. The juice was ameliorated with sucrose to give 23 °Brix and the sulfited and ameliorated juice (300 mL each) was poured into a clear transparent bottle (750 mL capacity). Fermentation was carried out using commercial dry wine yeast (300 mg/L, Lalvin EC-1118) at 20°C in the dark (the bottle was kept from exposure to light by covering with two layers of aluminum foil), or under irradiation with a D65 fluorescent lamp (similar to natural sunlight in spectral distribution). The light intensity was 5,500 lx as measured with an illumination meter (T&D PHR-51). After the fermentation ceased, the must was allowed to stand overnight. Then, the almost clear upper portion after spontaneous precipitation was centrifuged at 5,514 × *g* for 15 minutes and the supernatant was used as finished wine. The wine was filtered through a 0.5-µm membrane filter (PTFE, Advantec) and the amounts of *trans*- and *cis*-resveratrols in the filtrate were determined.

Exposure of *trans*-resveratrol solution to light

As regards the effect of exposure of *trans*-resveratrol solution to light, a clear transparent 379-mL glass bottle, an emerald green 379-mL glass bottle for wine, and a brown 314-mL glass bottle for Japanese "sake" were used. Three hundred and fifty mL (or 300 mL for the brown bottle) of standard *trans*-resveratrol solution [20 mg *trans*-resveratrol in 1 L of a wine-like model solution (6 g of tartaric acid, 100 mL of ethanol, and 900 mL of water, adjusted to pH 3.03 with 50% NaOH)] was poured into a glass bottle. The bottle was capped with a silicone stopper and irradiated continuously at a distance of 15 cm with a fluorescent lamp (22 × 2 cm, 15 W × 2, 67,121 lx/day, Matsushita Electric Industrial Co., Ltd., the former name of Panasonic Corp., Osaka, Japan) at 18°C The accumulated illumination intensity was measured with an illumination meter and expressed as lx/day. During irradiation, samples were collected periodically and centrifuged at 20,753 × *g* for 10 minutes at 15°C. The supernatant obtained was filtered through a 0.5-µm membrane filter (PTFE, Dismic-13JP, Advantec) and the amounts of *trans*- and *cis*-resveratrols in the filtrate were determined. The bottles used were smashed into small pieces (approximately 1.5 × 3 cm) and the effect of the color of the glass bottles used on light transmission at different wavelengths was investigated by spectrophotometric measurements in the %T mode (Hitachi U-2000).

Samples were periodically collected and filtered through a 0.45-µm membrane filter (PTFE, Advantec) for resveratrol analysis.

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

trans-Resveratrol was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). The preparation of *cis*-resveratrol and the determination of *trans*- and *cis*-resveratrols were done according to the method of Sato et al. (1997).

Results and Discussion

Changes in resveratrols during fermentation under various kinds of light

Koshu must containing a large amount of *trans*-resveratrol and stored in a clear transparent bottle was fermented for 15 days under continuous illumination with a conventional fluorescent lamp, an incandescent lamp, or a D65 fluorescent lamp that is similar to natural light in spectral distribution, and this was followed by the determination of various wine components, including *trans*-resveratrol (Fig. 1). No decrease in the amount of *trans*-resveratrol was noted and no *cis*-resveratrol was formed when the fermenting must was not exposed to light. When fermented under 1,500 lx of each lamp, the amount of *trans*-resveratrol decreased with time and *cis*-resveratrol was formed (Fig. 1). The average amounts of *cis*-resveratrol formed between 10 and 15 days after the start of fermentation were 3.1 mg/L under the D65 fluorescent lamp, 1.97 mg/L under the fluorescent lamp, and 0.87 mg/L under the incandescent lamp, and a decrease in the amount of *trans*-resveratrol, which corresponded to the amount of *cis*-resveratrol formed, was observed.

The fermentation rate, the pH, the absorbance at 280, 320, and 520 nm of the fermenting must, and the amounts of total phenols, non-flavonoid phenols, and flavonoids were not influenced by the kind of lamp used (data not shown).

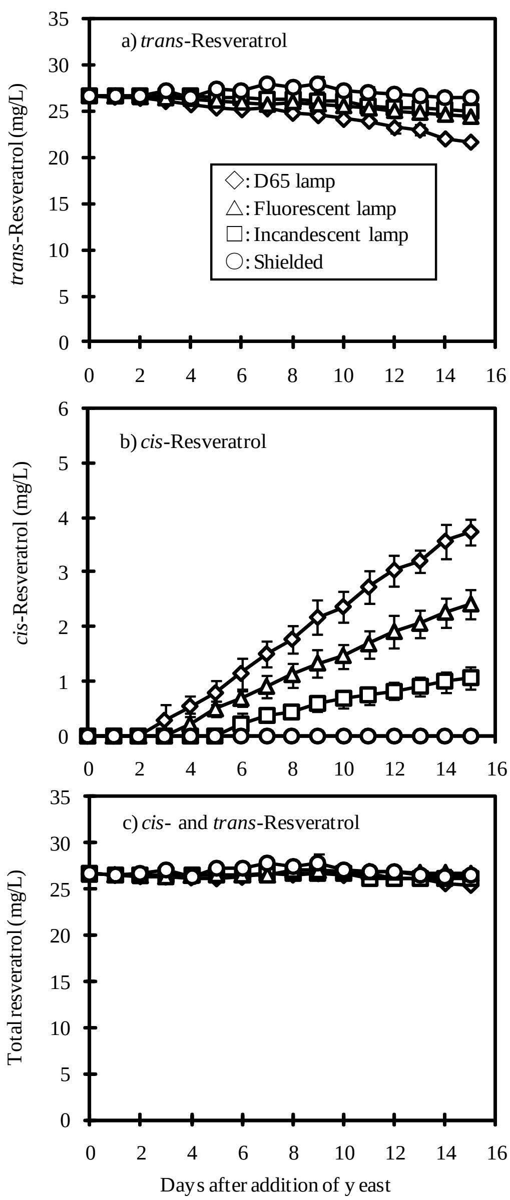

Fig. 1 Effect of exposure to light on the amounts of *trans*- and *cis*-resveratrols and of total resveratrols in must.

Effect of glass bottle color on the amount of *trans*-resveratrol in a wine-like model solution during storage

Figure 2 shows the effect of glass bottle color (clear transparent, emerald green, and Brown) on light transmission at different wavelengths. Emerald green and brown-colored glasses are known to filter out UV light that is responsible for the undesirable photochemical reaction in wine.

Changes in the amount of *trans*-resveratrol in the bottles exposed to fluorescent lamp were examined. Although the amount of *trans*-resveratrol in the emerald green bottle decreased and a certain amount of *cis*-resveratrol was formed, the total amount of *trans*- and *cis*-resveratrols decreased by approximately 35% compared with the initial amount of *trans*-resveratrol. On the 200th day of light irradiation, approximately half of the initial amount of *trans*-resveratrol remained, and approximately 30% of it was converted into *cis*-resveratrol. In the case of storage in the clear transparent bottle, a large decrease in the amount of resveratrol was observed: a rapid decrease in the amount of *trans*-resveratrol was noted and its amount was 2.94 mg/L on day 20 of light irradiation. The amount of *cis*-resveratrol reached a maximum on the 27th day, after which it gradually decreased. On the 200th day of light irradiation, the amounts of *cis*-resveratrol and total resveratrols (*cis* and *trans*) were

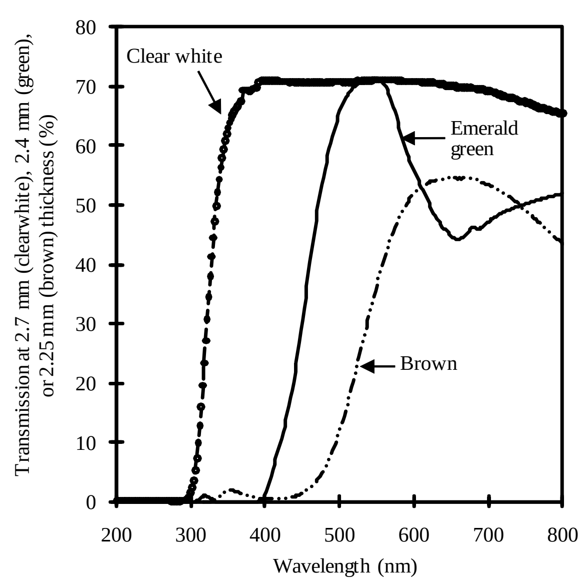

Fig. 2 Effect of glass bottle color on light transmission at different wavelengths.

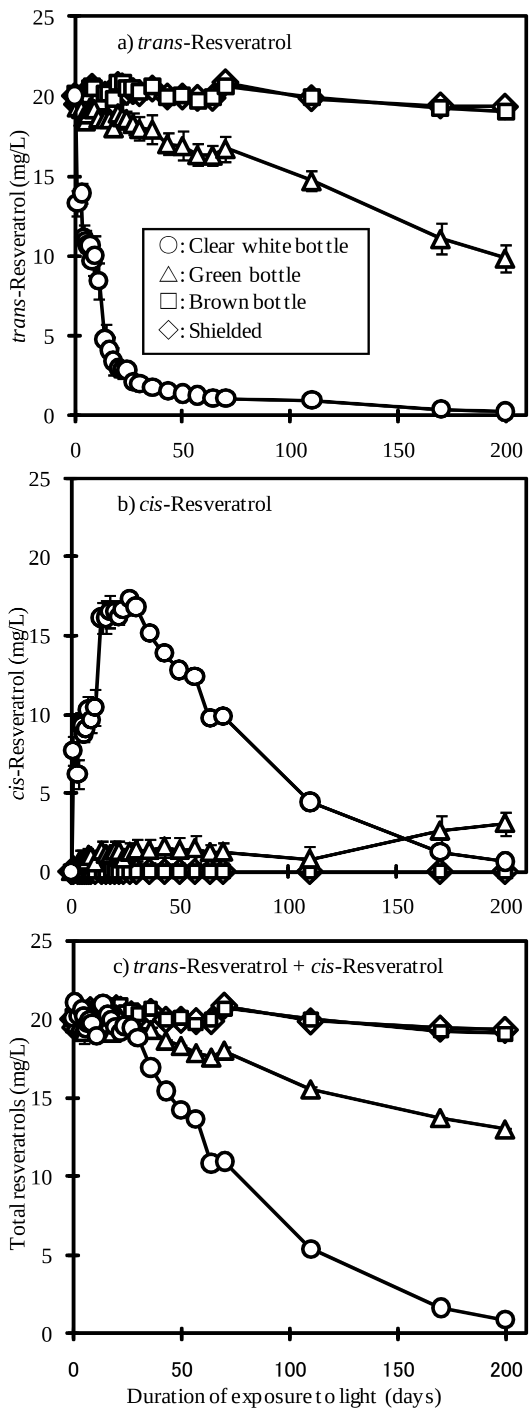

Fig. 3 Effect of exposure of standard *trans*-resveratrol solution in colored bottles to fluorescent light on the amounts of *trans*- and *cis*-resveratrols and of total resveratrols.

as small as 0.67 mg/L and 0.91 mg/L, respectively. *trans*-Resveratrol may have been oxidized or polymerized (Xing et al. 1997, Montsko et al. 2008). Montsko et al. reported that 3,4',5-trihydroxy-diphenylacetylene was formed as a secondary product during the photoisomerization of *trans*-resveratrol into *cis*-resveratrol by UV irradiation at 365 nm (Montsko et al. 2008). Their results well corresponded to ours and together, the results indicate that light of 350 to 450 nm wavelength promote the isomerization or oxidation of *trans*-resveratrol. From these results, resveratrol-rich wine should be stored in a brown bottle in the dark.

The initial amount of *cis*-resveratrol was zero and the initial amounts of *trans*-resveratrol in the juice (Fig. 1) and in the wine-like model solution (Fig. 3) were 26.6 mg/L and 20.1 mg/L, respectively. The amounts of *trans*- and *cis*-resveratrols were 21.6 mg/L and 3.74 mg/L, respectively, on the 15th day of fermentation under exposure to light (under a D65 fluorescent lamp) (Fig. 1), whereas those were 4.45 mg/L and 16.2 mg/L, respectively, on the 15th day of storage under light irradiation (Fig. 3). The percentage decrease of *trans*-resveratrol was approximately 19% in the former case and 78% in the latter case. Despite the similar initial amounts of *trans*-resveratrol in both figures (Fig. 1 and Fig. 3), the decrease in the amount of *trans*-resveratrol (the increase in *cis*-resveratrol amount) was larger in the wine-like model solution (no components other than water, ethanol, authentic *trans*-resveratrol, tartaric acid, and a very small amount of NaOH for pH adjustment were present) (Fig. 3) than in the rose wine produced using liquid must after heating of crushed grapes (note that Koshu grapes have pink skin) (Fig. 1). It seems that the large amounts of polyphenols in rose wine prevented the conversion of *trans*-resveratrol into *cis*-resveratrol because polyphenols may have high UV absorbing ability.

Conclusions

1. *trans*-Resveratrol was stable during fermentation in the dark. Fermentation under fluorescent light or incandescent light decreased the amount of *trans*-resveratrol in wine and a certain amount of *cis*-resveratrol was formed. The *trans*-resveratrol that disappeared corresponded to the amount of *cis*-resveratrol formed.

2. *trans*-Resveratrol solution was stored in clear transparent, emerald green, or brown glass bottles under exposure to fluorescent light. The amounts of *trans*-resveratrol in the bottle that was covered with two layers of aluminum foil

and the brown bottle did not change. In contrast, *trans*-resveratrol disappeared completely when stored in the clear transparent bottle, and disappeared appreciably when stored in the emerald green bottle.

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Literature cited

Clare, S. S., G. Skurray, and R. A. Shalliker. 2004. Effect of pomace－contacting method on the concentration of *cis*- and *trans*-resveratrol and resveratrol glucoside isomers in wine. Am. J. Enol. Vitic. 55: 401-406.

Jeandet, P., R. Bessis, and B. Gautheron. 1991. The production of resveratrol (3,5,4'-trihydroxystilbene) by grape berries in different developmental stages. Am. J. Enol. Vitic. 42: 41-46.

Jeandet, P., R. Bessis, B. F. Maume, P. Meunier, D. Peyron, and P. Trollat. 1995. Effect of enological practices on the resveratrol isomer content of wine. J. Agric. Food Chem. 43: 316-319.

Mattivi, F., F. Reniero, and S. Korhammer. 1995. Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. J. Agric. Food Chem. 43: 1820-1823.

Montsko, G., M. S. Pour Nigfardjam, Z. Szabo, K. Boddi, T. Lorand, R. Ohmacht, and L. Mark. 2008. Determination of products derived from *trans*-resveratrol UV-photoisomerisation by means of HPLC-APCI-MS. J. Photochem. Photobiol. A: Chem. 196: 44-50.

Roldán, A., V. Palacios, I. Caro, and L. Pérez. 2003. Resveratrol content of Palomino fino grapes: Influence of vintage and fungal infection. J. Agric. Food Chem. 51: 1464-1468.

Romero-Pe´rez, A. I., R. M. Lamuela-Raventos, C. A.-Lacueva, and M. C. de la Torre-Boronat. 2001. Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. J. Agric. Food Chem. 49: 210-215.

Sato, M., Y. Suzuki, T. Okuda, and K. Yokotsuka. 1997. Contents of resveratrol, piceid, their isomers in commercially available wines made from grapes cultivated in Japan. Biosci. Biotech. Biochem. 61: 1800-1805.

Takeuchi, S., S. Ruhman, T. Tsuneda, M. Chiba, T. Taketsugu, and T. Tahara. 2008. Spectroscopic tracking of structural evolution in ultrafast stilbene photoisomerization. Science. 322: 1073-1077.

Sun, B., A. M. Ribes, M. C. Leandro, A. P. Belchior, and M. I. Spranger. 2006. Stilbenes: Quantitative extraction from grape skins, contribution of grape solids to wine and variation during wine maturation. Anal. Chim. Acta. 563: 382-390.

Urhovsek, U., S. Wendelin, and R. Eder. 1997. Effects of various vinification techniques on the concentration of *cis*- and *trans*-resveratrol and resveratrol glucoside isomers in wine. Am. J. Enol. Vitic. 48: 214-219.

Versari, A., G. P. Parpinello, G. B. Tornielli, R. Ferrarini, and C. Giulivo. 2001. Stilbene compounds and stilbene synthase expression during ripening, wilting, and UV treatment in grape cv. Corvina. J. Agric. Food Chem. 49: 5531-5536.

Waldeck, D. H. 1991. Photoisomerization dynamics of stilbenes. Chem. Rev. 91: 415–436.

Xing, K. Z., N. Johansson, G. Beamson, D. T. Clark, J-L. Brédas, and W. R. Salaneck. 1997. Photo-oxidation of poly(p-phenylenevinylene). Advanced Materials. 9: 1027-1031.

Yokotsuka, K., T. Okuda, M. Hisamoto, H. Tajima, and T. Takayanagi. 2008. Effect of clarification and stabilization treatments on *trans*-resveratrol content of Delaware rose wine produced from UV-C irradiated grapes. J. ASEV Japan 19: 25-32.

［研　究　報　文］

発酵並びに貯蔵中のマスト及びワインの *trans*-リスベラトロールの *cis*-リスベラトロールへの光誘起異性化

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多量の *trans*-リスベラトロールを含む甲州種マストを透明なガラスボトルに入れ、太陽光と類似のスペクトル分布の D65 蛍光灯の照射下で発酵を行った。発酵の進行に伴い、*trans*-リスベラトロール量は減少し、一方 *cis*-リスベラトロールが生成され、その生成量は *trans*-リスベラトロール量と相当した。標準 *trans*-リスベラトロールを含むワイン様モデル溶液を、異なった 3 種の色（無色透明、エメラルド・グリーン、あるいは琥珀色）のガラスボトルのいずれかに入れ、200 日間蛍光灯に暴露した。エメラルド･グリーンのボトル中の *trans*-リスベラトロール量は減少し、ある程度の *cis*-リスベラトロールが生成された。他方、無色透明ボトルに入れた *trans*-リスベラトロールは完全に消失した。ワイン製造や貯蔵中の照射条件によるが、*cis*-リスベラトロールは *trans*-リスベラトロールから光化学的に生成されるかもしれない。