White Wine with Red Wine-like Properties: Increased Extraction of Grape Skin Polyphenols Improves the Antioxidant Capacity of the Derived White Wine

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Lower antioxidant activity in white wines in comparison to red wines lies in the low grape-skin-derived polyphenol content. This paper reports the analysis of the antioxidant capacities of white wine samples obtained along two different processing procedures directed to enrich the wine with polyphenols. White wine samples derived from whole squeezed grapes stored for increasing periods of time (up to 18 h) contained increasing concentrations of polyphenols (from 0.35 to 0.55 mmol/L) and, in parallel, exhibited increased capacity to scavenge free radicals and to inhibit copper ion-induced low-density lipoprotein (LDL) oxidation. However, addition of increasing concentrations of alcohol (up to 18%) to the whole squeezed grapes remarkably augmented the extraction of grape skin polyphenols into the wine up to 1.25 mmol/L, resulting in an increased capacity of the wine to scavenge free radicals and to inhibit LDL oxidation, to an extent similar to that of red wine. The extent of LDL oxidation inhibition was directly related to the wine polyphenolic content ($r = 0.986$). It is concluded that processing white wine by imposing a short period of grape skin contact in the presence of alcohol leads to extraction of grape skin polyphenols and produces polyphenol-rich white wine with antioxidant characteristics similar to those of red wine.

Keywords: *Red wine; white wine; polyphenols; LDL oxidation*

INTRODUCTION

The hypothesis that the oxidation of low-density lipoprotein (LDL) is a major cause of atherosclerosis raised an extensive investigation into the role of antioxidants against LDL oxidation (*1*, *2*).

Consumption of flavonoids in the diet was shown to be inversely associated with morbidity and mortality from coronary heart disease (*3*−*5*). Red wine is a rich dietary source for polyphenols (*6*−*8*), and polyphenolic flavonoids extracted from red wine were shown to protect LDL against oxidation (*9*−*11*). The "French paradox", that is, the low incidence of cardiovascular events despite a diet high in saturated fat, was attributed to the regular drinking of red wine in southern France (*12*). Ingestion of red wine was shown to be associated with increased antioxidant serum activity (*13*) and increased resistance of LDL to oxidation ex vivo (*14*−*16*). We have recently demonstrated that consumption of red wine or purified catechin (flavanol) or quercetin (flavonol) resulted in a reduced susceptibility of LDL to oxidation (*17*) and in an attenuate progression of atherosclerosis in atherosclerotic apolipoprotein E deficient mice (*17*).

Phenolic compounds in red wine are derived from the grape's skin, as well as from grape seeds, grape stems, or grape pulp, all of which are important sources of flavanols that are transferred to the wine during maintenance together with the grape juice at the first stage of wine fermentation. On the contrary, white wines are usually made from the free running juice, without the grape mash, having no contact with the grape skins. This is thought to be the main reason for the relatively low polyphenol content and for the lower antioxidant activity of white wine in comparison to red wine (*13*, *18*−*22*). Thus, we have hypothesized that processing of white wine in a way that will enrich it with the grape skin polyphenols will also increase the antioxidant capacity of white wines, making them more beneficial healthwise, like red wine. To test this hypothesis, we have analyzed the antioxidant capacities of white wine samples obtained along two processing procedures. First, whole squeezed grapes were stored for increasing periods of time before the removal of the grape's skins. Second, increasing concentrations of alcohol were added to the whole squeezed grapes to improve the extraction of grape skin polyphenols and, hence, the wine's antioxidant capacity against LDL oxidation.

MATERIALS AND METHODS

Wine Sample Preparation. Fifteen tonnes of whole squeezed Chardonnay grapes, which were cultivated in Mazkeret Batia, central Israel, were stored for up to 18 h, and samples were taken out at 2 h intervals. Then, the grapes' skins and other solid material were removed by filtration, using a system of serial filters starting with a rough filter followed by more delicate filters with smaller pores, ending with a very small pore filter, which removes all solid materials,

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including yeasts and bacteria. Then yeasts were added, and the juice was allowed to ferment into wine. The yeasts used were a specific train of *Saccharomyces cerevisiae*, D-47, which were purchased from Lauemand Inc. (Lalvin), Montreal, Canada. The Chardonnay dry white wine obtained after this procedure is classified as "table wine".

In a second study, 500 kg of whole squeezed Muscat grapes cultivated in Mazkeret Batia, central Israel, were incubated for 18 h with increasing concentrations of alcohol ranging between 0 and 18%.

The alcohol used was distilled from fermented grape wine to yield 94% ethyl alcohol. Then, the grapes' skins and other solid materials were removed by a filtration system as described above, yeast were added, and the juices were allowed to ferment into wine.

The yeasts used in this part of the study were *S. ceverisiae* L-2226, a specific strain resistant to high concentrations of alcohol, and they were purchased from Lauemand Inc. The final alcohol concentrations in the wines that were preincubated with 2, 4, 10, 12, 16, and 18% of alcohol were 13.4, 15.4, 16.0, 16.9, 17.9, and 18%, respectively, and the sugar contents in these wines were 10.6, 11.5, 12.6, 12.5, 12.5, and 13.7%, respectively. All of the obtained wines were sweet "dessert wines" (not table wines).

The Cabernet Sauvignon red wine used in the present study was an Israeli commercially available wine (Cabernet Sauvignon, Barkan, 1996). Usually, Cabernet Sauvignon wine is made by letting the crushed grapes ferment for up to 2 weeks.

Polyphenol Determination. Total polyphenol concentration in wine samples was determined spectrophotometrically with the phosphomolybdic phosphotungstic acid reagents (*23*). Quercetin was used as standard for the calibration curve.

Free Radical Scavenging Capacity. The free radical scavenging capacity of the wine samples was analyzed by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Aliquots of the tested wine samples were mixed at the indicated concentration, with 1 mL of 0.1 mmol DPPH/L (in ethanol) in a cuvette. The time course of the change in the optical density at 517 nm was then kinetically monitored.

Human LDL Isolation. LDL was isolated from human control subject plasma by discontinuous density gradient ultracentrifugation (LDL separation). The LDL was dialyzed against saline with Na_2 EDTA (1 mmol/L). Prior to oxidation, LDL was diluted in phosphate-buffered saline (PBS) to 100 mg of protein/L and dialyzed against PBS at 4 °C to remove the EDTA. LDL protein concentration was determined by using the Folin phenol reagent assay (*24*).

LDL Oxidation. Oxidation of LDL was carried out in a shaking water bath at 37 °C. LDL (100 mg of protein/L) was preincubated with the concentrations of wine specified in each experiment, for 30 min at 37 °C, followed by 3 h of incubation at 37 °C with freshly prepared $CuSO_4$ (5 μmol/L). LDL oxidation was terminated by refrigeration at 4 °C, and the oxidation rate was immediately determined by measuring the amount of thiobarbituric acid reactive substances (TBARS) (*25*).

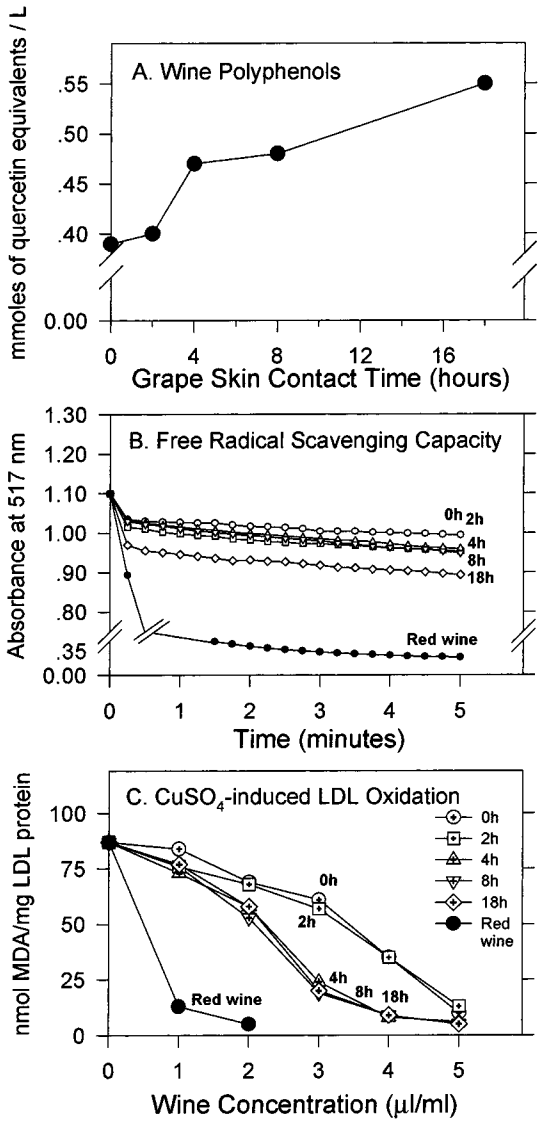

Figure 1. Effect of grape skin contact time on polyphenol content and antioxidant capacity of white wine. Whole Chardonnay squeezed grapes were incubated for up to 18 h, after which time the juice was separated from the grape skins and allowed to ferment into wine. (A) Polyphenol concentrations in wine samples were determined. (B) Aliquots of 25 μL/mL from each wine sample were added to a DPPH solution (0.1 mmol/L), and the optical density at 517 nm was monitored every 15 s. (C) Increasing concentrations of wine samples were added to LDL (100 mg of protein/L) that was incubated with 5 μmol/L $CuSO_4$ for 2 h at 37 °C. LDL oxidation was measured by the TBARS assay.

RESULTS

Effect of Grape Skin Contact Time on Polyphenol Content and Antioxidant Capacity of Wine. Incubation of whole squeezed grapes for increasing periods of time (between 2 and 18 h) resulted in a gradual increase in the white wine's polyphenol content (Figure 1A). A maximal increase of 41% (from 0.36 to 0.55 mmol of quercetin equivalent/L) was obtained after skin contact for 18 h.

The polyphenol content in red wine used in this study was 5.5 mmol of quercetin equivalent/L, which is ~14-fold higher than the polyphenol concentration in the running grape juice-derived white wine and 10-fold higher than the polyphenol concentration in wine derived after skin contact for 18 h. Next, we examined the capacity of these white wine samples to scavenge free radicals and to inhibit LDL oxidation induced by copper ions, in comparison to red wine. The addition of red wine (25 μL/mL) to the DPPH solution induced a rapid decrease (of 76%) in the optical density at 517 nm, which reached a plateau within 5 min (Figure 1B). Analysis of the free radical-scavenging capacity of the white wine samples revealed that wine derived after 18 h of grape skin contact had the greatest capacity to scavenge free radicals (19% reduction in the absorbance at 517 nm, reaching a plateau after 5 min, in comparison to only 10% reduction induced by the free running juice) (Figure 1B).

The antioxidative effect against copper ion-induced LDL oxidation of the wines, which were derived from the whole squeezed grapes incubated for different

periods of time, increased gradually in a dose-dependent manner. Wine derived after 2 h of grape skin contact inhibited LDL oxidation similarly to the free running grape juice, with an IC_{50} (the concentration needed to inhibit LDL oxidation by 50%) of 3.5 μL/mL, whereas wine derived after 4, 8, or 18 h of grape skin contact exhibited the maximal antioxidative effect, with an IC_{50} of 2.3 μL/mL, in comparison to the potent red wine with an IC_{50} of 0.8 μL/mL (Figure 1C).

At a concentration of 5 μL/mL, the copper ion-induced LDL oxidation was completely inhibited by all wine samples, similarly to the effect exhibited by 2 μL/mL of red wine (Figure 1C).

Effect of Alcohol Addition to Whole Squeezed Grapes on the Wine Polyphenol Content and Its Antioxidant Capacity. To evaluate whether alcohol can improve polyphenol extraction from the grape skin into the wine, we have added to the whole squeezed grapes increasing concentrations of alcohol and stored the mixture for 18 h.

The polyphenol content in the wine samples increased gradually and was alcohol concentration-dependent (Figure 2A). A maximal increase of 60% was obtained in the wine sample, which was preincubated with 18% alcohol, in comparison to the polyphenol content in wine derived from the whole squeezed grapes, which were incubated for 18 h without alcohol. Analysis of the polyphenol composition in the polyphenol-enriched white wine was performed by high-performance liquid chromatography (HPLC) using known standards. Polyphenols were identified as gallic acid, procyanidin dimers, catechin, epicatechin, polymeric phenols (tannins), phenolic acids (caftaric acid, caffeic acid, coutaric acid, and *p*-coumaric acid), flavonols (myricetin and quercetin in their glycosylated and unglycosylated forms), procyanidin B-2 and B-3. Quantitatively, the major polyphenol was the tannins fraction, which represented ~30% of total polyphenol content. The concentration of wine polyphenols achieved after preincubation of the whole squeezed grapes with 18% alcohol was only 4.4-fold lower than the levels of polyphenols measured in red wine (Figure 2A). In parallel, these wine samples exhibited a gradual increased capacity to scavenge free radicals (Figure 2B) and to inhibit copper ion-induced LDL oxidation (Figure 2C). A maximal reduction (by 79%) in the optical density at 517 nm of the DPPH solution was induced by the white wine sample derived from the whole squeezed grape juice that was preincubated with 18% alcohol, similar to the free radical-scavenging capacity exhibited by a similar concentration of red wine (Figure 2B). Furthermore, a maximal inhibition of copper ion-induced LDL oxidation (by 87%) was induced by the wine sample derived from the whole squeezed grape juice that was preincubated with 18% alcohol, very similar to the inhibition (94%) exhibited by red wine (Figure 2C).

To rule out the possibility that the inhibition in LDL oxidation was induced by the alcohol, we have added 18% alcohol to the grape juice separated from grape skins after storage of the whole squeezed grapes for 18 h. This wine sample induced a reduction of 34% (from 1.066 to 0.700) in the optical density of DPPH solution and inhibited LDL oxidation by 57% (from 94 to 39 nmol of TBARS/mg of LDL protein), similar to the effects exhibited by the wine sample derived from the whole squeezed grapes stored for 18 h without alcohol (Figure 2). Taking altogether all of these results, it is suggested

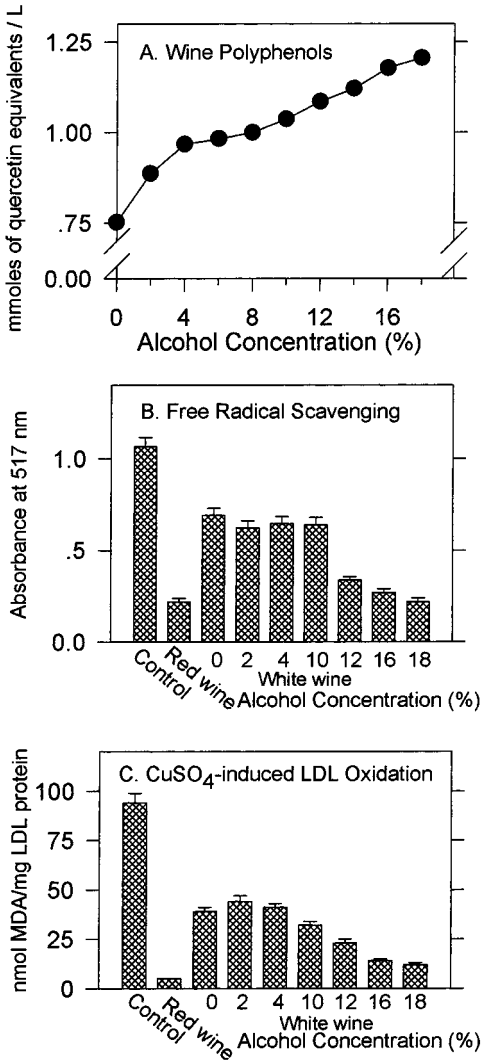

Figure 2. Effect of alcohol concentration added to whole squeezed grapes on wine polyphenol content and on its antioxidant capacity. Whole squeezed Muscat grapes were incubated for 18 h with increasing concentrations of alcohol up to 18%, after which the juice was separated from the grape skins and allowed to ferment into wine. (A) Polyphenol concentration in the wine samples was determined. (B) Aliquots of 25 μL/mL from each wine sample were added to a DPPH solution (0.1 mmol/L), and the optical density at 517 nm was recorded after 5 min. (C) Wine samples at a final concentration of 2 μL/mL were added to LDL (100 mg of protein/L) and incubated with 5 μmol/L $CuSO_4$ for 2 h at 37 °C. LDL oxidation was measured by the TBARS assay.

that the antioxidant capacity of the wine directly correlates with its polyphenol concentration. The extent of LDL oxidation inhibition is indeed directly proportional to the wine polyphenolic content (Figure 3, $r = 0.986$).

DISCUSSION

Red wines have a much higher concentration of polyphenols than white wines, and this is related to a much longer contact time of the grape skins during the fermentation process. The high polyphenol content in red wine contributes to its increased antioxidant potential in comparison to white wine. In the present study, we produced white wine enriched with polyphenols by

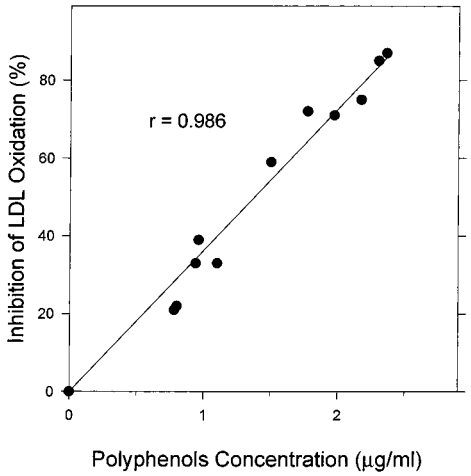

Figure 3. Linear regression analysis between the total polyphenol concentration of wine and the wine-induced inhibition of LDL oxidation.

imposing a short period of grape skin contact (18 h) in the presence of alcohol. This procedure resulted in extraction of the grape skin polyphenols into the wine, and the polyphenol-enriched white wine exhibited antioxidant characteristics similar to those of red wine. The resulting white wine contained a final alcohol concentration of 18% and a sugar content of 13.7%. Therefore, this wine is categorized as sweet "dessert white wine".

First, we have produced white wine samples that were exposed to grape skin contact for increasing periods of time by incubating the whole squeezed grapes for up to 18 h before separating the free running grape juice from the grape skins. The longest period of grape skin contact applied during this process was only 18 h so as not to affect the wine quality and color. This can explain why the curve of polyphenol concentration versus contact time (Figure 1A) or the curve of polyphenol content versus alcohol concentration (Figure 2A) does not reach a plateau. Elaboration of red wines, on the contrary, imposes skin contact for up to 2 weeks, during which time the polyphenol content curve reaches a plateau.

During this short period of time (18 h), no significant fermentation occurs and the juice was alcohol-free. This procedure resulted in a commonly used table white wine with a moderate increase in the white wine polyphenol content, and in parallel, the antioxidant potential of the white wine increased. However, these results were still far from the antioxidant capacity exhibited by red wine.

Because the procedure applied for red wine preparation utilizes grape skin contact for a much longer period of time (up to 10−20 days), during which time fermentation occurs, resulting in alcohol production, we hypothesized that alcohol may augment the extraction of polyphenols from the grape skin into the wine. To test this hypothesis, we have performed a second procedure for the preparation of polyphenol-rich white wine by adding alcohol to the whole squeezed grapes.

Our results clearly demonstrate that white wine obtained from the whole squeezed grapes that are incubated for 18 h with 18% alcohol contained a remarkable concentration of polyphenols and exhibited a significant antioxidant capacity, almost similar to that of red wine.

The antioxidant capacity of the above white wine was directly proportional to the wine's polyphenol content, in agreement with previous findings (*19*, *26*). Nevertheless, the polyphenol content in this white wine was still 4.4-fold less than that found in the red wine used in this study.

However, this white wine exhibited antioxidant activity to an extent similar to that of red wine. These results suggest that the polyphenol-enriched white wine contains polyphenols with higher antioxidant activity against LDL oxidation and higher free radical-scavenging capacity than do polyphenols in red wine. It is accepted that diverse polyphenols possess different antioxidant capacities, which are related to their chemical structures (*27*−*29*). For example, quercetin was shown to inhibit LDL oxidation to a greater extent than catechin, although both of these polyphenols possess a similar arrangement of OH groups. However, quercetin is a more potent antioxidant against LDL oxidation due to the 2−3 double bond and the 4-oxo structure in its C ring. Vinson et al. (*22*) has also demonstrated that white wine was a better inhibitor of LDL oxidation than red wine when both wines were compared on an equal polyphenol content basis. These results were further confirmed by Lamuela-Raventos et al. (*19*), who demonstrated that white wine phenols have an antioxidant capacity comparable to that of red wine phenols. These differences stem from the fact that, qualitatively, red wines, unlike white wines, contain anthocyanins and polymerized tannins, which are relatively weak antioxidants. Thus, it is possible that polyphenol-enriched white wine will exhibit an antioxidant capacity close to that of red wine, even though the total polyphenol content in the white wine is lower than that present in the red wine.

In summary, we have demonstrated the production of white wine with red wine-like antioxidant characteristics by increasing the content of polyphenols in the wine. This was achieved by imposing grape skin contact for a short period of time in the presence of alcohol, both conditions augmented the extraction of grape skin polyphenols into the wine.

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