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Synergetic Activity of Catechin and Other Antioxidants

Cédric T. Saucier and Andrew L. Waterhouse*

Department of Viticulture and Enology, University of California, One Shields Avenue, Davis, California 95616

The antioxidant synergy between (+)-catechin and other wine or biological antioxidants (Trolox, ascorbate, SO_2, uric acid) was measured in vitro using the Folin−Ciocalteu (FC) and metmyoglobin assays. Although the two assays are based on very different reagents (i.e., metal salts versus organic and biochemical reagents), the individual antioxidants showed similar relative activities in both systems. In addition, interaction studies showed simple additive effects in all cases except with the (+)-catechin/SO_2 mixture, which showed a remarkable synergetic effect in both assays.

Keywords: *Antioxidant; interaction; synergy; wine; free radical; catechin; SO_2; ascorbic acid*

INTRODUCTION

The effect of wine consumption on human health, in particular heart diseases, has been documented by a large number of epidemiological studies. The most famous (Renaud et al., 1992) showed that the consumption of red wine in some cities of France could explain low rates of coronary heart disease (CHD) mortality in the face of high saturated fat intake. This phenomenon was called the "French paradox" and may be explained by the antioxidant effect of phenolic compounds in red wine (Frankel et al., 1995).

This effect has been described many times, both for wine samples (Maxwell et al., 1994) and for many of the pure compounds found in wines (Urizzi et al., 1999; Pietta et al., 1998; Paganga et al., 1996).

It has been postulated that in some instances combinations have greater antioxidant activities than would be expected on the basis of their individual effects (Meyer et al., 1998; Vivas et al., 1997; Teissedre et al., 1996). It has also been observed that others may protect or recycle some compounds (i.e., like vitamin E sparing by vitamin C). Because both wine and the bloodstream clearly contain additional antioxidants [e.g., sulfites (Ribereau-Gayon et al., 1998; Yao et al., 1994; Ji et al., 1995)], it seemed appropriate to investigate second-order antioxidant effects. Catechin was chosen because it is an abundant single flavan-3-ol found in red wines (Ritchey and Waterhouse, 1999; Goldberg et al., 1998). It is also very closely related to condensed tannins, the most abundant source of polyphenols in wine, because it is one of the monomer subunits in these polymers (De Freitas et al., 1998; Souquet et al., 1996; Prieur et al., 1994). Its bioavailability seems very probable because similar compounds have been found in the bloodstream after tea or chocolate consumption (Lee et al., 1995; Richelle et al., 1999).

MATERIALS AND METHODS

Chemicals and Model Solutions. All reagents were of analytical grade. (+)-Catechin was Ref 2210 from Fluka (Buchs, Switzerland). Uric acid (Ref 17129-0250) and Trolox (Ref 21894-0050) were from Acros (Springfield, NJ). Ascorbic acid (Ref A-61 100) and other chemicals were from Fisher Scientific (Fair Lawn, NJ). Compounds were dissolved in a 12% ethanol solution buffered at pH 3.2 with tartaric acid (5 g/L) and sodium hydroxide. For solubility reasons uric acid was dissolved in a pH 7 ammonium phosphate buffer (10 mM).

Folin−Ciocalteu (FC). We used the method of Singleton and Rossi (1966) downscaled for microcuvettes, and the results are expressed directly in absorbance units at 700 nm.

In each analysis, 1.58 mL of water was pipetted into

* Corresponding author [telephone (530) 752-477; fax (530) 752-0382; e-mail alwaterhouse@ucdavis.edu].

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semimicrocuvettes, followed by 20 μL of a standard solution, sample solution, or water, and the solutions were well mixed. Then 100 μL of FC reagent was added to each cuvette, and the solutions were mixed again. After 30 s and before 8 min, 300 μL of a 20% sodium carbonate solution was added. The solutions were left at room temperature for 2 h. Then the absorbance of the developed blue color was determined at 700 nm. The amount of light absorbed is proportional to the amount of oxidizable material present, that is, phenolic compounds.

Metmyoglobin Assay. This test was carried out following the instructions supplied in a kit from Randox Laboratories (Antrim, U.K.): total antioxidant status (TAS). The method involves incubation of the chromogen 2,2′-azobis(ethylbenzothiazolinsulfonate) (ABTS) with a peroxidase (metmyoglobin), and hydrogen peroxide produces the green radical cation $ABTS^+$. The color development can be measured at 600 nm. Added antioxidants inhibit the reaction and cause a suppression of color production to a degree that is proportional to the concentration of antioxidants. The assay is calibrated using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and results are expressed in millimolar TAS units with a range of 0−2.5 mM.

Determination of Synergetic Effect. Potential synergetic effects were evaluated by comparing the antioxidant status calculated (sum of the individual compound's effect) and measured (combination of compounds at the same concentration: catechin, 200 μm; other antioxidant, 100 mg/L; experiments in triplicate). Interaction between antioxidants was tested statistically with a regular two-sided hypothesis at 0.1% significance level (Statlab software, version 3.0, SLP Infoware).

RESULTS AND DISCUSSION

In our experiments, we chose to use one phenolic compound (i.e., catechin) at a fixed concentration (200 μM). Catechin is indeed a typical flavanol of red wines (Ritchey and Waterhouse, 1999; Goldberg et al., 1998), and the goal of our study is to seek possible synergies between flavanol and other antioxidants present in wine or of biological interest (ascorbic acid, SO_2, uric acid, vitamin E equivalent). The in vitro antioxidant properties for phenolic compounds in general are well documented [see Rice-Evans et al. (1997) for a review]. For each antioxidant tested, FC and metmyoglobin tests were performed in solutions containing the compounds alone or in mixture with catechin. Figure 1 shows the results obtained with ascorbic acid. The results show that both catechin and ascorbic acid are antioxidants and that the mixture of the two compounds results in an additive effect of their antioxidant capacities. Indeed, the bars corresponding to the results obtained on mixtures are almost the same as those calculated by summing the effects of each compound alone. Uric acid and Trolox showed the same additive pattern. For each compound, it is then possible to calculate their antioxidant capacity expressed in Trolox equivalents (TEAC; Rice-Evans et al., 1995) The results are summarized in Table 1.

Our results are very similar to those found in previous studies (Rice-Evans et al., 1995) except for catechin, which, under our conditions, had a TEAC of 5.5, which is double the literature value. This result is surprising but may be explained by an effect of the tatrate buffer used. It is possible that tartaric acid acts in synergy with catechin, but this has to be confirmed. The other surprising result is that, at least at the low concentrations tested, sulfur dioxide alone had no antioxidant activity. This result is in accordance with previous studies (Vivas et al., 1995), which showed that sulfur dioxide is a very poor scavenger of superoxide anion.

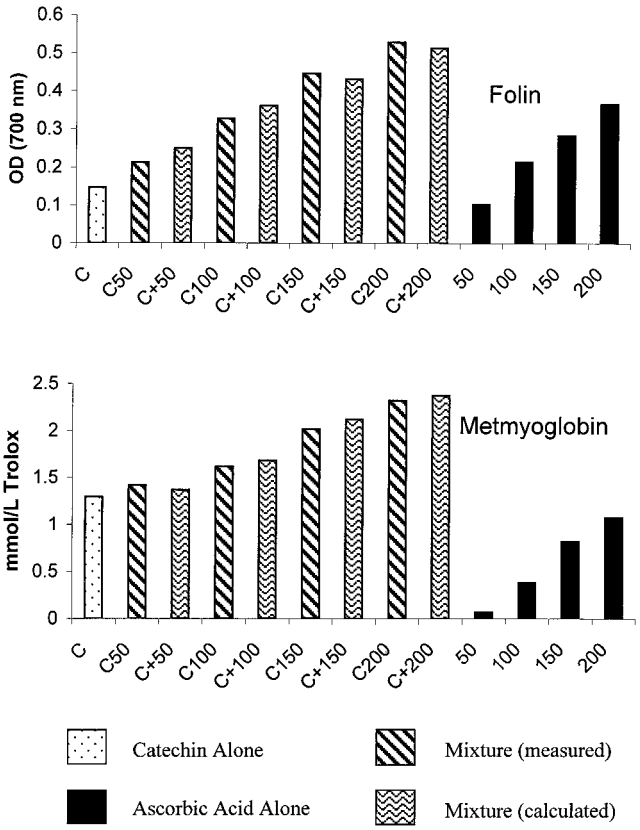

Figure 1. Interaction of (+)-catechin (200 μm) with ascorbic acid (50−200 mg/L). Numbers on horizontal axis refer to concentrations of ascorbic acid in mg/L.

Table 1. Trolox Equivalent of Studied Compounds Expressed in TEAC for the Compounds Alone and Behavior in the Presence of Catechin (Average of Triplicate Analyses ± SD)

product	SO_2	uric acid	Trolox	ascorbate	catechin
TEAC (mmol)	0	1.2 ± 08	1.1 ± 0.09	1.0 ± 0.05	5.5 ± 0.20
effect (+200 μM catechin)	synergy[a]	additive	additive	additive	

[a] $p < 0.001$.

However, our results are different on the question of the interaction of sulfur dioxide with phenolic compounds. Figure 2 shows the results obtained on catechin alone and mixed with sulfur dioxide. The results show that sulfur dioxide alone gave no significant response in either the FC or metmyoglobin tests. Indeed, the negative values obtained are not significant.

However, when sulfur dioxide was mixed with catechin, it remarkably enhanced its antioxidant properties. This was seen with both FC and metmyoglobin tests, as the measured values are much higher than the calculated sum of the two compounds. This is clearly a synergetic effect between these two types of compounds. In the case of the FC method, this effect has already been described in the literature (Somers and Ziemelis, 1980) because this test is widely used as a method of phenolic estimation (Singleton and Rossi, 1966). These studies found that because of this synergy, the procedure has to be used with caution, because SO_2 is an interference and will give high values for phenolics, especially with white wines which have lower level of phenolics (Somers and Ziemelis, 1980). Our work confirms this synergetic effect and shows that it is also present when a free radical scavenging test (metmyo-

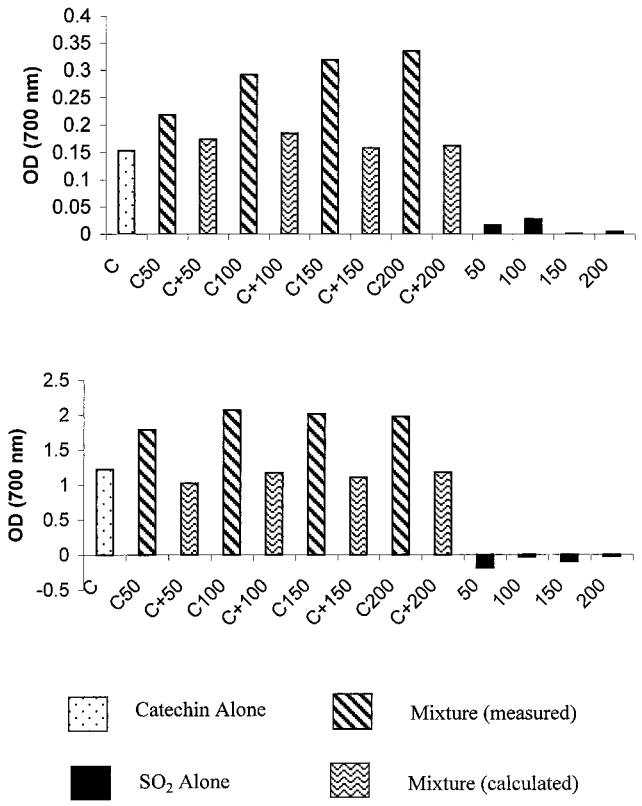

Figure 2. Interaction of (+)-catechin (200 μm) with SO_2 (50−200 mg/L). Numbers on horizontal axis refer to concentrations of SO_2 in mg/L.

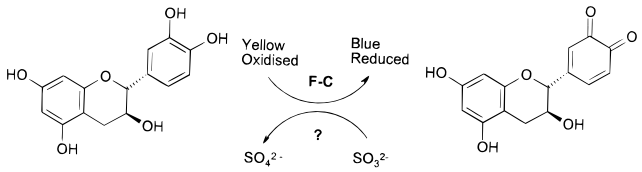

Figure 3. Possible explanation for the antioxidant synergetic effect between (+)-catechin and sulfite.

globin) is used. In fact, the two methods gave very similar responses as can be seen in Figures 1 and 2. The correlation between the FC test and antioxidant tests such as ABTS (Rice-Evans et al., 1996), β-carotene bleaching (Velioglu et al., 1998; Vinson et al., 1998), and, more recently, DMPD (Fogliano et al., 1999) was shown to be statistically significant for fruits, grains, tea, and wine samples. The correlation found can be explained by the fact that phenolic compounds are good hydrogen donors in the presence of other hydrogen radicals (Rice-Evans et al., 1996) but are also able to reduce the valence of phosphomolybdic and phosphotungstic acids in the FC test by giving their electrons to them (the FC test is carried out in a basic medium so the protons will be easily consumed).

The mechanism of the synergy observed with SO_2 remains unknown. It is possible that the quinone oxidation products may be recycled back to the phenol by the oxidation of sulfite to sulfate (Figure 3). The phenol could then be considered a catalyst in the oxidation of SO_2 in these two assays.

CONCLUSION

The antioxidant property of catechin mixed with various other antioxidants was assessed by using two different methods (FC and metmyoglobin). These two procedures gave very similar results and showed that uric acid, Trolox (vitamin E analogue), and vitamin C had similar activities when they were alone in solution. They also have an additive effect when mixed with catechin. SO_2, on the other hand, gave a strong synergetic antioxidant effect with catechin when measured by both the FC and metmyoglobin procedures. These results justify the common use of SO_2 in enology as an antioxidant as all wine phenolics are potent hydrogen donors. The common practice of using less SO_2 in red wines than in white wines is justified by the amount of phenolics, which is 10 time less (100−300 mg/L) than for red wines (~1−3 g/L).

The observed synergy may also be important in human health because oxidative processes are involved in many diseases. Future research should be focused on understanding the effects of these antioxidants in enology as well as human health.

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