

Total Phenolic Content, Antioxidant Activity, and Cross-Cultural Consumer Rejection Threshold in White and Red Wines Functionally Enhanced with Catechin-Rich Extracts

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ABSTRACT: White and red wines spiked with catechin-rich green tea extract and grape seed extract were assessed for phenolic content, antioxidant activity, and cross-cultural consumer rejection thresholds in relation to wine as a functional food. Health functionality is an important factor in functional foods, and spiking pure compounds or plant extracts is an effective method to increase or control functionality. The total phenolic content and antioxidant activity were measured in wines spiked to different extract concentrations, namely, control and 50, 100, 200, 400, and 800 mg/L, to confirm the dose–response curves in both white and red wines. Consumer rejection thresholds (CRTs) were established for spiked wines in a Korean and in an Australian population. Our results showed that the green tea extract and grape seed extract increased the antioxidant activity dose dependently, and the CRTs varied considerably between the Korean and the Australian groups, with Koreans preferring wines spiked with green tea extract and Australians showing a preference for wines spiked with grape seed extract. These results have implications for producing wine products that are enhanced in phenolic compounds and targeted to different cultural groups.

KEYWORDS: consumer rejection threshold (CRT), total phenolic content, DPPH antioxidant activity, Australian wine consumers, Korean wine consumers

■ INTRODUCTION

Functional foods are those that improve health or prevent certain diseases and are becoming increasingly popular with health-conscious consumers.¹ The functionality may be enhanced by a variety of means including the addition of functional ingredients to food. For example, grape seed extract, rich in polyphenol antioxidants, is now an ingredient in energy bars. Such an addition of plant extracts to increase health benefits is becoming more common in the food industry, especially those extracts that contain polyphenols, since this group of compounds has received much attention for potential health benefits.²

Consumer acceptance of health-enhanced wine is important. Lampila et al.³ found that focus group participants in Finland, The Netherlands, and France expressed that dark-colored fruit and wine are the most appropriate products to enhance flavonoid intake among juices, cakes, jams, cider, raisins, frozen berries, ice cream, and wine. Participants also were ready to accept the flavonoid-enhanced products and were willing to pay more even if the price was slightly higher than conventional food. Barreiro–Hurle et al.⁴ also found that Spanish consumers were willing to pay more for resveratrol-enhanced wine.

Wine contains abundant polyphenols, and many studies have reported that wine may be beneficial in preventing diseases such as heart disease.⁵ In particular, we have identified catechin, quercetin, and resveratrol as three compounds in wine that have received much attention in the literature.⁶ However, like all natural products, the levels of polyphenols in wine vary, and this is one factor that limits the acceptance of wine as a functional food. Climate, viticultural, and vinification practices are all known to impact concentrations of phenolic compounds in wine.⁷ Some of these factors are controllable, such as the

extraction time of juice, whereas others are more difficult or even impractical to control (e.g., rainfall). The only practical means of ensuring consistent levels of polyphenols, to the extent required for food functionality, is to spike them into wine postvinification.

Spiking does solve a good deal of the functionality challenge, as it allows a consistent amount of functional ingredient in the final product. However, selecting an appropriate agent that effectively increases the health functionality is important. Catechin-rich extract of green tea and grape seed were chosen for this study for a number of reasons, including reported potential health benefits of the high catechin content;⁸ they dissolve well in wine without any further treatment, unlike resveratrol and quercetin; and they are commercially available in food grade. However, some phenolic compounds in these extracts are bitter and astringent⁹ and will alter the flavor of the wine. Because flavor is one of the most important factors in consumer choice of functional foods,¹⁰ enhancing functional properties of wine through spiking must not be at the expense of taste. In this context, maximizing phenolic compounds in a range that keeps wine acceptable to consumers is important.

However, consumer preference varies across cultures, especially in the collectivistic Asian and individualistic Western cultures.¹¹ For example, Chinese consumers prefer fruity red wine with a degree of sweetness,¹¹ whereas Australians predominantly prefer dry wine.¹² Wine-purchasing patterns are also different between these two cultural groups; for

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Table 1. Total Phenolic Content of Green Tea Extract and Grape Seed Extract Spiked Wines^a

concn	green tea extract spiked (mg/L GAE)		grape seed extract spiked (mg/L GAE)	
	white wine	red wine	white wine	red wine
control	510.00 ± 0.0	2806.43 ± 75.8	524.29 ± 10.10	2692.14 ± 75.8
50 mg/L	563.57 ± 5.1	2813.57 ± 75.8	567.14 ± 10.10	2813.57 ± 65.7
100 mg/L	631.43 ± 10.10	2842.14 ± 75.8	610.00 ± 0.00	2895.71 ± 101.00
200 mg/L	756.43 ± 15.2	3063.57 ± 15.2	720.71 ± 5.1	2995.71 ± 70.70
400 mg/L	988.57 ± 10.10	3142.14 ± 15.2	881.43 ± 20.20	3038.57 ± 40.40
800 mg/L	1410.00 ± 30.30	3502.86 ± 171.8	1252.86 ± 20.20	3374.29 ± 80.80

^aTotal phenolic contents presented as mean values ± SDs of duplicate analyses.

example, Chinese consumers exhibit higher levels of risk avoidance in wine purchasing than Australians.¹¹ Because of these differences, it is important to establish consumer rejection thresholds (CRTs)¹³ in different cultural groups to determine if there are any cultural influences in threshold levels. In this study, a group of Koreans were chosen as representative of Asian cultures, and a group of Australians were chosen to represent Western cultures.

Because of the link between functionality and taste, it is important to establish to what extent a wine may be spiked before the taste is compromised. Our study determined the CRTs for Korean and Australian consumers in red and white wines spiked with green tea or grape seed extracts. Furthermore, the total phenolic content and antioxidant activities of spiked wines were measured to establish dose–response curves for the different levels of spiking. Such information may be used to guide winemakers to develop new products with enhanced and consistent functionality targeted to different consumer groups.

MATERIALS AND METHODS

Description of Wine Samples. The white wine sample was a popular Australian Chardonnay wine, purchased from a local liquor store, described on label as an “Unwooded Chardonnay, A contemporary fruit driven dry white”. Chemical analysis showed that the wine contained the following: alcohol, 12% alcohol/vol, pH 3.24; titratable acid, 5.5 g/L; acetic acid, 0.33 g/L; total glucose and fructose, 1.64 g/L; glycerol, 10.79 g/L; and L-malic acid, 2.28 g/L. The red wine sample was a popular Australian Cabernet Sauvignon wine, purchased from a local liquor store, described on label as “a soft fruit driven wine with plum and cherry flavors complemented by soft oak characters. An appealing wine with good length and firm tannins”. Chemical analysis showed that the wine contained the following: alcohol, 13% alcohol/vol, pH 3.40; titratable acid, 5.9 g/L; acetic acid, 0.41 g/L; total glucose and fructose, 4.35 g/L; glycerol, 10.84; and L-malic acid, 0.14 g/L.

Additive Materials and Reagents. Food-grade green tea extract was purchased from Shaanxi Sciphar Biotechnology Co. Ltd., China. Food-grade grape seed extract was purchased from New Zealand Extracts Ltd. Sodium carbonate was purchased from Labserv Biolab (Aust) Ltd. Folin–Ciocalteu's (FC) phenol reagent (2 N) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were all purchased from Sigma-Aldrich (United States). Deionized water was used in the analysis.

Methods. Determination of Total Phenolic Content. The FC reagent assay was used to determine the total phenolic content in wine. A wine sample (diluted 1 in 10 with water; 25 μ L) was added to a vial containing 725 μ L of water. FC reagent (diluted 1 in 10 with water; 300 μ L) was added, and after 1 min, aqueous sodium carbonate solution (10% w/v; 450 μ L) was added. The vial was vortexed briefly and allowed to stand for 1 h at room temperature. The absorbance was read at 760 nm. Results are expressed by reference to a six-point regression curve (0–1000 mg/L gallic acid) as milligrams of gallic acid equivalents (GAEs) per liter of wine.¹⁴

DPPH Radical Scavenging Activity Test. The radical scavenging activity was determined based on the reduction of the stable free radical DPPH by phenolic compounds.¹⁵ The reaction takes place when 1400 μ L of DPPH (60 μ M) was mixed with 100 μ L of the sample (at a dilution of 1 in 50 in water) at room temperature. After a reaction time of 2 h, absorbance values at 517 nm were measured. Results were expressed in mM Trolox using the dose–response curve described by this substance.¹⁶

CRT and Demographic Questionnaire. The CRT method was adopted from methodology described in the literature^{13,17} and is summarized here briefly. Food-grade green tea extract or grape seed extract was spiked into white and red wine samples using a measuring cylinder to yield final extract concentrations of 50, 100, 200, 400, or 800 mg/L. The minimum and maximum concentrations were determined by previous pilot studies and additional bench trials using two wine experts. Two wine samples of 20 mL were provided to participants, one being the spiked wine and one being the control or original wine. Wine glasses were coded with three digit randomized numbers and presented to subjects in order of increasing concentration. Participants were asked to indicate which of the two wines they preferred. Both wines were prepared at room temperature 1 h prior to the execution of the experiment and served at room temperature. Participants completed a pencil and paper questionnaire on basic consumption patterns and demographics before the tasting and then tasted a set of samples and circled which wine they preferred. Participants were instructed to wait 30 s between tasting each sample in a set with a 2 min break enforced between each set of wines. Participants were also instructed that all samples should be expelled, such that minimal wine would be ingested. Participants received 10 samples per session and completed a total of four sessions. Therefore, each participant completed a total of 10 paired comparisons across two separate days for each spiking extract.

Participants. Groups of 40 Korean and 40 Australian participants were recruited twice: one for green tea extract spiked white and red wines and again for grape seed extract spiked white and red wines. Korean participants were recruited from a Korean community in Sydney from which participants were recruited from selected youth, middle age, and old age groups. The age range of Korean participants was 18–74 years, and their residential period in Australia was from 2 weeks to 38 years. No second or further generations were included in this study. The relationship between wine preference changes and residential period for the Korean participants was not determined as it is not in the scope of this study. Australian participants were recruited from the Wagga Wagga campus of Charles Sturt University. The age range of Australian participants was from 19 to 68 years. Effort was made to not recruit wine science students or staff to generate the results from a general sample of Australians. On the basis of wine-drinking experience, consumption patterns, and self-reported wine preferences, the Wagga Wagga sample was consistent with a general Australian wine-drinking population reported elsewhere.¹⁸ All participants were required to be over 18 years old and consume wine at least once per month. This study was approved by the Charles Sturt University Ethics in Human Research Committee and conforms to the provisions of the Declaration of Helsinki; the protocol number is 2009/129.

RESULTS AND DISCUSSION

Determination of Total Phenolic Content and DPPH Antioxidant Activity. The total phenolic content of green tea extract and grape seed extract spiked wines was measured using the FC method, and the total phenolic content increased dose dependently in white and red wines (Table 1). The green tea extract increased the total phenolic content by 177% in spiked (800 mg/L) white wine and 25% in red wine, showing a much greater change for white wine than red. Grape seed extract also increased the total phenolic content by 139% in spiked (800 mg/L) white wine and 25% in red wine, such that the change was similar to that reported for green tea extract spiked wines.

Understanding the dose response of the green tea and grape seed extracts in wine over the concentration range of interest is important. In particular, we needed to establish if or where the total phenolic content reached a maximum value, that is, whether a point is reached where further addition of extract does not result in increased total phenolic content. Figures 1

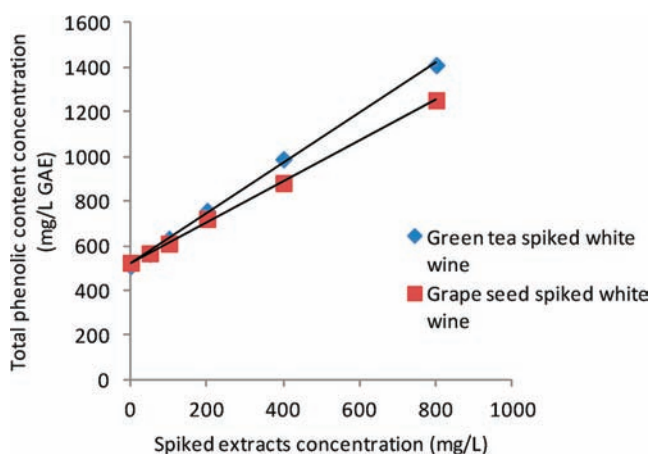


Figure 1. Total phenolic content of green tea extract and grape seed extract spiked white wine. Green tea spiked white wine (slope = 1.13, $R^2 = 0.999$). Grape seed extract spiked white wine (slope = 0.91, $R^2 = 0.999$).

and 2 show that spiked extracts increased the total phenolic content approximately linearly in white and red wines such that

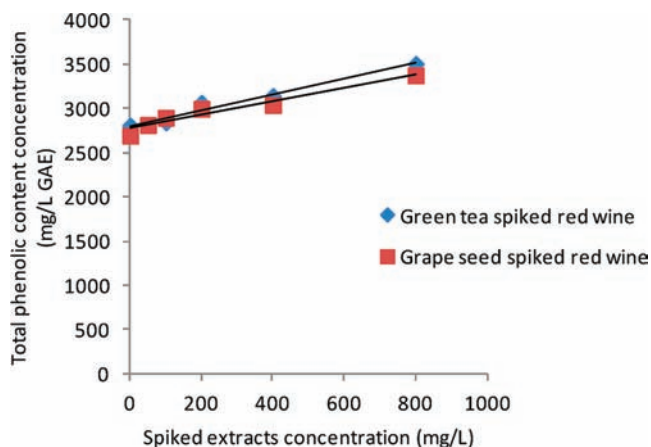


Figure 2. Total phenolic content of green tea extract and grape seed extract spiked red wine. Green tea spiked red wine (slope = 0.89, $R^2 = 0.971$). Grape seed extract spiked red wine (slope = 0.76, $R^2 = 0.945$).

functionality increased across the concentration range and no plateau effect was observed.

In white wine, the percentage increase in total phenolic content was greater than in red wine. This could be due to the fact that the initial total phenolic content in white wine was lower than in red wine such that relative increase (in percentage terms) is greater in white wine. Indeed, our results show that baseline levels of total phenols in red wines are more than five times higher than those of white wines, indicating the naturally greater concentrations of phenolic compounds in red wines as compared to white wines. The fact that the slopes of the dose–response curves observed for red and white wines are different indicates that the spiking extracts interact differently in red and white wine systems (Figures 1 and 2). A possible explanation for this is that red wine may contain components that bind the polyphenols from the extracts such that they do not react as effectively with the FC reagent. While in absolute terms red wine has higher total phenols, in relative terms, spiking is more beneficial in white wine.

Green tea extract increased the total phenolic content to a greater extent than grape seed extract in white wine. This is likely to be related to the composition of the particular phenolic compounds in the different extracts. Rababah et al.⁸ found that green tea extract had approximately the same total phenolic content as grape seed extract, although the antioxidant activity was significantly higher for the grape seed extract. Conversely, Pietta et al.¹⁹ reported that green tea extract had higher antioxidant activity than grape seed extract. Thus, it would appear that extracts from different suppliers have different total phenolic content and antioxidant activities. In terms of functional food products, spiking with a well-characterized extract of known total phenolic content would be essential to ensure consistency of functionality.

Antioxidant activities of green tea extract and grape seed extract spiked white and red wines also showed a dose-dependent increase in activity (Table 2). The highest spiking level (800 mg/L) of green tea extract increased the DPPH antioxidant activity by a considerable 297% in white wine and only 25% in red wine. Grape seed extract spiked wines also increased DPPH antioxidant activity, although at a lesser rate of 136% in white and 19% in red wines. The increase provided by green tea extract was much greater than that of grape seed extract in white wine, but their increases were not considerably different in red wine, similar to the results obtained for total phenolic content reported above.

The antioxidant activities of green tea and grape seed extracts have a similar pattern to the total phenolic content, but green tea extract increased the antioxidant activity more in white and red wines. In our results, the total phenolic content went up by 177% in green tea extract spiked white wine, while the antioxidant activity went up by 297% in the same wine. This may be because compounds other than phenolic compounds in the green tea extract contributed to the antioxidant activity. It is known that not all plant or vegetable extracts linearly correlate between total phenolic content and antioxidant activity. Babbar et al.²⁰ found that Kinnow peel extract was the highest in antioxidant activity in spite of its total phenolic content being low in the tested fruit extracts. The authors of this study suggested that it may be because other constituents may contribute to the antioxidant activity.

Determination of Rejection Thresholds for Green Tea and Grape Seed Extract Spiked Wines for Korean and Australian Consumers. The CRTs for green tea extract and

Table 2. DPPH Antioxidant Activity of Green Tea Extract and Grape Seed Extract Spiked Wines^a

concn	green tea extract spiked (mM TE)		grape seed extract spiked (mM TE)	
	white wine	red wine	white wine	red wine
control	3.60 ± 0.10	17.30 ± 0.4	3.59 ± 0.10	17.16 ± 0.4
50 mg/L	4.41 ± 0.00	17.68 ± 0.30	3.73 ± 0.00	17.51 ± 0.20
100 mg/L	5.01 ± 0.10	18.25 ± 0.30	3.96 ± 0.20	17.89 ± 0.30
200 mg/L	6.53 ± 0.10	18.78 ± 0.1	4.73 ± 0.00	18.32 ± 0.00
400 mg/L	9.41 ± 0.1	20.23 ± 0.10	5.87 ± 0.00	18.94 ± 0.1
800 mg/L	14.28 ± 0.00	21.59 ± 0.00	8.46 ± 0.1	20.44 ± 0.2

^aDPPH antioxidant activities presented as mean values ± SDs of duplicate analyses.

Table 3. CRT Levels of Green Tea Extract or Grape Seed Extract Spiked Wines and the Concentration of Green Tea Extract or Grape Seed Extract in Two Standard Drinks

samples	material	wine type	alcohol/VOL (%)	rejection threshold (mg/L)	amount of added extract in two standard drinks (20 g of alcohol) ^{32a} (mg)
Korea	green tea extract	white	12	550	116.2
Australia	green tea extract	white	12	236.4	49.9
Korea	green tea extract	red	13	377.8	73.7
Australia	green tea extract	red	13	342.9	66.8
Korea	grape seed extract	white	12	320	67.6
Australia	grape seed extract	white	12	400	84.5
Korea	grape seed extract	red	13	400	78.0
Australia	grape seed extract	red	13	600	117.0

^aTwenty grams of alcohol is contained in a volume of alcohol given by: $V = 20 \div [\% \text{ alcohol} \times \text{density of ethanol (0.789)}] \times 100$. The amount of added extract in V is given by: $\text{mass extract} = V \times \text{rejection threshold} / 1000$.

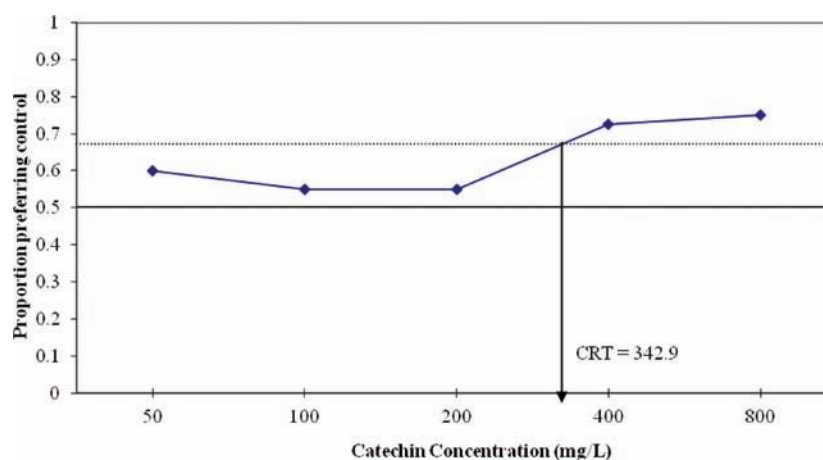


Figure 3. Proportion of respondents preferring the control for each green tea extract level. The dotted line is calculated assuming $\alpha = 0.05$ (0.66 preferring the control) using the binomial distribution for paired comparison tests ($n = 40$). The CRT is determined from the point at which the theoretical preference line intersects with the dotted line.

grape seed extract, spiked into white and red wines, for Korean and Australian consumers were determined (Table 3). The rejection threshold of green tea extract spiked white and red wines for Koreans was 133 and 10% higher than that of Australians, respectively. However, the rejection threshold of grape seed extract spiked white and red wines for Australians was 25 and 50% higher than that of Korean consumers, respectively. Figure 3 shows a representative proportion of Australian respondents, preferring the control for each green tea extract spiked red wine.

This is the first study to determine CRTs for wines spiked with polyphenol-rich extracts and the first study to look at cultural variation in the threshold. Recently, Saliba et al.¹⁷ determined the rejection threshold of wine spiked with eucalyptol. These authors suggested that while taste preference

is highly individual, familiarity plays an important role in food preference. This may explain why Korean consumers accepted higher levels of green tea extract spiked into white and red wines as compared to Australian consumers. Green tea is mainly consumed in Asian countries such as Japan, China, and Korea,²¹ such that Koreans may be more familiar with green tea flavor than Australians. The current study did not assess green tea drinking patterns of either the Australian or the Korean sample; future research in this area could include that as a dependent variable. Also, preference ranking of locally produced Korean wine was relatively high due to familiarity of flavor; its ranking was the second among six different wines (one local and five imported).²² The study suggested that "In South Korea, 'Campbell Early' is mainly consumed as a raw grape and thus its flavor is very familiar to Korean consumers."

The CRT of Australian consumers was higher in grape seed extract spiked wines than that of Korean consumers. This also may be due to Australians being more familiar with grape seed tannins as Australians drink wine much more than Koreans; the wine consumption of Korea and Australia is 0.86 and 22.4 L per capita, respectively.^{22,23}

The fact that the CRT for green tea extract spiked white wine was considerably higher in the Korean cohort as compared to the Australian cohort may also be due to the characteristics of white wine. The phenolic content of white wine is much lower than that of red wine, which is often perceived (to varying degrees) as more bitter and astringent. In this context, green tea extract can impact the taste greatly. Pickering et al.²⁴ showed that the detection threshold for 2-isopropyl-3-methoxypyrazine fluctuated depending on the wine. On the other hand, the CRT for both groups in green tea extract spiked red wine was not much different. This may be due to the typical bitter and astringent red wine flavor that masks the bitterness from green tea extract. Saliba et al.¹⁷ also noted that preference is likely to change depending on other factors such as masking flavors. The relatively flat preference line up until 200 mg/L shown in Figure 3 suggests that respondents either could not detect the spiked material or did not find it objectionable. A pilot scale detection threshold experiment was conducted by the current authors and determined that the detection threshold of both extracts used in the current experiment was between 50 and 100 mg/L for both red and white wine; hence, the minimum spiking level used was 50 mg/L. Previous research has shown that grape seed extract can be detected in red wine at 150 and 100 mg/L for white.²⁵ Given that the current experiment used wine experts to determine the detection thresholds and consumers to determine the CRTs, it is likely that CRT values below around 200 mg/L are due to the spiked material not being detected.

Concentration of Spiked Phenolic Compounds in Two Standard Drinks (20 g of Alcohol). With the increasing health focus of modern consumers, the terms “standard drink” and “moderation” became important vernacular for wine consumers because the level of alcohol consumption is critically linked to health, with low levels possibly linked to positive outcomes and higher consumption levels unquestionably linked to negative health outcomes. Di Castelnuovo et al.²⁶ performed a meta-analysis on 26 health-related wine studies and found that 10 studies supported the J-shaped relationship between alcohol intake and heart disease. As quantity of alcohol consumption is critically related to health benefits, delivering an effective dose of health beneficial compounds in moderation, recognized as two standard drinks per day,⁶ is important.

Table 3 shows that the delivered quantities of these two extracts vary. Koreans can tolerate nearly twice the amount of green tea extract than Australians in white wine. On the other hand, Australians can tolerate 1.5 times more grape seed extract than Koreans in red wine. Importantly, our results demonstrated that depending on the CRTs, white wine can deliver more spiked extract in two standard drinks than red wine.

From a functional food perspective, assessing health benefits of these two extracts in two standard drinks is important as functionality is one of the most important factors in food selection. As *in vivo* testing is beyond the scope of this study, we compared our results with those from the wine and health literature. For example, a green tea extract consumption of 18.6 mg/day increased plasma antioxidant activity,²⁷ while 500 mg/day changed triglyceride and high-density lipoprotein (HDL).²⁸

Our results showed that the green tea extract concentrations for Korean and Australian groups in two standard drinks are well over the effective level of antioxidant activity as suggested in these studies.

Assessing safety with delivered extracts is critical as functional foods must be safe for human consumption. Human intervention studies have shown that the consumption of 800 mg of green tea extract per day or 400 mg twice a day was found to be safe.²⁹ A 670 mg amount of green tea catechins consumed for 3 weeks did not cause adverse effects or impaired liver and kidney functions.²⁸ All results in the current study are under these safe concentrations. Generally, grape seed extract is recognized as a safe substance,³⁰ but human intervention studies for this extract are not available. However, indicatively, a rat study showed that 5000 mg/kg body weight of grape seed extract did not elicit acute toxic effects.³¹ Our results are well under this safe concentration.

Function-enhanced alcoholic beverages are well accepted by Korean and Australian consumers. Green tea-enriched Soju, a popular Korean alcoholic beverage, is selling in the Korean market, and a wine with enhanced grape derived resveratrol is selling in the Australian market. Depending on the market requirements and legislative framework, winemakers can increase the concentration of grape phenolic compounds using different methods as there are many ways to increase these compounds in viticultural or vinification practices. Spiking is one of these methods. The Andalusian Agricultural Research Institute in Spain has started developing resveratrol-enriched red wine through the winemaking practice.⁴

Our study suggests that wine enhanced with green tea extract is more likely to meet consumer taste preferences for Korean consumers. On the other hand, grape seed extract is more preferable for Australian consumers. This result provides quantitative guidance to winemakers on how much these two extracts can be added to wine before consumers find the taste objectionable. With these practically applicable results, a perception study of the healthfulness of wine needs to be done for the targeted market as each country may have its own degree of perception for functionality-enhanced wine.

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ABBREVIATIONS USED

DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAEs, gallic acid equivalents; TE, Trolox equivalents; VOL, volume; FC, Folin–Ciocalteu; CRT, consumer rejection threshold; ALC, alcohol; STD, standard deviation; HDL, high-density lipoprotein.

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