Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 108 (2008) 824–832
Food Chemistry
www.elsevier.com/locate/foodchem

Extracts from red muscadine and cabernet sauvignon wines induce cell death in MOLT-4 human leukemia cells

Susanne U. Mertens-Talcott [a], Susan S. Percival [b], Stephen T. Talcott [a,*]

[a] Department of Nutrition and Food Science, Texas A&M University, Centeq A #220F, TAMU 2254, 1500 Research Parkway A, College Station, TX 77843-2254, United States
[b] Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL 32611, United States

Received 22 August 2007; received in revised form 1 October 2007; accepted 15 November 2007

Abstract

Red wine contains a diversity of polyphenolic compounds that exert beneficial health effects including anti-cancer effects. This trial evaluated the anti-proliferative potential of red muscadine (*Vitis rotundifolia*) and red cabernet sauvignon (*Vitis vinifera*) wines in cell culture. Chemical properties of wines were determined by HPLC-PDA analysis and concentrated extracts of each wine were evaluated before and after glycosidic hydrolysis in MOLT-4 leukemia cells. Cell growth and the induction of apoptosis were evaluated after exposure to various extract dilutions. Wine extracts reduced cell viability up to 68% and cell numbers up to 50% after 48 h with muscadine extracts being more effective than cabernet sauvignon. Caspase-3 activity was induced similarly by all extracts in a dose dependent manner. Cell cycle arrest in the G_2/M phase was observed for both muscadine and the non-hydrolyzed cabernet sauvignon extract. Collectively, extracts from both wines exerted anti-cancer effects in leukemia cells.

Keywords: Red muscadine wine; Red cabernet sauvignon wine; Polyphenolics; Anti-cancer; Red wine; Apoptosis

1. Introduction

The polyphenolic content of wines has been the focus of increased interest due to potential health benefits associated with moderate consumption. Among fruits and vegetables, red grapes contain high polyphenolic levels and their anti-cancer activity is in part due to their ability to quench reactive oxygen species and the protection of critical cellular components such as DNA, proteins, and lipids from oxidative insult (Potter, 1997; Potter & Steinmetz, 1996; Scalbert, Manach, Morand, Remesy, & Jimenez, 2005). Polyphenols and phenolic-rich extracts from wine have been found to reduce neoplastic transformation and to exert anti-cancer activity by decreasing growth, inducing apoptosis, altering cell cycle kinetics, and interfering with intracellular signal transduction events in cancer cells (Briviba, Pan, & Rechkemmer, 2002; Coates et al., 2007; Damianaki et al., 2000; Elattar & Virji, 1999; Franke, Cooney, Custer, Mordan, & Tanaka, 1998; Iijima, Yoshizumi, & Ouchi, 2002; Katsuzaki et al., 2003; Shishodia, Sethi, Ahn, & Aggarwal, 2007). However, only few comparisons have been made among different *Vitis* species in which inherent chemical composition differences may have a significant impact on the purported benefits of red wines.

The two red wines examined in this study included the more traditional and widely distributed cabernet sauvignon wine (*Vitis vinifera*) and red muscadine wine of the cultivar Noble (*Vitis rotundifolia*). Both grapes contain relatively high concentrations of polyphenolic compounds compared to other fruits and vegetables, including hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, and flavonoids including anthocyanins, quercetin, myricetin, and kaempferol (Ector, Magee, Hegwood, & Coign, 1996; German & Walzem, 2000; Ghiselli, Nardini, Baldi, & Scaccini, 1998;

* Corresponding author. Tel.: +1 979 862 4056; fax: +1 979 458 3704.
E-mail address: stalcott@tamu.edu (S.T. Talcott).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.11.037

Talcott & Lee, 2002). However, obvious differences in phytochemical composition differentiate these grapes, including the presence of ellagic acid and its derivatives, the presence of anthocyanin 3,5-diglucosides, a higher proportion of *o*-diphenolic substituted anthocyanins, and an overall higher concentration of total polyphenolics in the muscadine grape (Pastrana-Bonilla, Akoh, Sellappan, & Krewer, 2003; Talcott et al., 2002). Cultural practices during vinification vary among processors and for each grape variety, and are likely to impact levels of polyphenolic compounds in each wine. The antioxidant, anti-cancer, and anti-inflammatory properties of muscadine grapes have previously been demonstrated (God, Tate, & Larcom, 2007; Greenspan et al., 2005), however, the anti-cancer properties of this grape have not been compared to *V. vinifera* varieties.

The objective of these trials was to understand the potential mechanisms of anti-cancer effects observed in acid-hydrolyzed and non-hydrolyzed extracts from the wines with regard to proliferation and apoptosis in a leukemia cell culture model (MOLT-4). Analysis of polyphenolics and hydrophilic antioxidant potential were used to compare compositional differences that might be related to the magnitude of the observed effects. Results of this study provide insight into the chemical differences between red muscadine and cabernet sauvignon wines in their ability to affect cancer cell proliferation.

2. Materials and methods

2.1. Wines and extraction of phenolics

Wines were prepared from cabernet sauvignon (*V. vinifera*) and muscadine (*V. rotundifolia*) grapes (cv. Noble) under similar conditions at the University of Arkansas, Fayetteville, AR, and the University of Florida, Gainesville, FL, respectively. Cabernet sauvignon grapes were grown on an experimental farm in Fayetteville, AR, while the red muscadine grapes were obtained from a vineyard in central Florida. The grapes were crushed, destemmed, and allowed to ferment on the skins for 7 days at 13 °C. The soluble solids for muscadine grapes were chaptalized to 21% prior to fermentation. The musts were pressed and fermentation continued to dryness (<0.05% sugar) at 13 °C. The wines were then treated with 100 mg/L of potassium metabisulfite, cold stabilized at 3 °C for 60 days, racked, and filtered through a 2 cm bed of diatomaceous earth. The cabernet sauvignon wine was subsequently transported to the University of Florida and held at 13 °C until analysis.

Polyphenolics were extracted and concentrated from 100 mL of cabernet sauvignon and muscadine wines after 1.5 years of storage at 6 °C. Wines were diluted with an equal volume of water and passed over a pre-conditioned Waters C_{18} Sep-Pak Vac 20 cc mini-column (Water Corporation, Mass, USA) under a mild vacuum. Water was passed through the column to remove residual polar compounds and retained polyphenolics were eluted with methanol that was subsequently removed by rotary evaporation (<40 °C). The polyphenolics were then redissolved in 10 mL of 20% dimethylsulfoxide (DMSO) in water. A portion of these extracts was subjected to acid hydrolysis in 2 N hydrochloric acid containing 50% methanol at 95 °C for 1 h to cleave glycosidic moieties, and likewise partitioned from C_{18} cartridges to remove residual acid. All extracts were concentrated tenfold compared to the original wines.

2.2. Chemical analysis of wines and wine extracts

The original wines and wine extracts were analyzed for total anthocyanins (pH-differential; mg/L cyanidin 3-glucoside equivalents), total soluble phenolics (Folin-Ciocalteu assay, mg/L gallic acid equivalents; GAE), and antioxidant capacity (oxygen radical absorbance capacity; μmol Trolox equivalents/mL) modified from (Ou, Hampsch-Woodill, & Prior, 2001; Swain & Hillis, 1959; Wrolstad, 1976). Individual polyphenolics of each wine were quantified using a Waters 2695 HPLC system with a Waters ODS-2 column (4.6 × 250 mm) using the solvent conditions previously used (Talcott et al., 2002). Compounds were identified based on spectral characteristics and compared and quantified against authentic standards when available whereas flavonoids were quantified in quercetin equivalents.

2.3. Cell culture

MOLT-4 cells (ATCC, Manassas, VA), an acute lymphoblastic leukemia T cell line, were maintained in RPMI-1640 containing 10% FBS, 2 mmol/L L-glutamine, 100,000 units/L penicillin, 0.1 g/L streptomycin, 0.25 mg/L fungizone and 0.05 g/L gentamycin as previously described (Mertens-Talcott, Bomser, Romero, Talcott, & Percival, 2005). Total cell counts and viability percentages of MOLT-4 cells were determined by trypan blue dye exclusion in a Neubauer hemocytometer. Camptothecin, a potent topoisomerase-I-inhibitor and inducer of apoptosis, was used as a positive control. Hydrolyzed and non-hydrolyzed wine extracts were added to 7.5×10^8 cells/L and incubated for 12, 24, and 48 h. Extract-dilutions ranged from 1:100 to 1:5000. The effect of DMSO was tested in a control culture for all assays.

2.4. Cell cycle kinetics

Cell cycle analysis of MOLT-4 cells was performed with propidium iodide stained cellular DNA (0.05 g/L in 38 mmol/L sodium citrate buffer) after debris removal as previously described (Mertens-Talcott, Talcott, & Percival, 2003). Flow cytometric analyses were performed on a FACScan flow cytometer (BD Immunocytometry Systems, San Jose, CA) at 488 nm excitation and 620 nm emission wavelengths. Resulting histograms were analysed by

determining the percentage of cells in each phase with the ModFit LT software, (Verity Software House, Inc., Topsham, ME).

2.5. Active caspase-3 assay

In order to quantify the percentage of apoptotic cells, MOLT-4 cells were stained with a phycoerythrin-conjugated antibody against the active form of caspase-3 according to the manufacturer's protocol (BD BioSciences, San Jose, CA). The antibody specifically targets the active form of caspase-3 and does not bind to the inactive pro-caspase forms. Analysis was performed by flow cytometry on a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Results were quantified as the percentage of active caspase-3-positive cells of the total cell population.

2.6. Statistical analysis

Statistical analyses were performed with a 2-way ANOVA ($p < 0.05$) using JMP-software (SAS Institute Inc., Cary, NC, 1996). Differences were deemed significant at $p < 0.05$ using the Student's post-hoc *t*-test.

3. Results and discussion

3.1. Chemical analysis of wines and wine extracts

Despite appreciable differences in phytochemical composition between the two wines, each contained an equivalent concentration of total soluble phenolics and antioxidant capacity suitable for a direct comparison to each other (Table 1). Methods to prepare the wine extracts concentrated each wine by a factor of 10.

Total anthocyanins were significantly higher in muscadine wine, extracts, and in hydrolyzed extracts. The Noble cultivar of muscadine grapes is exceptionally high in anthocyanins which are found in a 3,5-diglycoside form compared to mono-glucosides in cabernet sauvignon. The color intensity observed for the non-acylated 3,5-diglycosides is also greatly influenced by anthocyanin cofactors in solution (Talcott, Brenes, Pires, & Pozo-Insfran, 2003), yet 3,5-diglycosides are typically unstable during storage with a lower antioxidant capacity and higher propensity to oxidation and browning reactions (Sims, 1985; Timberlake, 1980). However, concentrations of total anthocyanins found in the muscadine wine were found to be unaltered after 18 months of storage at 10 °C (data not shown). The anthocyanins in cabernet sauvignon were half as much as found in the muscadine grape yet the concentrated extracts retained more color intensity following isolation, also potentially due to enhanced co-pigmentation by naturally occurring polyphenolics.

Hydrolyzed extracts from the two wines had significantly lower anthocyanins, total phenolics, and antioxidant capacity than non-hydrolyzed extracts due to the conditions of hydrolysis. These conditions are known to destroy certain hydroxybenzoic acids and hydroxycinnamic acids, while flavonols and anthocyanins are generally retained, but converted to their less-stable aglycone form. Extracts were kept under nitrogen and used immediately in the cell culture assays to prevent significant oxidative degradation. Losses due to hydrolysis for muscadine and cabernet sauvignon wine were similar for total anthocyanins at 27% and 23%, total soluble phenolics at 11% and 18%, and antioxidant capacity at 53% and 59%, respectively. The decrease in antioxidant capacity after hydrolysis was greater than what could be accounted for by losses in phenolics and anthocyanins, indicating potential disruption of interactions among the polyphenolics present.

HPLC analysis revealed higher flavonoid concentrations in muscadine wine compared to higher phenolic acid content in cabernet sauvignon. Muscadine wine was also appreciably higher in gallic acid and contains ellagic acid, unique among grapes, whereas cabernet sauvignon wine possessed more cinnamic acid derivatives such as caffeic, ferulic and *p*-coumaric acids (Fig. 1, Table 2). The predominant flavonoids in muscadine grapes were previously identified as myricetin, quercetin, and kaempferol with a rhamnoside moiety (Lee, Johnson, & Talcott, 2005). Despite these obvious differences in phytochemical composition, the antioxidant activity of the wines were not appreciably different, such that differences in cell culture models were a function of chemical composition, absorption by the cells, and surface interactions with the cells and not necessary the total antioxidant capacity of the wines and wine extracts.

Table 1
Polyphenolic content and antioxidant capacity of muscadine and cabernet sauvignon wines and wine extracts[a]

Description	Chemical analysis[a]		
	Total anthocyanins (mg/L)	Total soluble phenolics (mg/L)	Antioxidant capacity (μmoles TE[b]/mL)
Muscadine wine	440 ± 21	1295 ± 46	24.3 ± 1.1
Muscadine extract	7934 ± 18	10,269 ± 36	175 ± 6.5
Hydrolyzed muscadine extract	5815 ± 66	9116 ± 38	81.9 ± 3.3
Cabernet sauvignon wine	207 ± 1.9	1229 ± 51	23.3 ± 1.0
Cabernet sauvignon extract	2682 ± 18	9932 ± 22	165 ± 5.6
Hydrolyzed cabernet sauvignon extract	2070 ± 34	8102 ± 92	67.7 ± 4.1

[a] $n = 3$.
[b] TE = Trolox equivalents.

3.2. MOLT-4 cell culture experiments

Dilutions of concentrated wine extracts added to MOLT-4 cell culture were chosen based on physiological concentrations one might expect after moderate wine consumption. An absorption study showed peak plasma levels of ~10 mg/L GAE after consumption of 100 mL of red

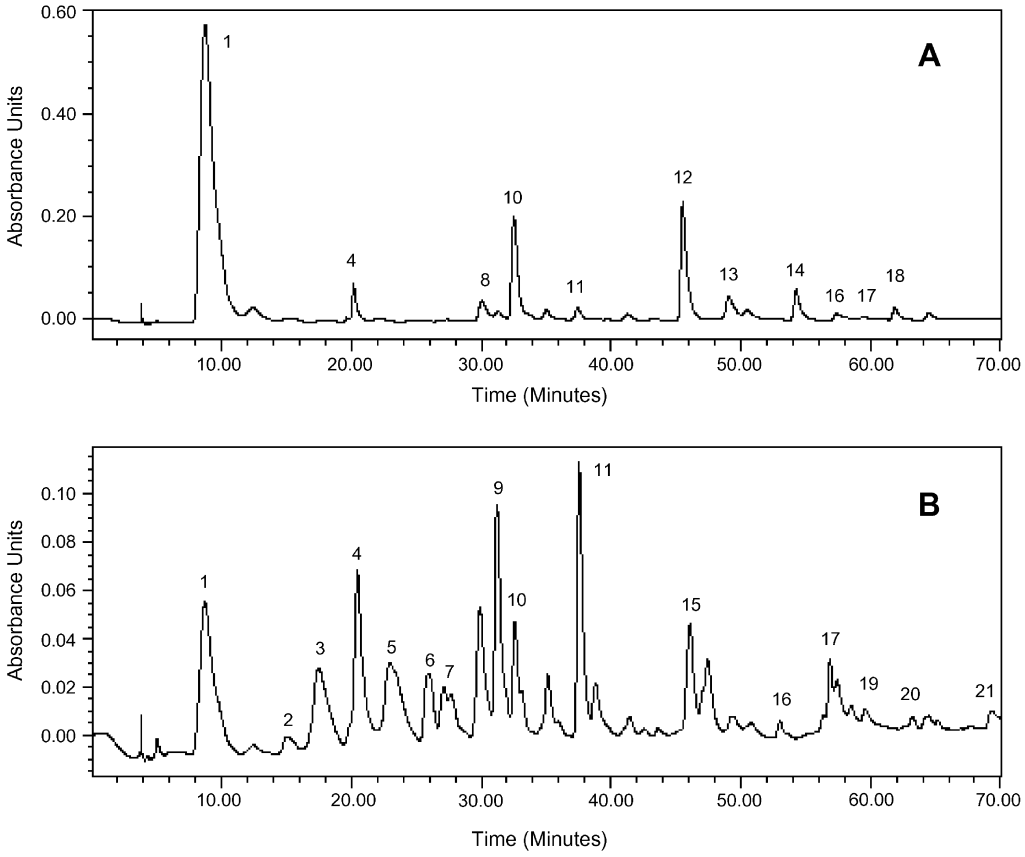

Fig. 1. HPLC chromatographs of non-hydrolyzed: (A) muscadine wine and (B) cabernet sauvignon wine. Note *y*-axis scale differences. Peak numbers correspond to tentative identification as shown in Table 2.

Table 2
Tentative identification and concentration (mg/L) of predominant polyphenolics present in non-hydrolyzed muscadine and cabernet sauvignon wines

Peak[a]	Compound	Muscadine	Cabernet sauvignon
1	Gallic acid	73.0	8.1
2	Protocatechuic acid	ND[b]	0.9
3	Caffeic acid derivative[c]	ND	8.5
4	Gallotannin	2.6	4.9
5	*p*-Coumaric acid derivative	ND	2.4
6	(+)-Catechin	ND	7.3
7	Caffeic acid	ND	6.6
8	Flavonoid glycoside[d]	16.9	ND
9	Syringic acid	ND	4.5
10	Gallotannin	11.3	10.9
11	*p*-Coumaric acid	0.7	3.6
12	Flavonoid glycoside	86.9	ND
13	Ellagic acid	8.5	ND
14	Flavonoid glycoside	23.8	ND
15	Flavonoid glycoside	ND	5.8
16	Flavonoid	7.1	5.1
17	Resveratrol	0.2	0.2
18	Flavonoid glycoside	8.4	ND
19	Ferulic acid derivative	ND	1.2
20	Flavonoid aglycone	ND	2.1

[a] Peak numbers correspond to those in Fig. 1.
[b] ND = not detected.
[c] Derivatives quantified in equivalents of their parent compound based on spectral similarities.
[d] Flavonoids identified based on spectral properties and quantified in quercetin equivalents.

wine (Duthie et al., 1998). Dilutions of the non-hydrolyzed muscadine and cabernet sauvignon wine extract ranged from 2.05–103 to 1.98–99 mg/L GAE (from the 1:5000 to 1:100 dilutions), respectively in order to create solutions with physiological relevance. Several studies have indicated that aglycones and their glycosidic conjugated forms can be absorbed after oral ingestion (Hsiu, Huang, Hou, Chin, & Chao, 2002; Morand, Manach, Crespy, & Remesy, 2000; Sesink, Arts, Faassen-Peters, & Hollman, 2003; Walle, Browning, Steed, Reed, & Walle, 2005; Walle, Otake, Walle, & Wilson, 2000; Zubik & Meydani, 2003) and might be biologically active. Therefore, this study investigated the efficacy of both, hydrolyzed and non-hydrolyzed extracts of red muscadine grape (MH and M) and Cabernet Sauvignon (CH and C) in cell culture. Treatments were compared to a 0.2% DMSO vehicle control culture which had no effect on vital cell parameters.

3.3. Cell counts and viability

Both hydrolyzed and non-hydrolyzed muscadine and cabernet sauvignon wine extracts reduced the growth of MOLT-4 cells at 48 h in a concentration-dependent manner (Fig. 2). M and MH reduced the total cell number by up to 41% and 27%, respectively compared to control cells. With C and CH, total cell number was reduced by up to 49% and 22%, respectively. Overall, the reduction of viabil-

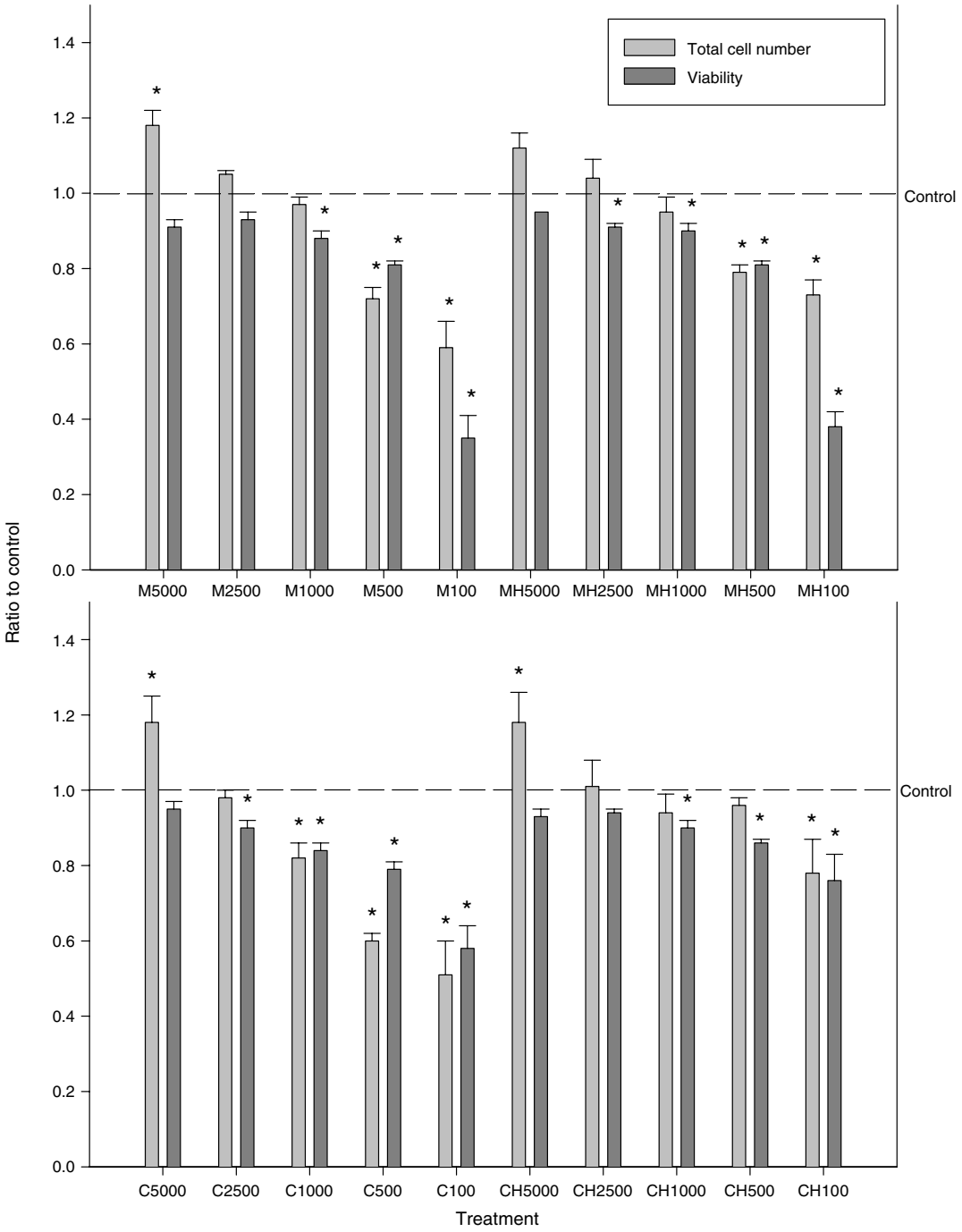

Fig. 2. Cell number and viability of MOLT-4 cells 48 h after treatment with wine extracts. Cell number and viability were determined by trypan blue exclusion in a Neubauer hemocytometer. The upper panel shows the muscadine non-hydrolyzed (M) and hydrolyzed (MH) at dilutions ranging from 1:100 to 1:5000. The lower panel shows the cabernet sauvignon non-hydrolyzed (C) and hydrolyzed (CH) at dilutions ranging from 1:100 to 1:5000. Bars that are significantly different from the control ($p \leqslant 0.05$) are marked with *.

ity was higher than for cell number. M and MH reduced viability by 65% and 62% and C and CH by 42% and 24%, respectively. The difference in efficacy in the reduction of cell number of the non-hydrolyzed compared to the hydrolyzed extracts may have been caused by the loss of antioxidant capacity. Conversely, this loss is not reflected when comparing data for viability. Overall, the reduction in cell growth and viability was comparable for both of the muscadine extracts and the non-hydrolyzed cabernet sauvignon extract. The hydrolyzed cabernet sauvignon extract was the least effective in altering cell growth and viability, coinciding with its lower antioxidant capacity and lower concentration of anthocyanins.

Polyphenolic compounds isolated from red wine including resveratrol, quercetin, ellagic acid, catechin, and anthocyanidins have been studied for their ability to reduce cell growth and proliferation of various cell types (Alkhalaf, 2007; Bianchini & Vainio, 2003; Dechsupa et al., 2007;

Haider, Sorescu, Griendling, Vollmar, & Dirsch, 2003; Yoshida et al., 1990). Several studies have examined the influence of a combination of phenolic compounds: the induction of cell death in cancer cells. Polyphenolic extracts from red wine inhibited proliferation in colon epithelial cells at μmolar concentrations (Briviba et al., 2002). Extracts from red and white wine have been shown to inhibit proliferation in HCT-15 cells, where anthocyanin-containing fractions where more effective than other fractions from the same wines (Kamei, Hashimoto, Koide, Kojima, & Hasegawa, 1998). Proliferation-inhibitory effects of physiologically relevant concentrations were also shown in breast cancer cell lines, where wine polyphenols decreased the growth (Damianaki et al., 2000). In this study, extracts from both wines were able to reduce cell growth at physiologically relevant concentrations.

3.4. Cell cycle kinetics

Cell cycle kinetics were determined at 24 h of incubation with each wine extract (Fig. 3). At low extract concentrations, no significant alterations in the cell cycle phases were observed. Both muscadine extracts induced minor but significant changes in cell cycle kinetics at the 1:500 dilution, with an increase in the percent of cells in the S phase. Incubation with the hydrolysed extract resulted in an additional 12% decrease in the G_0/G_1 phase. In contrast, non-hydrolysed cabernet sauvignon extract induced an 8-fold increase in the percentage of cells in the G_2/M phase at the 1:100 dilution compared to the control. Hydrolyzed cabernet sauvignon extracts had no significant effect on cell cycle kinetics.

Effects on cell cycle kinetics induced by single polyphenolic compounds from red wine were reported previously (Benitez, Pozo-Guisado, Alvarez-Barrientos, Fernandez-Salguero, & Castellon, 2007; Koide et al., 1997; Mertens-Talcott, Lee, Percival, & Talcott, 2006; Narayanan, Geoffroy, Willingham, Re, & Nixon, 1999; Richter, Ebermann, & Marian, 1999; Vijayababu et al., 2005). However, few studies have examined the effect of wine extracts on cell cycle kinetics. Anthocyanin extract and a flavonoid (non-anthocyanin) extract of red wine were found to induce cell cycle arrest in the S and G_2/M-phases (Kamei et al., 1998). Data from this study relate to those findings for both of the muscadine extracts and C. For this study, alterations of cell cycle kinetics cannot obviously be related to the chemical composition of the extracts; however, the lower antioxidant capacity and lower anthocyanin concentration found in hydrolyzed cabernet sauvignon extracts, compared to the other extracts, might explain their ineffectiveness in altering cell cycle kinetics.

3.5. Caspase-3-activity

All extracts induced activation of the apoptosis-executing enzyme caspase-3 in a concentration dependent manner

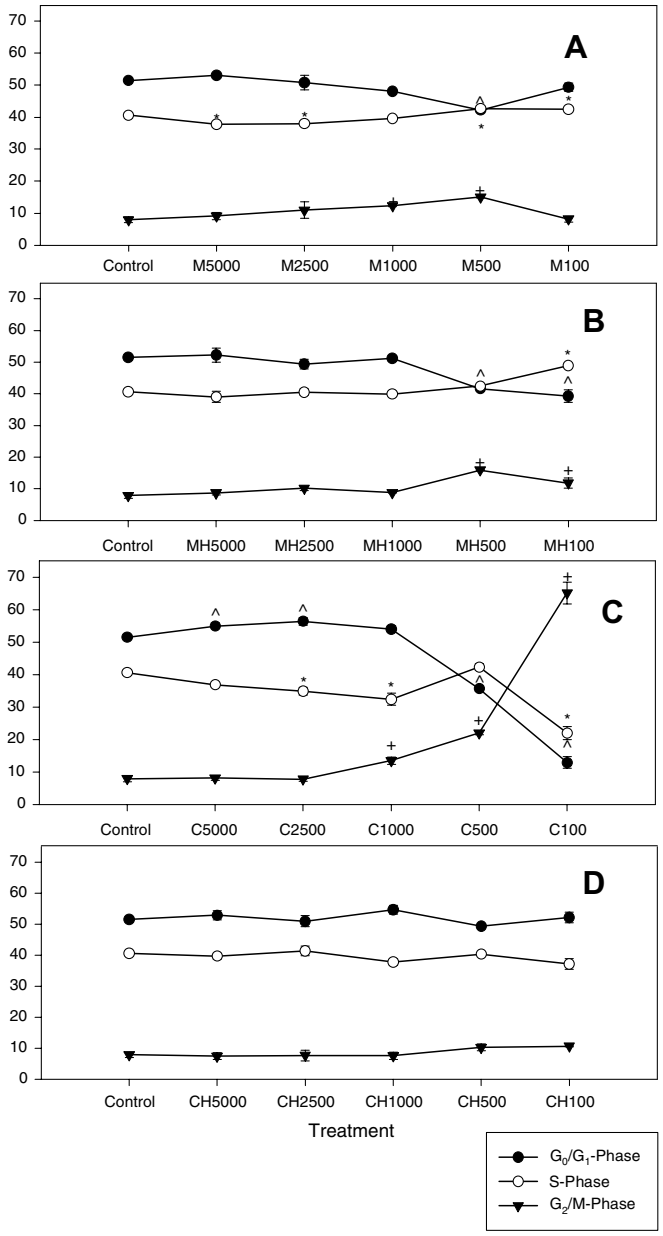

Fig. 3. Cell cycle kinetics of MOLT-4 cells 24 h after treatment with wine extracts. Panel A is 1:100 to 1:5000 dilution of muscadine extract (M); panel B is 1:100 to 1:5000 dilution of hydrolyzed muscadine extract (MH); panel C is 1:100 to 1:5000 dilution of cabernet sauvignon extract (C) and panel D is 1:100 to 1:5000 dilution of hydrolyzed cabernet sauvignon extract (CH). Values within the same cell cycle phase, which are significantly different from the control ($p \leqslant 0.05$) are marked with ^ (G_0/G_1-phase), * (S-phase), and + (G_2/M-phase).

(Fig. 4). Both muscadine extracts increased the percentage of cells with active caspase-3 at 1:1000 and lower dilutions to up to 26%. Both cabernet sauvignon extracts induced caspase-3 activity in up to 30% of cells. While M was highly potent in comparison to MH, C and CH in the other experiments, it induced caspase-3 activity only in 18% of cells at the 1:100 dilution. CH increased caspase-3 activity at all concentrations, indicating a broad range of effectiveness even at lower phenolic concentrations.

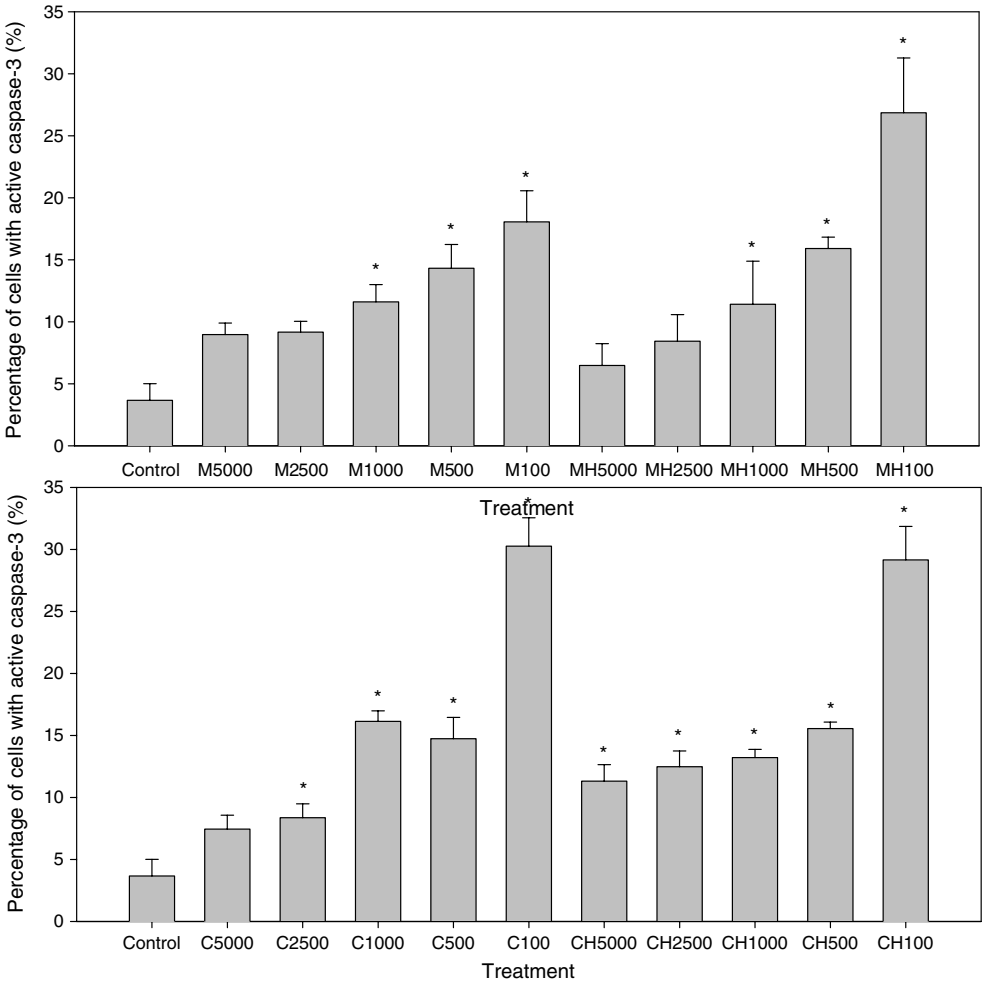

Fig. 4. Caspase-3 activity of MOLT-4 cells after 12 h treatment with wine extracts. Intracellular active caspase-3 was labeled with a specific PE-linked antibody and analyzed by flow cytometry. The upper panel is the muscadine non-hydrolyzed (M) and hydrolyzed (MH) at dilutions ranging from 1:100 to 1:5000. The lower panel is the cabernet sauvignon non-hydrolyzed (C) and hydrolyzed (CH) at dilutions ranging from 1:100 to 1:5000. Means that are significantly different from the control ($p \leqslant 0.05$) are marked with *.

Single compounds such as quercetin, ellagic acid, resveratrol, and gallic acid have been demonstrated to induce apoptosis in several cell lines at different concentrations (Dechsupa et al., 2007; Liesveld et al., 2003; Narayanan & Re, 2001; Richter et al., 1999). Differences in the magnitude of induced apoptosis was found to depend on the polyphenolic composition of the tested extracts, where the extracts containing flavonols and procyanidin oligomers of higher molecular weight were more effective in inducing apoptosis (Matito, Mastorakou, Centelles, Torres, & Cascante, 2003). Anthocyanins from red wine induced apoptosis in MOLT-4B cells (Katsuzaki et al., 2003), and grape seed extract in human prostrate carcinoma cells (Agarwal, Sharma, & Agarwal, 2000; Tyagi, Agarwal, & Agarwal, 2003). Results from this study demonstrate the pro-apoptotic effects of extracts from both wines.

4. Summary

In summary, data from this study indicate the anti-cancer activity of red muscadine and cabernet sauvignon wines, where extracts from both wines were fairly comparable in their anti-proliferative activity despite differences in their chemical composition.

The differences in polyphenolic composition and antioxidant properties were expected to result in significant differences in the anti-cancer activities of extracts from both wines. Conversely, overall similar effects were observed among extracts from both wine sources. Therefore, these data imply that antioxidant capacity and total polyphenolics had minor relevance regarding the overall anti-proliferative effects of these wine extracts.

Acknowledgements

We thank Dr. Justin Morris, Food Science Department, University of Arkansas, Fayetteville, AR, for the preparation of the cabernet sauvignon wine and Lakeridge Winery, Clermont, FL, for the donation of muscadine grapes. Ms. Rena Schonbrun, Food Science and Human Nutrition Department, University of Florida, is appreciated for her helpful assistance with the preparation of the muscadine

wine. We also would like to thank Dr. Cheryl A. Rowe and Ms. Meri Nantz, Food Science and Human Nutrition Department, University of Florida for their kind technical assistance.

References

Agarwal, C., Sharma, Y., & Agarwal, R. (2000). Anticarcinogenic effect of a polyphenolic fraction isolated from grape seeds in human prostate carcinoma DU145 cells: Modulation of mitogenic signaling and cell-cycle regulators and induction of G1 arrest and apoptosis. *Molecular Carcinogenesis, 28*(3), 129–138.

Alkhalaf, M. (2007). Resveratrol-induced apoptosis is associated with activation of p53 and inhibition of protein translation in T47D human breast cancer cells. *Pharmacology, 80*(2–3), 134–143.

Benitez, D. A., Pozo-Guisado, E., Alvarez-Barrientos, A., Fernandez-Salguero, P. M., & Castellon, E. A. (2007). Mechanisms involved in resveratrol-induced apoptosis and cell cycle arrest in prostate cancer-derived cell lines. *Journal of Andrology, 28*(2), 282–293.

Bianchini, F., & Vainio, H. (2003). Wine and resveratrol: Mechanisms of cancer prevention? *European Journal of Cancer Prevention, 12*(5), 417–425.

Briviba, K., Pan, L., & Rechkemmer, G. (2002). Red wine polyphenols inhibit the growth of colon carcinoma cells and modulate the activation pattern of mitogen-activated protein kinases. *Journal of Nutrition, 132*(9), 2814–2818.

Coates, E. M., Popa, G., Gill, C. I., McCann, M. J., McDougall, G. J., Stewart, D., et al. (2007). Colon-available raspberry polyphenols exhibit anti-cancer effects on in vitro models of colon cancer. *Journal of Carcinogenesis, 6*, 4.

Damianaki, A., Bakogeorgou, E., Kampa, M., Notas, G., Hatzoglou, A., Panagiotou, S., et al. (2000). Potent inhibitory action of red wine polyphenols on human breast cancer cells. *Journal of Cellular Biochemistry, 78*(3), 429–441.

Dechsupa, S., Kothan, S., Vergote, J., Leger, G., Martineau, A., Berangeo, S., et al. (2007). Quercetin, Siamois 1 and Siamois 2 induce apoptosis in human breast cancer MDA-mB-435 cells xenograft in vivo. *Cancer Biology and Therapy, 6*(1), 56–61.

Duthie, G. G., Pedersen, M. W., Gardner, P. T., Morrice, P. C., Jenkinson, A. M., McPhail, D. B., et al. (1998). The effect of whisky and wine consumption on total phenol content and antioxidant capacity of plasma from healthy volunteers. *52*(10), 733–736.

Ector, B. J., Magee, J. B., Hegwood, C. P., & Coign, M. J. (1996). Resveratrol concentration in muscadine berries, juice pomace, purees, seeds and wines. *American Journal of Enology and Viticulture, 47*, 57–62.

Elattar, T. M., & Virji, A. S. (1999). The effect of red wine and its components on growth and proliferation of human oral squamous carcinoma cells. *Anticancer Research, 19*(6B), 5407–5414.

Franke, A. A., Cooney, R. V., Custer, L. J., Mordan, L. J., & Tanaka, Y. (1998). Inhibition of neoplastic transformation and bioavailability of dietary flavonoid agents. *Advances in Experimental Medicine and Biology, 439*, 237–248.

German, J. B., & Walzem, R. L. (2000). *The health benefits of wine* (*Vol. 20,* pp. 561–593).

Ghiselli, A., Nardini, M., Baldi, A., & Scaccini, C. (1998). Antioxidant activity of different phenolic fractions separated from an Italian red wine. *46*(2), 361–367.

God, J. M., Tate, P., & Larcom, L. L. (2007). Anticancer effects of four varieties of muscadine grape. *Journal of Medical Food, 10*(1), 54–59.

Greenspan, P., Bauer, J. D., Pollock, S. H., Gangemi, J. D., Mayer, E. P., Ghaffar, A., et al. (2005). Antiinflammatory properties of the muscadine grape (*Vitis rotundifolia*). *Journal of Agricultural Food Chemistry, 53*(22), 8481–8484.

Haider, U. G., Sorescu, D., Griendling, K. K., Vollmar, A. M., & Dirsch, V. M. (2003). Resveratrol increases serine15-phosphorylated but transcriptionally impaired p53 and induces a reversible DNA replication block in serum-activated vascular smooth muscle cells. *Molecular Pharmacology, 63*(4), 925–932.

Hsiu, S. L., Huang, T. Y., Hou, Y. C., Chin, D. H., & Chao, P. D. (2002). Comparison of metabolic pharmacokinetics of naringin and naringenin in rabbits 1. *70*(13), 1481–1489.

Iijima, K., Yoshizumi, M., & Ouchi, Y. (2002). Effect of red wine polyphenols on vascular smooth muscle cell function–molecular mechanism of the 'French paradox'. *Mechanisms of Ageing and Development, 123*(8), 1033–1039.

Kamei, H., Hashimoto, Y., Koide, T., Kojima, T., & Hasegawa, M. (1998). Anti-tumor effect of methanol extracts from red and white wines. *Cancer Biotherapy and Radiopharmaceuticals, 13*(6), 447–452.

Katsuzaki, H., Hibasami, H., Ohwaki, S., Ishikawa, K., Imai, K., Date, K., et al. (2003). Cyanidin 3-*O*-beta-D-glucoside isolated from skin of black Glycine max and other anthocyanins isolated from skin of red grape induce apoptosis in human lymphoid leukemia Molt 4B cells. *Oncology Reports, 10*(2), 297–300.

Koide, T., Kamei, H., Hashimoto, Y., Kojima, T., Terabe, K., & Umeda, T. (1997). Influence of flavonoids on cell cycle phase as analyzed by flow-cytometry. *Cancer Biotherapy and Radiopharmaceuticals, 12*(2), 111–115.

Lee, J. H., Johnson, J. V., & Talcott, S. T. (2005). Identification of ellagic acid conjugates and other polyphenolics in muscadine grapes by HPLC-ESI-MS. *Journal of Agricultural Food Chemistry, 53*(15), 6003–6010.

Liesveld, J. L., Abboud, C. N., Lu, C., McNair, C., Menon, A., Smith, A., et al. (2003). Flavonoid effects on normal and leukemic cells. *Leukemia Research, 27*(6), 517–527.

Matito, C., Mastorakou, F., Centelles, J. J., Torres, J. L., & Cascante, M. (2003). Antiproliferative effect of antioxidant polyphenols from grape in murine Hepa-1c1c7. *European Journal of Nutrition, 42*(1), 43–49.

Mertens-Talcott, S. U., Bomser, J. A., Romero, C., Talcott, S. T., & Percival, S. S. (2005). Ellagic acid potentiates the effect of quercetin on p21waf1/cip1, p53, and MAP-kinases without affecting intracellular generation of reactive oxygen species in vitro. *Journal of Nutrition, 135*(3), 609–614.

Mertens-Talcott, S. U., Lee, J. H., Percival, S. S., & Talcott, S. T. (2006). Induction of cell death in Caco-2 human colon carcinoma cells by ellagic acid rich fractions from muscadine grapes (*Vitis rotundifolia*). *Journal of Agricultural Food Chemistry, 54*(15), 5336–5343.

Mertens-Talcott, S. U., Talcott, S. T., & Percival, S. S. (2003). Low concentrations of quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 human leukemia cells. *Journal of Nutrition, 133*(8), 2669–2674.

Morand, C., Manach, C., Crespy, V., & Remesy, C. (2000). Respective bioavailability of quercetin aglycone and its glycosides in a rat model 1. *12*(1–4), 169–174.

Narayanan, B. A., Geoffroy, O., Willingham, M. C., Re, G. G., & Nixon, D. W. (1999). p53/p21(WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells. *Cancer Letters, 136*(2), 215–221.

Narayanan, B. A., & Re, G. G. (2001). IGF-II down regulation associated cell cycle arrest in colon cancer cells exposed to phenolic antioxidant ellagic acid. *Anticancer Research, 21*(1A), 359–364.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *49*(10), 4619–4626.

Pastrana-Bonilla, E., Akoh, C. C., Sellappan, S., & Krewer, G. (2003). Phenolic content and antioxidant capacity of muscadine grapes 1. *51* (18), 5497–5503.

Potter, J. D. (1997). Cancer prevention: Epidemiology and experiment. *Cancer Letters, 114*(1–2), 7–9.

Potter, J. D., & Steinmetz, K. (1996). *Vegetables, fruit and phytoestrogens as preventive agents* (*Vol. 139*, pp. 61–90). IARC Scientific Publications.

Richter, M., Ebermann, R., & Marian, B. (1999). Quercetin-induced apoptosis in colorectal tumor cells: Possible role of EGF receptor signaling. *Nutrition and Cancer, 34*(1), 88–99.

Scalbert, A., Manach, C., Morand, C., Remesy, C., & Jimenez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition, 45*(4), 287–306.

Sesink, A. L., Arts, I. C., Faassen-Peters, M., & Hollman, P. C. (2003). Intestinal uptake of quercetin-3-glucoside in rats involves hydrolysis by lactase phlorizin hydrolase. *133*(3), 773–776.

Shishodia, S., Sethi, G., Ahn, K. S., & Aggarwal, B. B. (2007). Guggulsterone inhibits tumor cell proliferation, induces S-phase arrest, and promotes apoptosis through activation of c-Jun N-terminal kinase, suppression of Akt pathway, and downregulation of antiapoptotic gene products. *Biochemical Pharmacology, 74*(1), 118–130.

Sims, C. A. M. J. R. (1985). A comparison of the color components and color stability of red wine from Noble and Cabernet Sauvinon at various pH levels. *36,* 181–184.

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of Purmus domestica I. The quantitative analysis of phenolic constituents. *Journal of Science and Food Agriculture, 10*, 63–68.

Talcott, S. T., Brenes, C. H., Pires, D. M., & Pozo-Insfran, D. (2003). Phytochemical stability and color retention of copigmented and processed muscadine grape juice. *51*(4), 957–963.

Talcott, S. T., & Lee, J. H. (2002). Ellagic acid and flavonoid antioxidant content of muscadine wine and juice. *Journal of Agricultural Food Chemistry, 50*(11), 3186–3192.

Timberlake, C. F. B. P. (1980). Anthocyanins. In J. Walford (Ed.), *Developments in Food Colours 1*. London: Applied Science Publishers Ltd.

Tyagi, A., Agarwal, R., & Agarwal, C. (2003). Grape seed extract inhibits EGF-induced and constitutively active mitogenic signaling but activates JNK in human prostate carcinoma DU145 cells: Possible role in antiproliferation and apoptosis. *Oncogene, 22*(9), 1302–1316.

Vijayababu, M. R., Kanagaraj, P., Arunkumar, A., Ilangovan, R., Aruldhas, M. M., & Arunakaran, J. (2005). Quercetin-induced growth inhibition and cell death in prostatic carcinoma cells (PC-3) are associated with increase in p21 and hypophosphorylated retinoblastoma proteins expression. *Journal of Cancer Research Clinical Oncology, 131*(11), 765–771.

Walle, T., Browning, A. M., Steed, L. L., Reed, S. G., & Walle, U. K. (2005). Flavonoid glucosides are hydrolyzed and thus activated in the oral cavity in humans. *Journal of Nutrition, 135*(1), 48–52.

Walle, T., Otake, Y., Walle, U. K., & Wilson, F. A. (2000). Quercetin glucosides are completely hydrolyzed in ileostomy patients before absorption. *130*(11), 2658–2661.

Wrolstad, R. E. (1976). *Color and pigment analysis in fruit products*. p. 624.

Yoshida, M., Sakai, T., Hosokawa, N., Marui, N., Matsumoto, K., Fujioka, A., et al. (1990). The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. *FEBS Letters, 260*(1), 10–13.

Zubik, L., & Meydani, M. (2003). Bioavailability of soybean isoflavones from aglycone and glucoside forms in American women 1. *77*(6), 1459–1465.