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LIQUID-LIQUID EXTRACTION AND ANALYSIS OF THE ANTIOXIDANT RESVERATROL FROM WINE VARIETALS OF DIFFERENT GEOGRAPHIC VINEYARDS

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Abstract

The objective of this study was to extract, isolate and analyze the antioxidant resveratrol from various red, rosé and white wines. Three different growing locations were selected for this study. These vineyards include: US west (Oregon/Washington), US east (New York) and France. Merlot, Rosé and Chardonnay from each vineyard location were selected as the wines for analysis and comparison. The hypothesis that red wines contain the highest concentration of resveratrol followed by rosés and then white wines was studied. The extraction and quantification of resveratrol was conducted in order to compare the amount of this antioxidant found in the different wine varietals. In order to extract resveratrol, liquid-liquid phase techniques were implemented. The concentration of the antioxidant was quantified using ultraviolet spectroscopy at a wavelength of 310 nm. The analysis of the data collected from this study will suggest which type of wine and vineyard location contained the most resveratrol.

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Introduction:

Resveratrol (3,5,4'-trihydroxystilbene) is a naturally occurring polyphenolic phytoalexin that has *cis* and *trans* conformations. This compound is produced in more than 70 plant species, including the skin of dark fruit, such as grapes (1, 2). Resveratrol serves in response to environmental stress in plants, such as attacks by pathogens, UV radiation, mechanical injury and heavy metal pollution (3). The *trans*-isomer of resveratrol (Figure 1) has potential to be a beneficial treatment for inflammatory diseases (4), atherosclerosis, hypertension, diabetes, obesity, and aging (5-10). Resveratrol has also been studied for its benefits as an anti-cancer agent (11). The chemopreventive activity of resveratrol has been investigated in various tumor cell lines (12). Resveratrol has antimutagenic effects inhibiting all three main stages of carcinogenesis: tumor initiation, promotion, and progression (12, 13). This process was shown in culture by inhibiting the development of preneoplastic lesions in carcinogen-treated mouse mammary glands (12). It has also been found to have antioxidant properties. Specifically, resveratrol inhibited free radical formation in a dose-dependent manner in cultured mouse hepatoma cells (12). Researchers discovered that resveratrol has adverse effects on human tumorigenic cells, which are shown during initiating apoptotic cell death in HL60 leukemia cells and T47D breast carcinoma cells (14). In addition to its effects on cancer, many studies have also supported that resveratrol has the ability to slow the progression of other illnesses, such as cardiovascular disease (15). These effects of resveratrol, given in various concentrations, have been successfully demonstrated with *in vitro* experimentation (3). However, due to the low bioavailability of resveratrol, *in vivo* studies are less consistent (4). In naturally occurring sources, such as various wines and juices of dark fruits, resveratrol has been studied to determine its potential medicinal properties (5). Extraction and quantification methods have been used to measure the concentrations of resveratrol in various wines (16-20). Specifically, red wine has been studied for its benefits in reducing mortality from coronary artery disease (21, 22). It has been reported that the French population has decreased levels of cardiovascular disease (CVD) despite the similarities in dietary and lifestyle habits to other populations with heightened risks of CVD (22, 23). This phenomenon is known as the "French Paradox" (22, 23). The conformation of *trans*-resveratrol is prominent in red wines as well as other phenols, such as quercetin (24). It is supported that both *trans*-resveratrol and quercetin inhibit thrombin-induced and ADP-induced platelet aggregation, as opposed to ethanol, which only reduced thrombin-induced platelet aggregation (24). These effects have been observed more in red wines than in white wines due to the differences in resveratrol concentrations (25). Resveratrol is highly concentrated in the skins of grapes (25, 26). One study reported that the concentrations of the major form of resveratrol, the *trans*-isomer, generally ranged between 0.1 and 15.0 mg/L in red wines (26). Moreover, resveratrol is a biologically significant molecule that can be found in the skins of dark fruits that have chemopreventive effects, as well as benefits in reducing cardiovascular disease and antiplatelet aggregation.

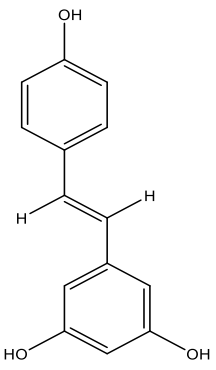

Figure. 1 Chemical structure of resveratrol (trans-3,5,4'-trihydroxystilbene)

Based on previous studies, our research will focus on liquid-liquid extraction of resveratrol from red, rosé and white wines from different growing regions. This study will compare extract concentrations for each varietal wine and growing region. After extraction, the concentration of resveratrol will be calculated from the absorbance measurement at 310 nm on a UV spectrophotometer (27). This analysis will suggest which type of wine contained the most resveratrol.

Methods:

The liquid-liquid phase extraction method (17) was conducted as follows: 250 mL samples of each wine varietal were evaporated for 25 minutes on a rotary evaporator with the condenser set at 4°C and the water bath at 40°C. This process was used to remove ethanol from each wine sample (N=27). The remaining wine sample was then poured into a 500 mL separatory funnel and washed twice with of 83mL of chloroform (CAS number 67-66-3). At the end of these washes, the organic phase was discarded. The remaining aqueous phase was extracted three times using 42 mL of ethyl acetate (CAS number 141-78-6). After each extraction, the organic phase was collected and combined, each aqueous phase was then discarded. The combined ethyl acetate extracts were washed twice with 83mL of saturated sodium chloride. The resulting aqueous phase was discarded. The samples were left uncovered overnight to allow the ethyl acetate to evaporate, which completed the resveratrol isolation. The remaining crystals were reconstituted in 200-300 mL of a 1:1 volume/volume solution of HPLC grade acetonitrile (CAS number 75-05-8) and HPLC grade water. The absorbance was obtained from the reconstituted resveratrol solution at a wavelength of 310 nm (Perkin-Elmer Lambda 25, Pittsburgh, PA). All solvents and reagents were from Sigma Aldrich; St. Louis, MO.

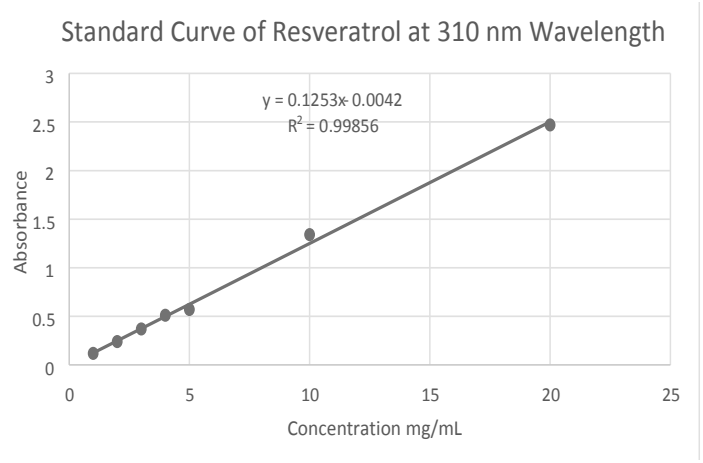

Figure 2. Standard curve for resveratrol of absorbance versus concentration at 310nm.

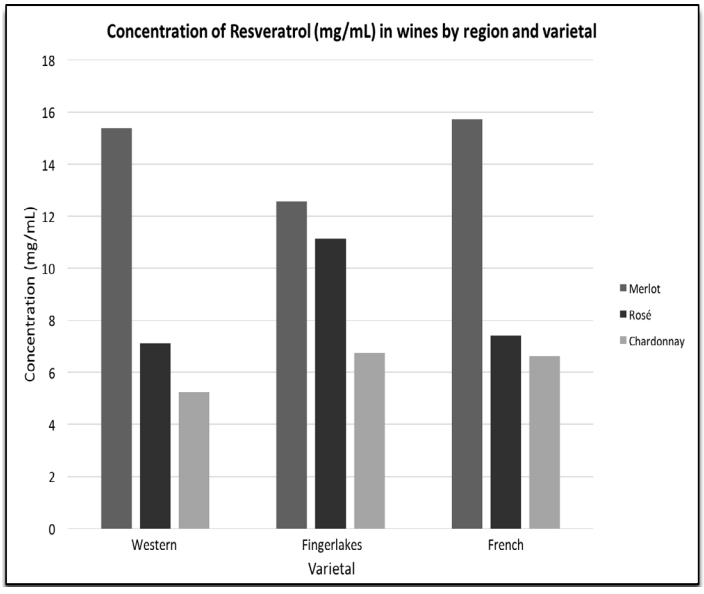

Figure 3. Concentration of resveratrol in each wine varietal and region.

Discussion:

Figure 2 represents the standard curve for resveratrol of absorbance versus concentration at 310 nm. The standard concentrations ranged from 1.0 mg/mL to 20.0 mg/mL. The standard curve linear formula (y = 0.1253x -0.0042) was used to convert the sample absorbance (y) from the ultraviolet spectrophotometer readings to concentration (x) in mg/mL. It should be noted that resveratrol, as well as other phenolic compounds in wine, can absorb in the same ultraviolet region of 310 nm (17, 27). In this work, resveratrol was used as an exemplary phenolic compound to represent total phenolic compounds and tannins, rather than a quantitative assay of resveratrol.

Figure 3 shows the general correlation between region and varietal, as well as a significant downward trend for resveratrol concentration in each region. For each region, the resveratrol concentrations significantly decrease from the merlot varietal to the rosé varietal to the chardonnay varietal. The merlots have an overall higher concentration with an average of 14.56 mg/mL. The rosés have a significantly lower average of 8.56 mg/mL. The chardonnays have even lower concentration levels with an average of 6.20 mg/mL. Among the merlots, the highest average concentrations are from the Maison Nicolas Winery, based out of France, at 15.73 mg/mL ± 1.68 and the Canoe Ridge Vineyards from Washington State at 15.39 mg/mL ± 1.95. Fulkerson Merlot from Finger Lakes, New York had the lowest concentration with an average of 12.57 mg/mL ± 0.53. Among the rosés, Goose Watch Winery, from the Finger Lakes, had the highest concentration at an average of 11.14 mg/mL ± 2.30, followed by Maison Nicolas Winery at an average concentration of 7.41 mg/mL ± 0.35. Primarius Winery from Oregon had the lowest concentration at an average of 7.12 mg/mL ± 0.66. Among the chardonnays, Wagner Vineyards from Finger Lakes had the highest concentration at an average of 6.74 mg/mL ± 1.18, followed by Maison Nicolas Chardonnay with an average of 6.62 mg/mL ± 0.45, and Washington Hills Winery from Washington had the lowest concentration with an average of 5.23 mg/mL ± 1.17.

The low standard deviations suggest that the extractions and data measurements from these trials were reproducible. However, the Nicolas Merlot, Western Merlot, and Finger Lakes Rosé samples had larger standard deviations, which may be due to extraction technique and different wine bottle samples. Each wine varietal sample were left uncovered to allow the ethyl acetate to evaporate off, which may have caused one or more samples to become contaminated due to air exposure. Exposure to UV light could have an effect on any decomposition of resveratrol as well. The concentration differences between the regions could be due to the grape growing conditions and/or the fermenting process of the wines at each winery. Moreover, this experiment supports our hypothesis that red wines contain a higher concentration of resveratrol as well as other phenolic compounds when compared to rosé and white wines. The next step in verification of the current data for this study will be to analyze each varietal sample using High Pressure Liquid Chromatography, HPLC, to further quantify and compare the presence of resveratrol

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