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EXTRACTION AND ANALYSIS OF THE ANTIOXIDANT RESVERATROL FROM VARIOUS R.W. KNUDSEN FAMILY JUICES

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Abstract:

This study aimed to extract, isolate, and analyze the antioxidant resveratrol from various juices. The R.W. Knudsen Family of juices were utilized because their products are 100% juice, containing no additives or preservatives. Resveratrol was quantified to compare the amount of this antioxidant found in the different juices. Liquid-liquid phase techniques were implemented to extract resveratrol. The antioxidant concentration was quantified using ultraviolet spectroscopy at 310 nm. The analysis of the data collected from this study suggests which juice contains the most resveratrol. The antioxidant potential was measured using a Ferric Reduction Antioxidant Potential (FRAP) Assay.

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INTRODUCTION

Resveratrol (*trans*-3,5,4'-trihydroxystilbene), Figure 1, is a naturally occurring polyphenolic phytoalexin with *cis* and *trans* conformations[1]. This compound is produced in more than 70 plant species, including the skin of dark fruit and vegetables, such as grapes[2,3]. Resveratrol responds to environmental stress in plants, such as attacks by pathogens, UV radiation, mechanical injury, and heavy metal pollution[4]. The *trans*-isomer of resveratrol has the potential to be a beneficial treatment for inflammatory diseases[5], atherosclerosis, hypertension, diabetes, obesity, and aging[6-11]. Resveratrol has also been studied for its benefits as an anti-cancer agent[12]. The chemo-preventive activity of resveratrol has been investigated in various tumor cell lines[13]. Resveratrol has antimutagenic effects inhibiting all three main stages of carcinogenesis: tumor initiation, promotion, and progression[13,14]. Specifically, resveratrol shows its antioxidant properties by inhibiting free radical formation in a dose-dependent manner in cultured mouse hepatoma cells[13]. Researchers discovered that resveratrol has adverse effects on human tumorigenic cells, which were observed during initiated apoptotic cell death in HL60 leukemia cells and T47D breast carcinoma cells[15]. In a study by Lee and associates, *trans*-resveratrol affected procarcinogen and toxin-producing cytochrome P450 enzymes, such as CYP1B1, CYP1A1, and CYP1A2[16]. Researchers concluded that a time-dependent and concentration-dependent relationship existed between *trans*-resveratrol and the catalytic activity of CYP1B1 and CYP1A1. A direct decline in the catalytic activity of the noted enzymes occurred as the exposed concentration of resveratrol was steadily increased. In addition, a mechanism-based relationship was displayed by trans-resveratrol's ability to disrupt the substrate conformation associated with the CYP1A2 enzyme[16].

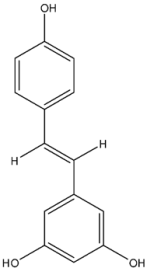

Figure. 1 Chemical structure of resveratrol (*trans*-3,5,4'-trihydroxystilbene)[21]

In addition to its effects on cancer, many studies have also supported that resveratrol can slow the progression of other illnesses, such as cardiovascular disease[17]. In naturally occurring sources, such as various juices of dark fruit, resveratrol has been studied to determine its potential medicinal properties[6]. In previous studies, extraction and quantification methods have been used to measure the concentrations of resveratrol in different wine varietals[18-23]. Specifically, red wine has been studied for its benefits in reducing mortality from coronary artery disease[24,25]. The population of France has seen decreased levels of cardiovascular disease (CVD) despite the similarities in dietary and lifestyle habits to other populations with heightened risks of CVD[25,26]. Trihydroxystilbene synthase produces the compound resveratrol to defend against pathogenic invasions and respond to ultraviolet radiation[27].

The Cardiovascular Research Center, University of Connecticut Health Center reports that resveratrol promotes cardiovascular health by inhibiting platelet aggregation, thereby preventing atherosclerosis[28]. Decreased collagen-induced platelet aggregation was shown with resveratrol treatment (0.15-0.25 mmol/L). Also, resveratrol has provided anti-inflammatory activity by inhibiting cyclooxygenase 1 (COX 1) and cyclooxygenase 2 (COX 2). The suppression of T-cell and B-cell activity was also exhibited[28].

Based on previous studies, our research will focus on liquid-liquid extraction of resveratrol from different R. W. Knudsen fruit and vegetable juices. After extraction the concentration of resveratrol will be calculated from the absorbance measurement at 310 nm on a UV spectrophotometer. This study will compare resveratrol extract concentrations for each type of juice with their measured reduction potential. The Ferric Reduction Antioxidant Potential (FRAP) assay will be utilized for reduction potential measurement. This analysis will suggest which type of juice contains the most resveratrol as well as the highest reduction potential.

METHODS

Liquid-liquid phase techniques were implemented to achieve resveratrol extraction and isolation[29]. Three trials were completed on five R.W. Knudsen Family Juices (Nexus Capital Management): cranberry, blueberry, black cherry, beet, and tart cherry. The liquid-liquid phase extraction method was conducted as follows;

each bottle of juice was allowed to warm to ambient temperature (N=27). A 250 mL juice sample was then poured into a 500 mL separatory funnel and washed twice with 100 mLs of chloroform. At the end of these washes, the organic phase was discarded. The remaining aqueous phase was extracted three times using 100 mLs of ethyl acetate. After each extraction, the organic phase was collected and combined. After these extractions, the aqueous phase was discarded. The combined ethyl acetate extracts were washed twice with 100 mLs of saturated sodium chloride. The resulting aqueous phase was discarded. The samples were left uncovered overnight in a hood, to allow the ethyl acetate to evaporate, which completed the resveratrol isolation. The remaining crystals were reconstituted in 250 mLs of a 12% solution of HPLC grade acetonitrile and water. The absorbance was obtained from the reconstituted resveratrol solutions at a wavelength of 310 nm (Perkin-Elmer Lambda 25). A Ferric Reduction Antioxidant Potential (FRAP) assay kit assessed each extract's oxidation potential[30-34]. All solvents and reagents were from Sigma Aldrich; St. Louis, MO.

DISCUSSION

Figure 2 represents a standard curve for resveratrol (98%, Thermo Fisher Scientific) of absorbance versus concentration at 310 nm. The standard concentrations ranged from 1.0 mg/mL to 20.0 mg/mL. The standard curve was used to convert the sample absorbance to concentration (mg/mL) of resveratrol for each juice extract.

Figure 3 analyzes the data collected from this study for the mean of three trials for each juice extract. The results suggest that Cranberry contains the highest concentration of resveratrol, at mean and standard deviation of 36.29 +/- 0.027 mg/mL. Blueberry had the second-largest resveratrol concentration, 21.86 +/- 0.063 mg/mL. Tart Cherry contained 17.73 +/- 0.062 mg/mL, the third greatest concentration. Moreover, Black Cherry had the fourth highest concentration, 13.99 +/- 0.102 mg/mL. Beet contained the lowest concentration in our study at 0.1613 +/-0.0091 mg/mL.

Figure 4 reports the antioxidant reduction potential using the Ferric Reducing Antioxidant Potential (FRAP) Assay. The analysis of our juice extracts resveratrol concentration directly correlates with the type of juice's antioxidant potential. For example, cranberry has the highest amount of resveratrol and the highest reduction potential, making it the best antioxidant in this study.

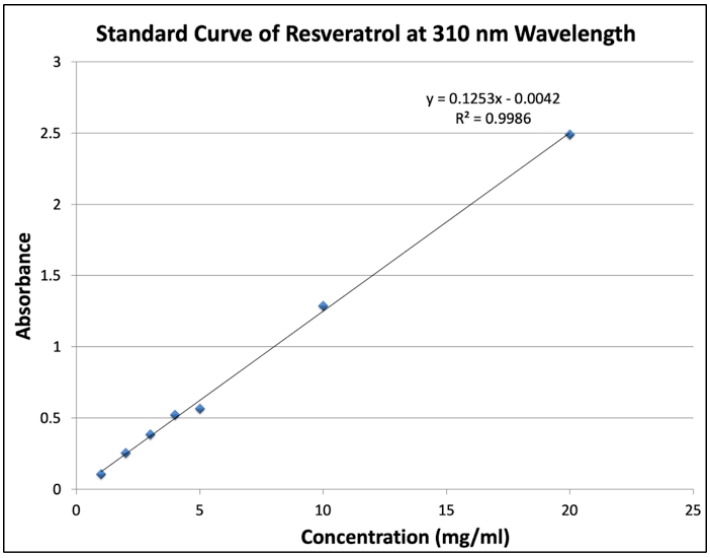

Figure 2: Standard Curve of Resveratrol

The low standard deviations suggest that the extractions and data measurements from these trials were reproducible. However, the black cherry extracts had larger standard deviations, which may be due to extraction technique. Moreover, this experiment supports our hypothesis that dark fruit juices contain a higher concentration of resveratrol as well as other phenolic compounds. The next step in verification of the current data for this study, will be to analyze each extracted sample using High Pressure Liquid Chromatography, HPLC, to further quantify and compare the presence of resveratrol, along with any other compounds that may absorb at 310 nm..

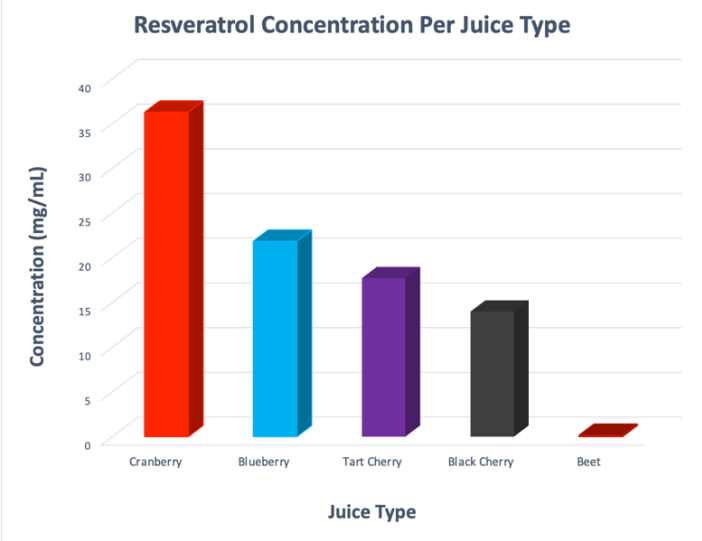

Figure 3: Resveratrol Concentration (mg/mL) Per Juice Type

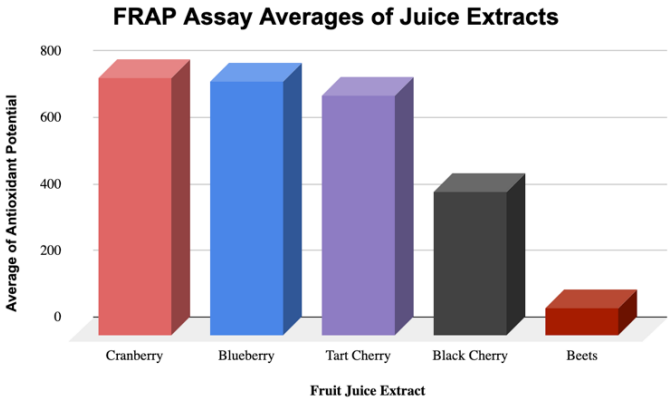

Figure 4: Antioxidant Potential Average

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