GRAPE PIE AS A FUNCTIONAL FOOD:

EXAMINING RESVERATROL CONTENT

**Purpose**

 In this lab, a well-known and appreciated dessert – the pie – was transformed into a functional food fortified with the phytochemical specifically found in various varieties of red grapes, resveratrol. The goal of this lab was to create a grape pie with the pleasing sensory characteristics common in a typical pie, though using atypical ingredients to lend to a more nutritionally sound and resveratrol-rich product.

**Background**

According to the CDC’s *Health, United States, 2012: In Brief*, the leading causes of death in adults continue to be heart disease and cancer. Specifically, in 2010, death from heart disease accounted for approximately 307,000 deaths in men and 290,000 deaths in women.[[1]](#endnote-1)1 Heart disease is highly correlated with risk factors such as high blood pressure and obesity. In 2010, nearly half of the adult hypertensive population in the US had uncontrolled high blood pressure.[[2]](#endnote-2)1 Furthermore, the prevalence of obesity continues to climb over the years, with 35.9% of adults and roughly 18% of adolescents at or above the 95th percentile for BMI.[[3]](#endnote-3)1

While conventional medicine has certainly showed its strengths in treating various chronic illnesses and beyond, homeopathic medicine has as well and continues to. The subsequent influence has been a shift from purely supplements and chemically-derived medicines to reliance on the natural compounds that exist in whole foods. These natural compounds may include essential nutrients – vitamins and minerals – and fiber, but also phytochemicals, whose benefits may even exceed those of vitamins and minerals. According to the Phytochemical Information Center (PIC) of the Produce for Better Health Foundation (PBH), phytochemicals are present in all edible portions of plants (i.e. fruits and vegetables), specifically concentrated in the skin and sometimes seeds.[[4]](#endnote-4) The role of phytochemicals in the body depends not only on the type of phytochemical, but also the way in which the plant cultivation process alters phytochemical concentration. However, it has been shown that phytochemicals in whole foods work concurrently with vitamins, minerals, and fiber to promote health and lower disease risk, perhaps more so than medication alone. The important aspect to note is the increased emphasis on prevention rather than dealing with treatments for various illnesses down the road.

The family of phytonutrients known as polyphenols are among the most abundant antioxidants in the body. While nonessential, they have been shown to play a significant role in reducing oxidative stress associated with cardiovascular disease and various forms of cancer, among other roles. Within the polyphenol family lies a particular stilbene known for its presence in red wine. This phytonutrient, Resveratrol (*trans-3,5,4'-trihydroxystilbene*), is responsible for the “French Paradox” observed in the Mediterranean population, who have maintained a low prevalence of cardiovascular disease despite a diet rich in saturated fat.[[5]](#endnote-5) The subsequent research has shown a wide range of results, though a significant amount does support notion identified in the French Paradox that moderate wine consumption can reduce oxidative stress associated with cardiovascular disease. Beyond reducing oxidative stress, research on resveratrol has indicated a variety of other mechanisms in which this antioxidant can work in the body, including inhibition of platelet aggregation, vasodilation, anti-inflammation, activation of sirtuins, and induction of cancer cell apoptosis.[[6]](#endnote-6)3 Put a simpler way, resveratrol has been shown to have cardioprotective, neuroprotective, anticancer, anti-inflammation, anti-aging, and anti-microbial properties.[[7]](#endnote-7)4

Resveratrol is present in grape skins and seeds, red wine, peanuts, and *vaccinium* berries (i.e. cranberries, blueberries, bilberries, etc.), as well as many herbal medicines such as Itadori tea made from the Japanese knotweed plant.[[8]](#endnote-8)5 According to Dey et al., the use of grape extracts containing resveratrol to promote health can actually be traced back over 2000 years ago in Indian culture.[[9]](#endnote-9)3 In these times though, the grape extracts were referred to as “darakchasava” and the presence of polyphenols like resveratrol were not discovered until years later via high-performance liquid chromatography analysis.[[10]](#endnote-10)3 It was in the 1990s that resveratrol gained popularity in the natural medicine sphere when the “French Paradox” came to be.[[11]](#endnote-11)3

The chemical structure of resveratrol consists of two aromatic rings attached via ethylene, with the rings also containing three additional hydroxyl groups. The compound exists in both cis- and trans-isomeric forms though benefits to health are primarily known about the trans-isomeric form of resveratrol (see Figure 1). Resveratrol can be efficiently absorbed in the body, lending to its multitude of positive health effects. However, its rapid metabolism into glucuronide and other sulfate metabolites by the liver following oral administration lends to low bioavailability.[[12]](#endnote-12)5 Generally, this metabolism occurs within 2-24 hours though Bode et al. found that this may be highly dependent on interindividual differences in gut microbiota, which can influence how much of the compound is excreted in urine versus absorbed.[[13]](#endnote-13)6 Results of their study actually indicated the extent of urinary excretion of trans-resveratrol ranging from 36.3% to 82.9% of the orally administered dose after a 24-hour period.[[14]](#endnote-14)6 The significance of this study was that it stressed the importance of being knowledgeable of the health-promoting effects of resveratrol on the body, but also the effects of the metabolites of resveratrol – which could potentially be very different. Murtaza et al. cited that the bioavailability of resveratrol can be enhanced with fatty acids (i.e. albumin) due to its lipophilic nature, and also that other polyphenols such as quercetin can enhance its bioavailability by preventing excessive sulfating in the liver.[[15]](#endnote-15)7 Enhancing the bioavailability of resveratrol and other polyphenols is a topic of research gaining popularity, for the ability to increase bioavailability could subsequently increase the extent to which it is beneficial in the body.

Figure 1 – Structures of trans- and cis-resveratrol (Dey et al., 2011, p.71)

As a significant polyphenol in wine, it must be noted that resveratrol exists in much more significant concentrations in red, rather than white, wine due to the wine-making process. Specifically, as cited by Murtaza et al., approximately 1.8 g/L of antioxidants are present per bottle of red wine while a bottle of white wine contains 0.2-0.3 g/L of antioxidants.[[16]](#endnote-16)7 In processing and fermentation, polyphenols are released from the skin and seeds of grapes, so the removal of skin and seeds prior to making white wine significantly limits the polyphenol concentrations.[[17]](#endnote-17)7

Although red wine is a noteworthy functional food containing resveratrol, the concentration of resveratrol is significantly dependent on the variety of grape used and the environment in which it was cultivated in. In a study on the resveratrol content in a variety of Italian wine grapes, it was found that Barbera cultivars showed the greatest trans-resveratrol content, followed by Franconia, Negroamaro, Corvina, and Marzemino.[[18]](#endnote-18)8 However, these varieties are uncommon to the average wine consumer. Vincenzi et al. did note that their study indicated average resveratrol concentrations in common wine varieties such as Merlot, Pinot Noir, and Cabernet Sauvignon – with Merlot being significantly higher than the others.[[19]](#endnote-19)8 Despite these findings, Vincenzi et al. claimed that clusters of grapes extracted from the same vineyard of the same variety sometimes showed quite different concentrations of resveratrol, indicating that other environmental factors, such as sun exposure, come into play as well.[[20]](#endnote-20)8

Although substantial discussion surrounds red wine and health, it is important to note that resveratrol concentrations may actually be greater in other forms of grapes than wine varieties. In a study assessing resveratrol content in grape berry skins of 75 cultivars of grapes (39 table grapes, 17 wine grapes, 11 juice grapes, and 5 rootstock grapes), it was found that rootstock cultivars had the highest amount of resveratrol in berry skins, followed by some juice cultivars – namely Concord juice grapes.[[21]](#endnote-21)9 These findings are significant in that they show that resveratrol can be consumed in relatively decent quantities in functional foods other than alcohol. The reason why red wine is promoted is that it is a substance formed by highly concentrating the grapes – just as pure Concord grape juice is formed.

Oxidative stress and an increase in inflammation are closely related to issues arising in cardiovascular disease, atherosclerosis, diabetes mellitus, chronic obstructive pulmonary disease, and cancer. It occurs in situations where there is an excess release of reactive oxygen species (ROS) that are responsible for activating inflammatory mediators, provoking further oxidative stress.[[22]](#endnote-22)10 Resveratrol and other polyphenols as antioxidants possess the potential to reduce ROS and other free radical activation of oxidative stress and inflammation, and also increase levels of anti-inflammatory cytokine genes.[[23]](#endnote-23)11 According to Chuang et al., the abundance of hydroxyl groups attached to polyphenol aromatic rings are what possess this ability.[[24]](#endnote-24)11 Furthermore, the activation of Sirtuins (SIRT1) – a class of regulatory proteins associated with anti-aging – by resveratrol leads to a reduction in inflammation.[[25]](#endnote-25)11

Exhaustive exercise bouts can also lead to ROS production, and subsequent lipid and protein oxidative damage to muscle tissue. In a study by Dalla Corte et al., organic grape juice was assessed for its ability to reduce oxidative stress induced by an exhaustive exercise bout in rats.[[26]](#endnote-26)12 Results indicated that organic grape juice intake proved to be effective in protecting brain, skeletal muscle, and blood from oxidative stress in these situations.[[27]](#endnote-27)12 However, in another study performed by Gliemann et al. where healthy, inactive men were given resveratrol supplementation and assigned a high-intensity exercise training program, the opposite was found.[[28]](#endnote-28)13 More specifically, resveratrol actually abolished positive effects of exercise on cardiovascular risk factors such as LDL and triglyceride concentrations in the blood.[[29]](#endnote-29)13 The conflicting results obtained by these two studies demonstrate the need for more evidence of resveratrol’s cardioprotective capacity in humans, rather than just rat models – an issue that is prevalent concerning much of the resveratrol research to date.

The presence of ROS is also relevant in discussing neuroinflammation associated with neurological disorders, such as traumatic injury, stroke, depression, and other neurodegenerative diseases. Microglia in the Central Nervous System (CNS) act at the onset of injury, secreting pro-inflammatory agents such as prostaglandins, chemokines, cytokines, proteinases, nitric oxide (NO), and reactive oxygen species (ROS), which in gross accumulation can contribute to further neuronal damage.[[30]](#endnote-30)9 In a study by Zhang et al. on the neuroprotective role of resveratrol, it was reported that resveratrol inhibited microglial activation and the subsequent release of pro-inflammatory agents in rat cortical microglia.[[31]](#endnote-31)9 This further represents evidence of anti-inflammatory mechanisms in which resveratrol acts.

Inflammation is also common when it comes to chronic conditions such as diabetes mellitus, where regulation of glucose and insulin are not working optimally. Mullin cited evidence that resveratrol can decrease serum glucose levels, increase the time it takes for glucose to peak, and decrease insulin resistance succeeding consumption of a meal.[[32]](#endnote-32)10 Insulin resistance is mediated through inflammatory mechanisms, therefore the previous evidence provided for resveratrol and other polyphenols having an anti-inflammatory role is very relevant here as well. Furthermore, Chuang et al. cited a number of research studies all suggesting that grape polyphenols inhibit pro-inflammatory factors – which normally increase inflammatory gene expression – and also inhibit negative regulators of insulin signaling.[[33]](#endnote-33)11 Due to the fact that diabetes mellitus and obesity are highly correlated – among other conditions discussed including cardiovascular disease and atherosclerosis – these polyphenol benefits can act in a wide range of chronic disease circumstances.

Cancer may be considered a different category from the chronic diseases readily discussed above, though resveratrol has also been shown to play a significant role here. In acting as an anti-cancer agent, resveratrol has been shown to induce apoptosis of cancerous lymphoma cells via multiple pathways and altering gene expression.[[34]](#endnote-34)15 More specifically in the case of breast cancer, resveratrol has been shown to exert either estrogenic or anti-estrogenic effects depending on the concentration of the dose administered. According to Castillo-Pichardo et al., resveratrol given at high concentrations can have an inhibitory effect on estrogens while resveratrol given at low concentrations can have a promotional effect on estrogens – encouraging tumor growth and metastasis.[[35]](#endnote-35)4 The significance of these study results lie in the fact that most dietary sources of resveratrol – wine, grapes, peanuts, etc. – do not provide especially large concentrations of resveratrol, and it is in these low concentrations that research indicates adverse effects in breast cancer. On the other hand, research on other forms of cancer such as colorectal, lung, kidney, bone, and ovarian has demonstrated the therapeutic anticancer role that resveratrol can play, even when administered in a fairly low dose concentration – refuting the above evidence concerning the negative effects in breast cancer. Murtaza et al. cites that in cases of prostate cancer, it has been shown that resveratrol may work by increasing the release of ROS, inhibiting anti-apoptotic factors, and up-regulating pro-apoptotic factors.[[36]](#endnote-36)7 Evidence of a reduction of risk of prostate cancer by 60% has been observed in men who consume four glasses of red wine per week.[[37]](#endnote-37)7 For these reasons, the topic of resveratrol in relation to cancer requires a great amount of research for its mechanisms of action to be fully understood and for safe dose concentrations to be established in treating these conditions.

At this point in time, there has been no definitive answer in place as to how much resveratrol is a sufficient quantity to be consuming per day to reap the benefits researched thus far. Due to the relatively low concentration that exists in red wine and grapes, among other food sources, this may be the optimal way to introduce resveratrol into one’s diet in a safe manner. The interesting point to note ties back to the “French Paradox;” it may be possible that other polyphenols present in red wine are those which are contributing to low cardiovascular disease, more so than resveratrol alone. Additionally, the diet patterns in the Mediterranean, including a greater percentage of whole food consumption rather than processed foods, may be a significant confounding variable that should be further addressed.

**Materials**

*Ingredients*

½ package all-natural graham crackers

1 tbsp sesame tahini

1 1/2 tbsp pure maple syrup

1 tbsp water

1 ½ lb black seedless grapes[[38]](#footnote-1)\*

1/2c light brown sugar

Juice of ½ fresh lemon

1 tsp cinnamon

2 tbsp cornstarch

2tbsp cold water

*Additional Materials*

Food Processor

9-inch glass pie dish

Canola oil spray (*Pam* was used in this experiment)

Various utensils (measuring spoons/cups, mixing spoons, small knife, whisk)

Medium saucepan

1-2 medium mixing bowls (optional)

**Procedure**

First, the oven was preheated to 350 degrees in preparation for the graham cracker crust. The graham crackers were ground to a fine consistency in the food processor and then the sesame tahini, pure maple syrup, and water were added, processing until well-blended. This mixture was pressed firmly into a lightly greased (with canola oil spray) 9-inch glass pie dish and baked for 25 minutes until it appeared browned/golden.

While the crust cooled, the grape filling was made. The grapes were measured out accordingly, thoroughly washed under cool water, and pat dry with a paper towel. Then, the grapes were cut into roughly fourths and added to a medium saucepan. The grapes were brought to a boil over high heat, at which point the heat was turned down to medium and covered for 15-20 minutes stirring occasionally to prevent sticking. The sugar, lemon juice, and cinnamon were whisked together in a small bowl, added, and the mixture was cooked for an additional 2 minutes.

In another small bowl, the cornstarch was mixed thoroughly with the cold water to create a slurry and then poured into the simmering grapes. Continuous stirring was necessary to prevent clumping and ensure the creation of a clear, thick mixture after roughly two minutes passed. Then, the mixture was removed from the heat to cool. Once cool, the grape mixture was spooned into the pie crust and refrigerated 3-4 hours before tasting.

**Results**

On coming out of the oven, the crust maintained a crisp, cookie-like consistency. When cooled and filled with the grape filling, the crust supported it well. The Concord grape pie filling was slightly firm after chilling and had a pleasing gel-like consistency. The taste was sweet and tart, yet had a mild warmth from the cinnamon and light brown sugar. I evaluated the pie after tasting using a 5-point acceptance test for common sensory categories:

{(1) Dislike very much, (2) Dislike moderately, (3) Neutral, (4) Like moderately, (5) Like very much}

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Texture | Appearance | Smell | Flavor | Aftertaste | Overall Acceptability |
| Concord Grape Pie | 4 | 5 | 4 | 5 | 5 | 5 |

According to the Linus Pauling Institute, fresh grapes contain approximately 0.24-1.25 milligrams of resveratrol per cup (160g) of the fruit.[[39]](#endnote-38)14 If this is the case, the grape pie made with two cups of fresh black grapes could contain an average resveratrol content of 1.49 milligrams, which is approximately 0.18625 milligrams of resveratrol per slice. Considering the fact that many resveratrol supplements contain between 10 and 50 milligrams of resveratrol per dose, the quantity consumed in the pie is extremely small.

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| **Nutrition Facts****Jackie’s Grape Pie** |
|   8 Servings |
| http://assets3.sparkrecipes.com/spacer.gif |
| **Amount Per Serving** |
| http://assets3.sparkrecipes.com/spacer.gif |
| **Calories** | 133.7 |
|  |
|  |
| **Total Fat** | 2.3 g |
| http://assets3.sparkrecipes.com/spacer.gif |
|     |   Saturated Fat | 0.5 g |
| http://assets3.sparkrecipes.com/spacer.gif |
|     |   Polyunsaturated Fat | 0.4 g |
| http://assets3.sparkrecipes.com/spacer.gif |
|     |   Monounsaturated Fat | 0.7 g |
| http://assets3.sparkrecipes.com/spacer.gif |
| **Cholesterol** | 0.0 mg |
| http://assets3.sparkrecipes.com/spacer.gif |
| **Sodium** | 62.9 mg |
| http://assets3.sparkrecipes.com/spacer.gif |
| **Potassium** | 39.7 mg |
| http://assets3.sparkrecipes.com/spacer.gif |
| **Total Carbohydrate** | 29.2 g |
| http://assets3.sparkrecipes.com/spacer.gif |
|     |   Dietary Fiber | 0.7 g |
| http://assets3.sparkrecipes.com/spacer.gif |
|     |   Sugars | 17.3 g |
| http://assets3.sparkrecipes.com/spacer.gif |
| **Protein** | 1.2 g |
|  |
|  |
|   Vitamin A | 0.1 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Vitamin B-12 | 0.0 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Vitamin B-6 | 0.3 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Vitamin C | 2.5 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Vitamin D | 0.0 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Vitamin E | 0.2 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Calcium | 5.4 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Copper | 1.8 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Folate | 0.6 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Iron | 4.0 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Magnesium | 1.9 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Manganese | 3.8 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Niacin | 2.3 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Pantothenic Acid     | 0.2 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Phosphorus     | 3.3 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Riboflavin | 1.9 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Selenium | 0.1 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Thiamin | 3.1 % |
| http://assets3.sparkrecipes.com/spacer.gif |
|   Zinc | 1.8 % |
| http://assets3.sparkrecipes.com/spacer.gif |
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|  |
| \*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs. |

**Discussion of Results**

The first step in creating the grape pie – forming the crust – proved to be tedious in nature though made sufficiently easier with the use of a food processor. The proper amount of tahini and water was essential in creating a crust that was not too moist, but just moist enough to retain a shape when pressed into the pie dish. I utilized a hand roller over a piece of wax paper to flatten the crust in the pan and ensure that the roller did not stick.

The second step – creating the grape filling – was much simpler. Due to the seedless nature of the black grapes used, seeding was not necessary, which eliminated a potentially difficult process from the procedure. It should be noted that the resveratrol contained in the seeds is lost in this way, however the large amount of resveratrol in the delicate grape skins *is* consumed in the pie. The purpose of chopping the black grapes in fourths was to disperse the skins more evenly throughout the filling, but also to allow the fluid to more easily release during the heating process on the stove.

In using the slurry created with cold water and cornstarch, I quickly recognized the necessity to stir the mixture adequately after noticeable clumps began to form shortly after adding to the simmering grape mixture. A whisk remedied this well, breaking up any remaining clumps with relative ease.

The finished product was certainly an unconventional sort of pie – using a graham crust and a refrigerated grape filling – though very pleasant. The dark grapes lent a rich depth of flavor and a sweetness that required a relatively small amount of added sugar. I believe this amount could have even been reduced further without lessening the appeal of the pie.

In the future, a variation on this recipe could use a food processor or immersion blender to break down the skins more so and create a smoother product. This may lend to a more pleasing textural perception for some individuals. Also, while refrigerating the pie resulted in a slightly firm grape filling, I would be curious to see what freezing the pie would do to the sensory perceptions. In my opinion, frozen grapes have a rich, creamy texture as compared to unfrozen grapes, so this may add pleasing textural qualities as well.

**Conclusion**

Despite a few adverse findings, the benefits of resveratrol can be readily seen in its ability to act as a potent antioxidant in conditions such as cardiovascular disease, diabetes mellitus, and various forms of cancer. In the future, research into ideal concentrations, environmental factors, and side effects of use should be furthered. Also, due to the fact that much of the research concerning resveratrol’s activity in the body has been done in animal models, more research is necessary in the human population to bolster current hypotheses and reject others. If further supported, resveratrol could provide health professionals with an alternative, natural treatment solution outside the sphere of conventional medicine. For the time being, the benefits of functional foods such as grapes still stand strong, providing a host of essential nutrients, fiber, and antioxidants.

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38. \* Due to seasonal factors, black grapes were used in this experiment as opposed to Concord grapes which truly contain the greatest amount of resveratrol. The resveratrol concentration of the final product (as identified on page 10) was estimated based on approximate resveratrol concentration in general red/black table grapes, therefore it is a correct estimation for this recipe. [↑](#footnote-ref-1)
39. [↑](#endnote-ref-38)