

Antioxidant Capacity and Anticancer Properties of Red Raspberry

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Abundant evidence suggests that regular consumption of fruits and vegetables reduces the risks of chronic diseases such as cancer and cardiovascular disease. In the process of converting food into energy, the body naturally produces oxygen radicals, which can damage DNA and enzymes and oxidize cell membrane lipids. Accumulated damage from these radicals is thought to lead to cancer, heart disease, and other diseases. Natural antioxidants in foods help counteract these oxygen radicals and protect us from their damaging effects. Fruits and vegetables are especially rich in a variety of antioxidants called phytochemicals such as flavonoids, which give many fruits their characteristic colors. Intake of sufficient amounts of antioxidants helps the body balance the demands of producing the energy it needs and producing damaging oxygen radicals. Research on raspberries revealed high levels of phytochemicals, antioxidant activity, and anticancer activity.

Raspberries are high in phenolic phytochemicals, particularly flavonoids such as anthocyanin pigments, which give raspberries their deep red color. These

potent antioxidants, along with naturally high levels of Vitamin C, minerals, and fiber, make raspberries especially good for people. In this study, the ability of raspberry fruit extracts to inhibit liver cancer cell growth in a test tube and the total antioxidant activity of four raspberry varieties was investigated. We also looked at the relationship between their total phenolics and flavonoids content and their antioxidant and anticancer activities. In addition, the relationship between fruit color and antioxidant activity was examined. Ultimately, the relationship between the food we eat and our long-term health needs to be better understood. This research gives more insight into the health benefits of fruits and vegetables.

Phytochemical Content

Fresh Heritage, Kiwigold, Goldie, and Anne raspberry fruit were obtained from plantings on the New York State Agricultural Experiment Station (Cornell University, Geneva, NY). Heritage is a dark red, fall-bearing variety that was developed at the Experiment Station in the 1960s. It remains the most widely

This study suggests that the phytochemicals in raspberries have significant antioxidant and anticancer activity. Darker raspberries show greater antioxidant activity than lighter ones but not significantly greater anticancer activity. Clearly other as yet unknown compounds are also involved.

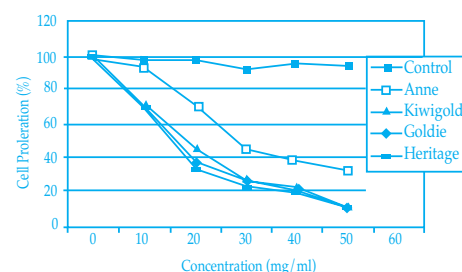


Figure 1.: Antiproliferative effects of four raspberry varieties versus apple and a control.

planted raspberry in the world today. Kiwigold and Goldie are both sports of Heritage with amber colored fruit. They display a mutation in the anthocyanin biosynthesis pathway that reduces the pigment content in the fruit. Both Kiwigold and Goldie are genetically identical to Heritage except for their anthocyanin content. Anne is a yellow-fruited variety developed at the University of Maryland. Anthocyanins are a type of flavonoid pigment common in most plants. Flavonoids are, in turn, part of a broader class of

TABLE 1

Total phenolics, flavonoids, fruit color, optical sensor reading ratios, antioxidant activity, and cancer cell antiproliferation activity of Heritage, Kiwigold, Goldie, and Anne raspberry fruit extracts.

Raspberry Variety	Total Phenolics (mg gallic acid equivalents/100g)	Total Flavonoids (mg catechin equivalents/100g)	Fruit Color	Optical Sensor (a/b)	Antioxidant Activity (EC ₅₀ ug/ml)	% Inhibition (50 mg/ml equivalent)	Inhibition EC ₅₀ (mg/ml)
Heritage	512.70 ±4.66	103.41 ±2.04	Dark red	4.92	312.06 ±28.27	87.96 ±0.19	14.83 ±0.14
Kiwigold	451.06 ±4.45	87.33 ±1.83	Amber	2.84	521.71 ±36.91	87.55 ±0.98	14.39 ±0.25
Goldie	427.51 ±7.51	84.16 ±1.82	Amber	2.24	544.52 ±12.30	89.43 ±0.11	11.69 ±0.61
Anne	359.19 ±3.35	63.53 ±0.65	Yellow	0.18	801.79 ±64.93	70.33 ±1.15	32.88 ±0.78

phytochemicals called phenolics. In previous research on apples (Liu et al., 2001) and other crops it was found that the total content of phenolics and/or flavonoids in fruits is a good indicator of total antioxidant activity. When the content of these compounds in raspberry was measured, Heritage proved to have the highest total phenolic and flavonoid content (513 mg/100g and 103 mg/100g fruit, respectively) of the varieties tested (Table 1). The total phenolic and flavonoid content of Kiwigold (451 mg/100g and 87 mg/100g fruit, respectively) and Goldie (428mg/100g and 84mg/100g fruit, respectively) were similar to each other but lower than Heritage (Table 1). Anne had the lowest total phenolic and flavonoid content (359mg/100g and 64 mg/100g fruit, respectively) (Table 1) of the varieties tested.

Raspberry Extracts and Cancer Cell Growth

To determine the effect of raspberry extracts on the growth of HepG2 liver cancer cells in a test tube, raspberry extracts were added to HepG2 liver cancer cells in amounts equivalent to 0, 1, 5, 10, 20, 30, 40, 50 mg of raspberry and incubated for 96 hours. After 96 hours, cell growth was determined. All varieties tested showed obvious growth inhibition. Cancer cell growth was inhibited in a dose dependent manner. As more extract was added, the cells grew less (Figure 1).

At the 50mg dose, Goldie, Heritage and Kiwigold all showed nearly 90% reduction of cancer cell growth (89%, 88% and 88%, respectively) (Figure 1, Table1). Even Anne, the raspberry variety showing the lowest anticancer activity, produced a 70% reduction in cancer cell growth (Figure 1, Table 1).

The dose needed to reduce cancer cell growth by 50% (EC_{50}) was also calculated. That is, a lower EC_{50} is indicative of greater inhibitory activity. Goldie extracts had the lowest EC_{50} of antigrowth activity (11.69mg/ml) of the varieties tested, but was not significantly different from Kiwigold (14.39mg/ml) or Heritage (14.83mg/ml) (Table1). Anne, having the highest EC_{50} of antigrowth activity (32.88mg/ml), showed significantly lower inhibitory activity (Table 1).

The anticancer activity was then compared to the total phenolic and flavonoid content of the raspberries. Heritage, Kiwigold, and Goldie, which all have a common genetic background, were different in their phenolic and flavonoid content but similar in their anticancer activ-

ity. This suggests that there are phytochemicals in addition to phenolics that are active against cancer cells. Further research into the phytochemical components of raspberry is needed to determine what these additional anticancer compounds are.

Total Antioxidant Activity

Antioxidants are thought to work in the body in a preventative manner by neutralizing destructive oxygen radicals. The potential for a food to counteract these oxygen radicals can be determined by measuring its total antioxidant activity. An assay called the Total Oxyradical Scavenging Capacity (TOSC) measures the ability of food extracts to counteract a known oxidizing chemical. This activity is expressed as the median effective dose (EC_{50}). The EC_{50} value in this test represents the dose needed to have a 50% effect in the assay much like an LD_{50} (Lethal Dose 50%) used to rate pesticide toxicity. A lower EC_{50} value indicates that a lower dose is needed to produce a 50% reduction in oxidation in the assay.

The total antioxidant activity of the phytochemical extracts from raspberry cultivars was measured by the TOSC assay. Heritage had the lowest EC_{50} of total antioxidant activity (312mg/ml) followed by Kiwigold (522mg/ml) and Goldie (545mg/ml) (Figure 2 and Table 1). Anne had the highest EC_{50} (802mg/ml) (Figure 2 and Table 1). The data shows that Heritage had the highest total antioxidant activity and Anne had the lowest antioxidant activity of the varieties tested.

Total Phenolic/Flavonoid Content Relationship to Antioxidant Activity

The total phenolic and flavonoid content correlated highly with total antioxidant activity in the raspberry varieties tested. Higher levels of phenolics and flavonoids corresponded to higher antioxidant activity (Table 1). This indicates that the phenolics and flavonoids in raspberry contribute significantly to their overall antioxidant capacity and that the levels of these compounds can be used as an indicator for the total antioxidant activity of various raspberry varieties.

Fruit Color as an Antioxidant Indicator

Various researchers have analyzed the total antioxidant activity of many fruits and vegetables. A trend seen is that

more highly pigmented fruits and vegetables have higher antioxidant activity (Figure 3). Black raspberries, blackberries, red raspberries, blueberries, and strawberries are red or purple in color and all contain high levels of anthocyanin pigments. Anthocyanins, which have high antioxidant activity, are some of the many flavonoids in raspberry and other fruits. This led us to believe that anthocyanin content, i.e. fruit color, could be used as an indicator of antioxidant activity.

To determine the relationship of fruit color to total phenolic/flavonoid content and antioxidant activity, the juice of each of the four raspberry varieties was measured with a colorimeter. It measures the relative brightness and color components of the sample as numerical values of a, b, and L. Previous research showed that, of the components measured, the ratio of a/b correlates well to anthocyanin pigment content in raspberries (Moore, 1997). Darker red varieties with higher antho-

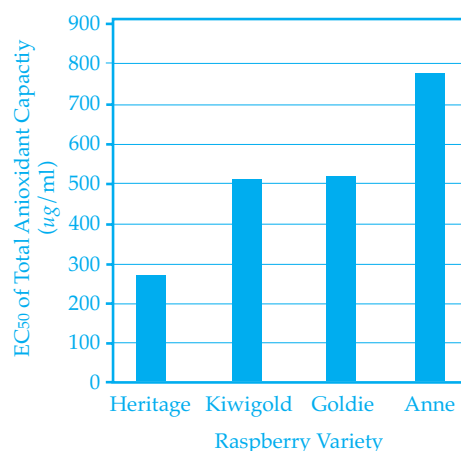


Figure 2: EC_{50} of total antioxidant capacity of four raspberry varieties. (A lower EC_{50} indicates higher antioxidant activity.) Anne has the lowest antioxidant activity, which corresponds to the highest EC_{50} value.

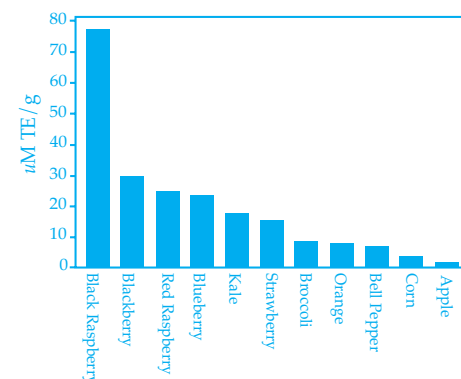


Figure 3: Antioxidant capacity of selected fruits and vegetables. (umole Trolox Equivalents (TE)/g) Trolox is a Vitamin E analog used in calculating relative antioxidant activity. (Payne, 2000)

cyanin content have a higher a/b ratio. These higher ratios also correspond to higher phenolic and flavonoid content in the varieties tested. Heritage, the darkest red berry, had the highest ratio (4.92) (Table 1). Kiwigold and Goldie were intermediate in color and in a/b ratio (2.84 and 2.24, respectively) and Anne had the lowest a/b ratio (0.18) with yellow fruit (Table 1). This was the same ranking as the total phenolic and flavonoid content of the varieties and the total antioxidant activity. This indicates that a darker red color can be used as an indicator of phytochemical content and antioxidant activity in raspberry. Using a hand-held colorimeter, instant field estimates of phytochemical content and antioxidant activity can be made. This tool will aid in the development of future varieties with enhanced health attributes.

Summary

It is well known that fruits and vegetables are good sources of antioxidants. Previously, most investigators focused mainly on the Vitamin C, Vitamin E, and b-carotene content of fruits. However the major components of fruit that act as antioxidants are phytochemicals such as phenolic compounds like flavonoids. Research shows that the Vitamin C in apples with skin accounts for only 0.4% of the total antioxidant activity indicating that almost all the antioxidant activity is from other phytochemicals in the fruit (Liu et al., 2001). Raspberries contain approximately 25 mg of Vitamin C per 100g of fruit. This accounts for a small portion of the antioxidant activity found in raspberry. Therefore, the total antioxidant ac-

tivity of raspberries is mainly from the other phytochemicals in the fruit rather than from the Vitamin C. The antioxidant activity may be explained by looking at the combination of different phytochemicals functioning additively or synergistically accounting for the total antioxidant activity of raspberries.

It seems that pigment content is a factor affecting antioxidant activity, but not a factor in the inhibition of cancer cell proliferation. Except for anthocyanin content, Heritage, Kiwigold, and Goldie are genetically identical. They had different levels of phenolics and flavonoids and varying antioxidant activity. However, extracts from all three varieties reduced cancer cell growth by nearly 90%. Therefore, it is assumed that phytochemicals other than anthocyanins in the raspberries were responsible for the inhibition of tumor cells.

This study suggests that the phytochemicals in raspberries have a significant antioxidant and anticancer activity. It is clear that raspberries higher in phenolic and flavonoid compounds have higher antioxidant activity. The darker colored raspberry varieties showed greater antioxidant activity than the lighter colored variety, Anne. The antioxidant activity of the raspberry was directly related to the total amount of phenolics and flavonoids found in the raspberry, but there was no relationship between anticancer activity and the total amount of phenolics/flavonoids found in the same raspberry. The inhibition of cancer cell proliferation is further influenced by some unknown compound(s) present in raspberry fruits. The additive and synergistic roles of phytochemicals may contribute significantly to the potent antioxidant ac-

tivity and the ability to inhibit tumor cell proliferation.

It is clear that choosing to eat fruits and vegetables directly affects your health. However, not all fruits and vegetables are created equal. An informed consumer realizes that eating a diverse selection of fruits and vegetables is the only way to ensure you obtain all of the nutrients your body needs. While the effectiveness of nutritional supplements still needs to be proven, it is certain that eating fruits and vegetables is good for you. For the good of us all, it is important for the grower and the consumer to be well informed about the benefits of a diet rich in fruits and vegetables.

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