The Properties of Cranberries that make them Effective as an Antioxidant against Cancer

An Honors Thesis (HONRS 499)

By

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Abstract:

Several antioxidants are found in cranberries and other products containing cranberries. The amount available within the products depends on the type and the distributor. Organic cranberry juices contain less sugar and more metal ions such as zinc. Less processing of organic juices also saves some of the antioxidant properties from being destroyed. Polyphenols have been documented to be in certain fruits and vegetables including high quantities in cranberries. A visual complex forms with certain polyphenols with the addition of ion samples including iron. Cranberries have more properties than just preventing cancer, they have also been shown to decrease risk related to kidney stone, urinary tract infections, lower LDL, help with ulcers, and prevent tooth decay.

Introduction:

Media and educational campaigns have begun to encourage people to eat “Five-a-Day”, which is five to nine servings of fruits or vegetables, to promote their health. This results from the apparent correlation of the consumption of fruits and vegetables with reduced risk for cancer and cardiovascular disease. Fruits and vegetables are rich in antioxidants, but it is not known which dietary factors are responsible for the overall
beneficial effects. Each plant contains hundreds of phytochemicals (plant chemicals), whose presence is dictated by hereditary factors. Only well-designed, long-term research can determine whether any of these chemicals, taken in a pill, would be useful for preventing specific diseases. Cranberries, with their rich deep red color, are suspected to be satiated with antioxidants, including a large amount of polyphenols and trace metals.

The term antioxidant arose in the 1920's to denote any substance that fought rust, or other forms of oxidation. Reactive oxygen species (ROS) have been linked to being formed by a variety of pathologies, including cancer and cardiovascular disease. Reactive oxygen species are molecules such as hydrogen peroxide (H$_2$O$_2$), ions like the hypochlorite ion (OCl$^-$), radicals like the hydroxyl radical (OH$^-$) (which is the most reactive of them all), and the superoxide anion (O$_2^-$) which is both an ion and a radical. A radical (also called a "free radical") is typically a cluster of atoms, one of which contains an unpaired electron in its outermost shell of electrons. This is an extremely unstable configuration, and radicals quickly react with other molecules or even other radicals to achieve the stable configuration of 4 pairs of electrons in their outermost shell (one pair for hydrogen). Reactive oxygen species can be formed by several different mechanisms. One way is the interaction of ionizing radiation with a biological molecule. A common path of formation is as an unavoidable byproduct of cellular respiration: some electrons passing down the respiratory chain are drawn away from the main path, especially as they pass through ubiquinone (a freely diffusible molecule) and go directly to reduce oxygen molecules to the superoxide anion (1). They can also be synthesized by dedicated enzymes in phagocytic cells like neutrophils and macrophages. These enzymes include NADPH oxidase and myeloperoxidase. (1)
Strong oxidants like the various ROS can damage other molecules and the cell structures of which they are a part. Among the most important of these are the actions of free radicals on the fatty acid side chains of lipids in the various membranes of the cell, especially mitochondrial membranes (which are directly exposed to the superoxide anions produced during cellular respiration). One of the insidious things about free radicals is that in interacting with other molecules to gain a stable configuration of electrons, they convert that target molecule into a secondary radical. So, a chain reaction begins that will propagate until two radicals randomly find each other, with each contributing its unpaired electron to form a covalent bond linking the two. A common example is the peroxyl radical, that may interact with another peroxyl radical on a nearby side chain, crosslinking them with a covalent bond, or with another nearby carbon-centered radical crosslinking them covalently. In both these cases, radical formation comes to an end, but with the result that the fatty acid side chains of membrane lipids may have become so deformed as to damage the membrane. The lipofuscin so characteristic of aging cells may be formed by these mechanisms. (1)

Cells have a variety of defenses against the harmful effects of ROS. These include two enzymes: superoxide dismutase (SOD) - which converts two superoxide anions into a molecule of hydrogen peroxide and one of oxygen; and catalase and several small molecules that are antioxidants, such as alpha-tocopherol (vitamin E), uric acid and vitamin C. These enzymes can break the covalent links that ROS have formed between fatty acid side chains in membrane lipids. Perhaps the long life span of some reptiles and birds is attributable to their high levels of uric acid, or vitamin C in the proper, effective
concentration. Pharmacy shelves are filled with antioxidant preparations that people take in the hope of warding off the damaging effects (perhaps including aging) of ROS. (2)

But it is important that the attempt to limit the production of ROS not succeed too well because ROS actually do have important functions to perform in the cell. Some examples include: the cells of the thyroid gland must make hydrogen peroxide in order to attach iodine atoms to thyroglobulin in the synthesis of thyroxine; macrophages and neutrophils must generate ROS in order to kill some types of bacteria that they engulf by phagocytosis; and neutrophils (but not macrophages) must kill off engulfed pathogens by using the enzyme myeloperoxidase which catalyzes the reaction of hydrogen peroxide (made from superoxide anions) with chloride ions to produce the strongly antiseptic hypochlorite ion. Chronic Granulomatous Disease (CGD) is a rare genetic disorder that demonstrates the importance of ROS in protecting us from many type of bacterial infection. It is caused by a defective gene for one of the subunits of NADPH oxidase. However, despite some advantages, in most cases the ROS are the problem, not the solution. (2)

By reducing ROS to harmless forms, antioxidants may prevent oxidative damage to biomolecules including lipids, proteins, and nucleic acids. There are several antioxidants that have been identified in living systems; they include enzymes, endogenous proteins and non protein species, and dietary antioxidants including tocopherols, carotenoids, and ascorbic acid. Epidemiological studies have shown that in humans low levels of antioxidants in blood plasma are correlated with elevated risk of disease (3) Although the levels of plasma antioxidants are related to risks, increased dietary intake of antioxidant vitamins does not necessarily reduce risk. (3) Studies of the
role of dietary antioxidants in maintaining health are complicated by the fact that levels of plasma antioxidants are a function not only of intake of nutrient antioxidants, but also of oxidative demand of the organism. (3)

ROS can be generated by either exogenous or endogenous reactions, thus causing an increased demand for antioxidants. Levels of plasma ascorbic acid are generally low in smokers, perhaps because oxidative metabolism of smoke by-products consumes the antioxidant. (3) Also the digestive tract is directly exposed to exogenous ROS when the diet is rich in polyunsaturated fats, which are susceptible to oxidations. (3) Endogenous ROS are produced in the digestive tract at sites of inflammation or mechanical irritation (3) or by Fenton-type reactions involving dietary iron or other metals. (3) One thing that could influence the demand for dietary antioxidants, such as ascorbic acids, is the level of the ROS in the digestive tract. If nutritive antioxidants are depleted by reaction with exogenously or endogenously formed ROS during digestion, they will be unavailable for uptake and transport to the serum.

Phytochemical, a current buzzword in the nutrition field, is defined by the American Cancer Society as non-nutrient plant chemicals that contain protective, disease-preventing compounds. A phytochemical that has been discussed quite often is polyphenol. Polyphenols are a group of chemical compounds that are widely distributed in nature. The basic structure of the backbone phenol can be found in Figure 1 (4). They are responsible for the colors of many flowers, for example delphinium, fuchsia, rose, and petunia, and of all red-berried fruit. Others are complex compounds present in bark, roots, and leaves of plants that are used for tanning hides and skins to give leather a softer texture. Others are simpler compounds present in most fresh fruit and vegetables, onions,
and tea. The term "polyphenols" was introduced some years ago to replace the term "vegetable tannin," and using the following definition: "Water-soluble phenolic compounds having molecular weights between 500 and 3000 and, besides giving the usual phenolic reactions, they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins" (5). However, the subsequent use of the term has been broadened to include lower molecular weight compounds, so that it now covers natural products with more than one phenolic group. Polyphenols are broken down into sub-categories, and these sub-categories are broken down even farther as their full function has become known. Dietary phenolics may play a role in reducing the risk of heart disease, cancer, and cataracts. Epidemiological studies generally, but not always, indicate a reduced risk of disease with high phenolic intake. A flow chart of phytochemicals can be found in Figure 3.

![Figure 1. Structure of phenol](image1.png)

![Figure 2. Basic structure of a flavonoid.](image2.png)
Some helpful antioxidants of the polyphenols group include the group of flavonoids, that are also important for food preservation. Another flavonoid called quercetin is found in onions, apples, tea and broccoli. This flavonoid is glycosylated in most plants, and the position and nature of the substitution of the sugar are species specific. This broad class of polyphenolic compounds are synthesized by plants as defenses against stresses such as invading organism, UV light, and tissue injury. Studies have shown that quercetin inhibits the cytochrome P450-catalyzed metabolism of
ethoxycoumarin and of ethylresorufin by HepG2 cells at concentrations as low as 0.1 μM. (3) Quercetin is a very potent inhibitor of Maloney mouse leukemia virus reverse transcriptase with an IC\textsubscript{50} value of 0.2 μM, and inhibits HIV reverse transcriptase with K\textsubscript{i} = 0.52 μM. (3) This Flavonoid also suppresses α-tumor necrosis factor-mediated stimulation of interleukin-8 expression partly by inhibiting the activation of the transcription factor, NF-kB. (3)

The activity of Flavonoids varies with each individual compound. Several structure-function relationships have been derived. They include:

1. Most of the free flavonols are efficient antioxidants in both the aqueous and lipid phases, but increased hydroxyl groups on the B ring tend to increase activity.

2. Glycosylation of flavonols decreases the antioxidant activity, but the effect is much more marked when the substitution is on the B ring. The antioxidant activity tends to decrease with increasing number of sugar moieties on one position.

3. Dimerization and trimerization of (epi)-catechin increases antioxidant activity, but the tetramer shows decreased activity. This could be a result of the increase in size of the molecule.

4. Galloylation of flavanols have been found to increase aqueous phase antioxidant activity but decreased lipid phase activity. (3)

Figure 4 shows the various subclasses of flavonoids, their basic structures, and various examples of the foods sources to which they can be found in.
<table>
<thead>
<tr>
<th>Examples of Flavonoid Subclasses</th>
<th>Basic Structure</th>
<th>Examples of Food Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavone</td>
<td><img src="image" alt="Flavone" /></td>
<td>Sweet red pepper, celery</td>
</tr>
<tr>
<td>Flavonol</td>
<td><img src="image" alt="Flavonol" /></td>
<td>Onion, apple, red wine, green tea, tomatoes</td>
</tr>
<tr>
<td>Flavanone</td>
<td><img src="image" alt="Flavanone" /></td>
<td>Citrus fruits</td>
</tr>
<tr>
<td>Flavanol</td>
<td><img src="image" alt="Flavanol" /></td>
<td>Cocoa, chocolate, green tea, grapes, apples,  red wine</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td><img src="image" alt="Anthocyanin" /></td>
<td>Cranberries, raspberries, blueberries</td>
</tr>
</tbody>
</table>

Figure 4 - The subclasses of flavonoids, the basic structure and the foods in which they are found.

Tannins, a form of polyphenols, act as a defense mechanism in plants against pathogens, herbivores, and hostile environmental conditions. In the manufacturing industry, tannins are used in the processing of leather. Tannins are extracted from various tree barks, with the Europeans preferring sumac and oak. Animal skins are composed of a protein called collagen (among other things). Bacteria and fungi readily degrade this protein. When tannins bond to the collagen, the crosslinked fibers are no
longer susceptible to attack. The tannin must effectively crosslink the protein, but must also have desirable color properties and meet many other requirements. Today, this process still occurs, but the tannins are normally extracted from vegetables. Generally, tannins induce a negative response when consumed by animals. These effects can be instantaneous, like astringency or a bitter or unpleasant taste, or can have a delayed response related to antinutritional/toxic effects. This is because polyphenols, such as tannins, have the ability to form complexes with proteins or related biopolymers. (7)

Lignins and tannins are abundantly the most widespread of all polyphenols in plants. As stated above, tannins are a group of polyphenols that are used in the commercial industry in tanning of leather and in the brewing industries. This group has had its pharmaceutical properties investigated to help determine the structural properties of tannins. Some interesting studies have been conducted regarding the iron complexation of tannins for plant defense mechanisms. There are an enormous amount of living things that require iron for other mechanisms to survive. The element Fe is involved in various essential cellular processes. Plants are not the only things fighting for iron resources. Pathogens and decay microorganism are also striving to obtain it. Tannins have a high affinity for iron which helps limit the availability to other organisms. Tannins appear to be very efficient in displacing iron from other ligands (3)

Studies dealing with bacteria and with the different strains of Erwinia chrysanthemi, a pectinolytic bacteria, show that they can cause soft rot on a wide range of plants. These studies showed that the binding of iron to tannins was a key property in the protection of the plant form soft rot. When the iron concentrations were high enough to provide both the plant and the bacteria, soft rot occurred. When there was only enough
Iron is an essential nutrient in the human body. One of the main components of hemoglobin, iron helps transport oxygen throughout the body and serves as a catalyst for respiration. The iron we consume in food is generally absorbed first by the upper organs such as the small intestines. It then travels through the liver on its way to the bone marrow to help form hemoglobin. A certain percentage is also distributed to the body cells, where it becomes an ingredient in nuclei chromatin and enzymes. Since it is so important, this research project focused on correlating it with the absorption of polyphenols.

The human diet contains several polyphenolic compounds which are obtained from eating fruits, vegetables, spices, pulses and cereals, with high amounts in wines, coffee, cocoa, and several varieties of teas. Some of these dietary intakes have been noted to prevent absorption of iron in the body from simple iron forms, but this was not noted when the iron form was more complex. There are two known low molecular weight phenolic compounds, gallic acid and chlorogenic acid, which are shown to inhibit absorption of iron, while catechin is shown to have no effect. (3)
Figure 5 - Shows the different areas of substitutions for the Catechin compound

Tea Catechin, traditionally called tea tannin, is the special component of tea-leaf with bitter and acerbic taste, as well as astringency. It has the effects of resisting oxidation, resisting mutation, preventing tumor, resisting the AIDS virus, lowering blood cholesterol, decreasing low density lipoprotein, inhibiting hyperpressure, inhibiting the congealing of blood platelets, controlling bacteria, and preventing allergies etc. Tea catechins are strong active materials, formed with catechin as the main structure combined with a gallic acid group. Those that exist in green tea are mainly (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (+)-epicatechin (EC) (as seen in Figure 6), (-)-gallocatechin gallate (GCG), and (-)-catechin. (8) Six different catechins can be found in Figure 5. The catechin that mainly exists in black tea is theaflavin, which is a doubly condensed catechin substance based on (-)-epigallocatechin-3-gallate (EGCG). The theaflvin has 4 isomers, which are theaflavin-3-gallate, theaflavin-3'-gallate, theaflavin-3, 3'-digallate and theaflavin. The catechin substances existing in Oolong tea are mainly catechin itself and catechin
Gallic acid or 3,4,5-trihydroxybenzoic acid, C₆H₂(OH)₃CO₂H, is a phenolic antioxidant which is a colorless crystalline organic acid found in gallnuts, sumach, tea leaves, oak bark, and many other plants, both in its free state and as part of the tannin molecule. Since gallic acid has hydroxyl groups and a carboxylic acid group in the same molecule, two molecules of it can react with each other to form a dimer ester, digallic acid. Gallic acid is obtained by the hydrolysis of tannic acid with sulfuric acid. When heated above 220°C, gallic acid loses carbon dioxide to form pyrogallol, or 1,2,3-trihydroxybenzene, C₆H₃(OH)₃, which is used in the production of azo dyes and photographic developers and in laboratories for absorbing oxygen. Its major use is in pharmaceutical industries for manufacture of trimethoprim, an antibacterial agent, which is usually given along with sulphonamide. Together they have a broad spectrum of action. The consumption coefficient of gallic acid in the manufacture of trimethoprim is 4.80. Gallic acid can increase its free radical scavenging ability when combined with Procyanidin and Epicatechin. Gallic acid is normally found in the break down of tannins. This polyphenol is best when used in conjunction with other antioxidants. Gallic acid is
also used in the enzymatic synthesis of gallic acid esters (e.g. propyl gallate), which is used mainly as a strong antioxidant in fats, oils and beverages. Further, it also has application in the leather industry and in pyrogallol manufacture. Gallic acid is commonly obtained by enzimatic hydrolisis of gallotannins and further solvent extraction. (9)

Proanthocyanidins (also called leukoanthocyanidins) are a class of natural polyphenolic bioflavonoids that are very widespread in nature. The structure of proanthocyanidins can be found in Figure 7. They are found in many herbs, fruits and vegetables, and often are the main precursors of the blue-violet and red pigments. More than 90% of the proanthocyanidins occur as chains of two, three or more proanthocyanidin molecules, which are called oligomeric proanthocyanidins (OPC). These OPC's are considered most potent for their health benefits (monomers, like catechin and epi-catechin). Proanthocyanidins are highly regarded for their strong antioxidant properties, and for their functions in supporting the body's connective tissues and capillary blood vessel system. Europeans have been studying and using various forms of these natural proanthocyanidins for several decades for their numerous beneficial effects. Proanthocyanidins are among the most powerful natural free radical scavengers yet discovered. As such, proanthocyanidins have their own unique place in the body's overall protection against harmful free radical damage. Proanthocyanidins appear to be especially effective in neutralizing highly reactive hydroxyl and singlet oxygen radicals. Both of these reactive oxygen species are involved in inflammatory processes. Proanthocyanidins also support and enhance the activity of vitamin C, and are known for their ability to help support the health of the body's capillary system and
connective tissues. Proanthocyanidins have been shown to bind with collagen fibers, thereby protecting them from premature degradation. This helps maintain the natural elasticity of collagen in skin, joints, arteries, capillaries, and other connective tissues.

(10)

Flavan-3,4-diols

![Flavan-3,4-diols](image)

Figure 7 – The structure of Proanthocyanidins and the structure of anthocyanidins

Ellagic Acid is a phenolic compound that has become a known as a potent anti-carcinogenic/anti-mutagenic compound. It also has anti-bacterial and anti-viral properties. Ellagic acid itself is not thought to be naturally present in plants. Instead, polymers of gallic acid and hexahydroxydipenooyl (HHDP) are linked to glucose centers to form the class of compounds known as ellagitannins. When two gallic acid groups
become linked side-by-side within a tannin molecule, an HHDP group is formed. Ellagic acid is the result when the HHDP group is cleaved from the tannin molecule and spontaneously rearranges. For example, the ellagitannins are present in red raspberries. Some articles in which ellagitannins have been quantified refer to ellagic acid because quantitation of ellagitannins is done by breaking them down into ellagic acid subunits and quantifying the subunits. The Meeker red raspberry is the best source of ellagic acid, followed by Chilliwack and Willamette. The Meeker variety is specific to the Pacific Northwest—grown primarily for commercial use in Washington State. The Chilliwack and Willamette varieties contain lesser variations of ellagic acid. Both of these varieties are grown in the Pacific Northwest and may be found in lesser quantities outside the United States. The availability to the body of ellagic acid from dietary sources has only been confirmed with red raspberries. Other foods such as strawberries, pomegranates, and walnuts contain far lesser amounts ellagic acid, and their bioavailability has not been confirmed. Ellagic acid acts as a scavenger to “bind” cancer-causing chemicals, making them inactive. It inhibits the ability of other chemicals to cause mutations in bacteria. In addition, ellagic acid from red raspberries prevents binding of carcinogens to DNA, and reduces the incidence of cancer in cultured human cells exposed to carcinogens. (11, 12)

Research in animal and laboratory models has found that ellagic acid inhibits the growth of tumors caused by certain carcinogens. Studies in humans are underway to determine the effect of long-term daily consumption of raspberries on cell activity in the human colon. Ellagic acid has been found to cause apoptosis (cell death) in cancer cells in the laboratory. The mechanism of how it works is not yet well understood. (13) Some research also claims that it prevents the binding of carcinogens to DNA, and strengthens
connective tissue, which may keep cancer cells from spreading. Ellagic acid has also been said to reduce heart disease, birth defects, liver fibrosis, and to promote wound healing. Many of these claims are currently under investigation. Ellagic acid has been demonstrated in animal models to inhibit tumor growth caused by carcinogens. A human study is being completed at the Medical University of South Carolina Hollings Cancer Center. Twelve participants, some of whom had undergone surgery to have cancerous polyps removed, ate one cup of red raspberries daily for a year, with some continuing for longer. The study was to determine if eating red raspberries could prevent colon cancer by both inhibiting the abnormal division of cells and promoting the normal death of healthy cells. The results of the study have not yet been published. Other studies have also found positive effects. A recent animal study found that ellagic acid protected mice against chromosome damage from radiation therapy. A separate study of ellagic acid indicated that it was effective at inhibiting tumor growth from esophageal cancer cells in mice. Animal studies may show a certain substance holds promise as a beneficial treatment, but further studies are necessary to determine if the results apply to humans. (11,12)

A flavonoid compound found in grapefruit, naringin, gives grapefruit its characteristic bitter flavor. Grapefruit processors attempt to select fruits with a low naringin content, and often blend juices obtained from different grapefruit varieties to obtain the desired degree of bitterness. Naringin is believed to enhance human perception of taste by stimulating the taste buds (some people consume a small amount of grapefruit juice before a meal for this reason). Naringin may be instrumental in inhibiting cancer-causing compounds and thus may have potential chemotherapeutic value. Studies have
also shown that naringin interferes with enzymatic activity in the intestines and, thus, with the breakdown of certain drugs, resulting in higher blood levels of the drug. A number of drugs that are known to be affected by the naringin in grapefruit include calcium channel blockers, estrogen, sedatives, medications for high blood pressure, allergies, AIDS, and cholesterol-lowering drugs. Caffeine levels and effects of caffeine may also be extended by consuming grapefruit or grapefruit juice. While the effect of naringin on the metabolism of a drug can increase the drug’s effectiveness, it can also result in dosages that are inadvertently too high. Therefore, it’s best not to take any drugs with grapefruit juice unless the interaction with the drug is known. In addition, the effects of drinking grapefruit juice is cumulative, which means that if you drank a glass of grapefruit juice daily with your medication for a week, the drug interaction would be stronger at the end of the week than at the beginning. (14)

**Vitamin C (Asorbic Acid)**

Asorbic Acid (Vitamin C) is essential in the formation of collagen a fibrous connective tissue. It is a natural antihistamine that helps in the reduction of symptoms in allergy and asthma patients. There is little doubt that a diet high in Vitamin C reduces the risk of cancer substantially. Its mode of action is multiple; it protects the genetic material from free radical damage, strengthens the immune system and increases resistance to harmful chemicals (15). Vitamin C can inhibit the conversion of a normal to a cancerous cell after exposure to a carcinogen. Cancer cells turn back into normal-appearing cells when you add vitamin C to the culture medium (16,17). It also blocks the initiation of cancer by X rays (18)

Vitamin C has been linked to a decrease in stomach cancer, which at one time was
the most prevalent type of cancer in the United States. The decrease has been associated with the production of frozen orange juice. Vitamin C in breakfast orange juice probably blocks the formation of carcinogens (called nitrosamines) in the stomachs of people eating bacon or ham and eggs (19, 20). In a randomized clinical trial in Russia, vitamin C, along with vitamins A and E were given to prevent postoperative complications in 197 people with stomach cancer. The complication rate dropped dramatically from 30.9 to 1.9 percent. (21) Japanese generally do not drink high amounts of orange juice, and concurrently they have a much higher rate of stomach cancer. It is believed that vitamin C would be of value in protecting against the destructive effects of malignant invasiveness by stabilizing the body and enhancing the formation of the cellular cement called collagen (22) In 1991 it was reported that 294 advanced cancer patients who received supplemental vitamin C at some stage in their illness were compared with 1,532 cancer patients not receiving supplements at the same hospital. Patients taking vitamin C had an average survival time of 343 days compared to that of 180 days for non-vitamin C patients (23). When cells treated with vitamin C were examined under an electron microscope they appeared to be disorganized, their internal structures altered, their membranes disrupted and they were surrounded by the body’s collagen cement (24, 25) It has been concluded that the role of vitamin C in the regulation of DNA repair enzymes and also demonstrates an antioxidant effect (26).

Studies have dealt with Vitamin C dosages and uses with various cancer treatments. The RDA is set at 60 milligrams, but a consensus is to increase it to between 120 to 200 milligrams. However, dosages above 400 milligrams have no evident improvement. Vitamin C has been proven to not interfere with chemotherapy. It has
been documented that vitamin C can protect normal cells from the damage cause by free radicals without interfering with the cytotoxicity of doxorubicin against tumors (27, 28).

**Zinc**

Zinc can play a role in the formation of powerful antioxidants; an adequate intake is needed daily to prevent a variety of diseases, one of which is cancer. Zinc is used to protect against damage from radiation therapy. It has been proven in experiments with animals, that when zinc was added to the synthetic antioxidant Amifostine, the effect was greater than with Amifostine alone (22). Experiments have documented the increased rate of cancer in rats with lower intake of zinc. When zinc was consumed in their daily diet and they were given a carcinogenic mineral, the cancer developed at a much lower rate in their testes when compared to the control with no zinc intake. NCI scientist concluded that dietary zinc deficiency appears to cause a generalized increase in the chronic toxic effects of cadmium(22). Zinc and cadmium are both in the same chemical family. Prostate cancer patients also have lower blood levels of zinc than patients with a non-malignant prostate condition. (22).

An experiment was conducted in Linxian, China, which is classified as the world’s capital for esophageal and stomach cancer. From 1982 to 1991 volunteers were given various food supplements, with dosages of zinc between two to three times higher than the RDA. The results that were obtained demonstrated that stomach cancer among those who received the vitamin A and zinc combination was decreased by an astonishing 62 percent, compared to those who did not receive these supplements. In addition, a combination of beta-carotene, vitamin E, and selenium caused a 42 percent reduction in esophageal cancer. People receiving supplements had lower overall cancer death rates as
Another Chinese study monitored the effects of zinc and licorice mixture on chemotherapy. Licorice has been used world-wide as a flavor agent and a medicine. Glycyrrhizic acid resides naturally in the root of the licorice plant, *Glycyrrhiza glabra*. Glycyrrhizic acid, which makes up from 4 to over 20% of the root, is not the only biologically active molecule. About 300 different polyphenols, which make up 1 to 5% of the root, are suspected antioxidants, perhaps even cancer-fighting compounds. Mice were given a combination of this mixture as well as cisplatin (a chemotherapy drug). The mixture significantly reduced kidney, blood, and testicular toxicity. At the same time, the mixture did not undermine chemotherapy, either in the animals or in the test tube.

Scientists from India published a study in 1995, on the use of a four-supplement pill—zinc, vitamin A, the B vitamin riboflavin, and selenium. Smoking is known to cause breaks in DNA (genetic material). They studied the effects of this supplement on broken DNA in heavy smokers. Out of nearly 300 volunteers, half were given the supplement pill and half the placebo. The end results were compiled at the end of one year, the frequency of DNA breaks decreased by 72 to 95 percent in the supplement group. No such effects were seen on the placebo group. There were complete remissions in 57 percent of the people who received the supplements, while only 8 percent of those receiving the placebo showed a similar response.

The key question is whether supplementation with antioxidants (or other phytochemicals) has been proven to do more good than harm. The answer is no, which is why the FDA will not permit any of these substances to be labeled or marketed with claims that they can prevent disease. The negative publicity has not deterred manufacturers from continuing to market antioxidants as though they have been proven
to be beneficial. Many have also responded by hyping new mixtures of beta-carotene and other carotenoids, which, they suggest, may provide the same benefits as fruits and vegetables. Many types of pills described as “concentrates” of fruits and/or vegetables are being marketed. However, it is not possible to condense large amounts of vegetable produce into a pill without losing fiber, nutrients, and many other phytochemicals [22]. Although some products contain significant amounts of nutrients, these nutrients are readily obtainable at lower cost from foods. (29)

The large class of flavonoids can be found in a wide variety of foods. Anthocyanidins such as cyaniding and delphinidin can be found in berries, grapes, fruit skins and true fruit juices. Catechins such as catechin and epicatechin can be found in true teas and not herbal teas. Flavanones including hesperetin and naringenin are commonly found in citrus fruits. While flavones including apigenin and luteolin are contained in grains and herbs and finally flavonols such as myricetin and quercetin are found in fruits, onions, and botanicals

Properties in other foods and beverages

Researchers have identified and separated monomeric and oligomeric flavanols present in cocoa and select chocolates (30). Cocoa and chocolate have been found to be abundant in the higher oligomeric compounds, procyanidins. Flavonoids found in chocolate include: the flavanols, which include (-) epicatechin and (+) catechin, and a related series of complex procyanidin oligomers built from this monomeric unit (30).

In vitro (test tube) and in vivo (in humans) studies have shown that cocoa flavonoids and certain chocolates may decrease low-density-lipoprotein (LDL) oxidation, may modulate platelet activation, and may positively affect the balance between certain
hormones, or eicosanoids. These actions can play a role in maintaining cardiovascular health (31-38).

One study found that chocolate liquor polyphenols (CLP), an enriched polyphenols fraction purified from chocolate liquor that is a major component of chocolate, inhibited the production of reactive oxygen species (ROS). (Polyphenols are a larger group to which flavonoids belong.) Decreasing ROS damage to cell membranes and biological molecules is believed to play an important role in maintaining overall good health throughout life (14). In other observations, in vitro studies suggest that cocoa flavonoids may have immunoregulatory effects that go beyond their antioxidant activity (39, 40).

Blueberry, a fruit closely related to the cranberry, has been shown to reverse certain aging characteristics. In a study found in the Journal of Neuroscience, researchers found that rats fed spinach and strawberries learned better than rats on a standard diet. Then they introduced a blueberry extract into the diet. The rats that got the supplement not only learned faster than other rats, but their motor skills improved. The rats were 19 months old (the equivalent of 60 to 65 years of age) Researchers fed them for another two months up to 70-75 years old. The blueberry-fed rats did better on standard rat tests, like making them swim in a water maze, or find an underwater platform in murky water. But they also did better on tests involving a spinning rod or an inclined rod which is a good test of coordination. Young rats six months old could stay on a rod an average of 14 seconds. Old rats fell off after six seconds. The blueberry-supplemented old rats could stay on for 10. The blueberries did not make the rats young again, but did improve their
skills considerably. When the rats’ brains were examined, the brain cells of the rats on blueberries communicated better. (41)

According to some alternative clinicians, menstrual problems typical of perimenopause can also be alleviated with large doses of flavonoids, including the powerful anthocyanins in bilberry extract. An Italian study found that a high intake of bilberry flavonoids (anthocyanins) may also decrease or even eliminate the soreness in fibrocystic breasts. This may be due mainly to the anti-inflammatory action of these phytonutrients and also to their probable ability to bind to Type II estrogen receptors and exert hormone-like effect counteracting too much estrogenic stimulation. (42)

There is extensive epidemiological evidence that populations consuming larger quantities of polyphenols show less obesity and lower cardiovascular, cancer and Alzheimer’s disease mortality. Some examples are: Eastern Asia, for instance, with its high intake of green tea, miso, ginger and a great variety of vegetables; and Southern France and other Mediterranean countries, with their high consumption of red wine and olive oil, both known to be rich in phenolic compounds. Chocolate too may have similar benefits, due to its high content of proanthocyanidins, similar to those found in red wine. Epidemiological studies tend to confirm a correlation between low risk for various degenerative disorders and high consumption of polyphenols. If red wine and chocolate do not agree with you, you can always reach for grape seed extract. (42)

We can only make an educated guess that blueberries and bilberry extract, as well as grape seed extract, with their highly effective antioxidant power, have anti-aging benefits and may even be able to extend life span if consumed daily in sufficient doses. This is highly likely in view of the documented antioxidant, chelating, hypoglycemic,
antiatherogenic, anticarcinogenic, anti-inflammatory, immunostimulating, and enzyme-modulating effects of polyphenols.

When considering the potential health benefits of flavonoids in foods and beverages, it is important to determine not only their presence, but also their bioavailability once ingested, since their nutritional significance will depend on their behavior in the digestive tract. One study analyzed plasma samples from healthy men after chocolate consumption and found that plasma concentrations of epicatechin increased markedly, reaching maximum levels between two and three hours. Thus, epicatechin was found to be readily absorbed from chocolate (43).

Tart cherries are another fruit that has been shown to have wonderful antioxidant properties. Tart cherries contain natural anti-inflammatory compounds called anthocyanins. In laboratory tests, Michigan State University research indicates that the tart cherry compounds are at least 10 times more active than aspirin. The advantage of tart cherries is that they are more effective without any of the adverse side effects of aspirin, such as stomach and kidney problems. In addition, other research has revealed that the production of a human hormone (prostaglandin) is the cause of joint pain. The production of this hormone is directly related to two enzymes (cyclooxgenase -1 and -2). Twenty tart cherries appear to provide enough anthocyanin (12 to 25 milligrams) to inhibit the enzymes that ultimately cause pain and inflammation. Tart cherries are an excellent source of antioxidant compounds. There are 17 compounds in tart cherries with antioxidant properties. The antioxidant activity of these tart cherry compounds, under the MSU evaluation system, is superior when compared to vitamin E, vitamin C and some synthetic antioxidants. Two of the antioxidant compounds (kaempferol and quercetin)
found in tart cherries also are found in several commercially prepared health products, which are used to improve memory, concentration and vision. Among the antioxidant compounds in tart cherries, there are four anthocyanins. Recent research indicates that these cherry anthocyanins may have the potential to inhibit the growth of colon cancer tumors. (44)

**Why Cranberries?**

There are thousands of naturally occurring components in fruits and vegetables that can help promote health and reduce risks for many common diseases. With regard to cancer prevention, the potential benefits of cranberry’s rich flavonoid profile have, understandably, received additional attention. Studies have shown that flavonoids isolated from cranberries can inhibit bacterial adhesion, the mechanism by which cranberry juice helps maintain urinary tract health (45), and possibly even help in the prevention of periodontal disease (46) and the formation of certain ulcers (47). Compounds in cranberries may also help prevent the progression of atherosclerotic plaques that lead to cardiovascular disease (48). Compared with 20 common fruits tested, cranberries have the highest fresh-weight content of flavonoids and related phenolic acids (49), making this berry a powerful source of potentially anti-cancer phytonutrients and antioxidants. While findings linking cranberry’s components to a decreased risk of cancer are preliminary, *in vitro* studies suggest cranberries contain components that can inhibit carcinogenesis. The polymeric proanthocyanidin fractions from lowbush blueberry, cranberry and lingonberry exhibited the greatest anti-carcinogenic activity. Other researchers found a proanthocyanidin-rich phenolic fraction from the American cranberry (*Vaccinium macrocarpon*) that demonstrated the most significant
anticarcinogenic activity (50). Of the seven flavonoids in high abundance in this active fraction, oligomeric proanthocyanidins, quercetin and myricetin were identified. Several laboratory studies have also investigated the role of the phenolic antioxidants ellagic acid and reversatrol, present in grapes and berries (such as cranberries) at high levels, in the inhibition of human cancer cells. Researchers found both compounds inhibited the growth of certain cancer cells, specifically colon and prostate cancer cells, by interfering with the expression of a number of genes required for cell growth and activating apoptotic-specific proteins that can trigger cell death (51). Research has shown that quercetin, a prominent cranberry flavonoid (52), inhibits the development of chemically-induced mammary and colon cancer (53).

According to the Alder Lake Cranberry Corporation, cranberries have several benefits including the prevention of urinary tract infections (UTI’s), anti-aging, preventing ulcers, promoting cardiovascular health and preventing cancer. Cranberries (Vaccinium macrocarpon) are the cousins of blueberries. Since most cranberry products are highly sweetened, it is advisable to try unsweetened varieties to gain the full benefits of the cranberry, especially the anti-tooth decay property. (54)

Besides being a heart-healthy source of antioxidants, cranberries were shown to decrease total cholesterol and LDL or “bad” cholesterol levels in a recent study conducted by the University of Wisconsin-Madison. According to Rutgers University Cranberries contain compounds that have an anti-adhesion or anti-stick mechanism that’s been shown to be effective in maintenance of urinary tract health. Preliminary research suggests this same anti-stick mechanism may work in the mouth and stomach, possibly helping to prevent gum disease and ulcers. A study conducted at the University of
Wisconsin-Madison found that feeding cranberry juice powder to animals with high cholesterol decreased total cholesterol and LDL cholesterol by 22 percent. (55)

Cranberries are rich in flavonoids. These phytonutrients have been shown to inhibit certain types of cancer. According to information obtained from the Cranberry Institute Dr. Catherine Neto of the University of Massachusetts, Dartmouth recently published a laboratory study in the Journal of Agricultural and Food Chemistry showing that certain cranberry extracts inhibited the growth of a variety of tumor cells, supporting earlier studies conducted elsewhere. While the effects in humans have not yet been established, this work will form the basis for future research. Cranberries are a rich source of the bioflavonoid quercetin. In a study published in the Journal Cancer Research, quercetin, which is present in many fruits and vegetables, was shown to be effective in inhibiting chemically induced colon and breast cancers. (56) More recently, researchers from the University of Western Ontario demonstrated that mice injected with human breast cancer cells had a significantly lower incidence of tumor development when fed cranberry components. In a presentation at Experimental Biology 2000, the annual meeting of life sciences researchers, Dr. Najla Guthrie and colleagues reported that cranberry consumption delayed tumor development and reduced the spread of tumors to the lungs and lymph system. (57) Guthrie concluded that the research found that mice that received cranberry juice and cranberry products had a significantly lower number of breast cancer tumors compared with the control group, and the development of tumors was delayed, and the results are very preliminary, this study may suggest that cranberry products could have cancer fighting properties in humans. (58, 59, 60)
New research suggests compounds found in cranberries may inhibit ulcer-causing bacteria from sticking to the stomach wall. From Life Extensions Magazine an article written in 2000 suggest that cranberries might be effective against Helicobacter pylori the bacteria implicated in stomach ulcers. In vitro research has shown that the anti-adhesion property of cranberries can help combat the ulcer-causing bacteria even when they have adhered to the lining of the stomach. Research was also presented on how this mechanism may be effective against bacteria in the mouth in the prevention of periodontal disease. (61, 62, 63) Apples, celery, cranberries, onions, red wine, and green and black tea are also high in natural chemicals known as flavonoids, which appear to inhibit *H. Pylori* growth and have many other health benefits. In fact cranberry juice specifically may have properties that help prevent *H. Pylori* from infecting the intestinal lining. (64)

Polyphenolic compounds found in cranberry may help to protect against neurodegenerative diseases, and the memory and coordination losses often associated with aging (54). Early findings suggest that eating plenty of high-ORAC fruits and vegetables—such as spinach and blueberries—(see Figure 8)—may help slow the processes associated with aging in both body and brain. Oxygen Radical Absorbance Capacity (ORAC)—measures the ability of foods, blood plasma, and just about any substance to subdue oxygen free radicals in the test tube. Cranberries score high on the antioxidant scale at 1750 ORAC units per 100 g (about 3.5 oz.) of fresh fruit. (66) In studies, eating plenty of high-ORAC foods: resulted in a raised the antioxidant power of human blood 10 to 25 percent, prevented some loss of long-term memory and learning ability in middle-aged rats, maintained the ability of brain cells in middle-aged rats to respond to a
chemical stimulus—a function that normally decreases with age, and protected rats’ tiny blood vessels—capillaries—against oxygen damage. (65-70)

Figure 8 – A chart containing the ORAC Values for various foods (71)

Cranberries and blueberries contain condensed tannins called Proanthocyanidins that prevent E. coli bacteria from attaching to cells in the urinary tract. These findings appeared in the *New England Journal of Medicine*. The cranberry plant transforms the flavonoids that contribute to the fruit’s bitter taste by removing part of the flavonoid molecule and replaces it with a sugar molecule. This has the effect of sweetening the fruit, making it more palatable as a food. This sugar molecule makes the cranberry effective as a nutrient within the urinary tract. In the human body, different cells have unique receptor sites. These sites can be thought of as a lock in a door requiring a unique key to open the lock. The sugar attached to the cranberry flavonoid seeks out an
acceptable receptor site to attach itself. In cranberries, the sugar unlocks a receptor site on the walls of the urinary tract. This explains some of cranberries' unique benefits. Cranberries contain a type of flavonoid that is capable of defeating the bacteria that cause urinary tract infections, and this flavonoid is attached to a sugar that seeks out the cells that line the urinary tract. (72) The National Kidney Foundation recommends drinking at least one large glass of cranberry juice a day to help maintain urinary tract health. This is a very helpful benefit because of the development of bacterial resistance. Another excellent reason for decreasing the use of antibiotics is that natural remedies are not harmless compounds. They have side effects also, such as diarrhea. The killing off of friendly bacteria often results in an overgrowth of harmful organisms such as Candida. Many women experience a miserable vaginal Candida infection practically every time they use antibiotics. The use of potent cranberry extract has no side effects, while producing multiple benefits. (42)

Cranberry juice may also be able to help reduce the risk of kidneys stones. A preliminary study suggests drinking a glassful of blackcurrant juice may make the risk of a certain type of kidney stone decrease. Researchers in Germany measured levels of compounds associated with kidney stones in the urine of 12 healthy men aged 18 to 38 and determined their urinary pH levels after they consumed plum, cranberry or blackcurrant juice on separate days. In the study, pH levels of urine increased after the study volunteers consumed blackcurrant juice, report researchers in the October issue of the European Journal of Clinical Nutrition. Low urinary pH, which indicates that the urine is more acidic, is associated with uric acid kidney stones. Uric acid kidney stones make up about 10% of kidney stones, which can also be comprised of calcium, oxalate or
other minerals. About 85% of stones are comprised mainly of calcium. Cranberry juice, on the other hand, decreased the urinary pH, suggesting that it may help to treat certain other types of kidney stones or urinary tract infections (UTIs), which affect more than half of women in the US, according to at least one survey. UTI symptoms can be mild, such as a frequent urge to urinate and pain on urination, or more severe, such as high fever, pain and blood in the urine. (73)

According to current statistics in America 35% of the population is expected to develop some form of cancer before they expire. Americans seem to be strongly keyed into different ways to prevent various diseases including cancer. To the majority of the population this information will be useful because almost everyone knows someone with cancer.

**Materials and Methods:**

*Preparation of Fresh Cranberries*

Various techniques were tried and their physical alterations on cranberries and grapes observed. The techniques used included oven drying at two different temperatures (100 Celsius and 140 Celsius), drying in desiccators, air-drying without light exposure, air-drying with light exposure and drying in a food dehydrator. It was concluded that the least destructive approach to prepare the cranberries into a powder would be to dehydrate them in the food dehydrator. Ocean Spray™ and Paradise Meadow Premium Cape Cod Cranberries were massed and then placed on a shelf in the food dehydrator. They were then dried using a food dehydrator (Harvest Maid – Model LD-1000). The dehydrator was set at 125 degrees Fahrenheit or 50 degrees Celsius for approximately 48 hours. The cranberries were then removed and massed and the percent water loss was calculated.
The cranberries were made into a fine powder by placing metal beads and the dried cranberries into a glass jar. Then the jar was manually shaken until a fine powder resolved. A good food processor or coffee grinder might be able to speed up the process slightly.

**Atomic Absorption**

Various brands of cranberry juice were used including three cranberry juice cocktails from Meijer, Wal-Mart and Ocean Spray, and two organic brands: Just Cranberry and Tree of Life, a concentrate. Dilutions of each were made of .1 ml, .25 ml, .5 ml, 1 ml, 5 ml, 10 ml, and 25 ml diluted with water to a volume of 100 milliliters for each brand of juice. The concentrated of Tree of Life was further diluted to .1 ml and diluted to 250 milliliters with water. They were also all tested full strength with no water added for some of the metals.

The standards were made by considering the linear working range for Na, K, Ca, Fe, Cu, Mg, Mn and Zn. The maximum concentration for the linear working range was used and then 3 more samples of each were made each decreasing in value by a factor of 2. For example, if the linear working range was 2µg/ml then the samples were made of 2µg/ml, 1µg/ml, .5µg/ml and .25 µg/ml (ppm). The standard samples were all double checked by making up another standard at each dilution and checking the concurrence of the results. A standard curve is made for each standard by graphing the the absorption or emission reading vs. concentration. The data that was collected from the samples are then found on the standard curve and the concentration is extrapolated.

**UV-Vis Diode Array Spectrophotometer**
Samples of polyphenols including: Naringin, Quercetin, Flavanone, Ellagic Acid, Catechin, Tannic Acid, 3-Hydroxyflavone, Gallic Acid, Flavone, and Epicatechin were purchased from various laboratory supply companies. Small amounts of polyphenols were used to find what chemical would work the best for the solvent. Acetone, dichloromethane and hexane were all tried alone, and then with mixtures of water. After reviewing the results straight acetone was chosen to be the solvent for all known polyphenols.

The stock polyphenols of Naringin and Quercetin, were diluted in acetone until the Absorbance on the UV-Vis Diode Array Spectrophotometer was close to 1.0 AU. The blank for this procedure was pure acetone. Serial dilutions of each sample were then made up in order to create a Beer's Law plot. Naringin was diluted down to .1g in 100 ml of acetone and then aliquots of this were diluted down in 100 ml volumetric flask. 20 ml, 15 ml, 10 ml and 5 ml samples were all removed and diluted down. These samples were than run on the Spectrophotometer and the results charted. Quercetin was diluted down by taking .05g of stock and diluting in a 100 ml volumetric flask with acetone. The molarity from the Naringin samples used are $8.613 \times 10^{-5}$, $1.722 \times 10^{-4}$, $2.583 \times 10^{-4}$ and $3.445 \times 10^{-4}$ the molarity for the Quercetin samples used are $1.476 \times 10^{-5}$, $2.953 \times 10^{-5}$, $4.430 \times 10^{-5}$, and $5.906 \times 10^{-5}$. Later, 10 ml samples of each dilution were removed and various metal ions will be added to this sample. These ions included: ferric iron, zinc to a different sample, and both iron and zinc to a third sample. Also a sample of ferrous iron was added to a fourth sample, while a sample was saved as the control, and a blank of acetone.
The samples were then analyzed to see which of the metal ions formed a complex. For the rest of the samples of polyphenols, ferrous sulfate and ferric sulfate were used as the ions. A .01 molar solution was made by taking .278g of Ferrous Sulfate and diluting in a volumetric flask with acetone, and a .01 molar solution of Ferric Sulfate by diluting .398 grams FeSO₄ in 100 mls of acetone. Approximately .5 ml or 10 drops of each solution were added to the various dilutions of polyphenols.

Aliquots of each polyphenol sample were place in small vials in sets of four. Ion was then added to each sample and visual changes noted. The amount of ion added was increased in increments of .5 ml ranging from .5ml to 2.0 ml drops. After viewing the data for the ferrous and ferric samples this portion was only used with Naringin and ferric sulfate.

Results:

<table>
<thead>
<tr>
<th>Drying Method</th>
<th>Description of berry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oven dried @ 140</td>
<td>Turned a dark brown color and the edges looked burnt, were not pliable</td>
</tr>
<tr>
<td>Oven dried @ 100</td>
<td>Turned a dark brown color and the edges looked burnt, were not pliable</td>
</tr>
<tr>
<td>Desiccators</td>
<td>Dried up but took over a week, and were still pliable so there was still water left to be removed</td>
</tr>
<tr>
<td>Room Air w/light</td>
<td>Dried up over several days</td>
</tr>
<tr>
<td>Room Air without light</td>
<td>Dried up over several days</td>
</tr>
<tr>
<td>Food Dehydrator</td>
<td>Dried in about 48 hours and deep red color still present, little change in structure of berry</td>
</tr>
</tbody>
</table>

Table 1 – Observations made of the physical changes of the dried cranberries.

<table>
<thead>
<tr>
<th>Ocean Spray #1</th>
<th>88.52%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocean Spray #2</td>
<td>86.91%</td>
</tr>
<tr>
<td>Ocean Spray #3</td>
<td>85.78%</td>
</tr>
<tr>
<td>Paradise Meadow #1</td>
<td>84.98%</td>
</tr>
<tr>
<td>Paradise Meadow #2</td>
<td>87.12%</td>
</tr>
<tr>
<td>Paradise Meadow #3</td>
<td>90.06%</td>
</tr>
</tbody>
</table>
Table 2 – Percent of water in samples of cranberries (sample size 300 cranberries)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocean Spray Average</td>
<td>87.07%</td>
</tr>
<tr>
<td>Paradise Meadow Average</td>
<td>87.39%</td>
</tr>
</tbody>
</table>

Table 3– The average percent of water in both brand names of cranberries tested.

Chart 1-7 – The linear graph of the AA samples, for various ions.
Mn with AA

\[ y = 0.0335x + 0.0021 \]
\[ R^2 = 0.9999 \]

Ca with AA

\[ y = 0.0576x + 0.0028 \]
\[ R^2 = 0.9977 \]
Table 4 – Analysis of Juice on AA (all data in ppm unless labeled)
<table>
<thead>
<tr>
<th>Berries</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Fe</th>
<th>Cu</th>
<th>Mg</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocean Spray Man</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ocean Spray Kay</td>
<td>0.337</td>
<td>6.5</td>
<td>1.41</td>
<td>0.27</td>
<td>0</td>
<td>0.615</td>
<td>0.027</td>
<td>0.033</td>
</tr>
<tr>
<td>Paradise Meadow Man</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Paradise Meadow Kay</td>
<td>0.167</td>
<td>5.7</td>
<td>1.11</td>
<td>0.24</td>
<td>0</td>
<td>0.548</td>
<td>0.014</td>
<td>0.028</td>
</tr>
<tr>
<td>Juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocean Spray Man</td>
<td>145.8</td>
<td>NA</td>
<td>NA</td>
<td>1.5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ocean Spray Kay</td>
<td>14</td>
<td>257.8</td>
<td>55.3</td>
<td>0.9</td>
<td>0</td>
<td>17.1</td>
<td>0.35</td>
<td>0.13</td>
</tr>
<tr>
<td>Meijer Man</td>
<td>145.8</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Meijer Kay</td>
<td>16</td>
<td>167.7</td>
<td>55.6</td>
<td>0.95</td>
<td>0</td>
<td>17.4</td>
<td>0.71</td>
<td>0.1</td>
</tr>
<tr>
<td>Walmart Man</td>
<td>145.8</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Walmart Kay</td>
<td>15</td>
<td>175.5</td>
<td>64.8</td>
<td>0.95</td>
<td>0.04</td>
<td>16.7</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Just Cranberry Man</td>
<td>73</td>
<td>480</td>
<td>135</td>
<td>11.413</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Just Cranberry Kay</td>
<td>60</td>
<td>512</td>
<td>156.4</td>
<td>6.25</td>
<td>0.13</td>
<td>26.3</td>
<td>0.81</td>
<td>0.32</td>
</tr>
<tr>
<td>Tree of Life Man</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>0.018 ppb</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tree of Life Kay</td>
<td>104</td>
<td>4725</td>
<td>558</td>
<td>15</td>
<td>0.89</td>
<td>87.5</td>
<td>12.5</td>
<td>2</td>
</tr>
</tbody>
</table>

Beer's Law for Naringin at 332nm
Charts 8-9 – Beer’s Law Plots for Naringin and Quercetin

Table #1 shows the results that were obtained from the various drying methods experimented with on grapes and cranberries. The grapes provided better observational data because they were not previously dried like the cranberries. The dried cranberries however still had a content of moisture remaining. Pictures of the dried cranberries along with pictures of grapes can be found in the appendix.

Table #2 shows the percentage of water loss after drying two different brands of cranberries in the food dehydrator. Cranberries are mainly water, and after drying, a light air filled shell remains. They are considerably different from the texture of a grape, and the remains have a texture similar to that of paper or dried leaves. Table #3 is an average of both brands of cranberries dried from the data found in Table #2.

Charts 1-7 show the various atomic absorption standard curves that were made using standard dilutions on the Atomic Absorption. Table #4 shows the final results of
all the data collected on the AA comparing the various brands of juices. It compares the data from the label and the results obtained from research. The values were obtained from the standard curves and the values obtained from the product were extrapolated to determine the ppm or ppb of each product. The Tree of Life cranberry juice concentrate has higher amounts compared to the other brands because the product has been concentrated down. There are very few differences in the Meijer, Wal-mart and Ocean Spray cranberry juice cocktails; one notable difference is that there is more potassium in the Ocean Spray brand and also less manganese. When comparing the juices to the whole fruit the berries have lower contents an all of the ions tested when compared to the juices.

Charts 8-9 show the Beer's Law plot for both Naringin and Quercetin. After graphing the data it was determined that Naringin has an \( \varepsilon \) value of 3306 and Quercetin's value is 26126. Each has their own wavelength with Naringin at 332nm and Quercetin's is set at 370nm.

In the appendix are the printouts from the data collected on the UV-Vis Diode Array Spectrophotometer. Visible color changes were noted in the Naringin polyphenol and Quercetin. Naringin turned a deep reddish-purple color when ferrous was added, and no visual change could be noted after the addition of zinc the original color of naringin was an off white color. Quercetin's yellow tinted color turned to a deep bright green after the addition of iron and no change was noted after the addition of zinc. The printouts show the slight effect of ferrous sulfate on cranberry juice in comparison with the other forms of iron. Ferrous sulfate had the most visible difference compared to the remaining iron samples. Ferric sulfate when added to the naringin showed an analysis at
approximately the same position as the same naringin sample missing the iron addition. The samples almost completely overlaid each other. While the ferrous sulfate samples had a difference from the original naringin sample, making a notable visible difference.

**Discussion:**

Polyphenols are destroyed at higher temperatures, causing concern during the manufacturing process. A food dehydrator was the least harsh process for drying the fresh cranberries but was also very time consuming. Since it is unclear where the majority of polyphenols in cranberries can be found future research can entail comparing seeds, with the rest of the berry. In grapes, for example, the skins and seeds have been documented to contain more polyphenols than the pulp of the fruit. For cranberries the same aspect may hold true or they might be contained throughout the berry. However removing the seeds from the cranberry will be a very tedious process. The amount of water in cranberries is very high for the type of berry. Cranberries are not as plush as most berries like grapes or raspberries, their hard texture limit one to believe little water content. Cranberries basically just have a fibrous shell left after all the water is removed, meaning water is the main content of the berry and then the fibrous tissue gives them their shape. For future research all the water content must be removed from the cranberry and a method must be found to remove the polyphenol content most probably an extraction method.

It appears that the less refining of cranberry juice provides for greater zinc content in the juice, or this could be simply a result of the geographic growing of the cranberry. Certain soils contain differences in the trace metals that are available to the plants that grow there. Also it was interesting to find out that Ocean Spray, Meijer and Wal-Mart labeled cranberry juice cocktail all have 10 times less sodium than what their label has
listed. After trying to contact the companies and gaining no information, it is assumed that this may be a mis-labeling, or there was another reason behind this perhaps. Sodium is a concern to patients of high blood pressure, making manufactures want to limit the amount they add to their products. Maybe they actually have lowered the amount of sodium they add to their product, while not changing the label.

From the analysis of 5 juice brands with metal ion content, organic cranberry juice is the route to take. Both organic juices had a higher amount of zinc, which is shown to help in the fight against cancer, and less sodium. Sodium is additive which be closely monitored by people suffering from hypertension, so less is better. Organic brands also contain fewer preservatives and more natural, less refined products. In future research the amount of polyphenols in each brand needs to be examined both in quantity and quality. Preliminary studies of the pure juice showed no positive, definite identification of any polyphenol.

Ferrous sulfate, which was the ion used to form complexes with the polyphenols, is the same form as iron supplements taken by anemic patients. This form of iron is more readily absorbed by the body, making it more useful to humans. Ferric sulfate was also looked at with the flavanol naringin. Future research will include at looking at more metal ions and their effects. Some metals have different ion charges, so correlating the charge of the ion related to the reaction of the polyphenols content will be critical. It will be key to understand how it is absorbed into the body and which metal ions make it more readably absorbed. It will also be key to determine if there is a mole ratio for each polyphenol and ions. Preliminary research suggested a high mole ratio with ferrous sulfate and naringin possibly around 34 moles of iron per mole of naringin, since this was
preliminary data future research will be utilized to determine if the calculated number is correct.

Beer's law needs to be completed on more polyphenols to see the remaining epsilon values. Naringin has an $\varepsilon$ value of 3306 and Quercetin's value is 26126. Preliminary work was begun to find a molar ratio between the metal ions as they complex using the Diode Array Spectrophotometer. This method can also be utilized in future research.

Dramatic visible changes occurred when adding certain metal ions to the polyphenols samples of Naringin and Quercetin. When viewing this on the spectrophotometer it was difficult to determine where the dramatic difference occurred. Future research can include determining what happens in the UV-Vis spectrum during the complexing of the polyphenols with various metal ions, including if they all have the same reaction per charge or not. While trying to find analyze the Quercetin sample on the Spectrophotometer the color began to fade away at a very rapid rate, it needs to be determined in future research if time or light degraded the complex. Light is believed to be the main reason because while in the Spectrophotometer box, shielded from the light, the color of the sample continued to fade at a rate visible to the box.

In the future the main goal needs to be to quantitatively and qualitatively determining which polyphenols are indeed in cranberries and their products. A correlation between the manufacturing process and finished polyphenols content will be crucial for the consumer. When buying a product, one wants the best product for the purpose he or she is buying it. So if they are purchasing solely for the antioxidant benefits, fresh cranberries are better than processed juices.
Since there is little documentation on cranberries and their polyphenols, it will be interesting to see what the future holds for this research area. With every experiment another door was opened and ideas on how to try new things evolved. Hopefully in the near future, it will be clearly understood the full value of a cranberry and just how good polyphenols are for us as antioxidants.
References:
9. A. Garcia-Najera¹, A. Medina, Y. Castro, M. L. Reyes-Vega, L. A. Prado-Barragan, and C. N. AGUILAR¹. (1) Food Research Department, Universidad Autonoma de Coahuila, P.O. Box 252, Saltillo, Coahuila, 25000, Mexico


54. www.alderlakecranberry.com


56. www.Hopkinsmedicine.org

57. Experimental Biology 2000, April 18-21, 2000, San Diego, CA


Appendix

Raw data from the Atomic Absorption Analysis

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Diode Array Spectrophotometer Printouts – On the following pages
Cranberry Control

Cranberry + Zinc

Cranberry + Zinc, Iron
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<th>Valleys (nm)</th>
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Sample/Result Table

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Quercetin 1ml/100 ml → 0.05g
Sample + Ferrous Sulfate

Quercetin 3ml/100ml → 0.05g
Sample + Ferrous Sulfate

Absorbance (AU)

Wavelength (nm)
Naringin 20ml/100ml → 1g
Sample + Ferrous Sulfate

Naringin 20ml/100ml → 1g
Sample + Ferric Sulfate
Cranberries on the left and Grapes on the right, both baked in an oven at 100 degrees.

Cranberries on the left and Grapes on the right, both baked at 140 degrees.
Room Temperature with Sunlight

Cranberries on the left and Grapes on the right, at room temperature in the presence of sunlight.

Room Temperature

Cranberries on the left and Grapes on the right room lighting and temperature.