

Antioxidants of Apples

Jeannelle Boyer, Rui Hai Liu

Department of Food Science, Cornell University, Ithaca, NY

In the United States and most industrialized countries, cardiovascular disease and cancer are ranked as the leading causes of death. These diseases have been linked to lifestyle choices, one of the most important of which is diet. It has been estimated that eating a healthy diet could prevent 30 percent of all cancers. Many of us as children were told to “eat your vegetables because they are good for you,” and the adage, “an apple a day keeps the doctor away” is still quite popular. Recently, many studies have been providing scientific backing for both of these common phrases.

Hundreds of epidemiological studies have been performed examining the effects of diet on health. Although not all studies show a link between fruits and vegetables and health, a majority of studies show a positive correlation between fruit and vegetable consumption and a reduced risk of chronic diseases, such as cardiovascular disease and cancer.

Diet and Chronic Disease

In the early 1990s, researchers examined well over a hundred epidemiological studies related to diet and cancer. In 128 of 156 dietary studies, fruits and vegetables were shown to have significant protective effect against a variety of different cancers (1). Researchers found that individuals who consumed small quantities of fruits and vegetables were twice as likely to get cancer compared to those who ate larger quantities of fruits and vegetables. Very recently, a study showed that high consumption of fruits and vegetables was also associated with a reduced risk of breast cancer in woman in China (2). Fruit and vegetable consumption also appears to have a protective effect against coronary heart disease (3). In this study, approximately 84,000 women were followed for 14 years and 42,000 men were followed for eight years. Researchers found that those who ate the greatest quantities of fruits and

vegetables had a 20 percent lower risk for coronary heart disease. The lowest risk was seen in people who consumed more green leafy vegetables and fruits rich in vitamin C. Not only could a diet high in fruits and vegetables help prevent heart disease and cancer, but it might also help protect against a variety of other illnesses. For example, a diet high in fruits and vegetables may help protect against cataracts, diabetes, Alzheimer disease, and even asthma (4-6).

Much of the protective qualities of fruits and vegetables has been attributed to phytochemicals, which are the non-nutrient plant compounds such as carotenoids, flavonoids, isoflavonoids, and phenolic acids. Thousands of phytochemicals have been identified in foods, and many have not. Different phytochemicals have many different activities which may help protect against chronic disease. For example, phytochemicals have been found to inhibit cell proliferation, regulate inflammatory and immune response, and protect against lipid oxidation (7, 8).

Phytochemicals' major role is to protect against oxidation. We live in a highly oxidative environment, and many processes involved in metabolism may result in the production of more oxidants. Like all animals, humans have many antioxidant defense systems, but they are not perfect and oxidative damage can occur. Oxidative damage plays a role in cancer and heart disease. As oxidative damage accumulates, it plays a role in the aging process in general. Therefore, it is important to protect against oxidative damage as best as we can. It is estimated that there are 10,000 oxidative hits to DNA per cell, per day, in humans (6). Oxidants may also target lipids and proteins, and lipid oxidation is associated with heart disease. Since fruits and vegetables are high in antioxidants, a diet high in these foods should help prevent oxidative stress, and therefore may slow aging and help prevent chronic disease. These findings have led the National Research Council to recommend five or more servings of fruits and vegetables a day.

Apples are a significant source of antioxidants in people's diets in the US and Europe. In the US, 22 percent of the phenolics consumed from fruits come from apples, which makes apples the largest source. Since apples are so high in a variety of antioxidants, it is no surprise that apples, specifically, have been associated with a decreased risk of chronic disease.

Health Benefits of Apples

A major class of phytochemicals found commonly in fruits and vegetables are the flavonoids. The most common flavonoids found in fruits and vegetables are quercetin and its conjugates (9). Apples are a significant source of flavonoids in diets in the US and in Europe. In the US, 22 percent of the phenolics consumed from fruits are from apples, which makes apples the largest source. In Finland, apples and onions are the primary sources of dietary flavonoids. In the Netherlands, apples rank third behind tea and onions as the top sources of flavonoids.

Apples are commonly enjoyed by many cultures, and our lab has determined that they are a good source of antioxidants. When compared to other commonly consumed fruits in the US, apples had the second highest level of antioxidant activity (Figure 1). Apples ranked second for total concentration of phenolic compounds. Perhaps more importantly, apples had the highest proportion of free phenolics when compared to other fruits (10). This means these compounds are not bound to other compounds in the fruits, and the phenolics may be more available for

eventual absorption into the bloodstream.

Since apples are so high in antioxidants, it is no surprise that apples, specifically, are associated with a decreased risk of chronic disease.

Three studies have specifically linked apple consumption with a decreased risk for cancer. Women who consumed at least one serving a day of apples and pears had a reduced risk of lung cancer (11). In another study, it was found that apples and onions, both good sources of quercetin, were associated with a reduced risk of lung cancer, and that those who consumed the least amount of apples and onions had the greatest risk of getting the disease (12). In Finland, yet another study associated high apple consumption with decreased risk of lung cancer (13).

A recent study has shown that apple and pear consumption has been associated with a decreased risk of asthma (4). Apple consumption has also been associated with a decreased risk of coronary heart disease in women (14). In the Netherlands, apple and pear consumption was associated with a decreased risk of obstructive pulmonary disease (15). In a Finnish study, death from heart disease in women was reduced in groups of women with the highest consumption of apples (16). Apple, tea, and onion consumption was also associated with a reduced risk of death from coronary heart disease in men in the Netherlands (17). A reduced risk of Type II diabetes was associated with apple and berry consumption in another major Finnish study (18).

In the laboratory, apples and the compounds in them have properties that may explain their effects in protecting against disease. Our lab has found that apples, and especially apple peels, have powerful antioxidant activity and can greatly inhibit the growth of liver cancer and colon cancer cells (19, 20). Based on results from all of these studies, it appears that apples may play a significant role in reducing the risk of a wide variety of diseases.

Phytochemicals in Apples

Apples contain a large concentration of flavonoids, as well as a variety of other phytochemicals. The concentration of these phytochemicals may depend on many factors, such as cultivar, harvest and storage, and processing. The variety and concentration of phytochemicals also

varies greatly between apple peel and apple flesh.

Some of the most well-studied antioxidant compounds in apples include quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, catechin, epicatechin, procyanidin, cyanidin-3-galactoside, coumaric acid, chlorogenic acid, gallic acid, and phloridzin.

The levels of all compounds vary greatly among different varieties of apples, and between the peel and the flesh. The compounds commonly found in apple peels are the procyanidins, catechin, epicatechin, chlorogenic acid, phloridzin, and the quercetin conjugates. In the apple flesh, there is some catechin, procyanidin, epicatechin, and phloridzin, but these compounds are found in much lower concentrations in the flesh than in the peel. Quercetin conjugates are found exclusively in the peel of the apples. The only compound found in higher concentrations in the flesh is chlorogenic acid (21). In general, it can be concluded that apple peels contain a higher concentration of antioxidant compounds.

Because the apple peel contains more antioxidant compounds, it can be inferred that the apple peel may have higher antioxidant activity and higher bioactivity than the apple flesh. Early research from our lab showed that peeled apples had less antioxidant activity than apples with the peel. Apples with the peel were also better able to inhibit cell

proliferation when compared to apples without the peel (20). The cell proliferation assay is a measure of the ability of a compound or fruit extract to inhibit the growth of tumor cells. Therefore, the greater the ability of a compound to inhibit cell growth, the greater the potential it has for anticancer activity. More recent work from our lab has shown that apple peels contain anywhere from two to six times (depending on the variety) more phenolic compounds than in the flesh, and two to three times more flavonoids. The antioxidant activity of the peel was also much greater, ranging from two to six times greater when compared to the flesh, depending on the variety of the apple (19).

Effects of Variety and Ripening on Phytochemical Content

Researchers in our lab have found quite a range in total phenolic and total flavonoid content among different varieties of apples. Of four common varieties used for applesauce (Rome Beauty, Idared, Cortland, and Golden Delicious), Rome Beauty had the highest phenolic content and Cortland had the lowest. Rome Beauty also had the highest flavonoid content while Cortland had the lowest. However, Idared contained much higher anthocyanins than any of the other varieties (19). Anthocyanins are the antioxidant compounds that give fruit a

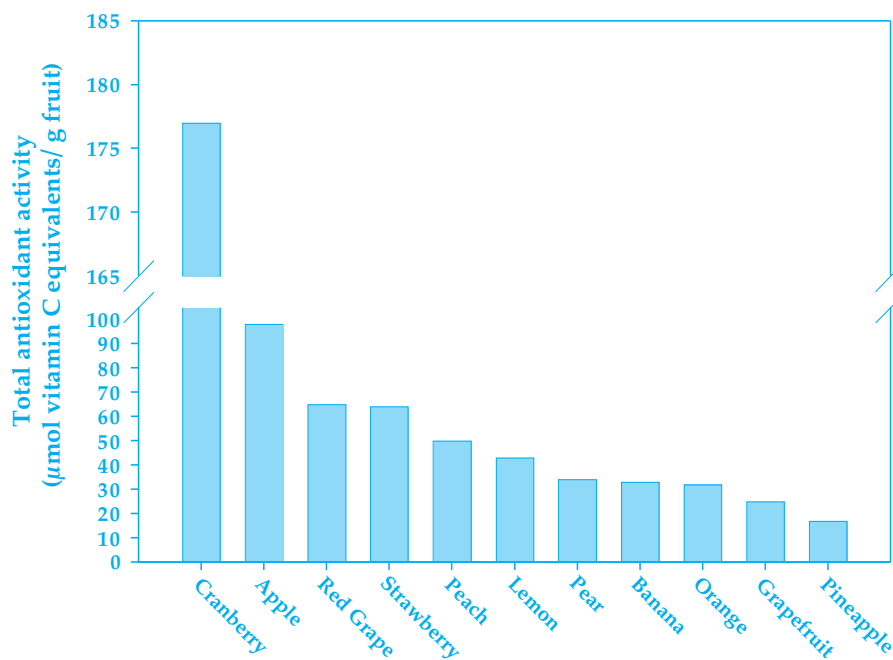


Figure 1. Antioxidant activity of various fruit extracts.

red or blue color. Out of 10 varieties commonly consumed in the US, Fuji apples had the highest total phenolic and total flavonoid compounds (Figures 2 and 3). Red Delicious were also quite

high. The apples containing the lowest amounts of phenolics and flavonoids were the NY647 and Empire, respectively.

Antioxidant activity also varies among the different varieties of apples, and this is a reflection of the phytochemical differences between varieties. There is a correlation between phenolic content of apples and antioxidant activity. The apple varieties with higher phenolics tended to have higher antioxidant activity.

Other researchers have found similar variations in phytochemical content between different cultivars of apples. Van der Sluis et al (2001) analyzed Jonagold, Golden Delicious, Cox's Orange, and Elstar, and found that Jonagold contained the highest concentration of quercetin glycosides, catechins, and chlorogenic acid when compared to the other varieties. Golden Delicious had the second highest concentration, while Cox's Orange and Elstar had the lowest concentrations. Escarpa and Gonzalez (1998) measured compounds in Golden Delicious, Reinata, Red Delicious, and Granny Smith, and found that, of these four varieties, Golden Delicious had the lowest concentration of many different flavonoids. Reinata had the highest level of flavonoids, but Granny Smith and Red Delicious were also very high. Another group looked solely at procyanidin content of four varieties of apples and found that Granny Smith and Red Delicious had the highest procyanidins while McIntosh and Golden Delicious had the lowest (22).

In addition to apple variety, there are a number of other factors that can affect the phytochemical profile of apples. Development and ripening of the fruits may impact phytochemicals in apples. In Jonagold and Elstar, quercetin glycosides, phloridzin, catechins, and chlorogenic acid concentrations were highest early in the season, and decreased to a steady level during maturation and ripening (23). Anthocyanins in Elstar and Jonagold started high and decreased in mid-season, but, before maturation, the concentrations rose rapidly. Interestingly, this increase in anthocyanin content occurred only in fruit grown in the outer part of the canopy, not in the inner canopy. The amounts of quercetin glycosides in both Jonagold and Elstar were also greater in fruit grown in the outer canopy (24). Awad (2000) also found that sun-exposed fruits (both Jonagold and Elstar) had greater levels of anthocyanins and quercetin glycosides when compared to shaded fruits, giving more evidence that exposure to sunlight affects production of these two

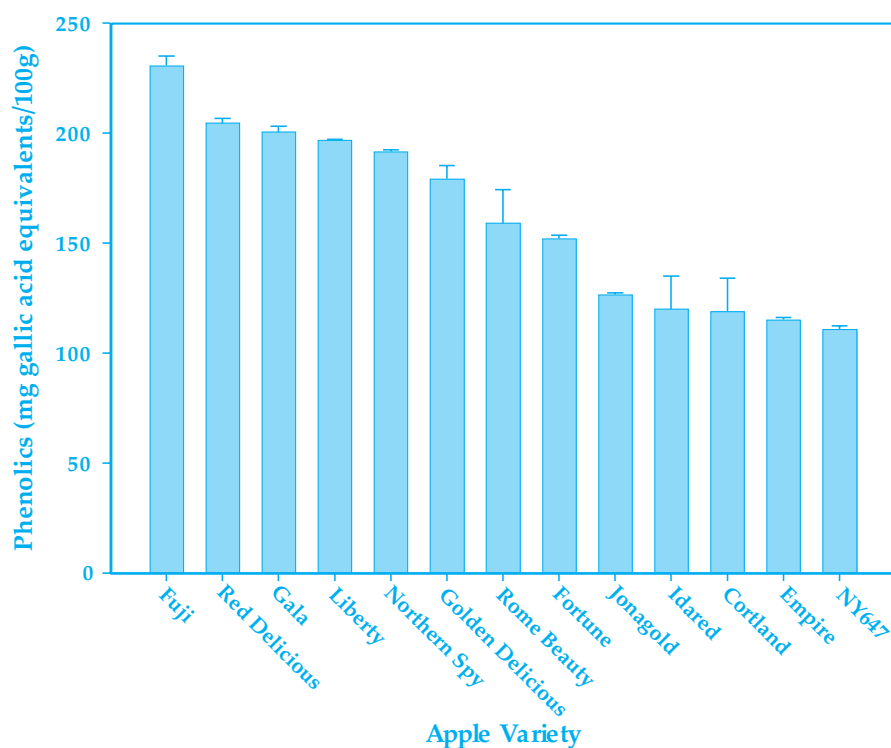


Figure 2. Total phenolic content of apples (mean $\pm$ SD, n=3).

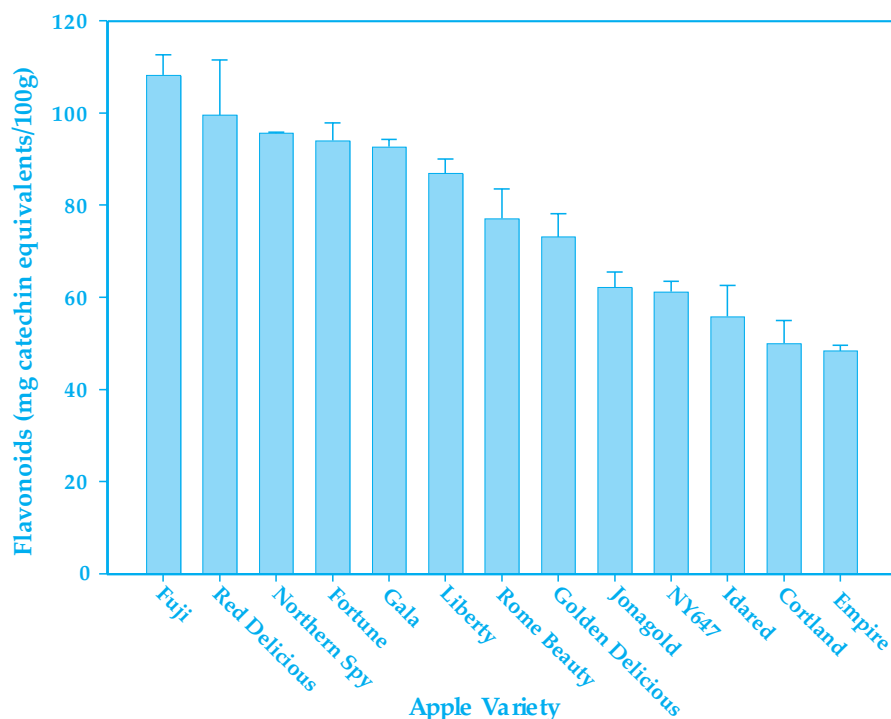


Figure 3. Total flavonoid content of apples (mean $\pm$ SD, n=3).

compounds (24). In general, it can be concluded that improving light exposure for apples may help increase the production of certain phytochemicals. There was no sunlight effect on phloridzin, catechin, and chlorogenic acid.

The effect of different nutrients on flavonoids and chlorogenic acid in apples has also been examined. Awad (2002) found that fertilizing with nitrogen was associated with decreases in anthocyanins, catechins and total flavonoids, and also with decreased percentage of blush in the fruit peels. Also in Elstar, fertilizing with calcium was associated with an increase in anthocyanins and total flavonoids.

Researchers examined the effect of applying different chemicals that may enhance ripening on the formation of various phytochemicals. Ethephon increased anthocyanin production, but did not increase chlorogenic acid or any of the other phytochemicals studied. Gibberellins and (s)-trans-2-amino-4-(2-aminoethoxy)-3-butenic acid hydrochloride (ABG-3168) both decreased anthocyanin production, but did not have an effect on other compounds studied. The application of other chemicals, such as alar, cycocel, seniphos, shikimic acid, plantacur-E and galactose did not have an effect on any of the phytochemicals examined (25, 26).

Effects of Storage and Processing on Phytochemical Content

Phytochemical content in apples is not significantly affected by storage. Quercetin glycosides, phloridzin, and anthocyanin content of Jonagold, Golden Delicious, Red Delicious, Elstar, and Cox's Orange were not affected by 52 weeks of storage in controlled atmospheric conditions. Chlorogenic acid and total catechins decreased slightly in Jonagold. Total catechin concentration decreased slightly in Golden Delicious, but chlorogenic acid concentrations remained stable (27). After 25 weeks of cold storage, there was no decrease in chlorogenic acid in any variety of apple, but catechin content decreased slightly in Golden Delicious, Elstar, and Cox's Orange. Both types of storage had no effect on antioxidant activity in any variety of apple examined. Another group looked specifically at the effects of storage on apple peel phenolics and found that

storage at 0°C for 9 months had little effect on phenolic content (28).

Processing can also affect phytochemical content. Apple juice obtained from Jonagold apples by pulping and straight pressing had only 10 percent the antioxidant activity of fresh apples. Juice obtained after pulp enzyming resulted in juice that had only 3 percent antioxidant activity. After pulp enzyming, the juice contained 31 percent less phloridzin, 44 percent less chlorogenic acid, and 58 percent less catechin. Most of the compounds remained in the apple pomace (29). Millions of pounds of waste apple peels are generated in the production of applesauce and canned apples in New York each year. Since apple peels contain the majority of the antioxidants when compared to the flesh (19), our lab has looked into the possibility of using apple peels as a value-added ingredient. Apple peels were blanched and then dried under a variety of conditions (oven-dried at a range of temperatures between 40°C and 80°C, air-dried, or freeze-dried). The freeze-dried samples had the greatest total phenolic and flavonoid content, and the total phenolic and flavonoid content was actually greater than in the fresh peels. Apple peel powder had strong antioxidant activity and also greatly inhibited cell proliferation (30). Apple peel powder may be used in various food products to increase their phytochemical content and antioxidant activity.

Summary

As researchers find more and more evidence to support the role of phytochemicals in decreasing the risk of chronic disease, some have hoped to find a magic bullet that prevents chronic disease. However, when researchers attempt to link specific chemicals with health benefits, they are often less successful than when comparing health benefits and total food consumption. This is because apples, as well as other fruits and vegetables, contain a complex mixture of thousands of phytochemicals.

After years of research, it is still impossible to identify all the compounds let alone thoroughly understand how all of these compounds interact. For example, we know that the antioxidant activity in 100 g of apples is equivalent to about 1500 mg of vitamin C, and the amount of vitamin C in 100 g of apples is only about 5.7 mg (20). Vitamin C is a powerful antioxidant, but this research

shows that nearly all of the antioxidant activity from apples comes from a variety of other compounds. Vitamin C in apples contributed less than 0.4 percent of total antioxidant activity. A dose of 500 mg of vitamin C has actually increased oxidative damage to DNA in humans. In addition, intervention studies with vitamin C supplements have shown no health benefits in the prevention of cancer and cardiovascular disease. Since the effects of high levels of other single antioxidants are unknown, it is not recommended that pure supplements be ingested, until further research is completed (8). In the meantime, we continue to support the recommendation that people eat at least five servings of fruits and vegetables a day. Evidence supports that apples are especially beneficial to consume.

References

1. Block, G.; Patterson, B.; Subar, A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* 1992, 18, 1-29.
2. Malin, A.; Qi, D.; Shu, X.; Gao, Y.; Friedmann, J.; Jin, F.; Zheng, W. Intake of fruits and vegetables and selected micronutrients in relation to the risk of breast cancer. *Int. J. Cancer* 2003, 105, 413-418.
3. Joshipura, K.; Hu, F.; Manson, J.; Stampfer, M.; Rimm, E.; Speizer, F.; Colditz, G.; Ascherio, A.; Rosner, B.; Spiegelman, D.; Willett, W. The effect of fruit and vegetable intake on risk of coronary heart disease. *Ann. Intern. Med.* 2001, 134, 1106-1114.
4. Woods, R.; Walters, H.; Raven, J.; Wolfe, R.; Ireland, P.; Thien, F.; Abramson, M. Food and nutrient intakes and asthma risk in young adults. *Am. J. Clin. Nutr.* 2003, 78, 414-421.
5. Willett, W. Balancing life-style and genomics research for disease prevention. *Science* 2002, 296, 695-698.
6. Ames, B.; Shigenaga, M.; Hagen, T. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci.* 1993, 90, 7915-7922.
7. Hollman, P.; Katan, M. Absorption metabolism and health effects of dietary flavonoids in man. *Biomed. and Pharmacother.* 1997, 51, 305-310.
8. Liu, R. H. Health benefits of fruits and vegetables are from additive and synergistic combinations of

- phytochemicals. *Am. J. Clin. Nutr.* 2003, 78suppl., 517s-520s.
9. Hollman, P.; Arts, I. Flavonols, flavones, and flavanols-nature, occurrence and dietary burden. *J. Sci. Food Agri.* 2000, 80, 1081-1093.
 10. Sun, J.; Chu, Y.; Wu, X.; Liu, R. H. Antioxidant and antiproliferative activities of common fruits. *J. Agric. Food Chem.* 2002, 50, 7449-7454.
 11. Feskanih, D.; Ziegler, R.; Michaud, D.; Giovannucci, E.; Speizer, F.; Willett, W.; Colditz, G. Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *J. Natl. Cancer Inst.* 2000, 92, 1812-1823.
 12. Le Marchand, L.; Murphy, S.; Hankin, J.; Wilkens, L.; Kolonel, L. Intake of flavonoids and lung cancer. *J. Natl. Canc. Inst.* 2000, 92, 154-160.
 13. Knekt, P.; Jarvinen, R.; Seppanen, R.; Heliovaara, M.; Teppo, L.; Pukkala, E.; Aromaa, A. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *Am. J. Epidemiol.* 1997, 146, 223-230.
 14. Sesso, H.; Gaziano, J. M.; Liu, S.; Buring, J. Flavonoid intake and risk of cardiovascular disease in women. *Am. J. Clin. Nutr.* 2003, 77, 1400-1408.
 15. Tabak, C.; Arts, I.; Smit, H.; Heederik, D.; Kromhout, D. Chronic obstructive pulmonary disease and intake of catechins, flavonols, and flavones. *Am. J. Respir. Crit. Care Med.* 2001, 164, 61-64.
 16. Knekt, P.; Jarvinen, R.; Hakkinen, R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary mortality in Finland: a cohort study. *Br. Med. J.* 1996, 312, 478-481.
 17. Hertog, M.; Feskens, E.; Hollman, P.; Katan, M.; Kromhout, D. Dietary antioxidant flavonols and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 1993, 342, 1007-1111.
 18. Knekt, P.; Kumpulainen, J.; Jarvinen, R.; Rissanen, H.; Heliovaara, M.; Reunanen, A.; Hakulinen, T.; Aromaa, A. Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr.* 2002, 76, 560-568.
 19. Wolfe, K.; Wu, X.; Liu, R. H. Antioxidant activity of apple peels. *J. Agric. Food Chem.* 2003, 2003, 609-614.
 20. Eberhardt, M.; Lee, C.; Liu, R. H. Antioxidant activity of fresh apples. *Nature* 2000, 405, 903-904.
 21. Escarpa, A.; Gonzalez, M. High-performance liquid chromatography with diode-array detection for the performance of phenolic compounds in peel and pulp from different apple varieties. *J. of Chromat. A* 1998, 823, 331-337.
 22. Hammerstone, J.; Lazarus, S.; Schmitz, H. Procyanidin content and variation in some commonly consumed foods. *J. Nutr.* 2000, 130, 2086S-2092S.
 23. Awad, M.; de Jager, A.; van der Plas, L.; van der Krol, A. Flavonoid and chlorogenic acid changes in peels of Elstar and Jonagold apples during development and ripening. *Scientia Horticulturae* 2001, 90, 69-83.
 24. Awad, M.; Wagenmakers, P.; de Jager, A. Effects of light on flavonoid and chlorogenic acid levels in the peels of Jonagold apples. *Scientia Horticulturae* 2001, 88, 289-298.
 25. Awad, M.; de Jager, A. Relationships between fruit nutrients and concentrations of flavonoids and chlorogenic acid in Elstar apple peels. *Scientia Horticulturae* 2002, 92, 265-276.
 26. Awad, M.; de Jager, A. Formation of flavonoids, especially anthocyanin and chlorogenic acid in Jonagold apple peels: influences of growth regulators and fruit maturity. *Scientia Horticulturae* 2002, 93, 257-266.
 27. van der Sluis, A.; Dekker, M.; de Jager, A.; Jongen, W. Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. *J. Agri. Food Chem.* 2001, 49, 3606-3613.
 28. Goulding, J.; McGlasson, B.; Wyllie, S.; Leach, D. Fate of apple phenolics during cold storage. *J. Agric. Food Chem.* 2001, 49, 2283-2289.
 29. van der Sluis, A.; Dekker, M.; Skrede, G.; Jongen, W. Activity and concentration of polyphenolic antioxidants in apple juice. 1. effect of existing production methods. *J. Agric. Food Chem.* 2002, 50, 7211-7219.
 30. Wolfe, K.; Liu, R. H. Apple peels as a value-added food ingredient. *J. Agric. Food Chem.* 2003, 51, 1676-1683.

Rui Hai Liu is assistant professor of food science at Cornell University's campus in Ithaca, NY. His research program focuses on diet and cancer, functional foods/nutraceuticals for chronic disease prevention, and herbal remedies for cancer and hepatitis. He studies the health benefits of phytochemicals in fruits and vegetables. Jeanelle Boyer is a graduate student working with Dr. Liu.
