Antioxidant and Antiproliferative Activities of Selected New York Apple Cultivars

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No single antioxidant can replace the combination of natural phytochemicals in apples to achieve health benefits. The benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in fruits and vegetables. Consumers should obtain their antioxidants from fruits and vegetables for health improvement and disease prevention.

Cancer is the second leading cause of death in the United States. Dietary factors were thought to contribute to about one-third of those cancer deaths. Diets rich in fruits and vegetables have been associated with lower incidences in cancer and lower mortality from coronary heart disease. In addition to the presence of Vitamins C and E, fruits and vegetables are a rich source of phenolic acids and flavonoids, called phytochemicals, which also serve as antioxidants to counteract the prooxidant load of the body. These phytochemicals can have complementary and overlapping mechanisms of action, including modulation of detoxification enzymes, stimulation of the immune system, regulation of cellular signal transduction pathway, reduction of platelet aggregation, modulation of cholesterol synthesis and hormone metabolism, reduction of blood pressure, and antioxidant, antibacterial, and antiviral effects. The specific mechanisms of action of most phytochemicals in cancer prevention, although not yet fully comprehended, seem to be diverse. It has been reported that phytochemicals can inhibit carcinogenesis by induction of phase II enzymes, and inhibiting phase I enzymes, scavenging free radicals, protecting DNA from oxidized damages, regulating expression of tumor genes and tumor suppressor genes, suppressing the abnormal proliferation of early, preneoplastic lesions and by inhibiting certain properties of the cancer cell. It was estimated that 32 percent of cancer deaths might be avoidable by changes in the diet. Thus, the National Research Council (NRC) recommends eating 5 or more servings of a combination of vegetables and fruits. The purpose of this article is to report the results of ongoing research on antioxidant and antiproliferative activities of phytochemical extracts from selected New York apples. This research provides evidence that consumption of fruits and vegetables may play a significant role in reducing the risk of chronic diseases such as cancer.

Phytochemical Content

Total phenolic content of 10 different apple varieties both with and without skin are shown in Table 1. Fuji apples with skin had the highest total phenolic content (230.49 ± 4.4 mg/100 g apple) followed by Red Delicious, Gala, Liberty, Northern Spy, Golden Delicious, Fortune, Jonagold, Empire, and NY674. The total phenolic content in apples without skin was highest for Red Delicious (167.82 ± 1.7 mg/100g apple) followed by Northern Spy, Fortune, Gala, Fuji, Liberty, Golden Delicious, NY674, Jonagold, and Empire. Total phenolic content was higher in all varieties for apples with skin when com

TABLE 1

Total phenolic content of apples

	Total Phenolics (mg gallic acid eq./100g)	
	With skin	Without skin
Fuji	230.49 ± 4.4	131.39 ± 1.0
Red Delicious	204.49 ± 2.1	167.82 ± 1.7
Gala	200.39 ± 2.6	133.78 ± 1.7
Liberty	196.75 ± 0.32	127.95 ± 3.2
Northern Spy	191.50 ± 0.84	142.34 ± 0.83
Golden Delicious	179.19 ± 5.9	124.14 ± 3.6
Fortune	152.04 ± 1.5	137.60 ± 1.8
Jonagold	126.49 ± 0.88	102.49 ± 3.7
Empire	115.07 ± 1.1	71.61 ± 1.4
NY647	110.68 ± 1.5	117.42 ± 3.8

pared to apples without skin with the exception of NY674.

The total flavonoid content was highest for Fuji apples with skin (108.22 ± 4.4 mg/100 g apple) followed by Red Delicious, Northern Spy, Fortune, Gala, Liberty, Golden Delicious, Jonagold, NY674, and Empire (Table 2). For apple varieties without skin, Liberty had the highest flavonoid content (81.67 ± 3.8 mg/100 g apple), whereas Empire apples had the lowest value (25.25 ± 0.86 mg/100 g apple). Apples with skins had a higher total flavonoid content when compared to apples without skins for all 10 varieties analyzed.

TABLE 2

Total Flavonoids content of apples

	Total Flavonoids (mg catechin eq./100g)	
	With skin	Without skin
Fuji	108.22 ± 4.4	59.62 ± 2.9
Red Delicious	99.51 ± 1.2	67.60 ± 0.8
Northern Spy	95.62 ± 0.16	72.28 ± 0.47
Fortune	93.97 ± 3.9	55.03 ± 1.7
Gala	92.66 ± 1.6	60.93 ± 1.0
Liberty	86.86 ± 3.1	81.67 ± 3.8
Golden Delicious	73.13 ± 4.9	50.10 ± 0.55
Jonagold	62.13 ± 3.4	42.41 ± 3.2
NY647	61.16 ± 2.3	39.40 ± 3.9
Empire	48.36 ± 1.2	25.25 ± 0.86

Total Antioxidant Activity

The total antioxidant activity of apples was measured by Total Oxyradical Scavenging Capacity (TOSC) expressed as mmol Vitamin C equivalents per gram. TOSC values (mmol Vitamin C equivalents/g apple) of the different apple varieties both with and without skin were determined (Fig. 1). All of the apples tested exhibited a great antioxidant activity. For apples with skin, Northern Spy and Red Delicious had the highest TOSC value at 83.34 and 83.3 followed by Fuji, Gala, Liberty, NY674, Golden Delicious, Fortune, Jonagold, and Empire. Northern Spy apples without skin had the highest antioxidant activity (48.54) followed by Fuji, Red Delicious, Golden Delicious, Liberty, Gala, NY674, Fortune and Jonagold, whereas Empire apples without skin had the lowest antioxidant activity (19.66 TOSC). Overall, apples with skin had greater antioxidant activities (as indicated by TOSC values) than apples without skin for all varieties. There was a significant difference ($p < 0.05$) for TOSC values between apples with skin and apples without skin for Northern Spy, Red Delicious, Fuji, Gala, Liberty, NY674, Fortune, and Empire. Apple skins are known to contain higher amounts of phenolic compounds than the flesh. It was reported that the amount of phenolics in the skin of apples was several times higher than that of the flesh and that the quercetin glycosides (the most predominate flavonoid in apples) were only found in the skin. The higher phenolic and flavonoid content found in the apple with skins may have contributed to its higher antioxidant activity over the apples without skin.

Vitamin C is a powerful antioxidant and it is present in high concentrations in fruits. The Vitamin C content of raw Red Delicious apples with skin is 5.7 mg/100g.

The antioxidant activity of 1 g of Red Delicious apple with skin and without skin was 83.3 and 46.07 TOSC (mmol vitamin C equivalents/g, Fig. 2), respectively. The calculated antioxidant activity of Vitamin C in 1 gram of Red Delicious apple with skin was only 0.32 TOSC (mmol vitamin C equivalents/g). The Vitamin C in apple with skin accounts for only 0.4 percent of total antioxidant activity. Therefore, the majority of antioxidant activity of apple is not from Vitamin C but from other phytochemicals in apples. The combinations of different phytochemicals in apples may function additively or synergistically to be responsible for this potent antioxidant activity.

Relationship Between Total Phenolic/flavonoid Contents and Antioxidant Activity

The relationship between total phenolic, and flavonoid content and antioxidant activity (TOSC values expressed as µmol vitamin C eq./g) of apples is shown in Figure 3. There was a positive ($r^2 = 0.7298$) relationship between total phenolic content and total antioxidant activity of apples with a statistical significance ($p<0.001$, Fig. 3A). The relationship between flavonoid content and total antioxidant activity of apples was also positive ($r^2 = 0.7403$) with statistical significance ($p < 0.001$, Fig. 3B). This indicates that total phenolic and flavonoid contents have a significant contribution to the antioxidant activity of apples. The different mixtures of antioxidants in apples may have had different additive or synergistic effects to enhance the total antioxidant activity.

Effect of Apple Extracts on Cancer Cell Proliferation

Apple extracts from selected apple varieties were added to human liver cancer HepG2 cells to determine if the extracts could inhibit tumor cell proliferation. HepG2 cells were treated with extracts equivalent to 0, 1, 5, 10, 20, 30, 40, and 50 mg of apple for 96 hours. These levels were chosen based on preliminary experiments indicating doses that were not cytotoxic to cells and resulted in the inhibition of cell proliferation. Fuji apples with skin inhibited cell proliferation by 39.03 percent and 15.94 peercent for apples without skin at a dose of 50 mg/ml (Fig. 4). Red Delicious apples with skin reduced cell proliferation by 57.14 percent and 39.54 percent for

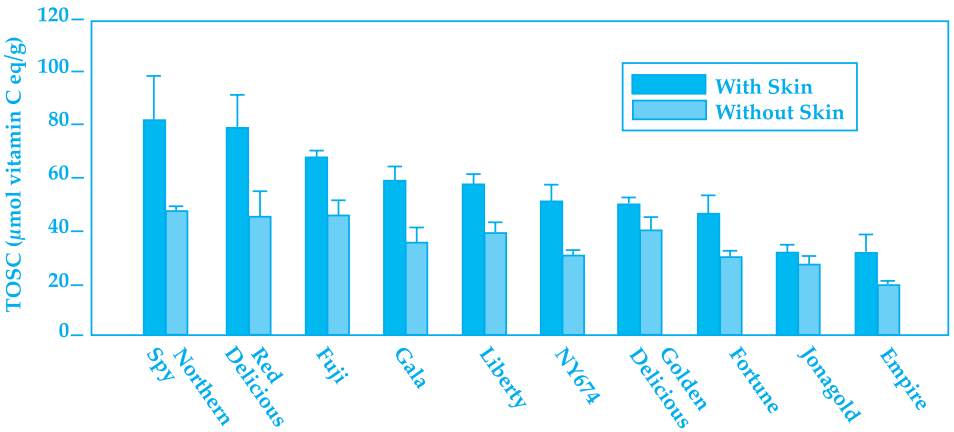
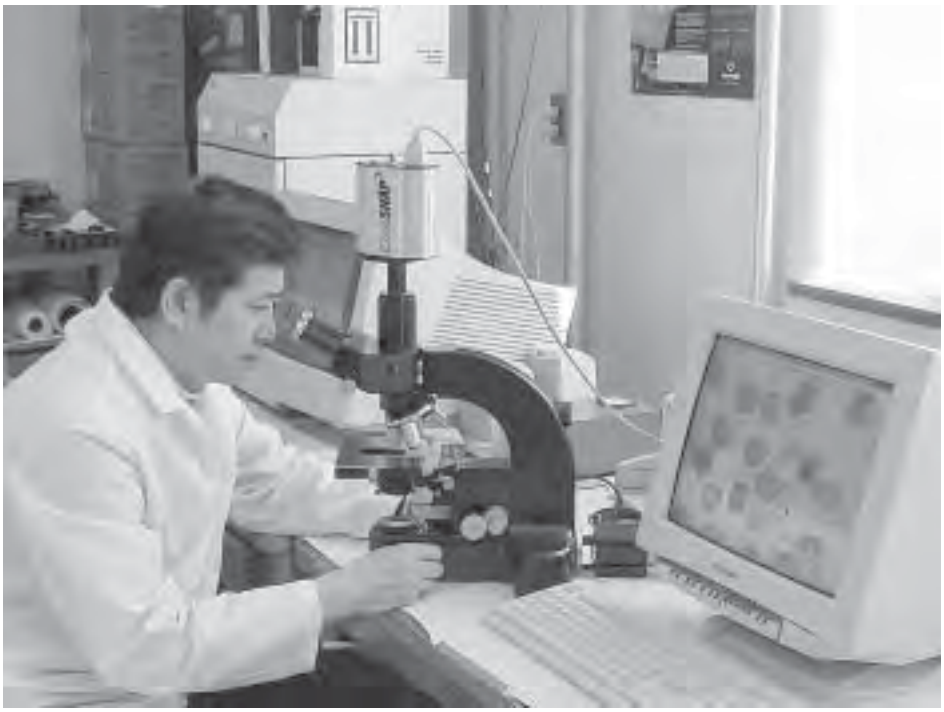


Figure 1. Total antioxidant activities of selected apple varieties (mean ± SD; n=3)



Dr. Rui Hai Lui examines human liver cancer Hep G2 cells.

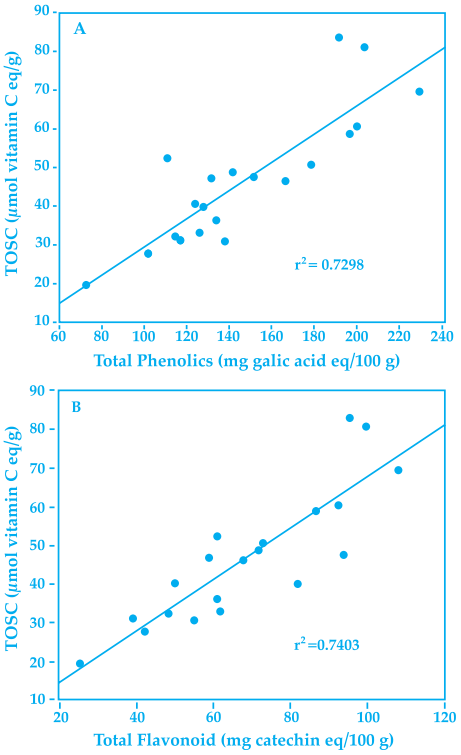


Figure 3. Relationship between (A) total phenolic, (B) flavonid content and antioxidant activity of selected apple varieties

apples without skin at a dose of 50 mg/ml. Gala without skin did not inhibit cell proliferation when compared to the control whereas Gala with skin inhibited cell proliferation 34.65 percent at 50 mg/ml. Northern Spy apples with skin and without skin did not inhibit cell proliferation when compared to the control.

The EC_{50} values for Red Delicious, Fuji, and Gala apple were calculated. A lower EC_{50} translates a higher activity to inhibit cancer cell proliferation. Red Delicious apples with skin had the lowest EC_{50} value at 86.90 mg/ml followed closely by Gala (92 mg/mL) and Fuji apples with skin (116 mg/mL).

There was a large variation in the effects of the different apple varieties on the inhibition of cell proliferation. Apples without skin were less potent in inhibiting HepG2 cell proliferation whereas apples with skins exerted greater inhibitions of cell proliferation.

In summary, our results demonstrated the combination of phytochemicals in apples is critical to its potent antioxidant activity and antiproliferative activity. Apple with skin displayed higher antioxidant and antiproliferative activities than apple without skin. The total phenolic and flavonoid content was positively related to antioxidant activity and inhibition of cell proliferation. Additionally, the minimal contribution of Vitamin C to the antioxidant activity of apples further supports the proposal that other phytochemicals, such as phenolic acids and flavonoids, significantly contribute to the *in vitro* antioxidant activity of apples. The benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in fruits and vegetables. Therefore, no single antioxidant can replace the combination of natural phytochemicals in fruits and vegetables to achieve the health benefits. These data provide direct supportive evidence for the Five-a-Day program and suggests that consumers obtain their antioxidants from fruits and vegetables for health improvement and disease prevention.

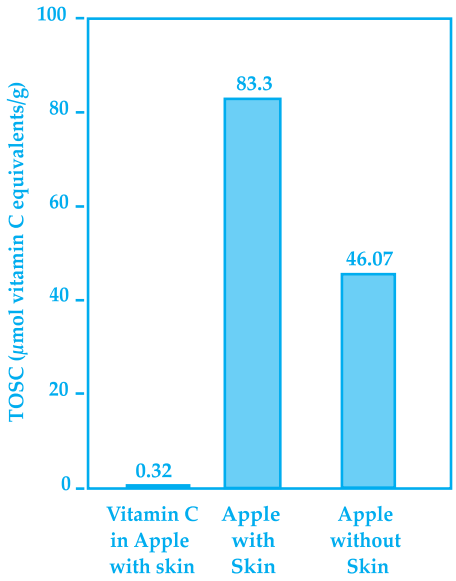


Figure 2. Total antioxidant activity of Red Delicious Apple (mean ± SD; n=3)

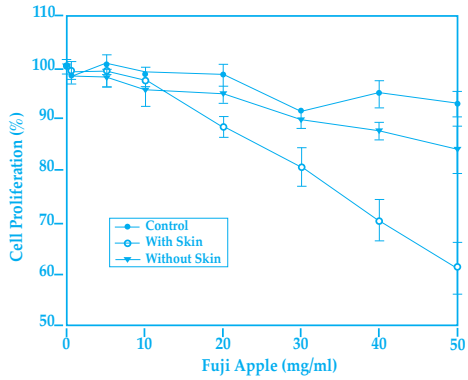


Figure 4. Inhibition of HepG2 cell proliferation by Fuji apple extracts (mean ± SD; n=3)

Rui Hai Liu is an assistant professor of food science in Ithaca who studies the antioxidant and anticancer activities of the phytochemicals in fruit. Marian Eberhardt is a technician who works with Rui Hai Liu. Marian Eberhardt is a graduate student working with Lui on the health benefits of apples. Chang Yong "Cy" Lee is a professor in the department of food science and technology at Geneva working on phytochemicals and their antioxidant properties.