

## Antioxidant Activity of Apple Peels

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Consumption of fruits and vegetables has been shown to be effective in the prevention of chronic diseases. These benefits are often attributed to the high antioxidant content of some plant foods. Apples are commonly eaten and are large contributors of phenolic compounds in European and North American diets. The peels of apples, in particular, are high in phenolics. During applesauce and canned apple manufacture, the antioxidant-rich peels of apples are discarded. To determine if a useful source of antioxidants is being wasted, the phytochemical content, antioxidant activity, and antiproliferative activity of the peels of four varieties of apples (Rome Beauty, Idared, Cortland, and Golden Delicious) commonly used in applesauce production in New York state were investigated. The values of the peels were compared to those of the flesh and flesh + peel components of the apples. Within each variety, the total phenolic and flavonoid contents were highest in the peels, followed by the flesh + peel and the flesh. Idared and Rome Beauty apple peels had the highest total phenolic contents ( $588.9 \pm 83.2$  and  $500.2 \pm 13.7$  mg of gallic acid equivalents/100 g of peels, respectively). Rome Beauty and Idared peels were also highest in flavonoids ( $306.1 \pm 6.7$  and  $303.2 \pm 41.5$  mg of catechin equivalents/100 g of peels, respectively). Of the four varieties, Idared apple peels had the most anthocyanins, with  $26.8 \pm 6.5$  mg of cyanidin 3-glucoside equivalents/100 g of peels. The peels all had significantly higher total antioxidant activities than the flesh + peel and flesh of the apple varieties examined. Idared peels had the greatest antioxidant activity ( $312.2 \pm 9.8$   $\mu$ mol of vitamin C equivalents/g of peels). Apple peels were also shown to more effectively inhibit the growth of HepG<sub>2</sub> human liver cancer cells than the other apple components. Rome Beauty apple peels showed the most bioactivity, inhibiting cell proliferation by 50% at the low concentration of  $12.4 \pm 0.4$  mg of peels/mL. The high content of phenolic compounds, antioxidant activity, and antiproliferative activity of apple peels indicate that they may impart health benefits when consumed and should be regarded as a valuable source of antioxidants.

**KEYWORDS:** Phenolics; flavonoids; anthocyanins; apple; antioxidant activity; cancer

### INTRODUCTION

The leading causes of death in the United States are cardiovascular disease and cancer. Doll and Peto (1) estimated that 35% (range 10–70%) of deaths from cancer could be avoided by dietary modifications. Willett (2) later narrowed the range to 20–42%, stating that roughly 32% of cancers could be prevented by changes in diet. Fruits and vegetables contain many compounds, including phenolics, thiols, carotenoids, tocopherols, and glucosinolates, that may exert chemoprotective effects through a variety of mechanisms (3). Increased intake of fruits and vegetables has also been associated with reduced risk of coronary heart disease (CHD) (4) and stroke (5). Flavonoids, commonly found in fruits and vegetables, have been linked to reduced risk or mortality from CHD (6). Such findings

have led the National Research Council (NRC) to recommend consuming five or more servings of fruits and vegetables a day.

Apples are a very significant part of the diet. From a Dutch Food Consumption Survey and previously analyzed flavonoid contents of fruits, vegetables, and beverages, Hertog et al. (7) determined that apples are the third largest contributors of flavonoids in the Dutch diet behind tea and onions. In Finland, along with onions, apples are the top contributors (8). Twenty-two percent of the fruit phenolics consumed in the United States are from apples, making them the largest source (9). Consumption of apples has been linked to the prevention of chronic disease. Apple intake has been negatively associated with lung cancer incidence in two separate studies (8, 10). It has also been related to reduced cardiovascular disease; coronary and total mortality (11), symptoms of chronic obstructive pulmonary disease (12), and risk of thrombotic stroke (13) have all been inversely associated with apple consumption.

Apples are a good source of phenolic compounds (14). The total extractable phenolic content has been investigated and

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ranges from 110 to 357 mg/100 g of fresh apple (15, 16). Previously, our research group found that peeled and unpeeled apples had high antioxidant activity and inhibited the growth of human cancer cells in vitro (14). Vitamin C was responsible for less than 0.4% of the antioxidant activity, indicating that other factors, such as phenolics, were the main contributors. The antioxidant and antiproliferative activities of unpeeled apples were greater than those of peeled apples. It is also known that the concentration of total phenolic compounds is much greater in the peel of apples than in the flesh (17–19). Both of these facts suggest that apple peels may possess more bioactivity than the flesh.

Apple peels are a waste product of applesauce and canned apple manufacture. The National Agriculture Statistics Service (NASS) (20) reported that 216 million pounds of apples were processed in this manner in New York state in 2000. We estimated that 16 million pounds of peels were generated. If apple peels show potential to improve health when consumed, their utilization should be investigated.

Therefore, the objective of this study was to evaluate the nutritional quality of apple peels. Total phenolic content, flavonoid content, anthocyanin content, antioxidant activity, and antiproliferative effect of apple peels were quantified, and the results were compared to those of the apple flesh and flesh + peel. Four apple varieties (Rome Beauty, Idared, Cortland, and Golden Delicious) commonly used in applesauce manufacture in New York state were evaluated.

## MATERIALS AND METHODS

**Chemicals.** Sodium nitrite, (+)-catechin, Folin–Ciocalteu reagent, and  $\alpha$ -keto- $\gamma$ -methiolbutyric acid (KMBA) were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium carbonate, sodium hydroxide, acetone, and potassium phosphate were obtained from Mallinckrodt (Paris, KY). Aluminum chloride, potassium chloride, and sodium acetate were purchased from Fisher Scientific (Pittsburgh, PA), and gallic acid was obtained from ICN Biomedical Inc. (Costa Mesa, CA). 2,2'-Azobis-(amidinopropane) (ABAP) was obtained from Wako Chemicals (Richmond, VA). The HepG<sub>2</sub> cells were from the American Type Culture Collection (ATCC) (Rockville, MD), and the MTS-based Cell Titer 96 nonradioactive cell proliferation assay was from Promega (Madison, WI). Williams Medium E (WME) and fetal bovine serum (FBS) were purchased from Gibco Life Technologies (Grand Island, NY).

**Samples.** Cortland and Idared apples were purchased from Cornell Orchards (Cornell University, Ithaca, NY). Rome Beauty and Golden Delicious apples were obtained from Red Jacket Orchards (Geneva, NY). All apples were kept in modified atmosphere storage until purchased. The apples were washed and dried before analysis. They were peeled, when necessary, with an Apple Master apple parer and cored. The flesh, flesh + peel, or peels were obtained from five randomly selected apples in each trial to minimize variation. The flesh was the edible portion of the apple without the peel. The flesh + peel was the edible portion of the apple with the amount of flesh and peel maintained in the same proportions as in the whole apple. The peels were the parts of the apple removed by the apple parer.

**Extraction.** The phenolic compounds of apples were extracted by a method similar to that reported previously by our laboratory (14, 21). Briefly, 50 g of apple flesh or apple flesh with the peel was blended with 200 g of chilled 80% acetone solution in a Waring blender for 5 min. The sample was then homogenized for 3 min using a Virtis 45 homogenizer. The slurry was filtered through Whatman No. 1 filter paper in a Buchner funnel under vacuum. The solids were scraped into 150 g of 80% acetone and homogenized again for 3 min before refiltering. The filtrate was recovered and evaporated using a rotary evaporator at 45 °C until less than 10% of the initial volume remained. The extract was made up to 50 mL with distilled water and frozen at -40 °C until analysis. Apple peels were extracted similarly using 25 g of peel. All extracts were made in triplicate.

**Determination of Total Phenolic Content.** The total phenolic contents of the apple samples were measured using a modified colorimetric Folin–Ciocalteu method (21). A volume of 0.5 mL of deionized water and 0.125 mL of a known dilution of the extract were added to a test tube. Folin–Ciocalteu reagent (0.125 mL) was added to the solution and allowed to react for 6 min. Then, 1.25 mL of 7% sodium carbonate solution was aliquoted into the test tubes, and the mixture was diluted to 3 mL with deionized water. The color developed for 90 min, and the absorbance was read at 760 nm using a MRX II DYNEX spectrophotometer (DYNEX Technologies, Inc., Chantilly, VA). The measurement was compared to a standard curve of prepared gallic acid solutions and expressed as milligrams of gallic acid equivalents per 100 g  $\pm$  SD fresh apple component for the triplicate extracts.

**Determination of Flavonoid Content.** The flavonoid content of the apple samples was measured using a modified colorimetric method (21, 22). A volume of 0.25 mL of a known dilution of extract was added to a test tube containing 1.25 mL of distilled water. To the mixture was added 0.075 mL of 5% sodium nitrite solution, and this was allowed to stand for 5 min. Then, 0.15 mL of 10% aluminum chloride was added. After 6 min, 0.5 mL of 1 M sodium hydroxide was added, and the mixture was diluted with another 0.275 mL of distilled water. The absorbance of the mixture at 510 nm was measured immediately using a MRX II DYNEX spectrophotometer and compared to a standard curve of prepared catechin solutions. The flavonoid content was expressed as milligrams of catechin equivalents per 100 g  $\pm$  SD fresh apple component for the triplicate extracts.

**Determination of Anthocyanin Content.** Monomeric anthocyanin content of the apple peels was measured using a spectrophotometric pH differential protocol (23, 24). The apple peel extracts were mixed thoroughly with 0.025 M potassium chloride pH 1 buffer in 1:3 or 1:8 ratio of extract to buffer. The absorbance of the mixture was then measured at 515 and 700 nm against a distilled water blank. The apple peel extracts were then combined similarly with sodium acetate buffer pH 4.5, and the absorbance of these solutions was measured at the same wavelengths. The anthocyanin content was calculated as follows:

$$\text{total monomeric anthocyanins (mg/100 g of fresh peel)} = \frac{A \times MW \times 1000}{(\epsilon \times C)}$$

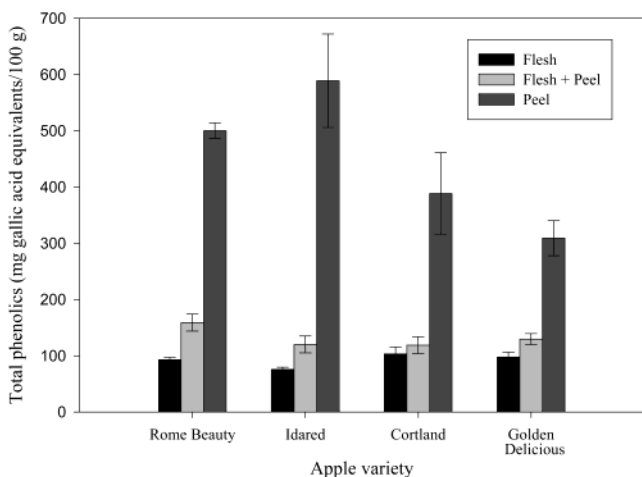
where  $A$  is absorbance =  $(A_{515} - A_{700})_{\text{pH } 1.0} - (A_{515} - A_{700})_{\text{pH } 4.5}$ ;  $MW$  is molecular weight for cyanidin 3-glucoside = 449.2;  $\epsilon$  is the molar absorptivity of cyanidin 3-glucoside = 26 900; and  $C$  is the concentration of the buffer in milligrams per milliliter. Anthocyanin content was expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g of fresh apple peel for the triplicate extracts.

**Quantification of Total Antioxidant Activity.** The total antioxidant activity of the apple components was determined using a modified total oxyradical scavenging (TOSC) assay (14, 25). In this assay, peroxy radicals are formed from 2,2'-azobis(amidinopropane) (ABAP) and oxidize  $\alpha$ -keto- $\gamma$ -methiolbutyric acid (KMBA) to form ethylene. The ethylene produced can be measured by gas chromatographic headspace analysis. Total antioxidant activity is measured by the degree of inhibition of formation of ethylene by the apple extracts. The antioxidant activity was measured at 15, 30, 45, and 60 min for four different concentrations to determine the TOSC values. The area under the kinetic curve was integrated to calculate the TOSC value at each concentration as follows:

$$\text{TOSC} = 100 - \left( \frac{\int SA}{\int CA} \right) \times 100$$

where  $\int SA$  is the integrated area from the sample reaction and  $\int CA$  is the integrated area from the control reaction. The median effective dose ( $EC_{50}$ ) was determined for each component (flesh, flesh + peel, and peel) of each apple variety from the dose–response curve of apple concentration versus TOSC. The antioxidant activity was expressed as micromoles of vitamin C equivalents per 1 g of apple. The TOSC values were stated as mean  $\pm$  SD for triplicate samples.

**Determination of Inhibition of HepG<sub>2</sub> Cell Proliferation.** The extracts were used to measure the ability of the apple peels to inhibit



**Figure 1.** Total phenolic content of apples (mean  $\pm$  SD,  $n = 3$ ).

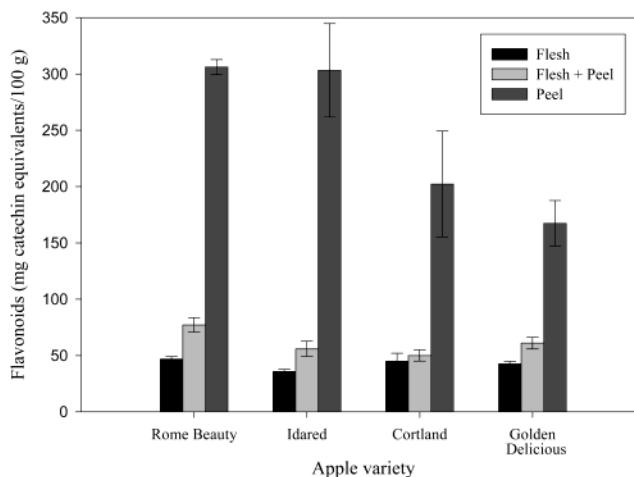
HepG<sub>2</sub> human liver cancer cell proliferation (14, 24). The cell cultures were exposed to various concentrations of the extracts during a 96-h growth period. Cell proliferation was measured by the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2H-tetrazolium (MTS) to formazan. This product absorbs light at 490 nm and was measured spectrophotometrically. One extract for each component of each variety was analyzed in triplicate, and the absorbance was measured using a MRX II DYNEX spectrophotometer. The effective median dose (EC<sub>50</sub>) was determined and expressed as milligrams of apple component per milliliter  $\pm$  SD.

**Statistical Analysis.** All data were reported as mean  $\pm$  standard deviation of three replicates. The results were compared by analysis of variance (ANOVA) using Minitab software (Minitab, Inc., State College, PA). Pairwise multiple comparisons were done by Tukey's significant difference test with the family error rate held at 0.05.

## RESULTS

**Content of Phenolic Compounds.** The total phenolic contents of the flesh, flesh + peel, and peel of the four apple varieties were determined (Figure 1). Of the peels, the total phenolic contents of Idared and Rome Beauty peels were highest ( $p < 0.05$ ) at  $588.9 \pm 83.2$  and  $500.2 \pm 13.7$  mg of gallic acid equivalents/100 g of peels, respectively, followed by  $388.5 \pm 82.4$  for Cortland and  $309.1 \pm 32.1$  for Golden Delicious apples. The flesh + peel values were  $159.0 \pm 15.1$ ,  $129.7 \pm 9.7$ ,  $120.1 \pm 15.0$ , and  $119.0 \pm 14.9$  mg of gallic acid equivalents/100 g of flesh + peel for Rome Beauty, Golden Delicious, Idared, and Cortland apples, respectively. These values were not significantly different ( $p > 0.05$ ). The total phenolic contents of the flesh of Cortland ( $103.2 \pm 12.3$  mg of gallic acid equivalents/100 g of flesh), Golden Delicious ( $97.7 \pm 8.9$ ), and Rome Beauty ( $93.0 \pm 4.1$ ) apples were similar ( $p > 0.05$ ). The flesh of Idared apples had a lower phenolic content ( $75.7 \pm 4.0$  mg of gallic acid equivalents/100 g of flesh) than the flesh of Cortland and Golden Delicious apples ( $p < 0.05$ ). The total phenolic content tended to be highest in the peel, followed by the flesh + peel and the flesh for all four apple varieties. The total phenolic contents of the peels were significantly higher than the flesh and flesh + peel values within all varieties ( $p < 0.05$ ), while the phenolic contents of the flesh samples were not significantly lower than the flesh + peels contents ( $p > 0.05$ ). The flesh and flesh + peel values were similar for Idared, Cortland, and Golden Delicious apples ( $p > 0.05$ ).

The soluble flavonoids of the apple components were measured (Figure 2). The peels of Rome Beauty and Idared apples had the highest flavonoid contents ( $306.1 \pm 6.7$  and  $303.2 \pm 41.5$  mg of catechin equivalents/100 g of peel, respectively).

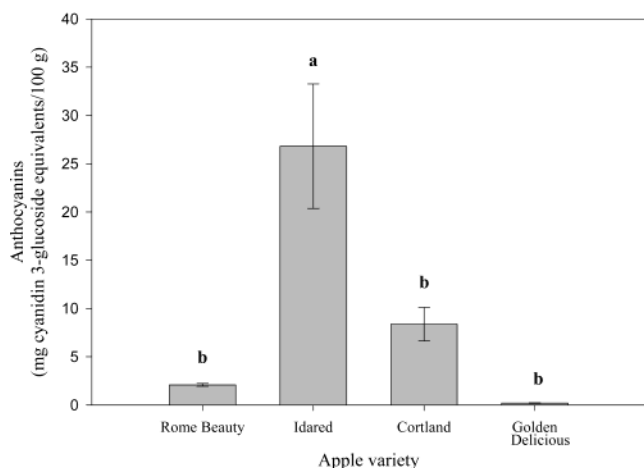


**Figure 2.** Flavonoid content of apples (mean  $\pm$  SD,  $n = 3$ ).

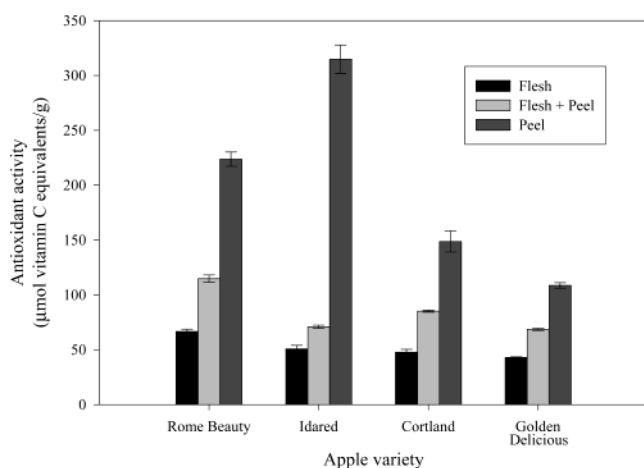
The flavonoid contents of Rome Beauty and Idared apple peels were not significantly different ( $p > 0.05$ ) and were higher than the contents of the peels from Cortland and Golden Delicious apples ( $p < 0.05$ ). The values for the peels of the Cortland and Golden Delicious apples were similar, at  $202.2 \pm 47.1$  for Cortland and  $167.4 \pm 20.2$  mg of catechin equivalents/100 g of peel for Golden Delicious apples ( $p > 0.05$ ). The flavonoid contents of the flesh + peel components of the apples were, in descending order,  $77.1 \pm 6.4$ ,  $61.0 \pm 5.1$ ,  $55.8 \pm 6.8$ , and  $50.0 \pm 4.9$  mg of catechin equivalents/100 g of flesh + peel for Rome Beauty, Golden Delicious, Idared, and Cortland, respectively. The Rome Beauty flesh + peel possessed significantly more flavonoids than the flesh + peel of the other varieties ( $p < 0.05$ ), which all had similar flavonoid contents ( $p > 0.05$ ). The flesh contained  $46.8 \pm 2.3$  (Rome Beauty),  $45.0 \pm 7.0$  (Cortland),  $42.5 \pm 2.3$  (Golden Delicious), and  $35.7 \pm 2.0$  (Idared) mg of catechin equivalents/100 g of flesh. Only the flavonoid contents of the flesh of Rome Beauty and Idared apples were significantly different ( $p < 0.05$ ). Within all varieties, the peels tended to contain the most flavonoids, followed by the flesh + peel and the flesh. The flavonoid contents of the peels were statistically higher than the flesh and flesh + peel values for each variety ( $p < 0.05$ ). Only for Rome Beauty apples was the flavonoid content of the flesh + peel significantly higher than that of the flesh ( $p < 0.05$ ).

The anthocyanins in the apple peels were quantified (Figure 3). The anthocyanin content of the flesh and flesh + peel were not analyzed, as apple flesh of these varieties does not contain anthocyanins. The anthocyanin content of Idared apple peels was the highest, at  $26.8 \pm 6.5$  mg of cyanidin 3-glucoside equivalents/100 g of peels ( $p < 0.05$ ), followed by Cortland ( $8.4 \pm 1.7$ ) and Rome Beauty ( $2.1 \pm 0.2$ ). Golden Delicious apple peels contained a trace amount of anthocyanins. The anthocyanin content of Idared peels was significantly higher than the content of the peels of the other varieties ( $p < 0.05$ ). There were no significant differences in the anthocyanin contents of Cortland, Rome Beauty, and Golden Delicious peels ( $p > 0.05$ ).

**Total Antioxidant Activity.** The total antioxidant activity of the peels was greater than that of the flesh or flesh + peel for all varieties (Figure 4). Idared peel possessed the greatest activity, with  $312.2 \pm 9.8$   $\mu$ mol of vitamin C equivalents/g of peel. The peels of Rome Beauty, Cortland, and Golden Delicious apples had total antioxidant activities of  $228.4 \pm 6.7$ ,  $159.0 \pm 4.3$ , and  $111.4 \pm 4.7$   $\mu$ mol of vitamin C equivalents/g of peel, respectively. All peel values of antioxidant activity were



**Figure 3.** Anthocyanin content of apple peels (mean  $\pm$  SD,  $n = 3$ ). Bar values with no letters in common are significantly different ( $p < 0.05$ ).



**Figure 4.** Antioxidant activity of apples (mean  $\pm$  SD,  $n = 3$ ).

significantly different ( $p < 0.05$ ). The flesh + peel of Rome Beauty apples had the highest antioxidant activity ( $131.6 \pm 0.8$   $\mu\text{mol}$  of vitamin C equivalents/g of flesh + peel) when compared to that component of the other apples ( $72.2 \pm 2.3$ ,  $83.5 \pm 2.3$ , and  $66.9 \pm 1.8$   $\mu\text{mol}$  of vitamin C equivalents/g of flesh + peel for Idared, Cortland, and Golden Delicious, respectively). The antioxidant activities of the flesh + peel components were statistically different ( $p < 0.05$ ) between varieties, except there was no difference between the values for Idared and Golden Delicious flesh + peel ( $p > 0.05$ ). In descending order, the flesh components had total antioxidant activities of  $68.02 \pm 1.47$  (Rome Beauty),  $50.4 \pm 2.2$  (Cortland),  $46.9 \pm 1.6$  (Idared), and  $43.5 \pm 0.4$  (Golden Delicious)  $\mu\text{mol}$  of vitamin C equivalents/g of flesh. The flesh antioxidant activity of Rome Beauty apples was significantly higher than that of the other varieties ( $p < 0.05$ ). There were no differences among the antioxidant activities of the flesh of Cortland, Idared, and Golden Delicious apples ( $p > 0.05$ ). The antioxidant activity was highest from the peels in all varieties, followed by the flesh + peel and the flesh ( $p < 0.05$ ).

**Inhibition of Cancer Cell Proliferation.** The effect of apple flesh, flesh + peel and peels on the growth of HepG<sub>2</sub> cells in vitro is summarized in **Figure 5**. The curves show that liver cancer cell growth was inhibited in a dose-dependent manner. The flesh and flesh + peel curves had similar slopes for most of the varieties; however, the flesh + peel of Rome Beauty apples was unusually inhibitory (**Figure 5A**). **Table 1** shows the EC<sub>50</sub> of the antiproliferative activity of different apple

varieties. Lower EC<sub>50</sub> values represent higher antiproliferative activities. Rome Beauty apples had the lowest EC<sub>50</sub> values for the peel and flesh + peel components, at  $12.4 \pm 0.4$  and  $26.5 \pm 0.3$  mg of apple/mL, respectively, indicating the most antiproliferative activity of the varieties examined ( $p < 0.05$ ). The differences in the activities of the peels between varieties were significant ( $p < 0.05$ ). All the values were similar for the flesh + peel ( $p > 0.05$ ), except Rome Beauty apples were statistically more inhibitory than Idared ( $p < 0.05$ ). Cortland and Golden Delicious flesh had similar EC<sub>50</sub> values ( $p > 0.05$ ). The inhibition of cell proliferation by Rome Beauty flesh and Idared flesh was not adequate to calculate their EC<sub>50</sub> values. The peels of each apple variety inhibited the growth of HepG<sub>2</sub> cells more than the flesh or flesh + peel, and the peels had low EC<sub>50</sub> values compared to the flesh and flesh + peel components. The EC<sub>50</sub> doses were significantly different between the peel, flesh + peel, and flesh of Rome Beauty, Cortland, and Golden Delicious apples ( $p < 0.05$ ). There was no statistical difference between the EC<sub>50</sub> values of the peel and flesh + peel of Idared apples ( $p > 0.05$ ). This may be due to the high variation of data collected from Idared apples.

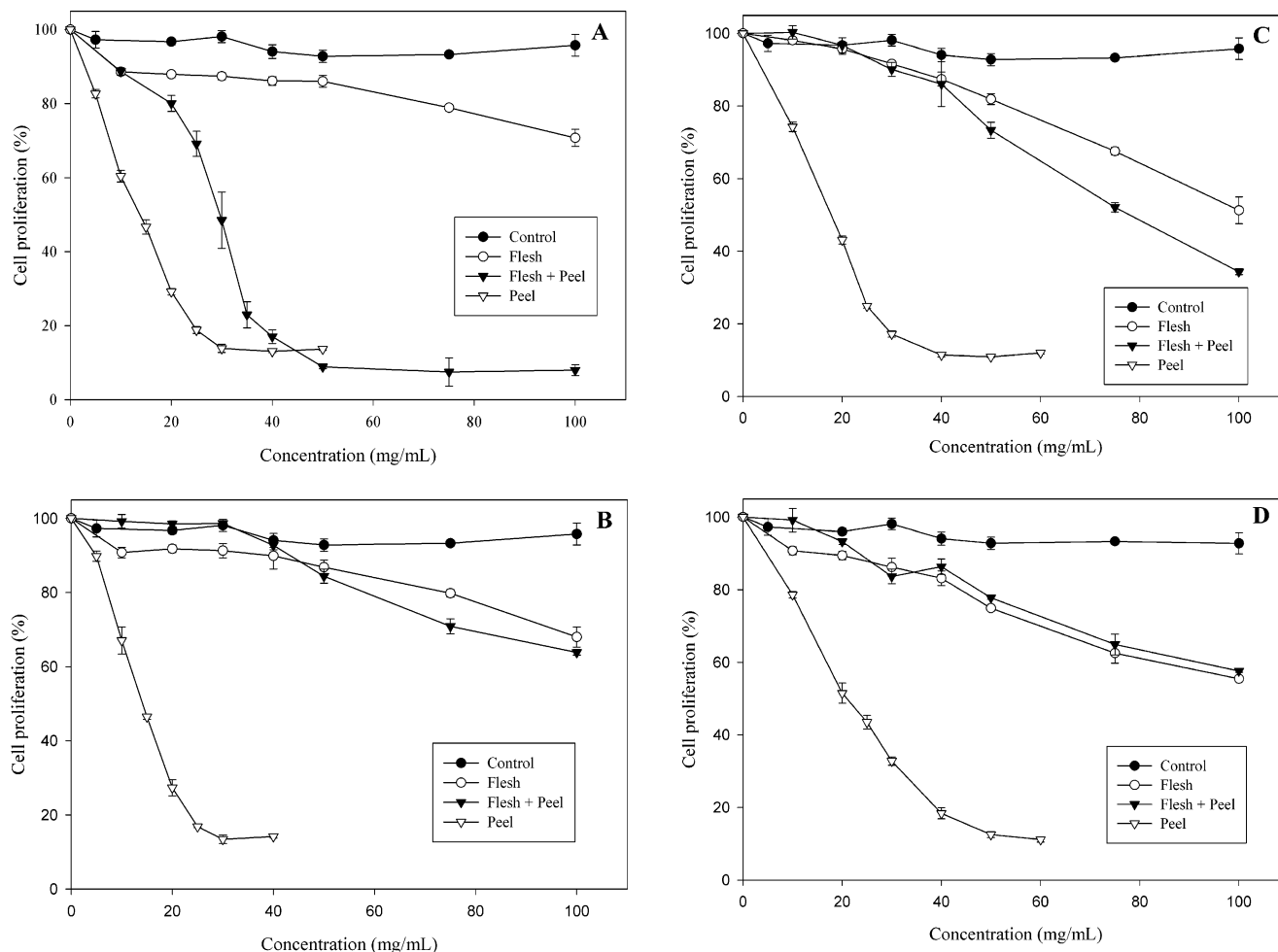
Correlations between total phenolics, total antioxidant activity, and cell proliferation EC<sub>50</sub> values were analyzed for the flesh, flesh + peel, and peels of the apples. Total antioxidant activity was highly correlated to cancer cell inhibition for the flesh + peel extracts ( $R^2 = 0.939$ ,  $p < 0.05$ ). There were no other significant relationships observed.

## DISCUSSION

Apple peels are a waste product from applesauce and canned apple manufacture. In the year 2000 in New York state, 216 million pounds of apples were processed in this manner (20), resulting in an estimated production of almost 16 million pounds of waste apple peels. Thus, a valuable source of nutrition may be removed from our food chain.

We have shown that apple peels possess high contents of phenolic compounds compared to other edible parts of the apple. The total phenolic and flavonoid contents for the flesh and flesh + peel samples were comparable to those previously reported (15, 16). Other research groups have also noted that apple peels had higher phenolic content than the flesh (17–19). The nature and distribution of these phenolics between the flesh and the peel of the apple is also different. Among others, the flesh contains catechins, procyanidins, phloridzin, phloretin glycosides, caffeic acid, and chlorogenic acid; the peel possesses all of these compounds and has additional flavonoids not found in the flesh, such as quercetin glycosides (17, 19, 26, 27). Of the catechins, only (+)-catechin and (–)-epicatechin are present in appreciable amounts in apples (28), with epicatechin being approximately twice as concentrated as catechin in the peels (26). The most common procyanidins, oligomers of epicatechin, are procyanidin B2, procyanidin B5, and procyanidin trimer (26, 29). Lister et al. (29) reported quercetin glycoside concentrations of 400–700 mg/100 g and 250–550 mg/100 g in Granny Smith and Splendour apple peels, respectively, with quercetin 3-galactoside (hyperin), quercetin 3-arabinofuranoside (avicularin), quercetin 3-rhamnoside (quercetin), and quercetin 3-xyloside (reynoutrin) being the four most common. Golding et al. (26) found similar levels of the flavonols in peels, recording values of 99–300 mg/100 g. Phloridzin and chlorogenic acid levels were quite low in the peel compared to those in the flesh (30).

The measured anthocyanin content of the apple peels was related to their appearance. The red color of apple peels is due to the presence of cyanidin 3-galactoside (31). The Idared apples



**Figure 5.** Inhibition of HepG<sub>2</sub> human liver cancer cell proliferation by phytochemical extracts of Rome Beauty (A), Idared (B), Cortland (C), and Golden Delicious (D) apples (mean  $\pm$  SD,  $n = 3$ ).

**Table 1.** EC<sub>50</sub> Values for the Inhibition of HepG<sub>2</sub> Human Liver Cancer Cell Proliferation by Phytochemical Extracts of Apples (Mean  $\pm$  SD,  $n = 3$ )

apple	flesh (mg/mL)	flesh + peel (mg/mL)	peel (mg/mL)
Rome Beauty	<sup>a</sup>	26.5 $\pm$ 0.3	12.4 $\pm$ 0.4
Idared	<sup>a</sup>	125.1 $\pm$ 58.8	13.6 $\pm$ 0.2
Cortland	103.9 $\pm$ 16.5	74.1 $\pm$ 4.0	15.7 $\pm$ 0.3
Golden Delicious	155.3 $\pm$ 11.7	107.7 $\pm$ 22.7	20.2 $\pm$ 0.7

<sup>a</sup> The EC<sub>50</sub> value could not be calculated from the dose-response curve.

were deep red in color and had the most anthocyanins. The Cortland apples were bright red with green patches, and the Rome Beauty apples were pink. Both varieties had far less anthocyanins than the Idareds. Golden Delicious apple peels were almost devoid of anthocyanins, as expected by their lack of red pigmentation.

To our knowledge, this is the first time the total antioxidant activity of apple peels has been measured. The peels of the apple varieties under investigation exhibited high antioxidant activity compared to the flesh and flesh + peel ( $p < 0.05$ ). These antioxidant activities were high in relation to those of other fruits and vegetables tested by our research group. The peels from one average-sized Idared apple had an antioxidant activity equivalent to 820 mg of vitamin C. The antioxidant activity of the edible portion of apples has been ascertained in the past using the oxygen radical absorbance capacity assay (ORAC).

Wang et al. (32) ranked apples ninth out of 12 fruits, and Vinson et al. (9) placed them eighth out of 20. Using the TOSC assay, apples exhibited high total antioxidant activity, and the value varied between apple varieties (14). There was also a positive relationship between the phenolic content of apples and their antioxidant activity (16). Apple extracts are able to bind to plasma low-density and very low-density lipoproteins and inhibit their oxidation (9).

The antiproliferative ability of apple peels had not been investigated previously. The peels of Rome Beauty, Idared, Cortland, and Golden Delicious apples greatly inhibited the growth of liver tumor cells in vitro. The flesh + peel and flesh samples also showed inhibitory effects in most cases, though they showed much less antiproliferative activity than the peels. Liu et al. (16) likewise reported an antiproliferative effect from phytochemical extracts of Fuji, Gala, and Red Delicious apples on human liver cancer cells. Interestingly, Rome Beauty flesh + peel had a low EC<sub>50</sub> value compared to the flesh + peel of the other varieties, despite the lack of growth inhibition by the flesh alone. This may indicate some synergistic effects between the phytochemicals of the flesh and peel of this variety.

Our results show that eating apple peels may have health benefits for consumers. Apple peels are often discarded in the production of processed apple products, but clearly they possess high levels of antioxidant and bioactive compounds. Waste apple peels from applesauce and canned apple manufacture should be regarded as a valuable product. We believe they show potential as a functional food or value-added ingredient in the

future. As part of a diet rich in fruits, vegetables, and grains, apples and their peels may assist in the prevention of chronic disease.

#### ACKNOWLEDGMENT

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#### LITERATURE CITED

- (1) Doll, R.; Peto, R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* **1981**, *66*, 1192–1308.
- (2) Willett, W. C. Diet, nutrition, and avoidable cancer. *Environ. Health Perspect.* **1995**, *103*, 165–170.
- (3) Dragsted, L. O.; Strube, M.; Larsen, J. C. Cancer-protective factors in fruits and vegetables: biochemical and biological background. *Pharmacol. Toxicol.* **1993**, *72*, 116–135.
- (4) Josphipura, K. J.; Hu, F. B.; Manson, J. E.; Stampfer, M. J.; Rimm, E. B.; Speizer, F. E.; Colditz, G.; Asherio, A.; Rosner, B.; Spiegelman, D.; Willett, W. The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann. Intern. Med.* **2001**, *134*, 1106–1114.
- (5) Gillman, M. W.; Cupples, L. A.; Gagnon, D.; Posner, B. M.; Ellison, R. C.; Castelli, W. P.; Wolf, P. A. Protective effect of fruits and vegetables on development of stroke in men. *JAMA, J. Am. Med. Assoc.* **1995**, *273*, 1113–1117.
- (6) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* **1993**, *342*, 1007–1011.
- (7) Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr. Cancer* **1993**, *20*, 21–29.
- (8) Knekt, P.; Jarvinen, R.; Seppanen, R.; Heliövaara, 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.
- (9) Vinson, J. A.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quantity and quality in foods: fruits. *J. Agric. Food Chem.* **2001**, *49*, 5315–5321.
- (10) Le Marchand, L.; Murphy, S. P.; Hankin, J. H.; Wilkens, L. R.; Kolonel, L. N. Intake of flavonoids and lung cancer. *J. Natl. Cancer Inst.* **2000**, *92*, 154–160.
- (11) Knekt, P.; Jarvinen, R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary mortality in Finland: a cohort study. *Br. Med. J.* **1996**, *312*, 478–481.
- (12) Tabak, C.; Arts, I. C. W.; Smit, H. A.; Heederik, D.; Kromhout, D. Chronic obstructive pulmonary disease and intake of catechins, flavonols, and flavones: The MORGEN Study. *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 61–64.
- (13) Knekt, P.; Isotupa, S.; Rissanen, H.; Heliövaara, M.; Jarvinen, R.; Hakkinen, S. H.; Aromaa, A.; Reunanen, A. Quercetin intake and the incidence of cerebrovascular disease. *Eur. J. Clin. Nutr.* **2000**, *54*, 415–417.
- (14) Eberhardt, M. V.; Lee, C. Y.; Liu, R. H. Antioxidant activity of fresh apples. *Nature* **2000**, *405*, 903–904.
- (15) Podsedek, A.; Wilska-Jeska, J.; Anders, B.; Markowski, J. Compositional characterisation of some apple varieties. *Eur. Food Res. Technol.* **2000**, *210*, 268–272.
- (16) Liu, R. H.; Eberhardt, M. V.; Lee, C. Y. Antioxidant and antiproliferative activities of selected New York apple cultivars. *N. Y. Fruit Q.* **2001**, *9*, 15–17.
- (17) Burda, S.; Oleszek, W.; Lee, C. Y. Phenolic compounds and their changes in apples during maturation and cold storage. *J. Agric. Food Chem.* **1990**, *38*, 945–948.
- (18) Ju, Z.; Yuan, Y.; Liu, C.; Zhan, S.; Wang, M. Relationships among simple phenol, flavonoid and anthocyanin in apple fruit peel at harvest and scald susceptibility. *Postharvest Biol. Technol.* **1996**, *8*, 83–93.
- (19) Escarpa, A.; Gonzalez, M. C. High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. *J. Chromatogr.* **1998**, *823*, 331–337.
- (20) USDA, National Agriculture Statistics Service, 2001.
- (21) Dewanto, V.; Wu, X.; Adom, K. K.; Liu, R. H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010–3014.
- (22) Jia, Z.; Tang, M.; Wu, J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* **1999**, *64*, 555–599.
- (23) Boyles, M. J.; Wrolstad, R. E. Anthocyanin composition of red raspberry juice: influences of cultivar, processing, and environmental factors. *J. Food Sci.* **1993**, *58*, 1135–1141.
- (24) Liu, M.; Li, X. Q.; Weber, C.; Lee, C. Y.; Brown, J. B.; Liu, R. H. Antioxidant and antiproliferative activities of raspberries. *J. Agric. Food Chem.* **2002**, *50*, 2926–2930.
- (25) Winston, G. W.; Regoli, F.; Dugas, A. J.; Fong, J. H.; Blanchard, K. A. A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radical Biol. Med.* **1998**, *24*, 480–493.
- (26) Golding, J. B.; McGlasson, W. B.; Wyllie, S. G.; Leach, D. N. Fate of apple peel phenolics during cool storage. *J. Agric. Food Chem.* **2001**, *49*, 2283–2289.
- (27) van der Sluis, A. A.; Dekker, M.; de Jager, A.; Jongen, W. M. F. Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. *J. Agric. Food Chem.* **2001**, *49*, 3606–3613.
- (28) Arts, I. C. W.; van de Putte, B.; Hollman, P. C. H. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J. Agric. Food Chem.* **2000**, *48*, 1746–1751.
- (29) Lister, C. E.; Lancaster, J. E.; Sutton, K. H. Developmental changes in the concentration and composition of flavonoids in skin of a red and a green apple cultivar. *J. Sci. Food Agric.* **1994**, *64*, 155–161.
- (30) Awad, M. A.; de Jager, A.; van Westing, L. M. Flavonoid and chlorogenic acid levels in apple fruit: characterisation of variation. *Sci. Hortic.* **2000**, *83*, 249–263.
- (31) Awad, M. A.; de Jager, A. Flavonoids and chlorogenic acid concentrations in skin of ‘Jonagold’ and ‘Elstar’ apples during and after regular and ultra low oxygen storage. *Postharvest Biol. Technol.* **2000**, *20*, 15–24.
- (32) Wang, H.; Cao, G.; Prior, R. L. Total antioxidant capacity of fruits. *J. Agric. Food Chem.* **1996**, *44*, 701–705.

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