Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Phenolic Composition and Antioxidant Activity of Selected Apple Genotypes

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The phenolic composition of eleven apple genotypes was determined in the flesh and peel by high performance liquid chromatography (HPLC), total phenolic content (TPC) by the Folin-Ciocalteu method, and antioxidant capacity using ferric reducing antioxidant power (FRAP). HPLC analysis identified and quantified several groups of phenolic compounds: procyanidins, hydroxycinnamates, acids, anthocyanins, flavonols, and dihydrochalcones. Procyanidins were the most predominant group in both flesh and peel and contributed 52.4% and 44% of the total phenolic index (TPI), respectively. Quercetin glucosides were almost exclusively found in the peel, while cyanadin 3-galactoside was found only in red apple peel. The profile of phenolic compounds varied among the eleven genotypes and the peel showed higher concentrations than the flesh. Among the studied genotypes 'Reinette Russet' and 'SJCA38R6A74' had the highest and the lowest concentrations, respectively. The total phenolics (TPI/TPC) of both flesh and peel extracts correlated well with antioxidant capacity as estimated by the FRAP assay (R2 = 0.87, 0.76, 0.92, respectively), with the exception of TPC from the apple peel determined with FC (R2 = 0.52). The low chlorogenic acid and zero total flavanol content in flesh of 'SJCA38R6A74' genotype tended to be associated with no browning compared to other cultivars.

Introduction

Interest in phytochemical content and antioxidant activity of fruits and vegetables has been very high in recent years. Antioxidants such as vitamin C, vitamin E, or β -carotene are not the only compounds responsible for the antioxidant capacity of fruits and vegetables. Recent studies have shown that the majority of antioxidant activity in fruits or vegetables may originate from the polyphenolic compounds.

The presence of phenolic compounds in fruits and vegetables has been studied fairly well. In addition to their important functions in plant defense mechanisms and external stresses, they also affect the quality, colour and taste of fruits and their products like juice and fruit slice. In low concentration, phenolics may protect food from oxidative deterioration; however at high concentration, they (or their oxidation products) may participate in discoloration of foods. For example, the brown colour development (known as enzymatic oxidation) is mainly due to the polyphenol peroxides (PPO) activity and the amount of the polyphenol substrates.

As shown for apple fruits, the coloration after oxidation depends on the balance between the phenolics: hydroxycinnamates, flavanols and flavonols. Moreover, enzymatic browning of raw fruits and vegetables is generally considered to be an undesirable reaction because of the unpleasant appearance and concomitant development of off-flavors. In addition, this browning has been a major impediment to expanding the shelf life and marketability of fresh-cut fruits.

The aim of the present work was to quantify individual phenolic compounds of selected advanced apple lines and to quantify their total phenolic content and antioxidant activities compare to known cultivars. The results of this investigation will be used further in our apple breeding program to select parentages to make new crosses. Furthermore it is our intension to further investigate if there is a correlation between the antioxidant activity, shelf life and resistance to diseases.

Materials and Methods

Sample preparation extraction procedure. Eleven advanced apple lines and cultivars were picked at commercial maturity during 2003 harvest season at the Agriculture and Agri-Food Canada, Quebec. Fruit samples were peeled, cored, cut into small pieces, frozen in liquid nitrogen immediately, and stored at -80 °C until use.

Ten grams of fresh-frozen peel or flesh was taken from each apple genotype and the phenolic composition of apples was analyzed by HPLC as described previously (2). All samples were prepared and analyzed in duplicate. The results were expressed as µg g-1 fresh-frozen weight.

Total phenolic content. Total phenolic content (TPC) was determined as described previously (3) with slight modification.

Ferric Reducing/Antioxidant Power (FRAP) assay. The FRAP was determined according to the method of (1). The FRAP value of the samples was calculated on the basis of 500 µM L-Ascorbic acid.

Results

HPLC analysis identified and quantified several groups of phenolic compounds: hydroxycinnamic acids, flavan-3-ols, flavonols, dihydrochalcones and anthocyanins. Apple genotypes tested in this study contained various levels of total phenolics measured by both TPC and TPI, as well as individual phenolic compounds. Our results are similar to those already published by other investigators and show that apple peels possess higher contents of phenolic compounds than the flesh, which is in accordance with previous studies. Furthermore, the composition and distribution of these phenolic compounds between the flesh and peel were different among the varieties.

In all apples studied, procyanidins were the predominant phenolics group in both flesh and peel. They accounted for 52.4% and 44% of the total phenolics, respectively. 'Reinette Russet' apple contained the highest concentration, whereas 'SJCA38R6A74' was the only apple that contained no procyanidins in its flesh.

According to our results and those reported earlier, epicatechin and procyanidin B2 were the main contributors to the high total procyanidins content, and were in higher amounts in apple peel. Many researchers have reported that chlorogenic acid was the major phenolic compound in apples, and was the most abundant phenolic compound in the apple flesh.

In addition to the five-quercetin glycosides found in the peel, three other unknown flavonols were also detected in this study. Commonly, glycosylated quercetin is essentially located in apple peel. Only low concentrations were detected in the flesh, which is consistent with those reported previously. Quercetin glycosides in apple have been found to be about 13.2 mg per 100g FW and 21-200 mg kg-1 FW.

Consistent with the results obtained on the apple cultivars grown in New York state and in Ontario (2), we found that phloridzin concentration in the peel was higher than that of phloretin-3-xyloglucoside and the other two phloretin derivatives. It was two or more times higher than in the flesh, depending on the apple genotypes.

Anthocyanins were essentially located in apple peel. Cyanidin-3-galactoside is the major anthocyanin (2), present in red or partially red genotypes. 'SJC7713-1' and 'SJC649' genotypes were dark red and contained more cyanidin-3-galactoside than 'SJC7123-2', 'SJCA38R6A74', 'Gala', 'Galarina', 'Spartan', 'Cortland', and 'Summerland McIntosh'. As expected, no detectable anthocyanin was found in 'Reinette Russet' and 'SJC658' peels. 'Reinette Russet' apples were greenish-yellow sprinkled with lenticels and russet patches, whereas the 'SJC658' apples were yellow to greenish-yellow with a lightly blushed red on sun side.

TPC, as measured by the FC method, varied widely among genotypes. The TPC for the flesh and peel samples of the studied apples were comparable to those previously reported by (2). In contrast, the TPC reported by in French cider apple varieties was in the range of 110-600 mg epicatechin equivalent per 100 g FW in the cortex of fresh fruit, which was much higher with respect to the levels observed in this study.

TPI gave specific concentrations of different polyphenolic groups and individual compounds. Although TPC measured by the Folin-Ciocalteu procedure does not give a full picture of the quantity and quality of phenolic constituents in the extracts, this widely used method provides a rapid and useful overall evaluation of the phenolic content.

Figure 1 shows the browning tendency of selected apple genotypes 4 days after cutting keeping at ambient temperature. 'SJCA38R6A74' genotype showed no browning compared to the industry standard 'Cortland' and 'Reinette Russet', which showed the highest level of browning. The genotypes 'SJC7713', 'Galarina' and 'SJC649' were intermediate. Of particular interest was chlorogenic acid and total flavanol content in the flesh of 'SJCA38R6A74' genotype. The low chlorogenic acid and zero total flavanol content of 'SJCA38R6A74' may be related to low browning and require further investigation and research. Indeed, chlorogenic acid and flavanols have been shown to be the main substrates of polyphenol peroxidase, which is important in apple fruit processing. In the presence of oxygen and polyphenol oxidase, chlorogenic acid and flavanols are converted into its o-quinone, which further reacts with other phenolic compounds, resulting in the formation of yellow and brown pigment. (1) concluded that the tendency of apples studied to brown was closely related to the content of phenolics during maturation and storage. This result is especially interesting as direct evidence shows that 'SJCA38R6A74' could be selected on the basis of its poor sensitivity to enzymatic browning, which affects the commercial value of the fruits.

The literature provides variable figures for the total antioxidant capacity of apples assessed by different methods: Total Oxyradical Scavenging Capacity (TOSC), Oxygen Radical

Results (cont'd)

Absorbance Capacity (ORAC) and FRAP. Apples possessed strong antioxidant capacity in this study as demonstrated previously (2). These results clearly indicate that apples are an excellent source of phenolics and therefore possess an extremely high antioxidant capacity.

Correlation analysis showed a high correlation between total phenolics (HPLC/FC) and antioxidant capacity (FRAP) in both flesh and peel, except for TPC of apple peel determined with FC method in this study. Literature reports on the relationship between total phenolics and antioxidant activities are contradictory; while some authors have observed a high correlation, others found a weak correlation or no direct correlation. They concluded that it was probably due to the other unquantified phenolics and/or synergism among these compounds and major phenolics.

In general our results showed a significant variation in antioxidant capacity and phenolic composition in selected apple genotypes. This investigation clearly shows the potential value of certain advanced lines resistant to browning with better marketability for fresh-cut or dried apple products.

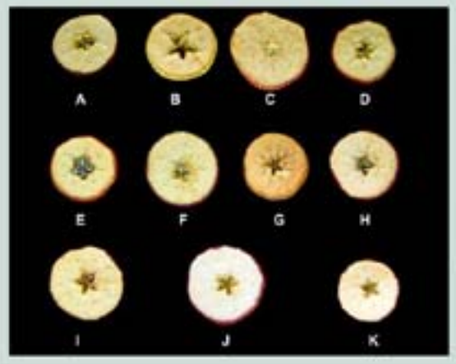

Fig. 1. Susceptibility of selected cultivars vs 'SJCA38R6A74' to browning 4 days after cutting at room temperature.

Cultivars	Total phenolic content[a] (µg/g) Flesh	Peel	FRAP[b] (µM) Flesh	Peel
SJCA38R6A74	136.8	592.2	752.4	11758.9
Cortland	162.5	698.2	1559.3	10527.1
SJC649	233.5	678.8	2672.5	9956.7
Gala	237.2	1809.9	2456.5	15804.9
Galarina	280.7	1838.3	2678.7	19201.4
Spartan	286.8	844.8	2889.6	10032.9
SJC7713-1	370.7	1151.7	3354.3	20089.3
M.d. Summerland	384.3	791.3	2245.7	12882.1
SJC7123-2	392.7	799.2	2183.5	11118.4
SJC658	436.8	1679.3	2179.5	12802.9
Reinette Russet	837.5	1204.9	13224.5	30587.9
F value	70.1	37.0	7.4	8.5
LSD 5%	69.4	126.9	2971	6042.3

Values are means of 4 replicates.

[a] Total phenols expressed as µg gallic acid equivalent (GAE) per gram fresh-frozen weight.

[b] FRAP: Ferric-Reducing Antioxidant Power expressed as µM FRAP

Table 1. Total phenolic content and antioxidant capacity (FRAP) of 11 advanced apples lines and cultivars.

Literature cited

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