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Total Phenolics and Antioxidant Properties of Cider Apple Cultivars

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Abstract

Fresh apple (*Malus* spp.) juice potentially contains similar phenolic compounds as the fruits from which it is derived, although some phenolic compounds may be lost or changed in quality during juice production, especially during pasteurization, which is necessary because apple juice is a chemically and microbiologically sensitive product. Juice was extracted and divided into pasteurized and non-pasteurized. Cultivars and species varied considerably in total phenolic content and radical scavenging capacity. The results indicated an approximate 55% relation between total phenolics and radical scavenging. Pasteurization of all samples caused a small but significant decrease in the total phenolic content and antioxidant capacity; however, *Malus sieversii* accessions were high in both and were less affected by pasteurization.

The availability of fresh, frozen and canned apple juice has increased significantly in recent years. Annual world production of apples is in the order of 40 million tons (5,16), and at least 5 million tons is processed into juice (12). Apples have long been recognized as a healthy fruit, but little research has been conducted on antioxidant properties of pasteurized apple juice. Recent studies of the importance of natural antioxidant activity against pathogenic free radicals and other active oxygen species have encouraged medical and food researchers to address antioxidant properties. Eberhardt et al. (3) estimated that because of its antioxidant activity, 100 g of fresh 'Red Delicious' apple has an antioxidant activity equivalent to 1,500 mg of vitamin C consumed as a dietary supplement. They also showed that whole-apple extracts inhibited the growth of colon and liver-cancer cells in vitro in a dose-dependent manner. Stushnoff et al. (15) found that the ABTS (-2,2'Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt) radical scavenging capacity correlated well with total phenolic content of apple juice. Moreover, Lea (8) mentioned that cider apple cultivars contain large amounts of phenolic compounds that are critical to the quality of manufactured ciders.

Studies have concentrated on the polyphenolic composition of apples (1,2,8,15) and less on their bioactivity. Hence, it is important to know if high temperature treatment has any effect on quality parameters and antioxidant levels. Since there is little information concerning changes in total phenolic content and antioxidant activity during the processing of apple juices, this study focused on these and hypothesized that pasteurization decreases the level of antioxidants and total phenolics in apple juice. The purpose of this research project was to characterize radical scavenging capacity and total phenolics of selected apple cultivars and a collection of *M. sieversii* accessions to aid in the development of apple juice rich in health-protecting compounds, and to test the effect of pasteurization on antioxidant stability.

Materials and Methods

Apples (*Malus* x *domestica* and *Malus sieversii*) were provided by Dr. Phil Forsline, USDA-ARS Plant Genetic Resources Institute, Agricultural Experiment Station Orchard in Geneva, NY in 2002. Juice was extracted from the whole fruit, including skin but excluding seeds, with a laboratory juicer (ACME Juicer Mfg. Co., Model # 6001). Juice samples were divided into two equal

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portions, one of which was pasteurized in a water bath for 30 min at 75°C (9). Both pasteurized and non- pasteurized samples were frozen at -80°C, and then freeze-dried (Virtis 25 LL Genesis). Both pasteurized and non-pasteurized juices were measured for total phenolics using Folin-Ciocalteu reagent (13), as slightly modified by Javanmardi et al. (6). Results were expressed as milligrams of gallic acid equivalent per gram freeze dried sample from three 10 ml replicates for each cultivar and treatment combination. The antioxidant capacity of apple juice was measured by ABTS assay (10,11), a procedure that measures the relative ability of antioxidant substances to scavenge the ABTS* free radical. The results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) per ml of juice.

Data were subjected to analysis of variance to test the effect of cultivar, pasteurization and their interaction using the GLM procedure (SAS Institute, 1999), and means were compared by Fisher's least significant difference (LSD) at $P \leq 0.05$. The Pearson's correlation coefficient between total phenolics and antioxidant capacity was obtained using the CORR procedure of SAS for pasteurized and non-pasteurized samples.

Results and Discussion

The variation in content of total phenolics among cultivars and species was significant ($P < 0.0001$). Pasteurization affected some cultivars more than others ($P < 0.0001$). In *Malus sieversii* accessions, however, pasteurization treatment did not significantly alter total phenolics ($P = 0.2408$).The total antioxidant potential or Trolox Equivalent Antioxidant Capacity (TEAC) of eleven apple juices (9 cultivars and 2 *M. sieversii* accessions) was determined by selecting high, moderate and low entries based upon the GLM procedure (LSD) for total phenolics. Taxa were separated into eleven groups, which were significantly different from each other for ABTS* + TEAC. Pasteurization of all cultivars and species had a significant effect ($P< 0.0001$) on radical scavenging capacity. Several of the *M. sieversii* species accessions had more total phenolics than the well-known cultivars. They were less affected by heat treatment than many of the cultivars, in which the level of total phenolics dropped after pasteurization (Tables 1 and 2). This finding is consistent with Spanos and Wrolstad (14), who found that polyphenol content depended on the cultivar of apple and extraction temperature. Moreover, all sources of unpasteurized cider apple juices are certainly better sources of antioxidants than pasteurized juices, which also have lower levels of TEAC antioxidant activity.

There was a positive linear relationship between total phenolic content and total antioxidant capacity ($r = 0.7431$, $P < 0.0001$), suggesting that phenolic compounds are likely sources of radical scavenging capacity. Higher antioxidant capacity was associated with a higher phenolic content. These data generally compare favorably with previous studies, which reported antioxidant activity of apple juices depended on their polyphenol content (6,4). One study indicated that no significant correlations might be found between the total phenolic content and radical scavenging capacity (17). Kahkonen et al. (7) pointed out that this might be because different phenolic compounds have different responses in the Folin-Ciocalteu method, or it might be that not all of the phenolic compounds are active radical scavengers or have the same matrix effect (15).

In conclusion the results support acceptance of the hypothesis that pasteurization decreases antioxidant levels, but because the magnitude of the reduction was so small, the added benefits from pasteurization in terms of food safety far outweigh any slight loss of antioxidant properties. Although the impact is not major, juice from those cultivars affected most in ABTS/TEAC analyses might benefit from use of a different method to eliminate microbial contamination that has less impact on ABTS/TEAC activity.

Table 1. Total phenolic (TP) and Trolox Equivalent Antioxidant Capacity (TEAC) of pasteurized and non-pasteurized apple juices as determined by a microplate based ABTS assay.

	Non-pasteurized		Pasteurized	
Cultivar	TP (mg/L)	TEAC (mM)	TP (mg/L)	TEAC (mM)
Frequin Rouge	1554.98 a	63.27 a	2212.31 a	41.30 a
Giant Crab	1242.26 b	47.94 b	1293.08 b	35.39 b
Fillbarrel	613.07 c	41.04 c	855.26 c	30.38 c
Okanagan	552.20 c	35.83 d	624.34 d	32.24 bc
Bellefleur de Brabant Rouge	367.72 d	18.38 g	572.94 d	12.52 e
Metais	360.89 d	n/d	353.76 ef	n/d
Robert's Crab	338.57 de	25.65 e	433.17 de	18.77 d
Granny Smith	314.10 def	24.48 ef	325.94 efg	14.81 e
Brown Thorn	303.58 def	n/d	219.68 fghij	n/d
Manito	270.56 defg	n/d	301.92 efghi	n/d
Binet Rouge	256.61 defgh	n/d	254.26 efghij	n/d
Kerr	220.23 efghi	22.57 f	309.51 efgh	13.62 e
Co-1-58	213.06 fghij	n/d	190.58 fghij	n/d
Crandall	188.45 ghij	n/d	175.58 fghij	n/d
Aargauer Jubilaums	180.64 hij	n/d	272.21 efghij	n/d
Haralson	132.63 ij	n/d	125.91 hij	n/d
Smith Jonathan	112.48 ij	n/d	159.63 ghij	n/d
Redspur Delicious	110.55 ij	n/d	133.59 ghij	n/d
Rome Beauty Law	107.92 ij	16.21 g	127.09 hij	11.54 e
Fuji Red Sport	95.27 j	n/d	106.43 j	n/d
Gala	94.56 j	n/d	115.75 ij	n/d

[z] n/d: not done

[y] means within columns followed by the same letter are not significantly different (Fisher's LSD, $P \leq 0.05$)

Table 2. Total phenolic (TP) and Trolox Equivalent Antioxidant Capacity (TEAC) of pasteurized and non-pasteurized Malus sieversii apple juices as determined by a microplate based ABTS assay.

	Non-pasteurized		Pasteurized	
Accession	TP (mg/L)	TEAC (mM)	TP (mg/L)	TEAC (mM)
Malus sieversii 4002.f	1886.97 ay	n/d	1747.73 a	n/d
Malus sieversii 3684.l	1237.50 b	n/d	1069.51 b	n/d
Malus sieversii 4002.o	1160.42 b	n/d	1208.77 b	n/d
Malus sieversii 3684.h	651.78 c	n/d	679.68 c	n/d
Malus sieversii 4002.e	607.18 cd	n/d	572.77 cd	n/d
Malus sieversii 4002.a	569.86 cd	33.60 a	530.93 cd	24.51 a
Malus sieversii 4002.p	525.17 de	n/d	527.46 cd	n/d
Malus sieversii 3684.e	456.03 e	28.20 b	447.91 de	20.56 b
Malus sieversii 3684.i	435.98 ef	n/d	418.56 de	n/d
Malus sieversii 3684.b	351.10 fg	n/d	308.16 e	n/d
Malus sieversii 4002.j	313.06 g	n/d	391.42 de	n/d

[z] n/d: not done

[y] means within columns followed by the same letter are not significantly different (Fisher's LSD, $P \leq 0.05$)

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Fresh-Cut Apple Slices: A Review

The future looks bright for fresh-cut apple slices, but the growth of this industry requires improvements in quality and reduction of production costs. The existing industry has grown from concept to reality in response to research on anti-browning dips, package technology, sensory analysis, post-harvest physiology, post-harvest pathology and food microbiology. Several examples are discussed of how these critical research inputs affected industry practice. Research in several disciplines will be required to resolve newly emerging issues. Traditional breeding effort and/or molecular technologies will be needed to provide non-browning fruit, and fruit with better processing characteristics such as small cores and improved flavor and nutrition retention after cutting. Also, pre-harvest factors such as phosphorus nutrition will need to be investigated further in terms of their effects on fruit tissue and membrane stability. Management of fungal pathogens is another key issue. The very nature of fresh-cut fruit requires a coordinated multi-disciplinary research strategy. See Toivonen, P.M.A. 2006. Fresh-cut apples: challenges and opportunities for multi-disciplinary research. Can. J. Plant Sci. 86:1361-1368.