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Phenolic Content and Antioxidant Activities of Commercial Apple and Pear Juices Representing Major World Producing Regions

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Abstract

Due to their popularity, apple and pear juices are an important contributor of phenolics to the human diet. Many different and often contradictory studies on the phenolic content of apples and pears and their juices exist in literature. Therefore, the overarching goal of this work was to examine the phenolic profile and antioxidant capacity of a database (n = 56) of commercially produced apple and pear juices representing the major world producing regions. The mean and standard deviation Total Phenolic Content (TPC) of the commercial pear juice samples was 246.4 ± 45.1 ppm Gallic Acid Equivalents (GAE). The mean and standard deviation TPC of the commercial apple juice samples was 294.7 ± 128.2 ppm GAE, and this mean value was not significantly different than that observed for pear juice (p-value > 0.05). In addition, changes in the phenolic profile of pear juice as a function of commercial processing conditions was examined chromatographically.

Keywords: Antioxidants; Apple Juice; Pear Juice; Phenolics; Total Phenolic Chromatographic Index

Introduction

The term phenolic encompasses a variety of structurally diverse compounds with the common feature of at least one hydroxyl group directly attached to an aromatic ring. Phenolics are secondary plant metabolites that are derived from phenylalanine and to a lesser extent tyrosine, which are widely distributed in plants with more than 8,000 having been reported in literature [1-3].

Phenolics are broadly divided into two main classes, the phenolic acids and the flavonoids; however, they are subdivided further within these main classes. For example, phenolic acids are divided into the hydroxybenzoic and hydroxycinnamic acids subclasses, and the flavonoids are divided into five major subclasses, namely the anthocyanins, flavanols, flavanones, flavones and flavonols. In addition, other minor subclasses also exist within the two main classes [1,4,5].

Phenolics have a variety of functions in plants such as imparting color, attracting or repelling insects, possessing both antimicrobial and antiviral activity, and providing UV protection [1]. Phenolics have been widely studied for their health promoting properties in humans, including antioxidant, anti-inflammatory and antiviral activities as well as the ability to inhibit carcinogenesis [6-12]. In addition to health benefits, the presence of phenolics in foods as antioxidants are beneficial in the food industry as they extend product shelf-life by delaying undesirable free radical reactions such as lipid oxidation [13,14].

Apple and pear are both members of the Rosaceae family along with other major fruit producing plants such as apricot and peach, which are collectively known as the pome fruits [15]. Apples are grown in a variety of world geographical regions and are second in terms of fruit utilization for juice behind oranges [16], whereas pear juice is widely used in juice blends. While the concentrations of phenolics in apple and pear are lower than in other fruits/berries such as cranberry or blueberry, their high consumption levels (approximately 2.6 billion liters of apple juice was consumed by Americans in 2012) makes them an important contributor of phenolics to the human diet [17].

Many different and often contradictory studies on the phenolic content of apples and pears and their juices exist in literature. These results may be explained by the roles that variety, fruit

maturity and ripeness, environmental growing conditions, storage, and processing have on both the fruit and fruit product (e.g. juice) phenolic composition and concentration [17-19]. The majority of published studies on apple and pear were conducted on whole fruit or on laboratory scale prepared juice and may not fully represent the phenolic/antioxidant profile of the commercial juices typically consumed. Therefore, the overarching goal of this work was to examine the phenolic profile and antioxidant capacity of a large (n = 56) database of commercially produced apple and pear juices representing the major world producing regions.

Materials and Methods

Samples: Thirty-two commercial pear juice and twenty-seven commercial apple juice concentrates (~70°Brix) representing three production years (2012-14) were analyzed in this study. Pear juice concentrates were obtained from Argentina, Chile, China, New Zealand and the United States of America. Apple juice samples were obtained from Argentina, Brazil, Chile, China and the United States of America.

Three sets of samples collected at various stages of pear juice processing (mash to concentrate) were received from two different commercial producers from South America. Processing stage samples were immediately heated at 90°C for 3 min in order to inactivate enzymes prior to shipping on ice. All samples were stored at -30°C until analyzed.

Chemicals: Amberlite XAD16N resin, apigenin, ascorbic acid, arbutin, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), caffeic acid, 5-O-caffeoylquinic acid (chlorogenic acid), (+)-catechin, p-coumaric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), (-)-epicatechin, ferulic acid, Folin and Ciocalteu's phenol reagent (2.0 N), formic acid, gallic acid, 4-hydroxybenzoic acid, 5-Hydroxymethylfurfural (HMF), isorhamnetin-3-O-glucoside, isoquercetin, narigenin, phloridzin, potassium persulfate, quercetin, resveratrol, rutin, sodium carbonate and Trolox (6-hydroxy-2,5,6,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma Aldrich Canada Ltd. (Oakville, ON, Canada). Acetonitrile (HPLC grade) and methanol (ACS grade) were purchased from Fisher Scientific (Ottawa, ON, Canada). The water used throughout this study was produced using a Milli-Q™ water system (Millipore Corp., Milford, MA, USA).

Total Phenolic Chromatographic Index (TPCI): The Total Phenolic Chromatographic Index (TPCI) of the apple and pear juice samples was determined employing an Agilent 1100 series HPLC system with a photodiode array (PDA) detector controlled by Chem Station LC-3D software (Agilent Technologies Canada Inc., Mississauga, ON, Canada). Juice samples were prepared by dilution with water to 11.5 ± 0.1 and 12.0 ± 0.1 °Brix (Auto Abbe Refractometer; Lecia Inc., Buffalo, NY, USA) for apple and pear juice, respectively. Samples containing particulates (i.e., mash samples) were centrifuged at 6000 rpm (Clinical 200 centrifuge, VWR International, Edmonton, AB, Canada) for 15 minutes and the supernatant was collected prior to dilution. Samples were syringe filtered (nylon, 0.2 µm pore size, 13 mm diameter, Chromatographic Specialties, Brockville, ON, Canada) into 2 mL amber HPLC vials (Chromatographic Specialties) prior to analysis. Phenolic separation was accomplished on an ODS-3 (250 x 4.6 mm; 5 µm, C_{18}, 100 Å) column (Phenomenex, Torrance, CA, USA) in series with a guard column (4 x 3 mm) of the same stationary phase. The sample injection volume was 60.0 µL. A linear gradient mobile phase system employing 10.0 mM aqueous formic acid (mobile phase A; pH 3.5) and 70% acetonitrile:30% mobile phase A (v:v; mobile phase B) was used for phenolic separation as follows: initial 100% A for 3.0 min, followed by a gradient to 4.0% B at 16.0 min, followed by a gradient to 10.0% B at 25.0 minutes, followed by a gradient to 15.0% B at 40.0 min, followed by a gradient to 20.0% B at 45.0 min, followed by a gradient to 23.0% B at 50.0 min, followed by a gradient to 25.0% B at 55.0 min, followed by a gradient to 30.0% B at 61.0 min, followed by a gradient to 50.0% B at 75.0 min, followed by a gradient to 80.0% B at 80.0 min, hold at 80.0% B for 5.0 min. The column was then re-equilibrated with 100% A for 7.0 minutes prior to the next injection. The total run time was 95.0 minutes.

Sample phenolic profiles were monitored at 254, 280 and 360 nm. Based upon their UV-vis spectra, chromatographic peaks were assigned to the following phenolic classes, simple phenols, hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavanols, dihydrochalcones, flavones and flavanones. Each phenolic class was quantified using a representative compound from that class as follows, simple phenols were represented by arbutin, hydroxybenzoic acids by gallic acid, hydroxycinnamic acids by chlorogenic acid, flavanols by epicatechin, flavonols by quercetin, dihydrochalcones by phloridzin, flavones by apigenin and flavanones by naringenin. Sample phenolics were quantified at 280 nm except for the flavonols, which were quantified at 360 nm. Standard curves were prepared and ranged from 0.1 to 100.0 ppm and had r^2 values ≥0.990. The concentration of each phenolic class was then summed to calculate sample TPCI. All samples were analyzed in duplicate.

Total Phenolic Content (TPC): The total phenolic contents of whole and fractionated juices were determined by the Folin-Ciocalteu method. To 1.25 mL of 0.2 N Folin-Ciocalteu reagent (diluted from 2.0 N stock with water), 250.0 µL of appropriately diluted sample (i.e., approximately 2.0 and 2.5°Brix for apple and pear juice, respectively) was added. Samples were vortexed (Fisher Scientific) for 15 seconds followed by the addition of 1.0 mL of 15.0% (w:v) sodium carbonate with vortexing for an additional 15 seconds. Samples were then held static in the dark at room temperature (23 ± 2°C) for 2.0 hours. Sample absorbance was measured at 765 nm employing a Genesys 10S UV-visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Sample

total phenolic content was reported as gallic acid equivalents by comparison to a concurrently analyzed standard curve of this compound in water at concentrations ranging from 10.0 to 50.0 ppm. Standard curves had r^2 values ≥0.999. All samples were analyzed in triplicate.

Juice Phenolic Fractionation: Six commercial juice concentrates (three each of apple and pear) were chosen as representatives of High (H), Medium (M) and Low (L) TPC (H: 250-311 ppm and 350-490 ppm; M: 205-249 ppm and 200-349 ppm; and L: 120-204 ppm and 80-199 ppm for pear and apple respectively). Sample phenolic fractions were produced using Amberlite XAD16N resin (Sigma-Aldrich) in conjunction with differing aqueous methanol concentrations (i.e. 0, 40 and 70%, v:v). Juice samples were prepared by water dilution of commercial concentrates to 23.0 ± 0.1 and 24.0 ± 0.1°Brix for apple and pear juice, respectively. The resin was initially hydrated in 50% aqueous methanol (v:v) for 30 minutes before being transferred into a glass column (30 x 0.5 cm) to produce a resin bed of approximately 30 mL. The resin bed was conditioned by sequential washing with 60 mL each of water and 90% aqueous methanol (v:v), followed by an additional 60 mL of water. A 5.0 mL aliquot of juice sample was then loaded onto the resin bed for phenolic fractionation. Water soluble sample components (e.g. carbohydrates and organic acids) were eluted with 60 mL (two bed volumes) of water, followed by sequential elution with 60 mL of 40% methanol (v:v) and 60 mL of 70% methanol (v:v) to produce phenolic fractions. Individual fractions were concentrated to approximately 1 mL by rotary evaporation (Büchi, Switzerland) before being transferred to 2.0 mL HPLC vials and freeze-dried (Heto Lab Equipment, Allerod, Denmark). Samples were stored at -18°C for further analysis. All samples were prepared in triplicate.

Trolox Equivalence Antioxidant Capacity (TEAC) assay for ABTS radical scavenging activity: In this method 2,2′-azinobis-3-ethylbenzthiazoline-sulfonic acid radical cations ($ABTS^{\bullet+}$) are generated by reaction with potassium persulfate. These radicals have an absorbance maximum at 734 nm and as they are scavenged by an antioxidant, this absorbance is reduced. The effectiveness of an antioxidant is assessed by its comparison to Trolox, a vitamin E analog [12,20].

A 7.0 mM ABTS stock solution was prepared by dissolving 38.5 ± 0.2 mg of ABTS in 10.0 mL of water. ABTS radical cations ($ABTS^{\bullet+}$) were produced by mixing 2.0 mL of stock solution with 1.0 mL of 7.0 mM potassium persulfate (18.9 mg/10.00 mL water) and this mixture was kept in the dark overnight at room temperature. The $ABTS^{\bullet+}$ solution was then diluted with 70% aqueous methanol (v:v) to give an absorbance reading of 0.75 ± 0.05 at 734 nm. Fresh $ABTS^{\bullet+}$ solution was prepared for each sample set.

Juice samples were diluted with water, and phenolic fractions with 70% aqueous methanol to produce data curves that had $ABTS^{\bullet+}$ radical scavenging activities ranging from ~10 to 80% (i.e. concentrations ranging from 1.5 to 24.0°Brix). The synthetic vitamin E analogue Trolox was used for $ABTS^{\bullet+}$ radical scavenging comparison and was prepared at concentrations ranging from 0.4 to 2.0 mM in 70% aqueous methanol (v:v).

Sample radical scavenging ability was determined by adding 10.0 µL of the appropriately diluted juice, phenolic fraction or standard to 1.0 mL of diluted $ABTS^{\bullet+}$ solution. The absorbance at 734 nm was recorded after 6 minutes of incubation in the dark for each sample and the percent inhibition was calculated as follows, where 10.0 µL of 70% methanol was used as the blank:

$$\%\ ABTS^{\bullet+}\ \text{inhibition} = [1 - (A_{734}\ \text{sample}/A_{734}\ \text{blank})] * 100$$

The % $ABTS^{\bullet+}$ inhibition was plotted as a function of sample concentration and the linear regression equation for each sample was determined. The % $ABTS^{\bullet+}$ inhibition of 1.0 mM Trolox was determined from the slope of the linear regression line. Sample linear regression equations were then used to determine the concentration of sample required to inhibit the same percentage of $ABTS^{\bullet+}$ as 1.0 mM of Trolox (Y_{TE}). The Trolox Equivalent Antioxidant Capacity (TEAC) was expressed as the equivalent activity of 1.0 mM Trolox in 100 mL of sample and was calculated as $100/Y_{TE}$. Sample analysis was performed in triplicate.

DPPH radical scavenging assay: In this method 2,2-diphenyl-1-picrylhydrazyl (DPPH) is dissolved in 70% methanol to produce the $DPPH^{\bullet}$ radical which absorbs at 515 nm (A_{515}) and when it is scavenged by an antioxidant its absorbance decreases/disappears. Therefore, by monitoring the decrease in A_{515} over time (typically, 15 or 30 minutes) the effectiveness of an antioxidant can be assessed [21,22].

A 500 µM DPPH solution was prepared by dissolving 9.8 ± 0.2 mg of DPPH in 50 mL of 70% aqueous methanol (v:v). Solutions were sonicated (Branson 2510 Ultrasonic Cleaner, Branson, MO, USA) for 20 minutes to ensure complete solubilisation and fresh solutions were prepared for each sample set. Juice samples were diluted with water, and phenolic fractions with 70% aqueous methanol to give final % DPPH radical scavenging activities from 10 to 80%. A 250.0 µL aliquot of appropriately diluted sample was added to 1.0 mL of DPPH solution and the mixture was vortexed for 10 to 15 seconds. The samples were kept static at room temperature in the dark for 15 minutes before their absorbance at 517 nm was determined. A control consisting of 250.0 µL of 70% methanol was also analyzed in conjunction with samples. Percent DPPH radical scavenging activity was calculated as follows:

$$\%\ \text{DPPH radical scavenging} = [1 - (A_{517}\ \text{sample}/A_{517}\ \text{control})] * 100$$

A plot of the % DPPH radical scavenging activity versus sample concentration for each sample was prepared and the concentration at 50% radical scavenging activity (IC_{50}) was deter-

mined from linear regression equations. Sample antioxidant activity was reported as $1/IC_{50}$. All samples were analyzed in triplicate.

Statistics: An analysis of variance (one-way ANOVA) with Tukey's HSD (honest significant difference) post hoc test was used to determine significant differences ($p<0.05$) between experimental means. All statistical analyses were performed using Social Sciences (SPSS) software (SPSS Inc., ver. 24.0, 2016, Chicago, IL).

Results and Discussion

Apple is ranked second in juice consumption after orange, with an estimated value of $82.8 million in the United States alone [23]. While pear juice consumption is lower than apple, it is often used as an ingredient in juice blends. Although apple and pear juices have lower concentrations of bioactive compounds when compared to cranberry or raspberry juice, their popularity and usage make them an important source of bioactive compounds such as phenolics in the North American diet [24]. Goals of this research were, (1) to examine the Total Phenolic Content (TPC) and antioxidant potentials of commercial apple and pear juice from the major world producing regions; (2) to relate TPC and Total Phenolic Chromatographic Index (TPCI) data to world producing regions; (3) examine the relationship between apple and pear juice free radical scavenging and phenolic structure; and (4) investigate the relationship between the different stages of commercial pear processing and their phenolic content by TPCI.

Total Phenolic Content (TPC) and Total Phenolic Chromatographic Index (TPCI): Due to its simplicity, reproducibility and compatibility with a wide range of foods, the F-C method is often used to obtain an estimate of the total phenolic content of fruits and juices [5,25]. The F-C reagent consists of a mixture of sodium molybdate and sodium tungstate that react with phenols to produce a blue color, which is measured at 765 nm [25,26]. The absorbance at 765 nm is then related to sample phenolic content by comparison to a standard, typically gallic acid, and is reported as gallic acid equivalents or GAE. Unfortunately, the F-C assay is subject to interference from other compounds commonly found in food products, such as amino acids, inorganic ions (i.e., Fe^{2+} and Mn^{2+}) and vitamins (i.e., Vitamin C), and other less ubiquitous compounds such as copper complexes, which can artificially inflate the total phenolic content value obtained by this method [26].

The TPC values for the 24-commercial apple and 32 commercial pear juice samples representing each of the major world geographical regions for these fruits/juices were determined by the F-C method and are shown in Tables 1 and 2. The mean and standard deviation TPC of the commercial pear juice samples was 246.4 ± 45.1 ppm GAE with a range of 120.9 to 311.1 ppm GAE. The mean and standard deviation TPC of the commercial apple juice samples was 294.7 ± 128.2 ppm GAE, and this mean value was not significantly different than that observed for pear juice (p-value > 0.05). While the mean TPC did not differ significantly between commercial apple and pear juice samples, apple showed a greater TPC range of 82.8 to 487.6 ppm GAE, and a higher maximum value (i.e. 487.6 vs. 311.1 ppm GAE). The observed larger range in TPC and greater standard deviation values for apple are most likely due to the number of different fruit varieties used in commercial apple juice production (i.e. greater than 14 varieties in a single blend in some cases) as compared to the two to three varieties normally used in pear juice [27], as it has been shown that the total phenolic content can vary with fruit variety [28,29].

Juice	Geographical Origin	Total Phenolic Content (TPC)[a]	Total Phenolic Chromatographic Index (TPCI)[b]	TEAC[c]	DPPH Radical Scavenging Activity[d]
1	Argentina	378.1	142.1	179.2	30.6
2	Argentina	101.6	108.3	45.5	6.1
3	Argentina	384.6	134.0	183.6	28.9
4	Argentina	487.6	178.1	216.9	37.8
5	Argentina	444.2	160.9	210.9	37.7
6	Brazil	183.3	88.0	88.6	11.0
7	Brazil	195.7	84.9	82.1	13.6
8	Brazil	193.5	83.5	89.9	12.9
9	Brazil	296.5	117.3	132.9	10.1
10	Brazil	290.4	119.6	134.6	10.5
11	Chile	393.9	132.5	190.2	30.5
12	Chile	350.7	131.1	196.1	29.6
13	Chile	375.4	159.1	149.8	24.9
14	Chile	417.0	150.0	160.9	27.0

15	China	83.0	33.6	33.3	4.9
16	China	214.3	203.2	79.1	12.5
17	China	82.8	41.5	33.8	6.5
18	China	160.3	102.8	59.1	10.1
19	China	194.8	118.4	88.6	12.9
20	USA	410.0	160.8	167.7	30.1
21	USA	175.5	88.9	79.1	13.5
22	USA	407.7	167.3	180.3	39.3
23	USA	419.3	194.9	222.0	38.2
24	USA	432.9	189.2	199.2	38.0
Mean		294.7 ± 128.2	128.8 ± 44.9	130.8 ± 60.8	21.5 ± 12.1
Min		82.8	33.6	33.2	4.9
Max		487.6	203.2	222.0	39.3
[a]Reported as ppm gallic acid equivalents (n = 3). [b]Reported as mg phenolics/L of juice (n = 2). [c]Expressed as mM Trolox/100 mL juice (n = 3). [d]Expressed as 1/IC50 (1/mL of juice for 50% DPPH radical inhibition; n = 3).					

Table 1: Mean and standard deviation data for Total Phenolic Content (TPC), Total Phenolic Chromatographic Index (TPCI), and Trolox Equivalence Antioxidant Capacity (TEAC) and DPPH radical scavenging assays of 24 commercial apple juices.

Juice	Geographical Origin	Total Phenolic Content (TPC)[a]	Total Phenolic Chromatographic Index (TPCI)[b]	TEAC[c]	DPPH Radical Scavenging Activity[d]
1	Argentina	247.6	193.1	99.8	14.1
2	Argentina	238.8	196.2	134.7	14.4
3	Argentina	238.7	185.5	122.9	15.2
4	Argentina	277.7	220.5	125.5	15.7
5	Argentina	264.4	215.8	133.8	17.5
6	Argentina	274.8	225.7	130.6	18.2
7	Argentina	281.7	229.1	159.6	18.8
8	Argentina	278.3	222.5	150.3	15.5
9	Argentina	295.7	224.9	147.4	15.6
10	Argentina	289.0	229.7	114.7	25.0
11	Argentina	298.5	244.4	148.7	15.9
12	Chile	248.1	222.4	148.9	12.5
13	Chile	265.9	235.9	140.2	14.6
14	Chile	260.0	222.2	144.5	12.9
15	Chile	269.7	227.5	143.4	12.5
16	Chile	257.0	224.8	125.6	11.9
17	Chile	256.7	223.7	123.5	12.8
18	Chile	273.5	232.5	157.0	13.8
19	China	187.7	172.4	167.3	6.6
20	China	311.1	401.4	375.2	14.4
21	China	181.6	178.4	172.2	10.3
22	China	250.9	305.6	360.4	10.0

23	New Zealand	203.6	147.0	149.8	11.7
24	New Zealand	120.9	120.1	76.9	5.5
25	New Zealand	167.5	108.8	92.6	5.5
26	New Zealand	279.9	257.9	188.0	17.3
27	New Zealand	288.8	287.9	225.5	13.5
28	USA	253.7	187.9	111.9	13.9
29	USA	159.4	100.6	93.8	7.5
30	USA	232.3	177.5	131.3	15.2
31	USA	191.4	156.2	110.7	11.0
32	USA	238.9	197.0	117.7	16.4
Mean		246.4 ± 45.1	211.7 ± 57.2	150.8 ± 63.9	13.6 ± 5.5
Min		120.9	100.6	76.9	5.5
Max		311.1	401.4	375.2	25.0

[a]Reported as ppm gallic acid equivalents (n=3).
[b]Reported as mg phenolics/L of juice (n=2).
[c]Expressed as mM Trolox/100 mL juice (n=3).
[d]Expressed as 1/IC50 (1/mL of juice for 50% DPPH radical inhibition; n=3).

Table 2: Mean and standard deviation data for total phenolic content (TPC), Total Phenolic Chromatographic Index (TPCI), and Trolox Equivalence Antioxidant Capacity (TEAC) and DPPH radical scavenging assays of 32 commercial pear juices.

Comparison of TPC results for pear juice samples showed that there were no significant differences based on fruit production and processing regions (Table 3). However, apple juice concentrates from China showed significantly lower mean TPC values (147.0 ± 61.7 ppm GAE) when compared to those from Argentina (359.2 ± 150.9 ppm GAE), Chile (384.2 ± 28.1 ppm GAE) and the United States (369.1 ± 108.6 ppm GAE). However, the mean TPC values for China were not significantly different from those obtained for apple juices from Brazil (231.9 ± 56.4 ppm GAE) (Table 4). These differences were most likely due to differences in the apple varieties used in juice production from this geographical region and the environmental growing conditions during fruit production. The limitations of this study include the small selection of commercial samples analyzed from each world production region (ranging from three to five samples) and the lack of information on the specific apple varieties used in juice production.

Geographical Origin[a]	Total Phenolic Content (ppm GAE)	Total Phenolic Chromatographic Index (ppm)	DPPH[b]	TEAC[c]
Argentina	359.2 ± 150.9[a]	144.7 ± 26.6[a]	28.2 ± 13.0[ab]	167.2 ± 70.0[a]
Brazil	231.9 ± 56.4[ab]	98.7 ± 18.1[a]	11.6 ± 1.5[ac]	105.6 ± 25.9[ab]
Chile	384.2 ± 28.1[a]	143.2 ± 13.7[a]	28.0 ± 2.6[ab]	174.2 ± 22.4[a]
China	147.0 ± 61.7[b]	99.9 ± 68.6[a]	9.4 ± 3.6[c]	58.8 ± 25.4[b]
United States	369.1 ± 108.6[a]	160.2 ± 42.4[a]	31.8 ± 10.9[b]	169.7 ± 54.6[a]

[a]Values marked with different letters within a column were statistically different ($p < 0.05$).
[b]Reported as mL of DPPH/mL of juice.
[c]Reported as mM Trolox equivalents/100 mL juice.

Table 3: Mean and standard deviation data for total phenolic content (TPC), Total Phenolic Chromatographic Index (TPCI), DPPH and TEAC of commercial pear juices by country.

Geographical Origina	Total Phenolic Content (ppm GAE)	Total Phenolic Chromatographic Index (ppm)	DPPHb	TEACc
Argentina	217.0 ± 18.0a	271.4 ± 21.4a	16.9 ± 3.1a	133.4 ± 17.4a
Chile	227.0 ± 5.3a	261.6 ± 8.7a	13.0 ± 0.9ab	140.4 ± 12.1a
China	264.5 ± 110.0a	232.8 ± 60.9a	10.3 ± 3.2b	268.8 ± 114.5b

New Zealand	184.4 ± 82.7a	212.2 ± 72.2a	10.7 ± 5.1b	146.5 ± 62.7a
United States	163.8 ± 38.5a	215.1 ± 38.8a	12.8 ± 3.6ab	113.1 ± 13.5a
[a]Values marked with different letters within a column were statistically different ($p < 0.05$). [b]Reported as mL of DPPH/mL of juice [c]Reported as mM Trolox equivalents/100 mL juice				

Table 4: Mean and standard deviation data for total phenolic content (TPC), Total Phenolic Chromatographic Index (TPCI), DPPH and TEAC of commercial apple juices by country.

The observed TPC mean (294.7 ppm GAE) and range (82.8 to 487.6 ppm GAE) values for commercial apple juice samples in this study were similar to the range of values (142 to 780 ppm GAE) previously reported for laboratory scale apple juices produced from different apple varieties [30].The apple varieties used for laboratory juice production were Granny Smith, McIntosh, Red Delicious and Spartan which are all used in commercial apple juice production, however, these varieties were analyzed separately where commercial juices utilize a blend of varieties. The same authors analyzed four commercial apple juice concentrates and found the total phenolic content of these samples ranged from 49 to 224 ppm GAE [30]. Although these values were on the lower end of the concentration range observed in our study, only one (i.e., 49 ppm GAE) was outside the range reported for the 27 commercial apple juices analyzed. The lower TPC values observed in the commercial apple juice samples in this study when compared to the laboratory produced juices can be explained by the harsher processing conditions in the former, which can result in phenolic structure changes and degradation.

Spanos and Wrolstadt (1990) [29] reported that the mean TPC of laboratory scale pear juices produced separately from Bartlett, Comice and d'Anjou pears ranged from 134 to 542 ppm GAE depending upon pear variety and processing conditions. The observed mean (246.4 ppm GAE) and range (120.9 to 311.1 ppm GAE) TPC values for the 32 commercial pear juice samples in this study were on the lower end of the aforementioned results. These differences in TPC can be explained by the harsher processing conditions (e.g. time and temperature) employed in commercial processing than those used to produce the laboratory scale samples. To the best of our knowledge, this work is the first to focus on the TPC values for an extensive sampling of commercial apple and pear juices, and the first to investigate the relationship between sample TPC value and world fruit geographical regions.

Sample TPCI is measured by determining its phenolic profile by High Performance Liquid Chromatography coupled with Photodiode Array Detection (HPLC-PDA). Phenolics are then grouped into classes based upon their characteristic UV-vis spectra and each class is quantified using a standard from that class. In order to obtain the final TPCI value the concentration of all phenolic classes are summed [5,31]. The phenolic classes assigned to samples in this study included, hydroxybenzoic acids, hydroxycinnamic acids and the flavonoids, specifically, flavanols, flavanones/dihydrochalcones, flavones and flavonols.

The 27 commercial apple juices analyzed in this study had a mean TPCI of 128.8 mg/L and ranged from 33.6 to 203.2 mg/L. The TPCI values of the 32 commercial pear juices had a mean value of 211.7 mg/L, and ranged from 100.6 to 401.4 mg/L. Representative chromatographic phenolic profiles as determined by HPLC-PDA for apple and pear juices are shown in Figure 1. The observed wide TPCI ranges for both commercial apple and pear juices are due to a number of factors including, but not limited to, fruit variety, environmental fruit growing conditions and ripeness, and juice processing and storage conditions [32-34]. The calculated TPCI mean values for apple and pear juices were found to be significantly different (p-value < 0.05). Previous research has shown that TPC values are not always correlated or may only show poor correlation with TPCI results, which is likely due to compounds which may interfere with the F-C method [30].

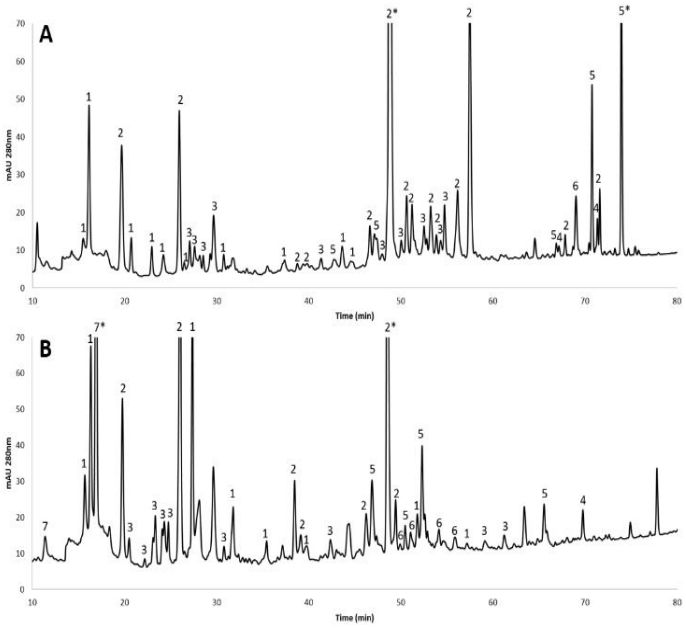

Figure 1: HPLC-PDA chromatograms of pear (A) and apple (B) juice. Where: 1) hydroxybenzoic acids; 2) hydroxycinnamic acids; 3) flavanols; 4) flavonols; 5) dihydrochalcones/flavanones; 6) flavones; 7) simple phenolics. Peak assignments: 2*: 5-O-caffeoylquinic acid; 5*: phloridzin; and 7*: arbutin.

Literature has focused on the TPCI of unprocessed fruit, often looking at the peel and pulp separately. In general, the peel of the fruit is reported to have a higher phenolic content than the pulp

as shown by TPCI range values for apple pulp and peel of 176 to 891 mg/kg and 663 to 4265 mg/kg, respectively; and for pear pulp and peel of 73 to 149 mg/kg and 386 to 2951 mg/kg, respectively [35,36]. The experimental mean TPCI range values for the commercial pear juice samples analyzed were comparable to the literature midrange of the pulp, and on the low end of the peel. The TPCI range observed for the commercial apple juice samples analyzed in this study were much lower than those reported for both apple pulp and peel. These observed TPCI differences were most likely due to the phenolic extraction solvents used for the pulp and peel samples of methanol:water (70:30 to 100:0; v:v). The use of a hydrophobic alcohol like methanol with a dielectric constant (i.e. polarity measure, where the higher the number the more polar the solvent) of 32.7 at 25°C and when mixed with water (dielectric constant of 78.5) gives70:30 methanol:water a dielectric constant of 47.1 [37]; are specifically used to extract both hydrophilic (e.g., hydroxybenzoic acids) and hydrophobic (e.g. flavonols) phenolic compounds from fruits such as pear and apple. Whereas, in commercial juice processing the focus is on increasing overall juice yield (i.e., total carbohydrates) rather than phenolic extraction, and water is the solvent.

The hydroxybenzoic and hydroxycinnamic acids had the largest contribution to the overall TPCI of the commercial apple juice (Table 5) with concentrations ranging from 10.1 to 71.8 ppm and 14.0 to 165.2 ppm corresponding to 5.5 to 40.3% and 33.9 to 81.3% of the overall TPCI, respectively. As examples, when the hydroxybenzoic acid content was low (i.e., 5.5% for AJ 23) the hydroxycinnamic acid was higher (56.4%), whereas AJ 7 had a lower contribution from hydroxycinnamic acids (33.9%) and a higher contribution from hydroxybenzoic acids (40.3%). The most abundant phenolic in apple juice was the hydroxycinnamic acid 5-O¬-caffeoylquinnic acid (5-CQA; chlorogenic acid), which ranged in concentration from 7.0 to 86.0 ppm. Following these two classes, the flavanols were the next most abundant with concentrations ranging from 2.1 to 53.6 ppm (5.0 to 52.2% of the TPCI) in apple juice. The remaining phenolic classes (flavonols, dihydrochalcones, flavones, flavanones) each individually accounted for less than 10% of apple juice TPCI.

Geographical Origin[a]	HBA[b] (ppm)	HCAc (ppm)	Flavanols (ppm)	Dihydrochlacones (ppm)	Flavanones (ppm)
Argentina	43.3 ± 19.9[a]	63.0 ± 4.8[a]	31.5 ± 6.0[a]	4.8 ± 2.6[abc]	1.6 ± 1.3[a]
Brazil	24.3 ± 1.9[b]	53.4 ± 14.5[a]	17.6 ± 3.7[b]	2.0 ± 0.5[bc]	1.1 ± 0.9[a]
Chile	25.5 ± 4.4[ab]	74.1 ± 2.8[a]	33.3 ± 9.5[a]	7.3 ± 0.7[ab]	1.4 ± 0.2[a]
China	19.2 ± 4.9[b]	64.8 ± 60.5[a]	13.2 ± 9.4[b]	1.9 ± 0.9[bc]	1.4 ± 0.7[a]
United States	12.4 ± 2.1[b]	82.9 ± 31.0[a]	49.0 ± 2.8[c]	9.8 ± 5.2[a]	9.8 ± 5.2[a]

[a]Values marked with different letters within a column were statistically different ($p < 0.05$).
[b]HBA: hydroxybenzoic acids.
[c]HCA: hydroxycinnamic acids.

Table 5: Mean and standard deviation of the different phenolic classes as identified by TPCI in commercial apple juices by country.

For the commercial apple juice samples, it was found that there was no significant difference between geographical regions based on their TPCI (Table 3). However, differences were observed between the different phenolic subclasses. For example, samples from Argentina had the highest hydroxybenzoic acid content (43.3 ± 19.9 ppm), thus being significantly different from all other geographical regions except Chile.

Arbutin was a major contributor to pear juice TPCI with concentrations ranging from 50.6 to 286.0 ppm [27], which corresponded to 28.4 to 75.9% of the overall TPCI. Additionally, the hydroxycinnamic acids were a significant contributor to pear juice TPCI in the majority of cases with concentrations ranging from 6.4 to 89.5 ppm, which corresponded to 4.2 to 36.6% of the overall TPCI. The most abundant hydroxycinnamic acid in pear was 5-O-caffeoylquinic acid with concentrations ranging from <0.1 to 45.7 ppm. Following arbutin and the hydroxycinnamic acids, the hydroxybenzoic acids and flavanols were the next major phenolic classes identified in pear juice with concentrations ranging from 7.0 to 43.7 ppm (5.1 to 20.0%) and 12.4 to 70.6 ppm (7.0 to 28.8%), respectively. The remaining phenolic classes each individually contributed <10% to total sample TPCI (Table 6).

Geographical Origin[a]	HBA[b] (ppm)	HCA[c] (ppm)	Flavanols (ppm)	Dihydrochlacones (ppm)	Flavanones (ppm)	Arbutin (ppm)
Argentina	15.4 ± 1.9a	71.4 ± 9.9a	38.4 ± 10.5a	7.3 ± 2.1a	2.7 ± 0.7a	69.4 ± 2.9a
Chile	18.6 ± 2.0ab	59.2 ± 7.7a	44.7 ± 3.6a	7.0 ± 1.4ab	4.4 ± 2.1a	80.9 ± 2.8a
China	27.7 ± 14.2b	17.5 ± 14.8b	25.9 ± 13.7a	2.1 ± 1.6b	1.3 ± 1.2a	192.1 ± 80.3b
New Zealand	15.9 ± 6.7a	26.2 ± 14.6bc	43.5 ± 23.1a	7.0 ± 6.4ab	3.0 ± 0.9a	85.2 ± 32.5a
United States	12.7 ± 3.4a	42.0 ± 16.9ac	30.7 ± 7.6a	7.7 ± 2.1ab	3.1 ± 2.0a	61.5 ± 7.3a

[a]Values marked with different letters within a column were statistically different ($p < 0.05$).
[b]HBA: hydroxybenzoic acids.
[c]HCA: hydroxycinnamic acids.

Table 6: Mean and standard deviation of the different phenolic classes as identified by TPCI in commercial pear juices by country.

As with the TPC, there was no statistically significant differences observed between the overall TPCI of samples from the different geographical regions for the commercial pear juices. However, phenolic subclasses were found to vary significantly between regions (Table 6). For example, the concentration of hydroxybenzoic acids were significantly higher in the pear juice samples from China (27.7 ± 14.2ppm) as compared to those from Argentina (15.4 ± 1.9 ppm), New Zealand (15.9 ± 6.7 ppm) and the United States (12.7 ± 3.4ppm). In addition, the hydroxycinnamic acid and dihydroxychalcone concentrations in the Chinese samples were significantly lower than all other geographical regions studied except for New Zealand (Table 6). The only other observed significant difference between regions was the high concentration of arbutin in the Chinese samples compared to all other regions. The high arbutin content (192.1 ± 80.3 ppm), coupled with the lower hydroxycinnamic acid (17.5 ± 14.8 ppm) and flavanol (25.9 ± 13.7 ppm) contents of the Chinese samples when compared to juices from the other geographical regions, is likely the reason why the overall TPCI of the samples studied were similar across all regions even though phenolic concentrations varied within specific classes.

In order to determine the effect of processing on the final phenolic content of commercial pear juice, the TPCI of three commercial pear juices at various stages of production were determined. These stages included the initial mash stage before the addition of processing enzymes (i.e., the crushed whole fruit), the mash after enzymatic treatment with carbohydrates and the final juice concentrate [38]. It was found that the TPCI increased throughout processing from 140.1 ± 46.4 ppm for mash, to 217.6 ± 35.7 ppm following enzyme addition, to 276.2 ± 96.9 ppm in the final concentrate. In addition, all phenolic classes, excluding the flavonols, increased in concentration from the mash to the final concentrate. Of interest was the fact that sample flavonols showed a decrease in concentration during processing in two of the samples and were not detected in the third, indicating that this phenolic class underwent destruction during these processing stages (from an average of 10.9 ppm in the mash to a final average of 4.9 ppm in the concentrate) and as such had minimal impact on final concentrate TPCI values due to their low concentrations. Flavonols have been previously shown to be sensitive to heat treatment [39].

During commercial juice production, carbohydrases (primarily pectinases) are added to improve juice yield by breaking down cell walls so as to increase the total soluble carbohydrate content and reduce the viscosity of the juice [38]. The observed increase in TPCI during laboratory pear processing with commercial enzymes was likely due to breakdown of the fruit cell walls releasing phenolics during the mashing stage. Based on this result, it is postulated that an increase in phenolics release from the fruit to the juice would also occur in commercial apple/pear juice production.

DPPH and ABTS Radical Scavenging Activity of Commercial Apple and Pear Juice: The average DPPH radical scavenging activity (Table 1) for commercial apple juice was found to be 21.5 ± 12.1mL of DPPH/Ml of juice with a range of 4.9 to 39.3mL of DPPH/mL of juice. While the commercial pear juice samples showed an average DPPH radical scavenging activity of 13.6 ± 5.5 with a range of 5.5 to 25.0mL of DPPH/mL of juice (Table 2). It was found that these results were significantly different with commercial apple juice having a greater DPPH radical scavenging ability that commercial pear juice.

The average TEAC values (Table 1) for the commercial apple juice samples was 130.8 ± 60.8 with a range of 33.2 to 260.5 mM Trolox/100 mL juice. While the average TEAC forthe commercial pear juices was 150.8 ± 63.9 with a range of 76.9 to 375.2 mM Trolox/100 mL juice (Table 2). These results were not significantly different from each other (p-value> 0.05).

Due to a lack of published results on the free radical scavenging ability of commercial apple and pear juices, and the differences in the way free radical scavenging results are reported and variations in the method (i.e., concentration of DPPH/ABTS used, reaction time, etc.) direct comparisons of the results from this study to those reported from laboratory scale experiments would lack scientific rigor. Therefore, a selection of commercial fruit juices (blueberry, cranberry, strawberry and watermelon; n = 1) were analyzed under the same reaction conditions as the apple and pear juices analyzed in this study. The resulting DPPH

scavenging activity results showed that cranberry showed the highest(447.0mL of DPPH/mL of juice), followed by strawberry (379.5mL of DPPH/mL of juice), blueberry (198.8mL of DPPH/mL of juice), apple (25.6mL of DPPH/mL of juice), pear (13.6mL of DPPH/mL of juice) and watermelon juice (4.5mL of DPPH/mL of juice).Free radical scavenging results employing the TEAC method for the same commercial juices showed that strawberry was the highest (1890.4 mM Trolox/100 mL juice), followed by cranberry (1423.2 mM Trolox/100 mL juice), blueberry (1029.4 mM Trolox/100 mL juice), pear (150.8 mM Trolox/100 mL juice), apple (146.7 mM Trolox/100 mL juice) and watermelon juice (36.0 mM Trolox/100 mL juice).

These results agree well with literature reports, which show that red/blue fruits and berries (such as cranberry) that are rich in phenolics are better radical scavengers than fruits with less color (such as apple and pear) that have lower phenolic contents and therefore lower free radical scavenging ability [40]. Differences in the ordering between the TEAC and DPPH methods is likely due to differences in the structures of the radicals scavenged. The ABTS•+ radical involves the transfer of two electrons from one or two different antioxidants while the DPPH assay is based on hydrogen transfer and proceeds via a single electron transfer mechanism, as such TEAC and DPPH results for the same sample may not show strong correlation [41].

Apple and pear showed significant differences in their DPPH and TEAC values based upon geographical region (Tables 3 and 4). It was found that the mean DPPH value for apple juice samples from Argentina (28.2 ± 13.0mL of DPPH/mL of juice), Chile (28.0 ± 2.6mL of DPPH/mL of juice) and the United States (31.8 ± 10.9mL of DPPH/mL of juice) were not significantly different. While samples from Brazil (11.6 ± 1.5mL of DPPH/mL of juice) were not significantly different from those from Argentina or Chile but were different from those of the United States. Finally, the Chinese samples showed the lowest overall mean DPPH radical scavenging activity (9.4 ± 3.6mL of DPPH/mL of juice), which was significantly different from those found for all other geographical regions excluding Brazil.

The mean TEAC value for samples from Argentina (167.2 ± 70.0mM Trolox/100 mL juice), Brazil (105.6 ± 25.9mM Trolox/100 mL juice), Chile (174.2 ± 22.4mM Trolox/100 mL juice) and the United States (169.7 ± 54.6mM Trolox/100 mL juice) were found to be not significantly different. However, the mean TEAC value for Chinese samples was the lowest (58.8 ± 25.4mM Trolox/100 mL juice) and was significantly different from all other geographical regions excluding Brazil.

For the pear juice samples, the mean DPPH radical scavenging activities of samples from Argentina (16.9 ± 3.1mL of DPPH/mL of juice), Chile (13.0 ± 0.9mL of DPPH/mL of juice) and the United States (12.8 ± 3.6 mL of DPPH/mL of juice) were not significantly different. Also, samples from Chile, China (10.3 ± 3.2mL of DPPH/mL of juice), New Zealand (10.7 ± 5.1mL of DPPH/mL of juice) and the United States did not show significant differences in mean DPPH values. Fewer statistically-based differences in mean TEAC values were observed for pear juice samples from different geographical regions with the exception of the Chinese samples, which had both the highest value of 268.8 ± 114.5mM Trolox/100 mL juice, and which was significantly higher than all other regions (113.1 to 146.5mM Trolox/100 mL juice; Table 4).

To gain an understanding of the role that individual classes of phenolics play in the overall antioxidant potential of commercial apple and pear juices, three representative samples for both apple and pear were selected based on low (AJ 17 and PJ 23), medium (AJ 12 and PJ 13) and high (AJ 23 and PJ 7) TPC and radical scavenging ability. These juices were initially separated into three fractions using Amberlite XAD16N resin, a water fraction, a 40:60 methanol:water fraction and a 70:30 methanol:water fraction. Results from these experiments are shown in Table 7. These fractions were then individually analyzed for their TPC, DPPH and ABTS radical scavenging ability. The water fraction contains the water-soluble components, including the carbohydrates and ascorbic acid. The remaining mobile phases were found to elute the majority of the sample phenolics from the column with the most hydrophobic eluting in the 70% methanol fraction and those of intermediate polarity in the 40% methanol fraction.

	Fraction	Total Phenolic Content (ppm GAE)	DPPH[a]	TEAC[b]
Pear Juice	Water	25.2	1.1	6.9
	40% methanol	117.4	5.5	76.2
	70% methanol	80.2	5.3	47.9
Apple Juice	Water	19.3	1.2	4.9
	40% methanol	77.9	5.4	46.4
	70% methanol	133.5	10.7	90.9
[a]Reported as mL of DPPH/mL of juice. [b]Reported as mM Trolox equivalents/100 mL juice.				

Table 7: Mean data for Total Phenolic Content (TPC), Total Phenolic Chromatographic Index (TPCI), DPPH and TEAC of fractionated apple and pear juices.

The water fractions of all samples (apple and pear) showed the lowest TPC and antioxidant activity for both the DPPH and TEAC methods. In general, for apple juice the highest TPC, DPPH and TEAC values were found in the 70% methanol fraction. For pear juice the reverse was true, on average the highest TPC, DPPH and TEAC values were found in the 40% methanol fraction.

The higher contribution of the 40% methanol fraction, as compared to the 70% methanol fraction, in the pear juices is likely

due to the higher concentration of phenolics of intermediate polarity in these samples (i.e., 5-O-caffeoylquinic acid) as can be observed in Figure 2. This is illustrated by the majority of phenolics eluting within 55 minutes where the acetonitrile content in the mobile phase was0-17.5%, whereas less polar compounds eluted after 55 minutes, where the acetonitrile content increased to56.0% (i.e. more hydrophobic mobile phase conditions). For apple juice samples, there were greater concentrations of hydrophobic phenolics (i.e., phloridzin) eluting later in chromatograms, which contributed to the higher observed TPC and antioxidant activity of the 70% methanol fraction.

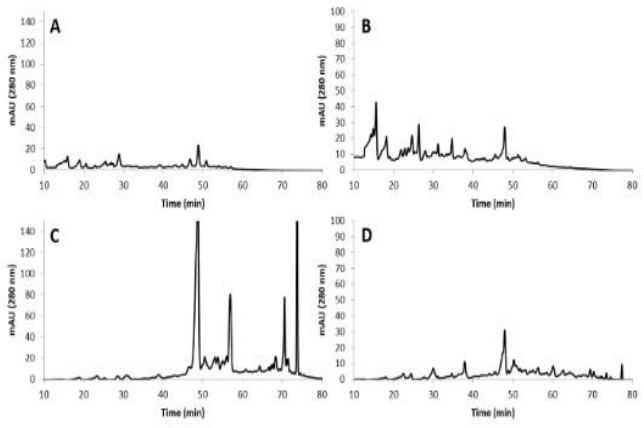

Figure 2: HPLC-PDA chromatograms of juice phenolic fractions. **A)** AJ 12, 40% methanol fraction; **B)** PJ 23, 40% methanol fraction; **C)** AJ 12, 70% methanol fraction; **D)** PJ 23, 70% methanol fraction.

Conclusions

Results from this study showed that commercial apple and pear juices contained a variety of phenolics, which were the main contributor to their TPC and TPCI values, and antioxidant activities (i.e. *in vitro* free radical scavenging ability). The phenolic content of commercial apple juice was 294.7 ± 128.2 ppm GAE by TPC and 128.8 ± 44.9 ppm by TPCI, whereas for commercial pear juice these values were 246.4 ± 45.1 ppm GAE and 211.7 ± 57.2 ppm, respectively. The free radical scavenging ability of apple and pear juices employing the DPPH and TEAC methods, gave values of 21.5 ± 12.1 and 130.8 ± 60.8 for apple, and 13.6 ± 5.5 and 150.8 ± 63.9 for pear, respectively. Phenolics have been extensively studied for their ability to quench reaction oxygen species (ROS), and these reactive compounds have been implicated in a number of human diseases including but not limited to cancer, and heart and neurodegenerative diseases.

References

1. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79: 727-747.
2. Tvermoes BE, Banducci AM, Devlin KD, Kerger BD, Abramson MM, et al. (2014) Screening level health risk assessment of selected metals in apple juice sold in the United States. Food Chem Toxico 171: 42-50.
3. Vermerris W, Nicholson R (2008) Families of phenolic compounds and means of classification. In: Vermerris W, Nicholson R, (eds.). Phenolic Compound Biochemistry. Springer, Berlin, Germany. Pg No: 1-32.
4. Gharras HE (2009) Polyphenols: food sources, properties and applications - a review. Int J Food Sci Technol 44: 2512-2518.
5. Green RC (2007) Physicochemical properties and phenolic composition of selected Saskatchewan fruits: buffaloberry, chokecherry and see buckthorn. Ph.D. Thesis, University of Saskatchewan, Saskatoon, SK.
6. Aron PM, Kennedy JA (2008) Flavan-3-ols: nature, occurrence and biological activity. Mol Nutr Food Res 52: 79-104.
7. Dos Santos MD, Almeida MC, Lopes NP, de Souza GE (2006) Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biol Pharm Bull 29: 2236-2240.
8. Joseph SV, Edirisinghe I, Burton-Freeman BM (2016) Fruit polyphenols: a review of anti-inflammatory effects in humans. Crit Rev Food Sci Nutr 56: 419-444.
9. Kweon MH, Hwang HJ, Sung HC (2001) Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllostachys edulis*). J Agric Food Chem 49: 4646-4655.
10. McDougall B, King PJ, Wu BW, Hostomsky Z, Reinecke MG, et al. (1998) Dicaffeoylquinic and dicaffeoyltartaric acids are selective inhibitors of human immunodeficiency virus type 1 integrase. Antimicrob Agents Chemother 42: 140-146.
11. Mori H, Tanaka T, Shima H, Kuniyasu T, Takahashi M (1986) Inhibitory effect of chlorogenic acid on methylazoxymethanol acetate-induced carcinogenesis in large intestine and liver of hamsters. Cancer Lett 30: 49-54.
12. Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radio Biolo Medic 20: 933-956.
13. Ahmad SR, Gokulakrishnan P, Giriprasad R, Yatoo MA (2015) Fruit-based natural antioxidants in meat and meat products: a review. Crit Rev Food Sci Nutr 55: 1503-1513.
14. Bonilla F, Mayen M, Merida J, Medina M (1999) Extraction of phenolic compounds from red grape marc for use as food lipid antioxidants. Food Chem 66: 209-215.
15. Cornille A, Giraud T, Smulders MJM, Roldán-Ruiz I, Gladieux P (2014) The domestication and evolutionary ecology of apples. Trends Genet 30: 57-65.

16. Bates RP, Morris JR, Crandall PG (2001) Principles and practices of small- and medium-scale fruit juice processing. FAO Agricultural Services Bulletin, University of Florida, USA.

17. Tanrıöven D, Ekşi A (2005) Phenolic compounds in pear juice from different cultivars. Food Chem 93: 89-93.

18. Nayak B, Liu RH, Tang J (2015) Effect of processing on phenolic antioxidants of fruits, vegetables, and grains - a review. Crit Rev Food Sci Nutr 55: 887-918.

19. Spanos GA, Wrolstad RE (1992) Phenolic profiles of apple, pear and white grape juices and their changes with processing and storage - a review. J Agri Food Chem 40: 1478-1487.

20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 26: 1231-1237.

21. Mishra K, Ojha H, Chaudhury NK (2012) Estimation of antiradical properties of antioxidants using the DPPH· assay: a critical review and results. Food Chem 130: 1036-1043.

22. Sharma OP, Bhat TK (2009) DPPH antioxidant revisited. Food Chem 113: 1202-1205.

23. United States Department of Agriculture (USDA) (2016) Noncitrus fruits and nuts, 2015 summary. National Agricultural Statistics Services, USA.

24. Chun OK, Kim DO, Smith N, Schroeder D, Han JT, et al. (2005) Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. J Sci Food Agri 85: 1715-1724.

25. Shaghaghi M, Manzoori JL, Jouyban A (2008) Determination of total phenolics in tea infusions, tomato and apple juice by terbium sensitized fluorescence method as an alternative approach to the Folin-Ciocalteu spectrophotometric method. Food Chem 108: 695-701.

26. Everette JD, Bryant QM, Green AM, Abbey YA, Wangila GW, et al. (2010) Thorough study of reactivity of various compound classes toward the Folin-Ciocalteu reagent. J Agric Food Chem 58: 8139-8144.

27. Willems JL, Low NH (2014) Authenticity Analysis of Pear Juice Employing Chromatographic Fingerprinting. JAgric Food Chem 62: 11737-11747.

28. Lee KW, Kim YJ, Kim DO, Lee HJ, Lee CY (2003) Major phenolics in apple and their contribution to the total antioxidant capacity. J Agric Food Chem 51: 6516-6520.

29. Spanos GA, Wrolstad RE (1990) Influence of variety, maturity, processing and storage on the phenolic composition of pear juice. J Agric Food Chem 38: 817-824.

30. Spanos GA, Wrolstad RE, Heatherbell DA (1990) Influence of processing and storage on the phenolic composition of apple juice. J Agric Food Chem 38: 1572-1579.

31. Escarpa A, González MC (2001) Total extractable phenolic chromatographic index: an overview of the phenolic class contents from different sources of foods. Eur Food Res Technol 212: 439-444.

32. Klimczak I, Małecka M, Szlachta M, Gliszczyńska-Świgło A (2007) Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. J Food Compos Anal 20: 313-322.

33. Sánchez-Rodríguez E, Ruiz JM, Ferreres F, Moreno DA (2012) Phenolic profiles of cherry tomatoes as influenced by hydric stress and rootstock technique. Food Chem 134: 775-782.

34. Teixeira A, Eiras-Dias J, Castellarin SD, Gerós H (2013) Berry phenolics of grapevine under challenging environments. Inter J Mol Sci 14: 18711-18739.

35. Escarpa A, González MC (2001) Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods. Analy Chim Acta 427: 119-127.

36. Tsao R, Yang R (2003) Optimization of a new mobile phase to know the complex and real polyphenolic composition: towards a total phenolic index using high-performance liquid chromatography. J Chromatogr A 1018: 29-40.

37. Albright PS, Gosting LJ (1946) Dielectric constants of the methanol-water systems from 5 to 55°. J Am Chem Soc 68: 1061-1063.

38. Willems JL, Low NH (2016) Oligosaccharide formation during commercial pear juice processing. Food Chem 204: 84-93.

39. Makris DP, Rossiter JT (2001) Domestic processing of onion bulbs (*Allium cepa*) and asparagus spears (*Asparagus officinalis*): effect on flavonol content and antioxidant status. J Agric Food Chem 49: 3216-3222.

40. Ryan L, Prescott SL (2010) Stability of the antioxidant capacity of twenty-five commercially available fruit juices subjected to an *in vitro* digestion. Food Sci Technol 45: 1191-1197.

41. Wootton-Beard PC, Moran A, Ryan L (2011) Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. Food Res Int 44: 217-224.