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Original Article

The partial compositional characteristics of apple juice from 175 apple varieties

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Abstract

The partial compositional characteristics were determined for apple juice from 175 non-commercial varieties of apples developed from scion wood collected from approximately 12 countries and several USA geographical areas. Juices from many of the varieties were high in malic acid and potassium. Mean values for many of the attributes did not match existing compositional database value means. However, some of the overall minimum and maximum values for the various attributes (i.e., Brix°, pH, ash, TA, sucrose, glucose, fructose, sorbitol, malic, citric, fumaric, sodium, and calcium) in this study compared reasonably well with existing compositional database values. Distribution of phenolics between the various varieties was highly variable with some juices containing little if any phenolic compounds. Chlorogenic acid and phloridzin were detected in all varietal samples while arbutin and HMF were not measurable. The data developed should be useful with other databases in describing authentic apple juice and in the development of future apple commercial varieties to target specific consumer requirements.

Keywords: Apple juice; Composition; Varieties; Carbohydrates; Phenolics; Acids; Database

1. Introduction

Several comprehensive reviews were published that discussed the chemical composition of authentic single strength apple juice (Mattick and Moyer, 1983; Withy et al., 1978; Lee and Wrolstad, 1988a) and commercially produced apple juice concentrate (Elkins et al., 1996). Various factors such as cultivar, growing region, climate, cultivar practices, harvest maturity (Drake and Eisele, 1997), storage atmosphere (Drake and Eisele, 1994), storage conditions

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(Drake et al., 2002; Drake and Eisele, 1999), shipping (Babsky et al., 1989) and processing (Spanos et al., 1990; Wrolstad et al., 1989) are known to affect the chemical composition of apple juice and apple juice concentrate. One of many objectives of these investigations was to define the parameters for the authenticity of apple juice based on such analysis as: Brix°, titratable acidity, ash, pH, proline, specific gravity, formal index values, sugars, non-volatile organic acids, minerals, amino acids, phenolics, and isotopic carbon ratios (Lee and Wrolstad, 1988c) to produce individual, combined, or matrix databases (Elkins et al., 1988; Lee and Wrolstad, 1988b; Wallrauch, 1988; Evans et al., 1983). Also, many of the juice compositional studies were limited to common or commercial varieties of apples which were developed to meet various marketing schemes and customer acceptance for sweetness, acidity, color, and texture (Way and McLellan, 1989).

The compositional data on apple juice reported in this study are unique in that the fresh fruit was obtained from a local plot in Selah, WA that contained over 400 varieties on 85 semi-dwarf orchard trees (Prater, 1996). Scion wood for grafting was collected from major growing regions of the world over a 15-year period. This included new and "antique", old, varieties—many of which are not easily available. There was sufficient fruit formed in the fall of 1997 to investigate the compositional characteristics of 175 varieties. Care was taken to maximize cooling and minimize treatment times to maintain the quality integrity of the samples. The compositional information can be used in conjunction with existing databases to better describe acceptable attribute ranges for authentic apple juice and in the development of commercial apple varieties that would target specific consumer requirements.

2. Materials and methods

Ten apples were picked for each variety based on historical harvest records and stored at 2°C. Each group of 10 fruit was washed, combined, and juiced through a Champion Juicer (Plastaket Mfg. Co., Lodi, CA) within 24 h of harvest. The subsequent juice was filtered through a large coffee filter. Approximately 300 mL of juice was treated for 4 h with 0.1 mL diazyme and 0.1 mL Clarex 5X (currently termed Crytalzyme 401 and Crystalzyme 100XL, respectively; Valley Research Inc., South Bend, IN) at approximately 15°C with occasional stirring. Subsequent enzyme treated juice was centrifuged to remove insoluble solids in a Sorval RC-5B refrigerated centrifuge with a SS-34 rotor (Dupont Instruments, Wilmington, DE) for 30 min at 10,000 rpm resulting in an average centrifugal force of approximately $8000 \times g$. Aliquots of the same were distributed into various vials and frozen prior to analysis until a sufficient number of samples were collected.

Samples were tested for Brix° (AOAC 932.12), pH (AOAC 981.12), ash (AOAC 940.26), and titratable acidity (AOAC 942.15) (AOAC, 1990). Sugars (Drake and Eisele, 1999) were determined using an HPLC system consisting of a Waters 510 pump (Waters, Milford, MA), a Waters 710B Wisp autosampler, a BioRad, column heater (BioRad Lab, Richland, CA) set at 80°C, and a BioRad Aminex HPX-87C monosaccharide analysis column fitted with a Carbo-C microguard column, and BioRad refractive index monitor. The mobile phase, consisting of 0.01% reagent grade calcium chloride prepared with deionized water, was used at a flow rate of 0.8 mL/min. Organic acids were determined using a modified AOAC 986.13 procedure. The HPLC system for organic acids analysis was similar to that used for sugar analysis except the detector was a

Waters 490 UV/visible set at a wavelength of 214 nm. Organic acid separation was achieved with two Phenosphere 5 micron, ODS(1), 250 × 4.6 mm columns (Phenomenex, Torrance, CA) in series connected to a reverse phase guard column. The mobile phase consisted of a 0.05 M phosphate buffer, pH 2.4, at a flow rate of 0.7 mL/min. The injection volume for both sugars and organic acids was 10 μL. The sugar standards were prepared by adding 2.00 g of each reagent grade glucose, sucrose, fructose, and sorbitol (Sigma, St. Louis, MO) to a 100 mL volumetric flask and diluting to 100 mL volume with DI water. Organic acid standards were prepared by adding 10 mg each of reagent grade shikimic acid and fumaric acid and 100 mg each of reagent grade quinic acid, malic acid, isocitric acid, and citric acid to a 100 mL volumetric flask and diluting to 100 mL volume with DI water.

Minerals were determined by atomic absorption spectrophotometry using a modified AOAC 985.35 procedure where 10.0 mL aliquots of juice were diluted to a final volume of 100 mL with 1 N HNO_3. Calibration curves for each mineral, wavelengths, dilutions, and flame parameters were optimized for a GBC 932AA in accordance with the instruments manufacturer's recommendations (GBC Scientific Equipment, Inc., Arlington Heights, IL).

The chloride and phosphate content were determined by ion chromatography with a HPLC system similar to that used for sugar analysis. The column heater was set at 30°C, and a Bio-Rad Conductivity Monitor was set at a sensitivity of 100 and range of 2000. A Hamilton PRP-X100, 250 × 4.1 mm (Alltech Associates, Inc., Deerfield, IL), was used for separation of the anions. The mobile phase consisted of 4 mM *p*-hydroxybenzoic acid and 2.5% methanol adjusted to a pH of 8.5 with LiOH. The flow rate was 1.5 mL/min, and sample injection volumes were 10 μL. Anion standards were obtained as Alltech Mix #5 (Alltech Associates, Inc., Deerfield, IL).

Phenolics were determined using a modified procedure of Spanos and Wrolstad (1992). The HPLC consisted of a binary system with two Waters 515 pumps, a Waters 717 plus autosampler, and Waters 996 Photodiode Array detector interfaced to a computer with Waters Millennium 32 Chromatography Software. The detector array was set for 200–400 nm at a 3.6 nm resolution for compound identification compared to known standards. A 280 nm wavelength was used for individual compound quantification. A Phenomenex Prodigy 5u ODS(3), 100 A, 250 × 4.6 mm, column with an ODS guard column was used for separation (Phenomenex, Torrance, CA). The mobile phase flow of 1.0 mL/min consisted of two solvents: A—0.05 M potassium phosphate, pH 3.00 and B—70% acetonitrile and 30% A (by volume). The gradient at start was set at 100% A followed by 3 min (100% A), 6 min (96% A and 4% B), 15 min (90% A and 10% B), 30 min (85% A and 15% B), 35 min (80% A and 20% B), 50 min (77% A and 23% B), 60 min (75% A and 25% B), 66 min (70% A and 30% B), 83 min (20% A and 80% B), and 85 min (100% A) with an additional 15 equilibrium time. Typical data collection time was 80 min at ambient temperature. All sample injection volumes were 20 μL. Standards were obtained from Sigma (St. Louis, MO): hydroxymethylfuraldehyde (HMF), arbutin, gallic acid, chlorogenic acid, catechin, caffeic acid, epicatechin, *p*-coumaric acid, ferulic acid, rutin, and phloridzin. Initial stock solutions of each analyte at 1000 ppm were prepared in 50/50 water/methanol, stored refrigerated, and covered from light. Subsequent dilutions were used to prepare a standard curve.

HPLC detectors were interfaced to a 4-channel computer controller card configured to EZChrom Chromatography Software (Scientific Software, Inc., San Ramon, CA). Calibration curves were obtained by diluting previously described standards. The limits of detection for each analyte based on the calibration curve were: sucrose, glucose, fructose, and sorbitol—0.02 g/100 mL;

quinic, malic, isocitric, shikimic, and citric acids—0.1 mg/100 mL; fumaric acid—0.01 mg/100 mL; sodium, potassium, magnesium, calcium, iron, chloride, and phosphate—0.1 ppm; and chlorogenic acid, catechin, caffeic acid, epicatechin, *p*-coumaric acid, ferulic acid, rutin, and phloridzin—0.1 ppm. Appropriate standards for each analysis were also tested prior to each group of samples to assure proper calibration.

Each attribute was determined once per variety. Results were reported at a standardized 11.5 Brix° except for the original juice Brix° and pH. The statistical mean, standard deviation, percent coefficient of variation, minimum, maximum, and a range value were calculated using Microsoft Excel Descriptive Statistics.

The scion wood source is followed in parentheses and indicative of the geographical area from which it was obtained and not necessarily where the variety was developed. Varieties tested were: Adams Pearmain (England), Akin or Aiken-Aken (ME, USA), Allington Pippin (MN, USA), Almata (Russia), American Beauty (MA, USA), Antonovka (Russia), Arkansaw or Mammouth Black Twig (AR, USA), Aromatic Russet (England), Ashmead's Kernel (England), Babich (Unknown), Bailey Sweet (NY, USA), Baldwin (MA, USA), Barry (NY, USA), Belle De Boskoop (Holland), Ben Davis (TN, USA), Benoni (MA, USA), Bently or Bentley's Sweet (VA, USA), Bevans Favorite (NJ, USA), Bietigheirmer (Germany), Black Ben Davis (TN, USA), Black Twig or Paragon (TN, USA), Braeburn (WA, USA), Bramley Seedling (England), Buckley Giant (WA, USA), Buff (NC, USA), Bullock Pippin (USA), Calville Blanc D'Hiver (France), Cannon Pearmain (VA, USA), Chehalis (WA, USA), Chenango Strawberry (NY, USA), Cinnamon Spice (Russia), Claygate Pearmain (England), Coe's Golden Drop (England), Cole's Quince (MA, USA), Collett (Europe), Corder (WV, USA), Cornish Gilliflower (England), Cox's Orange Pippin (England), Crow Egg (KY, USA), Dave's Old Red Delicious (USA), Deacon Jones (PA, USA), Detroit Red (Canada), Devonshire Quarrendon (England), Domine (MA, USA), Dr. Mathews (IN, USA), Earliest (Russia), Early Joe (NY, USA), Early Strawberry (NY, USA), Elstar (WA, USA), Eve's Delight (Unknown), Fall Pippin (USA), Fall Russet (MI, USA), Fameuse or Snow (France), Fayette (France), Fuji (WA, USA), Gala (WA, USA), Gano (TN, USA), Gloria Mundi (USA), Golden Delicious-Starkspur (WV, USA), Golden Harvey (England), Golden Pearmain (England), Golden Russet (England), Granny Smith (Australia), Green Chisel (TN, USA), Grimes Golden Pippin (WV, USA), Grindstone or American Pippin (MI, USA), Gruschovka (Russia), Hass or Horse Apple (NC, USA), Harvey (England), Hibernal (Russia), Hightop Sweet (MA, USA), Holland (OH, USA), Holland Red Winter (OH, USA), Hoover or Black Coal (SC, USA), Huntsman (MO, USA), Hyde King (IA, USA), Irish Peach (Ireland), Jonathon (NY, USA), July (Germany), July Tart (VA, USA), Kalama River (Russia), King David (GA, USA), King Solomon (GA, USA), Late Strawberry or Autumn Strawberry (NY, USA), Livland Reaspberry or Lowland Reaspberry (Russia), Lord Hindlip (England), Lord's Seedling (NY, USA), Lyscom or Bill Luce (MA, USA), Magog Redstreak (VT, USA), Malinda (VT, USA), Margil (England), McIntosh (Canada), McMahon's White (WI, USA), Melon (NY, USA), Mill Creek (USA), Mollies Delicious (MN, USA), Monroe Sweet (OH, USA), Motts Sweet (OH, USA), Moyer Prize (IN, USA), Nodhead (NJ, USA), Northern Spy (NY, USA), Northfield Beauty (VT, USA), Nutmeg Pippin (IN, USA), Palouse (WA, USA), Paradise Sweet or Winter Sweet Paradise (OH, USA), Parks Pippin or Park Apple (GA, USA), Peche Melba (France), Peck's Pleasant or Waltz Apple (RI, USA), Ping Sugar (PA, USA), Pomme Gris (France), Pound Pippin or Fall Pippin (USA), Priestley or Red Cathead (PA, USA), Rall's Janet or Neverfail

(France), Ramsdell's Sweet (CT, USA), Red Astrachan (Russia), Red Delicious-Old Striped (IA, USA), Red June or Carolina Red June (NC, USA), Red Rome (WA, USA), Red Royal Limbertwig (NC, USA), Red Spitzenburg (IL, USA), Rhode Island Greening (RI, USA), Ribston Pippin (England), Roxbury Russet or Leather Coat (England), Saint Johnsbury Sweet (VT, USA), Salome (IL, USA), Sam Young (Ireland), Schaufnase (Austria), Seaconk Sweeting (CT, USA), Sheepnose (Russia), Sheppard Sweet (CT, USA), Shockley or Grizzle (GA, USA), Smokehouse (PA, USA), Sops O Wine (ME, USA), Somerset of Maine (Europe), Spokane Beauty (WA, USA), Starr (NJ, USA), Stearns (IN, USA), St. Lawrence (Canada), Stone (MI, USA), Strawberry Pippin (England), Striped Astrachan (Sweden), Striped Rambo (PA, USA), Summer Limbertwig (NC, USA), Summer Rambo or Rambo (France), Summer Rose or Woolman's Early (NJ, USA), Surprise (England), Swayzie (Canada), Sweet Bough (IA, USA), Sweet Winesap (PA, USA), Twenty Ounce or Cayuga Red Streak or Twenty Ounce Pippin (NY, USA), Virginia Beauty (VA, USA), Virginia Crab or Hewes Crab (VA, USA), Wade (WI, USA), Wagener (NY, USA), Washington's Oldest Apple (WA, USA), Washington Royal or Palmer Greening (MA, USA), Washington Strawberry (NY, USA), White Pippin (KY, USA), White Winter Calville (France), White Winter Pearmain (England), Willliams or Williams Early (MA, USA), Wine Apple (DE, USA), Winesap (NJ, USA), Winter Banana-Spur (IN, USA), Winter John (Russia), Winter Rambo (MA, USA), Winthrop Greening (ME, USA), Wismer's Dessert (Canada), Wyken Pippin (England), Yar Mohammidi (Turkey), Yates (GA, USA), Yellow Bellflower or Lady Washington (NJ, USA), Yellow June or White June (SC, USA), Yellow Transparent (Russia), Zabergau Reinette (Germany).

3. Results and discussion

The apple juice compositional data in Table 1 represent 175 varieties, mostly non-commercial, of fruit from scion wood that was collected from approximately 12 countries. Since previously published compositional apple juice data was mainly limited to common or commercial varieties of apples, it was not surprising that there was a large variation for most of the attributes in this study as evident by the large percent coefficient of variation (Table 1). However, when considering the minimum and maximum values for the attributes of Brix°, pH, ash, TA, sucrose, glucose, fructose, sorbitol, malic, citric, fumaric, sodium, and calcium for the 175 varieties, they compare reasonably well with the overall values reported by Lee and Wrolstad (1988b) as part of a comprehensive literature review on the chemical composition of apple juice. Also, the minimum and maximum values for quinic and isocitric acids were also very similar to published results (Lee and Wrolstad, 1988b) even though there were several varieties in which these organic acids were not detected. The range values for quinic acid agree closely to that found in an apple juice concentrate characterization study by Elkins et al. (1996). Malic acid, the major acid of apple juice, was found in one variety at a concentration of 1738.2 mg/100 ml. Many of the varietal juices tended to be high acid types as demonstrated by the malic acid mean of 847.7 mg/100 ml and the overall varietal range of 1544.9 mg/100 ml at 11.5 Brix°.

The potassium content of many of the varieties was highly variable as shown by the wide range of 1946.4 ppm. The maximum value of 2712.3 ppm is almost a double magnitude value of that reported by Lee and Wrolstad (1988b) or Elkins et al. (1996); and the mean value of 1511 ppm is

Table 1
The mean, standard deviation, minimum, maximum, and range values for the composition of apple juice prepared from 175 apple varieties grown in Selah, WA, USA

Attribute	Units[a]	Mean	Std[b]	%CV[c]	Minimum	Maximum	Range
Brix°		14.24	1.80	12.6	10.26	21.62	11.36
pH		3.71	0.16	4.3	3.37	4.24	0.87
Ash	(% w/w)	0.25	0.05	20.0	0.12	0.39	0.27
TA	(% as malic)	0.87	0.28	32.2	0.23	1.82	1.59
Sucrose	(g/100 mL)	2.16	0.73	33.8	0.38	5.65	5.27
Glucose	(g/100 mL)	2.01	0.53	26.4	1.05	3.23	2.18
Fructose	(g/100 mL)	5.69	0.84	14.8	3.84	8.01	4.17
Sorbitol	(g/100 mL)	0.45	0.22	48.9	0.17	1.40	1.23
Fru/Glu Ratio		3.05	1.04	34.1	1.30	6.73	5.43
Quinic	(mg/100 mL)	41.8	24.8	59.3	ND[d]	152.0	152.0
Malic	(mg/100 mL)	847.7	280.7	33.1	193.3	1738.2	1544.9
Isocitric	(mg/100 mL)	3.8	4.9	128.9	ND	24.8	24.8
Shikimic	(mg/100 mL)	1.4	0.8	57.1	0.3	4.6	4.3
Citric	(mg/100 mL)	11.9	5.4	45.4	0.8	27.4	26.6
Fumaric	(mg/100 mL)	0.14	0.11	78.6	ND	0.89	0.89
Sodium	(ppm)	11.8	10.5	89.0	0.5	73.4	72.9
Potassium	(ppm)	1511.0	266.9	17.7	765.9	2712.3	1946.4
Magnesium	(ppm)	64.9	9.9	15.3	35.2	100.5	65.3
Calcium	(ppm)	41.9	13.6	32.5	18.7	80.3	61.6
Iron	(ppm)	0.1	0.2	200.0	ND	0.7	0.7
Chloride	(ppm)	1.4	2.2	157.1	ND	18	18
Phosphate	(ppm)	252.1	72.9	28.9	86	459	373
Chlorogenic	(ppm)	70.7	79.3	112.2	1.5	396.9	395.4
Catechin	(ppm)	1.2	6.1	508.3	ND	52.0	52.0
Caffeic	(ppm)	4.9	4.6	93.9	ND	31.8	31.8
Epicatechin	(ppm)	16.6	25.6	154.2	ND	148.5	148.5
p-Coumaric	(ppm)	4.9	3.3	67.3	ND	19.4	19.4
Ferulic	(ppm)	0.3	0.5	166.7	ND	2.4	2.4
Rutin	(ppm)	8.4	8.6	102.4	ND	45.5	45.5
Phloridzin	(ppm)	26.1	22.3	85.4	0.9	120.3	119.4

[a] All values standardized to 11.5 Brix° except for Brix° and pH.
[b] Std = standard deviation.
[c] %CV = percent coefficient of variation.
[d] ND = not detectable.

outside the suggested maximum potassium German RSK (Wallrauch and Faethe, 1988) value of 1500 ppm. In addition, the sodium (73.4 ppm) and phosphate (459 ppm) maximum values were well outside the German RSK suggested maximum value of 30 ppm for sodium and 300 ppm for phosphate. The chloride content of the varieties in Table 1 did meet the German RSK suggested guidelines standard of 50 ppm maximum.

Arbutin was not detected in any of the juice samples. Elkins et al. (1996) reported that in apple juice concentrates, 13 of 26 samples of the third year sampling contained 1–5 ppm arbutin at 11.5 Brix°. It has been suggested that arbutin might serve as a marker for the presence of pear juice in apple juice since arbutin is very stable through storage and processing (Wrolstad et al., 1989). However, Schieber et al. (2001) could only quantify arbutin in two of three pear cultivars suggesting that its significance as an appropriate marker for pear may be questionable. This study would suggest that arbutin may not be present in apple juice or at levels that could not be detected with the methodology.

HMF was also not detected in any of the juice samples. HMF is formed by acid-heat catalyzed degradation of sugars and often used as an index for heat, storage, and processing abuse (Bielig and Hofsommer, 1982). Samples in this study were not subjected to heat and samples were kept frozen prior to testing.

Phloridzin and chlorogenic acid were widely distributed and present in all varieties. The mean chlorogenic acid content of 70.7 ppm was approximately two-fold higher than that found in apple juice by Lee and Wrolstad (1988b) or in apple juice concentrate by Elkins et al. (1996). Also, the maximum value for chlorogenic acid was 396.9 ppm, much higher than in the same published studies. Chlorogenic acid is known to be susceptible to degradation by pholyphenoloxidase (Coseteng and Lee, 1987). The higher chlorogenic acid content could be due to sample preparation protocol as temperatures were kept predominately cool and time of preparation was kept to a minimum. The concentration of phloridzin in apple juice is relatively unknown. However, Elkins et al. (1996) reported that apple juice from concentrates of the third year of a 3 year sampling exhibited a phloridzin content minimum of 0.91 ppm, maximum of 30.4 ppm, and a mean of 10.7 ppm. The maximum phloridzin content in this study was 120.3 ppm (Table 1), approximately four-fold greater than in the previous published study, and the mean was 26.1 ppm.

Catechin, caffeic acid, epicatechin, *p*-coumaric acid, ferulic acid, and rutin were also widely distributed and not detected in many of the varieties. The distribution of many of the phenolics in apple juice appears to a function of extraction method, processing, and enzyme treatment as well as other factors (Spanos and Wrolstad, 1992). In this study, the juice contact time with peel and seeds material was very limited and temperatures were refrigerated except during the actual juicing process, which was conducted at ambient temperatures. The extraction of many of the phenolics as well as their solubility was more than likely a function of this process and as a result, may explain why the juice of many varieties contained little if any measurable phenolic compounds.

4. Conclusions

The composition characteristics were determined for apple juice from 175 non-commercial varieties of apples developed from scion wood collected from approximately 12 countries and several USA geographical areas. The mean values for many of the attributes did not match existing compositional database value means. However, some of the overall minimum and maximum values for the various attributes (i.e., Brix°, pH, ash, TA, sucrose, glucose, fructose, sorbitol, malic, citric, fumaric, sodium, and calcium) in this study compared reasonably well with existing compositional database values even though there were large quantitative variations

within some of the measured attributes. Many of the varieties examined tended to be high in acid and contained large amounts of malic acid, much more so than in previously published studies that examined predominately common or commercial apple varieties (Elkins et al., 1996; Lee and Wrolstad, 1988b). The potassium mean and maximum values were also greater in value than in the same published studies. The distribution of phenolics between the various varieties was highly variable with some juices containing little if any phenolic compounds. Chlorogenic acid and phloridzin were detected in all varietal samples while arbutin and HMF were not measurable in any sample.

Many of the mineral maximum values such as sodium, potassium, magnesium, and phosphate were greater than the German RSK guideline values even though these juice samples were from authentic apples. This study demonstrates that one or more measured attributes of a given variety may not meet some current database authenticity guidelines.

The entire database is available from the authors (teisele@treetop.com) upon request or at www.treetop.com in PDF file format.

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