Carboxylic Acid Composition of Varietal Juices Produced from Fresh and Stored Apples

Tibor Fuleki,* Estela Pelayo, and Rodrigo B. Palabay

Horticultural Products Laboratory, Horticultural Research Institute of Ontario, Box 7000, Vineland Station, Ontario, Canada L0R 2E0

Juices were produced from 11 apple cultivars from three regions of Ontario before and after storage in 2 years and analyzed for acids. The concentration ranges (mg/L) for fresh and stored apples, respectively, were as follows: malic, 4780−15 730 and 2470−13 390; citric, 263−538 and 321−714; quinic, 10−754 and 10−693; lactic, 10−203 and 17−219; succinic, 2−28 and 1−51; shikimic, 1−25 and 1−26; chlorogenic, 74−85 and 10−80; total, 5770−16 880, and 3330−14 760; titratable acidity, 2700−11 700 and 1500−9300; pH, 3.22−3.90 and 3.29−4.41; malic:citric, 16.0−39.6 and 7.0−26.8; sugar:acid, 10.0−37.7 and 12.3−66.0. Cultivar affected all except succinic and chlorogenic acids. Region and season had only minor influence. Malic, quinic, total, and titratable acids and H^+ decreased, while citric, lactic, and shikimic acids increased upon storage. Most commercial juices had acid compositions similar to those of the authentic ones, except fumaric acid was present and quinic, shikimic, and succinic acids were frequently absent.

Keywords: *Acids; juice; apple; cultivars; storage*

INTRODUCTION

Acids contribute to the taste and stability of foods and beverages. The major acid in apples is malic acid and its acid salt. Citric and quinic acids are also present in substantial quantities. The minor acids of apples have been identified as follows: acetic, ascorbic, caffeic, chlorogenic, citramalic, *p*-coumaric, *p*-coumaroylquinic, dicaffeoylquinic, ferulic, fumaric, galacturonic, glyceric, glycolic, glyoxylic, isocitric, lactic, mucic, oxalic, oxal-acetic, α-oxoglutaric, phosphoric, pyruvic, salicylic, shikimic, syringic, and succinic acids (Fernandez-Flores et al., 1970; Hulme and Rhodes, 1971; Robertson and Kermode, 1981; Steenkamp et al., 1983; Macheix et al., 1990; Schols et al., 1991). The identity of malic, citric, quinic, and succinic acids in apples had been confirmed using GC/MS by Chapman and Horvat (1989). Quantitative determination of individual acids is important in juice and cider making and is used as a measure of maturity, taste, spoilage (acetic), and authenticity of juice and cider.

More apple juice is consumed, not only in Canada but worldwide, than any other juice except that made from oranges. In recent years, it has become apparent that apple juice is subject to adulteration (Brause, 1992). The acid composition of apple juice provides a means for the detection of adulteration (Lee and Wrolstad, 1988a). Unlike in grapes (Fuleki et al., 1993), tartaric acid is absent while quinic acid is present in apples. Most pear cultivars contain significantly higher concentrations of quinic and citric acid than apple, and this can be used to detect the addition of pear juice to apple juice. Lee and Wrolstad (1988a) suggested that ratios such as malic/citric (M/C), quinic/citric, and malic/quinic could be used to detect adulteration of apple juice with pear juice. The ratios have lower standard deviation than individual acid concentrations, which would be an advantage in the application of confidence limits.

There is considerable literature on the major acid components of apple juice. The concentration ranges for the individual acids are summarized in Table 1 for authentic and commercial "pure apple juices". Ryan (1972) and Ryan and Dupont (1973) described the individual acid composition of 26 authentic commercially produced apple juices from four apple-growing regions of Canada. Zubeckis (1962) determined the titratable acidity (TA) and pH in seven apple and three crabapple cultivars grown in Ontario for 5 consecutive years, but there is no information available on the individual acid composition of apples produced in Ontario. Furthermore, most studies on the composition of apple juice did not evaluate juice produced from fruit kept in cold storage for longer periods, even though substantial quantities of stored apples are used by juice manufacturers. The present study was undertaken to rectify this situation. Although the project was initiated to provide a data base for authentication of fruit juices, it is expected that this information will also be useful to food technologists and pomologists as well.

* Author to whom correspondence should be addressed [fax (905) 562-3413].

MATERIALS AND METHODS

The source of apples, production of juice, and preparation of juice samples for analysis were described previously (Fuleki et al., 1994). The analytical procedures, apparatus, and reagents, as well as the statistical methods, were presented in an earlier paper on the acid composition of grape juice (Fuleki et al., 1993).

RESULTS AND DISCUSSION

Chromatogram of the acid fraction of an authentic juice made from fresh apples monitored at 210 nm is shown in Figure 1. Citric, malic, quinic, succinic, shikimic, lactic, and chlorogenic acids were identified by comparing their retention times and UV spectra to those of authentic standards (Fuleki et al., 1993). Since succinic and lactic acids coeluted with other compounds, their quantities were determined enzymatically (Fuleki et al., 1993).

In addition to the chromatographic peaks for the above compounds there was, in most authentic and commercial apple juices, a small peak eluting at around

Table 1. Acid Composition of Authentic and Commercial Pure Apple Juice As Reported in the Literature[a]

origin	no. of samples (cultivars)	malic, g/L	citric, mg/L	quinic, mg/L	lactic, mg/L	chlorogenic, mg/L	succinic, mg/L	shikimic, mg/L	total, g/L	TA,[b] g/L	pH	malic/citric	sugar[c]/acid	analyt method[d]	ref
France	6 (6)	2.36–9.86	140–240	na[e]	30–90	na	30–40	na	2.62–10.10	3.14–**15.86**	na	13.9–58.0	na	Ch	Tavernier and Jacquin (1947)
France	33 (33)	1.41–13.21	100–560	na	30–100	na	60–**100**	na	**1.53**-13.50	**0.88**-10.86	3.27–**4.43**	10.5–55.6	14.8–15.9	Ch	Tavernier and Jacquin (1952)
England	21 (19)	1.8–**13.6**[f]	**0**-200[f]	400–2300[f]	**0**-tr[f]	**tr-600**[f]	**0**-tr[f]	na	2.95–**14.65**[f]	1.4–13.6[f]	3.07–4.25	**1.4**-28.3	na	PC	Philips et al. (1956)
England	na	**1.01**-10.06	19–102	480–2400	na	tr-301	na	**tr-14**	na	na	na	na	na	CC,PC	Whiting and Coggins (1960)
Austria	12	4.3–8.0	<100–600	na	na	na	na	na	6.9–10.5	4.6–8.1	3.30–3.62	7.2–95.0	15.7–25.1	Ez	Kain (1969)
Austria	6	4.8–7.8	300–1000	na	na	na	na	na	6.5–9.6	3.8–7.2	3.22–3.68	6.0–26.0	16.1–29.5	Ez	Kain and Vlcek (1971)
Canada	20	5.45–7.60	<50–150	**200**-415	na	na	na	na	5.92–8.04	3.75–5.83	3.38–3.52	na	23.0[g]	GC	Ryan (1972)
Canada	6	6.40[h]	140[h]	280[h]	na	na	na	na	7.0[h]	na	na	45.7	na	GC	Ryan and Dupont (1973)
Germany	38[i]	6.2–10.6[i]	30–220[i]	na	0–**160**[i]	na	na	na	6.37–10.86[i]	5.1–9.3[i]	na	32.5–226.7	**13.8**-25.6	Ez	Wucherpfennig et al. (1977)
U.S.	19	2.80–9.00	<100–200	na	na	na	na	na	na	na	na	18.0–41.0	na	HPLC	Evans et al. (1983)
England	30 (3)	3.7–12.8	50–110	na	0–20	na	na	na	na	3.22–11.79	**2.9**-3.7	96.3[g]	16.2[g]	Ez, Ch	Burroughs (1984)
U.S.	4[i]	5.7[h]	1100–**1600**[h]	1500–2000[h]	na	na	na	na	na	na	na	3.6–5.2	na	HPLC	Coppola and Starr (1986)
Switzerland	1[i]	6.87	250	600	na	na	na	na	7.72	na	na	27.5	na	HPLC	Badoud and Pratz (1986)
Switzerland	1[i]	7.0	510	na	na	na	na	na	na	6.0	3.35	13.7	19.3	HPLC	Bloeck et al. (1986)
Sweden	16[i]	4.7–7.6	60–1380	na	na	na	na	na	5.02–7.95	4.49–7.30	na	4.7–108.6	18.7–24.9	HPLC	Fuchs et al. (1987)
U.S. (MI)	2 (2)	7.18–8.16[j]	40–86[j]	1467–**3582**[j]	na	na	na	5–13[j]	8.75–11.78[j]	5.6	3.43–3.50	83.8–204.1	21.6–23.4	HPLC	Lee and Wrolstad (1988a)
U.S. (WA)	2 (1)	3.84–5.25[j]	78–361[j]	1752–1775[j]	na	na	na	10–14[j]	5.98–7.07[j]	2.1–3.2	3.73–3.96	10.7–67.5	38.4–**56.2**	HPLC	Lee and Wrolstad (1988a)
New Zealand	1 (1)	6.39[j]	69[j]	664[j]	na	na	na	4[j]	7.14[j]	5.5	3.58	92.6	26.9	HPLC	Lee and Wrolstad (1988a)
Mexico	1 (1)	5.77[j]	10[j]	651[j]	na	na	na	11[j]	6.44[j]	4.2	3.96	**578.7**	33.3	HPLC	Lee and Wrolstad (1988a)

[a] Minimum and maximum values within the column are in boldface. [b] Titratable acidity (TA) expressed as malic acid. [c] Calculated as TSS (Fuleki et al., 1994)/TA (g/100 mL). [d] CC, column chromatography; Ch, chemical; Ez, enzymatic; GC, gas chromatography; HPLC, high-pressure liquid chromatography; PC, paper chromatography. [e] na, not available. [f] Calculated for fruit weight. [g] Calculated from mean values. [h] Range of means. [i] Commercial juice. [j] Calculated from normalized values.

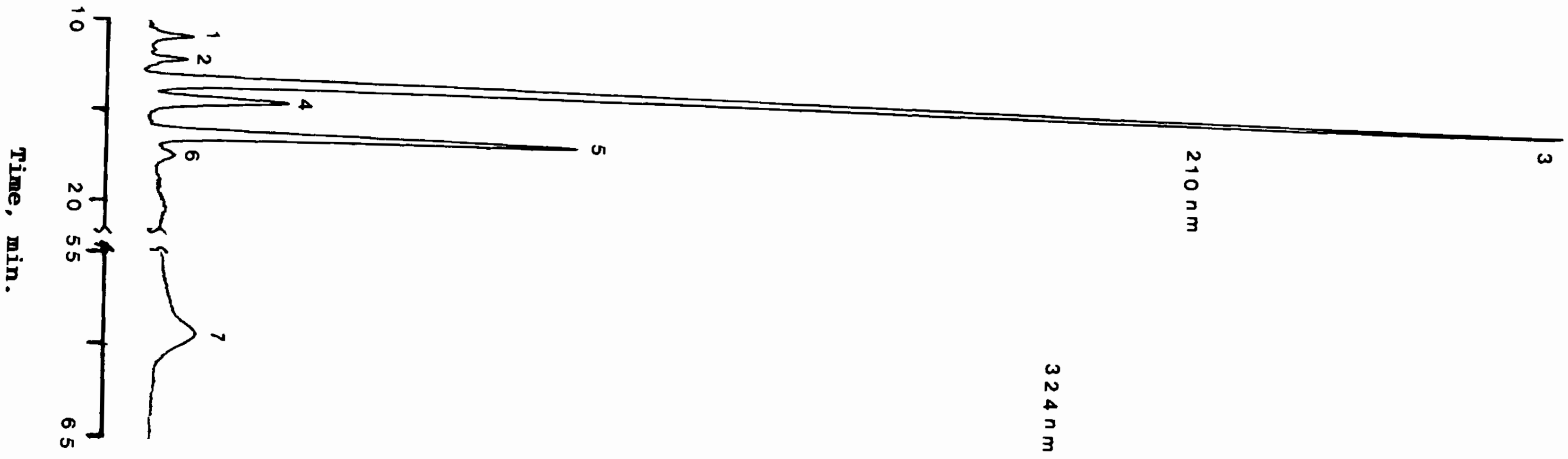

Figure 1. Separation of acids in the acid fraction of authentic apple cv. Mutsu juice on an ION-300 column. Identification of acid peaks: 1, citric; 2, unidentified; 3, malic; 4, quinic; 5, succinic and shikimic; 6, lactic; 7, chlorogenic.

12 min and having absorption maxima at 193 and 241 nm. Spectral analysis with the diode array detector indicated that there were two compounds present in this peak (Figure 2). The spectra of these unidentified compounds did not match any of the acids that were

Table 2. Acid Composition of Juice Produced from Fresh and Stored Fruit of Apple Cultivars Grown at Three Apple-Growing Regions of Ontario

cultivar	region[a]	storage[b]	year	malic, g/L	citric, mg/L	quinic, g/L	lactic, mg/L	chlorogenic, mg/L	succinic, mg/L	shikimic, mg/L	total, g/L	TA,[c] g/L	pH	malic/citric	sugar[d]/acid
Delicious	S	F	89	5.94	322	754	40	75	14	23	7.17	3.5	3.85	18.5	32.2
	S	S	89	5.09	404	693	33	75	11	23	6.33	2.8	3.98	12.6	40.8
	S	F	90	4.98	302	554	58	74	9	17	5.99	3.0	3.85	16.5	36.0
	S	S	90	3.73	396	431	128	74	51	18	4.83	2.2	4.08	9.4	49.2
	Sf	F	89	4.82	299	596	10	74	9	22	5.83	3.0	3.90	16.1	37.7
	Sf	S	89	4.25	404	451	59	76	14	26	5.28	2.8	4.08	10.5	38.8
	Sf	F	90	4.78	299	730	38	75	2	19	5.94	2.8	3.85	16.0	37.4
	Sf	S	90	3.82	351	603	53	75	5	22	4.93	1.9	4.03	10.9	57.6
	V	F	89	5.20	279	577	39	75	6	20	6.20	3.1	3.88	18.6	34.6
	V	S	89	4.55	350	583	56	74	10	20	5.64	2.5	3.95	13.0	42.3
	V	F	90	5.25	278	731	82	74	4	25	6.44	3.1	3.82	18.9	33.2
	V	S	90	4.42	334	624	105	74	20	25	5.60	2.4	3.99	13.2	43.7
Empire	S	F	89	9.93	340	271	32	75	9	6	10.66	6.9	3.40	29.2	17.4
	S	S	89	5.39	398	269	30	10	1	8	6.11	3.2	3.69	13.5	35.9
	S	F	90	7.03	332	273	63	74	13	9	7.79	5.0	3.38	21.2	20.4
	S	S	90	3.62	384	236	93	10	8	10	4.36	2.2	3.98	9.4	44.5
	Sf	F	89	11.01	403	267	18	78	2	6	11.71	7.8	3.35	27.3	17.1
	Sf	S	89	7.17	509	234	57	76	16	2	8.06	4.6	3.71	14.1	26.5
	Sf	F	90	8.35	366	212	71	77	13	6	9.10	5.4	3.41	22.8	23.0
	Sf	S	90	4.91	437	187	68	74	9	7	5.69	3.1	3.72	11.2	38.7
	V	F	89	10.41	263	193	76	74	18	5	11.04	7.5	3.38	39.6	17.1
	V	S	89	5.35	434	232	52	10	8	7	6.09	3.2	3.76	12.3	38.8
Golden Delicious	S	F	89	6.59	328	269	10	75	13	10	7.30	4.1	3.59	20.1	30.5
	S	S	89	4.16	434	267	59	75	9	12	5.02	2.2	3.96	9.6	53.2
	Sf	F	89	8.29	372	208	69	76	5	2	9.02	5.2	3.60	22.3	28.6
	Sf	S	89	4.85	465	171	104	75	9	9	5.68	2.6	4.04	10.4	45.8
	Sf	F	90	7.90	376	219	115	75	14	7	8.71	4.8	3.59	21.0	28.5
	Sf	S	90	5.24	494	213	106	75	7	12	6.15	2.6	3.91	10.6	26.7
	V	F	89	5.58	303	263	79	74	12	9	6.32	3.5	3.61	18.4	33.1
	V	S	89	4.32	423	212	87	74	13	9	5.14	2.2	4.03	10.2	57.3
	V	F	90	4.89	289	378	116	74	6	19	5.77	2.7	3.66	16.9	35.9
	V	S	90	2.47	351	253	130	75	40	13	3.33	1.5	4.41	7.0	66.0
Idared	S	F	89	9.56	329	173	28	77	14	2	10.18	6.5	3.42	29.1	18.3
	S	S	89	8.10	352	10	62	76	11	2	8.61	5.3	3.52	23.0	21.9
	S	F	90	9.16	312	10	56	75	17	3	9.63	6.5	3.29	29.4	17.7
	S	S	90	7.51	321	10	70	74	6	4	8.00	4.7	3.49	23.4	24.3
	Sf	F	89	8.71	318	177	80	75	14	6	9.38	5.9	3.47	27.4	18.6
	Sf	S	89	6.80	335	10	107	74	12	5	7.34	4.6	3.64	20.3	22.8
	Sf	F	90	12.41	374	166	105	79	28	2	13.16	8.3	3.23	33.2	14.8
	Sf	S	90	8.39	370	10	90	77	5	4	8.95	5.5	3.36	22.7	20.9
McIntosh	S	F	89	7.92	316	237	10	74	25	1	8.58	5.4	3.33	25.1	21.5
	S	S	89	4.49	326	289	17	75	14	7	5.22	2.8	3.68	13.8	19.6
	S	F	90	10.45	393	172	36	76	13	2	11.14	7.4	3.29	26.6	17.2
	S	S	90	6.03	383	202	35	75	48	7	6.78	4.0	3.65	15.7	27.3
	Sf	F	89	10.51	362	237	46	75	13	1	11.24	7.5	3.35	29.0	28.0
	Sf	S	89	7.30	367	248	52	74	16	3	8.06	4.9	3.55	19.9	14.3
	Sf	F	90	8.77	359	276	49	76	21	4	9.56	5.9	3.31	24.4	20.3
	Sf	S	90	5.62	359	237	44	76	24	1	6.36	3.4	3.56	15.7	35.0
Moira	Sf	F	89	8.90	346	280	102	75	13	6	9.72	6.7	3.38	25.7	16.4
	Sf	S	89	6.28	470	290	219	74	9	4	7.35	4.4	3.78	13.4	22.0
	Sf	F	90	11.15	382	263	194	75	21	3	12.09	8.0	3.28	29.2	13.8
Mutsu	S	F	89	7.59	313	303	56	75	14	8	8.36	5.2	3.45	24.3	23.8
	S	S	89	5.35	394	255	59	74	13	13	6.16	3.4	3.71	13.6	34.4
	S	F	90	7.62	315	158	44	74	7	7	8.23	5.1	3.42	24.2	25.1
	S	S	90	5.29	368	173	52	74	43	10	6.01	3.1	3.79	14.4	41.3
Northern Spy	S	F	89	12.73	430	337	76	85	20	4	13.68	9.1	3.38	29.6	15.8
	S	S	89	7.24	490	235	36	80	9	8	8.10	4.8	3.58	14.8	26.5
	S	F	90	9.85	369	228	46	76	13	4	10.59	7.3	3.28	26.7	17.1
	S	S	90	8.05	463	231	55	76	43	6	8.92	5.1	3.53	17.4	25.3
	Sf	F	89	11.71	370	281	61	79	18	6	12.53	8.4	3.38	31.7	13.6
	Sf	S	89	9.17	494	324	63	77	28	8	10.16	6.3	3.53	18.6	17.8
	Sf	F	90	12.50	423	273	114	78	16	6	13.41	8.8	3.25	29.6	15.2
	Sf	S	90	8.57	568	195	145	76	6	9	9.57	5.3	3.50	15.1	20.1
RI Greening	Sf	F	89	13.74	485	308	68	75	10	11	14.70	10.4	3.38	28.3	10.6
	Sf	S	89	12.07	451	328	96	74	39	14	13.07	9.0	3.49	26.8	12.3
	Sf	F	90	15.73	538	303	203	75	25	7	16.88	11.7	3.22	29.2	10.0
	Sf	S	90	13.39	714	339	218	75	14	10	14.76	9.3	3.29	18.1	12.3
Spartan	S	F	89	8.91	332	357	62	75	27	3	9.77	5.5	3.63	26.8	20.3
	S	S	89	5.09	616	328	72	74	11	7	6.20	2.6	4.04	8.3	46.0

Table 2. (Continued)

cultivar	region[a]	storage[b]	year	malic, g/L	citric, mg/L	quinic, mg/L	lactic, mg/L	chlorogenic, mg/L	succinic, mg/L	shikimic, mg/L	total, g/L	TA,[c] g/L	pH	malic/citric	sugar[d]/acid
	Sf	F	89	7.44	333	461	16	78	5	2	8.34	4.9	3.60	22.3	22.0
	Sf	S	89	4.88	482	463	56	79	16	5	5.98	3.0	3.98	10.1	37.6
	Sf	F	90	7.79	339	446	71	75	13	6	8.74	4.7	3.62	23.0	19.7
	Sf	S	90	5.96	579	342	120	75	26	7	7.11	2.9	3.94	10.3	31.6
Trent	Sf	F	89	9.96	303	373	74	74	2	6	10.79	7.4	3.36	32.9	13.1
	Sf	S	89	6.92	377	377	38	74	18	8	7.81	5.1	3.50	18.4	19.9
	Sf	F	90	11.38	332	432	85	75	18	6	12.33	8.3	3.24	34.3	13.1
	Sf	S	90	8.79	402	349	97	74	17	8	9.74	5.7	3.41	21.9	18.1
means (*n* = 77)				7.53	387	307	73	73	15	9	8.40	5.0	3.62	20.0	27.6
SD				2.82	83	165	44	13	11	7	2.80	2.3	0.27	7.5	12.4
min				2.47	263	10	10	10	1	1	3.33	1.5	3.22	7.0	10.0
max				15.73	714	754	219	85	51	26	16.88	11.7	4.41	39.6	66.0
lit. min (ref)[e]				1.01 (8)	0 (4)	200 (5)	0 (1,4,9)	tr (4,8)	0 (4)	tr (8)	1.53 (7)	0.88 (7)	2.9 (1)	1.4 (4)	13.8 (9)
lit. max (ref)[e]				13.6 (4)	1600 (2)	3582 (3)	160 (9)	600 (4)	100 (7)	14 (3, 8)	14.65 (4)	15.86 (6)	4.43 (7)	578.7 (3)	56.2 (3)

[a] S, Simcoe; Sf, Smithfield; V, Vineland. [b] F, fresh; S, stored. [c] Titratable acidity (TA) expressed as malic acid. [d] Calculated as TSS (Fuleki et al., 1994)/TA (g/100 mL). [e] 1, Burroughs (1984); 2, Coppola and Starr (1986); 3, Lee and Wrolstad (1988a); 4, Philips et al. (1956); 5, Ryan (1972); 6, Tavernier and Jacquin (1947); 7, Tavernier and Jacquin (1952); 8, Whiting and Coggins (1960); 9, Wucherpfennig et al. (1977).

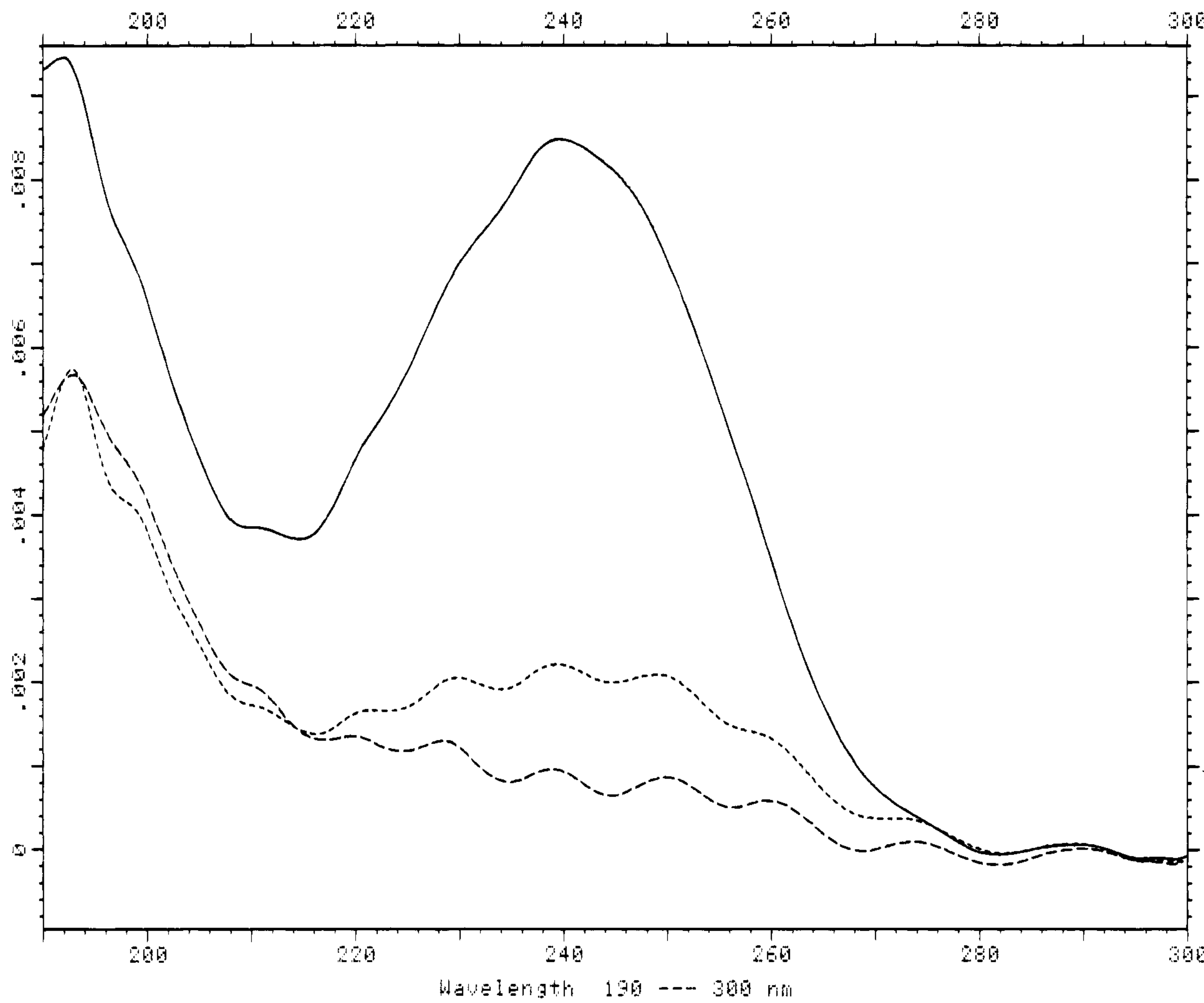

Figure 2. Spectral analysis of the unidentified peak in the acid fraction of Empire apple juice eluting at 12.2 min. Peak: center (—), early (– –), and late (- - -) eluting segment.

reported to be present in apples and elute between citric and malic acid (isocitric, tartaric, galacturonic, glyceric). The size of the peak was cultivar dependent, present in relatively large quantities in Empire, McIntosh, and Trent apples.

Authentic Varietal Apple Juice. The results of the analyses on authentic juices produced from fresh and stored fruit of 11 apple cultivars grown in three apple-growing regions of Ontario in 1989 and 1990 are presented in Table 2. Malic acid was the most abundant acid in every sample examined. A comparison of the results with those in the literature shows that the values reported here were within the literature ranges for most samples. In a few cases, the concentration of

Table 3. Effect of Cultivar on Acid Composition of Juice Produced from Fresh Apples Grown at Smithfield[a]

cultivar	N	malic, g/L	citric, mg/L	quinic, mg/L	lactic, mg/L	chlorogenic, mg/L	succinic, mg/L	shikimic, mg/L	total, g/L	TA,[b] g/L	pH	malic/citric	sugar[c]/acid
Delicious	2	4.80d	299e	663a	24b	75	6	21a	5.89d	2.90e	3.88a	16.1a	37.6a
Empire	2	9.68bc	385bc	240cd	45b	78	8	6bc	10.41bc	6.60bcd	3.38c	25.3bcd	20.1cde
Gldn Delicious	2	8.10c	374bc	214cd	92ab	76	10	5bc	8.87cd	5.00cde	3.60b	21.6b	28.6b
Idared	2	10.56bc	346bcde	172d	93ab	77	21	4bc	11.27bc	7.10bcd	3.35c	30.3de	16.7def
McIntosh	2	9.64bc	361bcd	257cd	48b	76	17	3c	10.40bc	6.70bcd	3.33c	26.7bcd	24.2bc
Moira	2	10.03bc	364bcd	272c	148a	75	17	5bc	10.91bc	7.35bc	3.33c	27.5de	15.1defg
Northern Spy	2	12.11ab	397b	277c	88ab	79	17	6bc	12.97ab	8.60b	3.32c	30.6de	14.4efg
RI Greening	2	14.74a	512a	306c	136a	75	18	9b	15.79a	11.05a	3.30c	28.8de	10.3g
Spartan	2	7.62cd	336cde	454b	44b	77	9	4bc	8.54cd	4.80de	3.61b	22.7bc	20.9cd
Trent	2	10.67bc	318de	403b	80ab	75	10	6bc	11.56bc	7.85b	3.30c	33.6e	13.1fg
signif[d]		**	***	***	*	ns	ns	**	**	**	***	**	***

[a] Means within each column followed by the same letter are not significantly different using Duncan's multiple-range test ($p = 0.05$). [b] Titratable acidity (TA) expressed as malic acid. [c] Calculated as TSS (Fuleki et al., 1994)/TA (g/100 mL). [d] *, **, ***, significant at $p \leq 0.05$, 0.01, and 0.001 confidence levels, respectively, by analysis of variance; ns, not significant.

Table 4. Effect of Growing Season on Acid Composition of Juice Produced from Fresh Apples Grown at Simcoe and Smithfield

location and year	N	malic, g/L	citric, mg/L	quinic, mg/L	lactic, mg/L	chlorogenic, mg/L	succinic, mg/L	shikimic, mg/L	total, g/L	TA[a] g/L	pH	malic/citric	sugar[b]/acid
Simcoe													
1989	6	8.95	342	346	40	77	16	7.3	9.77	6.10	3.47	25.9	21.5
1990	6	8.18	337	233	51	75	12	7.0	8.90	5.72	3.42	24.1	22.3
signif[c]		ns	ns	*	ns	ns	ns	ns	ns	ns	*	ns	ns
Smithfield													
1989	10	9.51	359	319	54	76	9	7.0	10.33	6.72	3.48	26.4	20.6
1990	10	10.08	379	332	105	76	17	7.0	10.99	6.87	3.40	26.3	19.6
signif[c]		ns	ns	ns	**	ns	**	ns	ns	ns	*	ns	ns
Simcoe and Smithfield													
1989	10	9.28	349	333	40	77	14	8.0	10.10	6.40	3.48	26.3	22.0
1990	10	8.83	353	289	64	76	15	7.0	9.63	6.04	3.41	24.6	21.9
signif[c]		ns	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns

[a] Titratable acidity (TA) expressed as malic acid. [b] Calculated as TSS (Fuleki et al., 1994)/TA (g/100 mL). [c] *, **, ***, significant at $p \leq 0.05$, 0.01, and 0.001 confidence levels, respectively, by analysis of variance; ns, not significant.

malic, lactic, shikimic, and total acids and the sugar/acid ratio (TSS/TA) were above and the quinic acid and TSS/TA values below those found in the literature. Rhode Island Greening, a high-acid cultivar, was responsible for the exceptionally high malic and total acid values and the extremely low TSS/TA ratios.

Effects of Cultivar. It is well established in the literature that cultivar will affect the amount of total acid as well as the proportion of individual acids in apples (Hartmann and Hillig, 1934; Smock and Neubert, 1950; Johnston and Hammill, 1968) and apple juice (Tavernier and Jacquin, 1947, 1952; Phillips et al., 1956; Brown and Harvey, 1971; Brause and Raterman, 1982; Blanco Gomis et al., 1988; Lee and Wrolstad, 1988b; Cilliers et al., 1990; Blanco et al., 1992).

The data presented here support this view. The acid composition of the cultivars was compared using Duncan's multiple-range test on the 1989 and 1990 data. The results from Smithfield (Sf), where we had the largest number of cultivars available (Table 3), showed that there were significant differences in malic, citric, quinic, lactic, shikimic, and total acids, TA, pH, M/C, and TSS/TA. A similar pattern emerged when the five cultivars that were available from both Sf and Simcoe (S) in 1989 and 1990 were compared. There were no significant differences among the examined cultivars in their chlorogenic and succinic acid contents. The cultivar Delicious was lowest in malic, citric, lactic, chlorogenic, and total acids, TA, H^+, and M/C, while it was highest in quinic and shikimic acids and TSS/TA. The cultivars Rhode Island Greening and Northern Spy had the highest concentrations of malic, citric, and total acids, TA, and H^+.

Malic acid represented 74–95% of the total acid in the samples examined. Citric acid was present in the second largest concentration in every cultivar except Delicious, Spartan (fresh), and Trent (fresh), for which the concentration of quinic acid was higher than that of citrate. In the literature (Table 1) the concentration of quinic acid was always higher than that of citric acid.

Effects of Growing Area. The effects of growing area on the acid composition of fresh and stored apples were compared with those cultivars that were analyzed from both locations (Sf and S) in the same year. The results showed no significant difference in acid composition between the juices produced from either fresh or stored apples grown at Sf and S locations.

The large-scale 3-year study sponsored by the Processed Apple Institute found significant differences in the TA of authentic varietal apple juice from eight states of the United States (Lee and Mattick, 1989). Since the number of cultivars studied in each state varied from one to seven, the differences found could be partly attributed to varietal differences. It should also be noted that the climatic differences among the surveyed states are considerably greater than those found among the apple-growing regions of Ontario.

Effects of Growing Season. Data on the influence of growing season on the acid composition of the juice from those apple cultivars that were available as fresh fruit in both years are presented by growing area in Table 4. The results show that while the quinic acid content and pH were significantly higher in 1989 at the S location, the lactic acid and H^+ concentration were significantly higher in 1990 in the 10 cultivars from Sf and in the five cultivars available from both Sf and S. Succinic

Table 5. Effect of Storage on Acid Composition of Apple Juice

storage	N	malic, g/L	citric, mg/L	quinic, mg/L	lactic, mg/L	chlorogenic, mg/L	succinic, mg/L	shikimic, mg/L	total, g/L	TA,[a] g/L	pH	malic/citric	sugar[b]/acid
1989 and 1990													
fresh	38	8.80	346	329	63	76	13	8	9.63	6.0	3.48	25.2	22.5
stored	38	6.17	428	287	80	70	17	10	7.07	3.9	3.76	14.6	33.1
signif[c]		***	***	**	*	*	ns	**	***	***	***	***	***
1989													
fresh	21	8.83	340	330	50	76	13	8	9.64	6.1	3.51	25.8	22.4
stored	21	6.13	427	299	67	69	14	10	7.02	3.9	3.77	14.6	32.1
signif[c]		***	***	ns	*	ns	ns	**	***	***	***	***	***
1990													
fresh	17	8.76	353	327	80	75	14	9	9.61	5.9	3.45	24.3	22.6
stored	17	6.22	428	273	95	71	22	10	7.12	3.8	3.74	14.5	34.3
signif[c]		***	***	*	ns	ns	ns	ns	***	***	***	***	***

[a] Titratable acidity (TA) expressed as malic acid. [b] Calculated as TSS (Fuleki et al., 1994)/TA (g/100 mL). [c] *, **, ***, significant at $p \leq 0.05$, 0.01, and 0.001 confidence levels, respectively, by analysis of variance; ns, not significant.

Table 6. Acid Composition of Commercial Pure Apple Juice[a]

brand	container[b] and size, mL	grade[c]	malic, g/L	citric, mg/L	quinic, mg/L	succinic and shikimic, mg/L	fumaric, mg/L	total, g/L	TA,[d] g/L	pH	malic/citric	sugar[e]/acid
domestic												
A	M, 1360	C	5.58	481	446	**12**	16	6.53	4.2	3.63	11.6	29.0
	G, 1000	C	8.32	451	267	4	**nil**	9.04	6.2	3.47	18.5	18.1
	T, 250	C	6.97	409	336	5	24	7.74	5.2	3.53	17.0	21.2
	T, 1000	C	7.74	**611**	288	6	17	8.66	5.9	3.48	12.7	18.6
B	G, 1000	ND	6.51	290	352	7	47	7.21	4.7	3.48	22.5	25.1
	M, 1360*	ND	7.51	336	**nil**	**nil**	23	7.87	8.2	3.48	22.4	14.5
	G, 1360	ND	7.16	298	nil	nil	16	7.47	7.8	3.45	24.0	15.0
C	M, 1360*	C	7.25	295	nil	nil	16	7.56	7.2	3.55	24.6	16.3
D	M, 1360	C	9.56	342	nil	nil	15	9.91	7.5	**3.23**	28.0	15.7
	P, 2000	C	5.87	306	285	5	nil	6.47	5.0	3.52	19.2	22.0
E	M, 1360	C	6.07	578	305	6	nil	6.95	4.6	3.51	10.5	24.8
F	M, 1360	C	7.40	315	353	9	nil	8.08	5.8	3.52	23.5	20.9
G	P, 1360	C	7.95	296	257	3	16	8.53	6.0	3.50	26.9	19.0
H	M, 1360	C	5.01	296	**467**	10	24	5.81	3.4	3.56	16.9	32.4
I	M, 1360	C	5.35	**tr**[f]	393	10	20	5.77	3.5	3.57	**535.0**	30.3
J	M, 1360	C	7.74	442	279	3	19	8.48	6.0	3.47	17.5	17.0
K	M, 1360	C	6.74	421	299	8	45	7.52	5.3	3.39	16.0	20.6
	G, 1360	C	4.71	395	nil	4	31	5.14	3.8	3.40	11.9	30.3
	M, 1360*	C	6.05	292	nil	nil	23	6.36	6.5	3.39	20.7	16.6
L	G, 1360*	C	6.63	267	nil	nil	13	6.91	6.9	3.48	24.8	15.2
M	G, 1360*	ND	5.62	328	nil	nil	nil	5.95	5.0	3.41	17.1	23.6
imported												
N	M, 1360	C	5.04	298	nil	nil	28	5.37	3.6	3.62	16.9	31.4
O	M, 1360	C	4.83	344	nil	tr	23	5.20	3.3	3.49	14.0	33.9
P	G, 1894	ND	6.26	344	368	9	18	7.00	6.2	3.60	18.2	18.2
Q	G, 236*	ND	**0.61**	321	nil	nil	**79**	**1.01**	**1.5**	**3.99**	**1.9**	**75.3**
R	G, 946*	ND**	6.20	321	nil	nil	13	6.53	6.2	3.32	19.3	21.1
S	G, 750*	C	**12.25**	425	nil	nil	nil	**12.68**	**13.0**	3.40	28.8	**10.6**

[a] Minimum and maximum values within the column are in boldface. [b] M, metal can; G, glass bottle; T, Tetra Pak; P, plastic bottle; *, single sample. [c] F, fancy; C, choice; S, standard; ND, not declared; **, organically grown. [d] Titratable acidity (TA) expressed as malic acid. [e] Calculated as TSS (Fuleki et al., 1994)/TA (g/100 mL). [f] tr, trace.

acid was also significantly higher in the apples from Sf in 1990. When the data for juices made from fresh, stored, and both groups of apples combined from all three locations were compared, then only the lactic acid was significantly higher in the 1990 season.

A study carried out in the Washington, DC, area with 216 cultivars, over a period of 6 years, showed that warm and sunny seasons favoring the accumulation of sugars also resulted in the highest concentrations of TA (Caldwell, 1928). Mattick and Moyer (1983) in their large-scale 3-year study found no significant differences from year to year in the TA of apple juice.

Effects of Storage. Since sortouts from storages and overstored apples are utilized for juice production, the composition of juice made from apples stored for 6 months was also studied. The acid compositions of juice made from 11 apple cultivars from three locations where both fresh and stored fruit were available were com-

Table 7. Acid Composition of Commercial Apple Juice from Concentrate[a]

brand	container[b] and size, mL	grade[c]	malic, g/L	citric, mg/L	quinic, mg/L	succinic and shikimic, mg/L	fumaric, mg/L	total, g/L	TA,[d] g/L	pH	malic/citric	sugar[e]/acid
domestic												
A	M, 1360	nd	4.69	283	**nil**	**nil**	61	5.03	2.8	3.72	16.6	43.2
B	M, 1360	nd	**3.70**	254	nil	tr[f]	19	**3.98**	**2.3**	**3.82**	14.6	**46.5**
C	T, 250	C	4.25	**191**	480	10	35	4.97	na[g]	na	22.3	na
D	G, 284*	C	5.28	326	nil	nil	19	5.63	5.4	3.39	16.2	18.1
E	G, 284	C	7.27	**803**	328	11	23	8.43	5.2	3.67	**9.1**	24.4
	G, 1000	C	6.63	289	242	11	18	7.19	4.7	3.67	22.9	27.0
F	T, 250	C	4.43	401	231	nil	63	5.13	3.4	na	11.1	30.6
G	T, 250	C	5.57	378	tr	3	**148**	6.10	5.0	3.22	14.7	23.0
H	T, 250	C	5.31	275	nil	nil	14	5.60	5.1	3.49	19.3	21.0
I	T, 250	C	7.07	256	430	**21**	40	7.81	4.8	na	27.6	23.5
J	T, 250	C	6.10	630	nil	3	43	6.78	5.2	3.28	9.7	20.6
K	T, 1000	C	5.87	294	nil	1	39	6.20	5.3	3.57	20.0	21.9
L	T, 250	C	5.87	487	**537**	10	36	6.94	4.5	3.72	12.1	27.6
M	T, 250	C	8.36	346	nil	tr	**nil**	8.70	5.9	3.35	24.2	21.2
	T, 1000	C	7.97	373	nil	tr	nil	8.34	5.8	3.37	21.4	20.7
	G, 1360	C	**10.08**	347	nil	nil	nil	**10.43**	6.8	3.29	**29.1**	18.7
N	G, 1360	C	8.92	496	233	1	39	9.69	6.6	3.39	18.0	19.1
O	T, 1000	C	6.75	370	tr	3	51	7.17	5.0	3.31	18.2	23.4
P	T, 1000	C	5.85	449	207	4	29	6.54	5.1	**3.18**	13.0	21.6
	T, 250	C	5.02	258	nil	tr	54	5.30	3.9	na	19.5	31.0
Q	G, 284*	C	5.76	326	nil	nil	16	6.10	5.3	3.60	17.7	20.2
imported												
R	G, 1890	nd	8.64	367	314	13	40	9.38	7.0	3.60	23.5	**15.6**
S	T, 254	nd	6.59	371	249	16	20	7.25	5.9	3.70	17.8	19.7
T	G, 1894	nd	5.51	322	310	9	33	6.16	5.1	3.73	17.1	21.2
V	G, 1420	nd	7.42	362	nil	nil	25	7.81	**7.4**	3.70	20.5	15.8

[a] Minimum and maximum values within the column are in boldface. [b] M, metal can; G, glass bottle; T, Tetra Pak; *, single sample. [c] F, fancy; C, choice; S, standard; ND, not declared. [d] Titratable acidity (TA) expressed as malic acid. [e] Calculated as TSS (Fuleki et al., 1994)/TA (g/100 mL). [f] tr, trace. [g] na, not available.

pared by year (Table 5). The combined results for 1989 and 1990 show significant differences in every one of the identified components and their ratios except succinic acid. There was a sizable reduction in malic and total acids contents as well as in TA, M/C, and H^+ concentration in every sample upon storage of the apples. The juices made from stored apples were also significantly lower in quinic acid in 1990 and in quinic and chlorogenic acids when data from the 2 years were combined. In contrast, the citric acid and TSS/TA in both years and the lactic and shikimic acids concentrations in the two combined years and in 1989 were significantly higher in the juices made from stored fruit.

There is general agreement in the literature regarding the decrease in TA (Smock and Neubert, 1950; Wills and McGlasson, 1968; Hansen and Rumpf, 1979; Lidster et al., 1984), malic acid (Hulme and Wooltorton, 1958; Wills and McGlasson, 1968; Hulme and Rhodes, 1971; Gorin et al., 1975; Gorin and Frijters, 1976; Ackermann et al., 1992; Blanco et al., 1992), and quinic acid (Hulme and Wooltorton, 1958; Wills and McGlasson, 1968; Blanco et al., 1992) contents in apples during storage. Beside sugars, malic acid, the dominant acid in apples, is the main substrate for respiration in apples (Hulme and Rhodes, 1971), which explains the decrease in malic acid and TA during storage. In some cultivars (Bramley's Seedling and Golden Delicious) an increase in citric acid content was reported (Hulme and Wooltorton, 1958), while in Glockenapfel a decrease was observed (Ackermann et al., 1992). Similar observations were reported for shikimic acid, which increased in the peel of Bramley's Seedling (Hulme and Wooltorton, 1958) and decreased in Jonathan (Wills and McGlasson, 1968) during storage. A closer examination of the data presented in Table 2 suggests that cultivar influences the metabolism of both citric and shikimic acids during storage. In most cultivars, both acids increased upon storage, but in McIntosh citric acid and in Delicious, Empire, Golden Delicious, Idared, and Moira shikimic acid concentrations frequently remained about the same or slightly decreased during storage.

The total acid determined by HPLC and the TA measurements for all analyzed authentic varietal apple juice samples (Table 2) correlated highly ($r = 0.97798$), but the latter measurement was always lower. Since TA measures only the free carboxylic groups while HPLC will account for the free as well as the bound acids (acid salts, salts), this was to be expected. Other authors (Table 1) also found that the TA was always lower than the total acid. A regression equation was established using all relevant data in Table 2 for the calculation of the total acid content in apple juice from

Table 8. Fumaric Acid Content of Commercial Food Grade Malic Acid

supplier	fumaric acid area, %	fumaric acid wt, %
A	36.7	1.55
B	38.3	1.61
C	47.9	2.36
D	50.6	2.67
E	43.2	1.98
F	52.8	2.87
G	43.9	2.03
H	50.5	2.63
I[a]	2.0	0.09
J[b]	22.0	0.77

[a] Experimental product, produced through fermentation. [b] Reagent grade chemical.

the TA measurement:

$$\text{total acid (g/L)} = \text{TA} \times 1.217 + 2.33$$

Commercial Apple Juice. To get an indication of the changes in acid content as a result of commercial processing and of the authenticity of juices available in Ontario, the acid compositions of commercial apple juices and ciders purchased locally in 1989 and 1990 were examined (Tables 6 and 7). The commercial juices were analyzed before the authentic ones, and some of the analyses were not carried out on these samples. Since the enzymatic analyses were omitted, succinic and shikimic acids are reported together and lactic acid was not determined. Data for chlorogenic acid, which requires a different wavelength of detection (324 nm) and longer chromatographic runs, also were not available. While quinic, succinic, and shikimic acids were always present in authentic apple juice (Table 2), they were frequently absent in the commercial juices (Tables 6 and 7). This suggests that commercial processing may have removed or destroyed these acids.

Most commercial pure apple juice had an acid composition (Table 6) similar to that found in pure authentic varietal juices (Table 2). In some cases, the values were outside the ranges reported here or in the literature for authentic apple juice. Sample Q had exceptionally low malic and total acids content, TA, H^+, and M/C and the highest fumaric acid concentration. These suggest that this juice may have been adulterated. Curiously, the TA in some of the pure juices, including this one, was higher than the total acids value. Most of the commercial "apple juice from concentrate" (Table 7) and sweet cider showed acid composition similar to those of the pure apple juices.

Since fumaric acid is always present in synthetic malic acid and its absorbance at 210 nm is much greater than that of malic acid, fumaric acid content had been proposed to detect the addition of malic acid to apple juice (Junge and Spadinger, 1982). To verify the fumaric acid content of synthetic malic acid, eight food grade samples were acquired from ingredient suppliers and analyzed with HPLC (Table 8). The samples contained 1.5−2.9% fumaric acid, but these translated to 37.4−53.1% of the total peak area at 210 nm due to the much higher absorbency of fumaric acid (Fuleki et al., 1993). Traces of a second impurity with absorption maxima at 210 nm, eluting around 10 min, were also present in in most food grade malic acid samples (Figure 3). Junge and Spadinger (1982) found 0.4−1.0% fumaric acid in synthetic malic acid obtained from five laboratory chemical suppliers. The results presented in Table 8 show lower fumaric acid content in reagent grade than food grade malic acid.

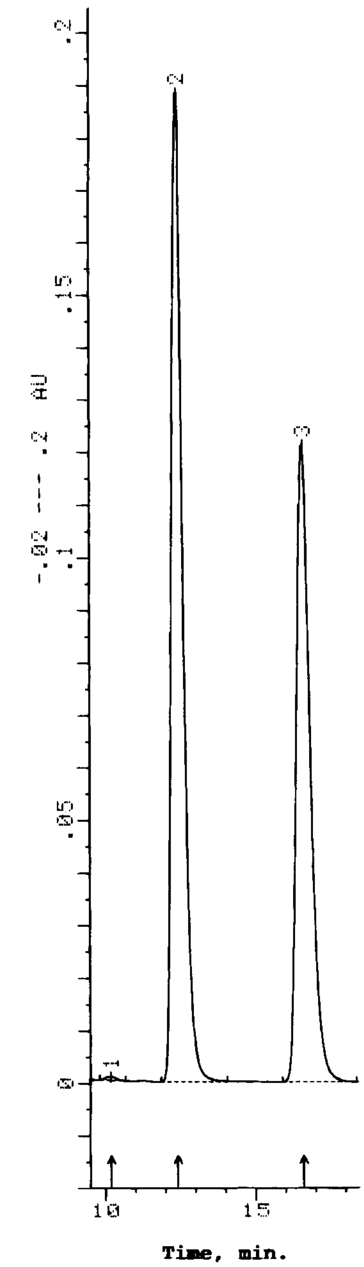

Figure 3. Chromatogram of a food grade synthetic malic acid monitored at 210 nm. Identification of peaks: 1, unidentified; 2, malic acid; 3, fumaric acid.

Traces of fumaric acid may be present in apples (Fernandez-Flores et al., 1970; Ulrich, 1970; Steenkamp et al., 1983), and it is produced in the juice and concentrate as a result of thermal stress during heat processing, concentration, and storage (Evans et al., 1983; Mattick, 1988). Junge and Spadinger (1982) suggested that greater than 3 mg/L fumaric acid in apple juice indicates the addition of synthetic malic acid. Zyren and Elkins (1985) and Mattick (1988) found 3.84 mg/kg and 4.0 mg/L fumaric acid, respectively, in authentic pure apple juice. Mattick (1988) found 16.2 mg/L fumaric acid in a reconstituted authentic apple juice prepared from a concentrate made under high-heat conditions. In light of the above data these authors questioned the validity of the 3 mg/L fumaric acid limit.

In this study fumaric acid was not detected in the authentic juices but it was present in most of the commercial products analyzed. The fumaric acid con-

Table 9. Acid Composition of Commercial Apple Juice Concentrate[a]

brand	malic, g/L	citric, mg/L	tartaric, mg/L	shikimic, mg/L	fumaric, mg/L	total, g/L	malic/citric
A	3.32	tr[b]	nil	nil	31	3.36	332
B	1.68	295	394	1	58	2.43	5.7
C	7.95	351	nil	nil	67	8.37	22.6
D	5.80	358	nil	nil	nil	6.15	16.2

[a] Concentrates were reconstituted to 10.5% TSS prior to analysis. [b] tr, trace (for calculations it was assumed to be 10 mg/L).

tent of pure apple juice, reconstituted apple juice, and sweet cider, respectively, ranged from 0 to 79, from 0 to 148, and from 0 to 83 mg/L. Most commercial products had considerably higher fumaric acid content than reported in the literature. This suggests that acidulation of apple juice/concentrate with malic acid is a widespread practice. This is not surprising, considering that most of the apple tonnage available for juice/concentrate production worldwide is from low-acid table cultivars.

Since most of the reconstituted apple juice is produced from imported concentrate, the acid composition of four such samples was determined (Table 9). One of the concentrates (B) contained a substantial quantity of tartaric acid, which indicates that it contained grape juice/concentrate or that it was acidulated with tartaric acid. All but one concentrate contained substantial quantities of fumaric acid, suggesting that synthetic malic acid was added during the production of these concentrates. Since high-acid concentrates command higher prices on the world market than low acid ones, there is financial incentive for increasing the acidity of the concentrate. The possibility also exists that acidulation covered up the addition of sugar to the concentrate (Mattick, 1988).

LITERATURE CITED

Ackermann, J.; Fischer, M.; Amadò, R. Changes in sugars, acids, and amino acids during ripening and storage of apples (cv. Glockenapfel). *J. Agric. Food Chem.* **1992**, *40*, 1131–1134.

Badoud, R.; Pratz, G. Improved high-performance liquid chromatographic analysis of some carboxylic acids in food and beverages as their *p*-nitrobenzyl esters. *J. Chromatogr.* **1986**, *360*, 119–136.

Blanco Gomis, D.; Moran Gutiérrez, M. J.; Gutiérrez Alvarez, M. D.; Mangas Alonzo, J. J. Application of HPLC to characterization and control of individual acids in apple extracts and ciders. *Chromatographia* **1988**, *25*, 1054–1058.

Blanco, D.; Morán, M. J.; Gutiérrez, M. D.; Moreno, J.; Dapena, E.; Mangas, J. Biochemical study of the ripening of cider apple varieties. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 33–37.

Bloeck, S.; Kreis, A.; Stanek, O. Comparative determination of aldehydes and ketones in apple juice after 2 years storage in inner protected aluminum and tin cans at 4 and 20 °C. *Alimenta* **1986**, *25*, 23–28.

Brause, A. Detection of juice adulteration—1991 perspective. *Fluess. Obst* **1992**, *59*, 178–182; (Fruit Processing) 48–51.

Brause, A. R.; Raterman, J. M. Verification of authenticity of apple juice. *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 846–849.

Brown, A. G.; Harvey, D. M. The nature and inheritance of sweetness and acidity in the cultivated apple. *Euphytica* **1971**, *20*, 68–80.

Burroughs, L. F. Analytical composition of english apple juices. *Proc. Int. Fed. Fruit Juice Prod.* **1984**, *18*, 131–138.

Caldwell, J. S. Chemical composition of apple juices as affected by climatic conditions. *J. Agric. Res.* **1928**, *36*, 289–365.

Chapman, G. W., Jr.; Horvat, R. J. Determination of nonvolatile acids and sugars from fruits and sweet potato extracts by capillary GLC and GLC/MS. *J. Agric. Food Chem.* **1989**, *37*, 947–950.

Cilliers, J. J. L.; Singleton, V. L.; Lamuela-Raventos, R. M. Total polyphenols in apples and ciders; Correlation with chlorogenic acid. *J. Food Sci.* **1990**, *55*, 1458–1459.

Coppola, E. D.; Starr, M. S. Liquid chromatographic determination of major organic acids in apple juice and cranberry juice cocktail: Collaborative study. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 594–597.

Evans, R. H.; Van Soestbergen, A. W.; Ristow, K. A. Evaluation of apple juice authenticity by organic acid analysis. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1517–1520.

Fernandez-Flores, E.; Kline, D. A.; Johnson, A. R. GLC determination of organic acids in fruits as their trimethylsilyl derivatives. *J. Assoc. Off. Anal. Chem.* **1970**, *53*, 17–20.

Fuchs, G.; Kuivinen, J.; Wretling, S. Study on apple juice. *Vaar Foeda* **1987**, *39*, 84–99.

Fuleki, T.; Pelayo, E.; Palabay, R. B. Carboxylic acid composition of authentic varietal and commercial grape juices. *J. AOAC Int.* **1993**, *76*, 591–600.

Fuleki, T.; Pelayo, E.; Palabay, R. B. Sugar composition of varietal juices produced from fresh and stored apples. *J. Agric. Food Chem.* **1994**, *42*, 1266–1275.

Gorin, N.; Frijters, J. E. R. Criterion of keeping quality in Golden Delicious apples. *Lebensm. Wiss. Technol.* **1976**, *9*, 183–184.

Gorin, N.; Rudolphij, J. W.; Heidema, F. T.; van der Vuurst deVries, R. G. Metabolites in Golden Delicious apples as possible parameters of acceptability. *J. Sci. Food Agric.* **1975**, *26*, 599–607.

Hansen, H.; Rumpf, G. Nutritionally important components of stored apples with special consideration to newly cultivated varieties. *Erwerbsobstbau* **1979**, *21* (1), 8–11.

Hartmann, B. G.; Hillig, F. Acid constituents of food products. Special reference to citric, malic and tartaric acids. *Assoc. Off. Agric. Chem. J.* **1934**, *17*, 522–531.

Hulme, A. C.; Rhodes, M. J. C. Pome fruits. In *The Biochemistry of Fruits and their Products*; Hulme, A. C., Ed.; Academic Press: New York, 1971; Vol. 2, pp 333–373.

Hulme, A. C.; Wooltorton, L. S. C. Determination and isolation of the nonvolatile acids of pome fruits and a study of acid changes in apples during storage. *J. Sci. Food Agric.* **1958**, *9*, 150–158.

Johnston, F. B.; Hammill, M. M. The non-volatile organic acids of some fresh fruits and vegetables. *Can. Inst. Food Technol. J.* **1968**, *1*, 3–5.

Junge, C.; Spadinger, C. Proving an addition of [L(−) and DL] malic acid in apple and pear juices by determining the quantity of fumaric acid. *Fluess. Obst* **1982**, *49*, 46–47, 57–62.

Kain, W. Analyses of authentic fruit juices from the 1968 crop year. *Mitt. Klosterneuburg* **1969**, *19*, 372–383.

Kain, W.; Vlcek, G. Analyses of authentic fruit juices from the 1969 crop year. *Mitt. Klosterneuburg* **1971**, *21*, 207–212.

Lee, C. Y.; Mattick, L. R. Composition and nutritive value of apple products. In *Processed Apple Products*; Downing, D. L., Ed., Van Nostrand Reinhold: New York, 1989; pp 303–322.

Lee, H. S.; Wrolstad, R. E. Detection of adulteration in apple juices. In *Adulteration of Fruit Juice Beverages;* Nagy, S., Attaway, J. A., Rhodes, M. E., Eds.; Dekker: New York, 1988a; pp 343–376.

Lee, H. S.; Wrolstad, R. E. Apple juice composition: sugar, nonvolatile acid, and phenolic profiles. *J. Assoc. Off. Anal. Chem.* **1988b**, *71*, 789–794.

Lidster, P. D.; Sanford, K. A.; McRae, K. B.; Stark, R. Apple juice quality and recovery from McIntosh apples stored in controlled atmospheres and air. *Can. Inst. Food Sci. Technol. J.* **1984**, *17*, 86–91.

Macheix, J. J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990.

Mattick, L. R. An evaluation of the methodology for determining the authenticity of apple juice and concentrate. In

Adulteration of Fruit Juice Beverages; Nagy, S., Attaway, J. A., Rhodes, M. E., Eds.; Dekker: New York, 1988; pp 175–193.

Mattick, L. R.; Moyer, J. C. Composition of apple juice. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1251–1255.

Murata, T.; Minamide, T. Studies on organic acid metabolism and ethylene production during controlled atmosphere storage of apples (*Mallus pumila* Miller *cv.* Rolls). *Plant Cell Physiol.* **1970**, *11*, 857–863.

Phillips, J. D.; Pollard, A.; Whiting, G. C. Organic acid metabolism in cider and perry fermentations. I. A preliminary study. *J. Sci. Food Agric.* **1956**, *7*, 31–40.

Prabha, T. N.; Salimath, P. V.; Patwardhan, M. V. Primary metabolites and organic acid metabolism in apple (*Malus sylvestris*) fruit callus culture. *J. Sci. Food Agric.* **1990**, *51*, 381–389.

Robertson, G. L.; Kermode, W. J. Salicylic acid in fresh and canned fruit and vegetables. *J. Sci. Food Agric.* **1981**, *32*, 833–836.

Ryan, J. J. Chemical composition of Canadian apple juice. *J. Assoc. Off. Anal. Chem.* **1972**, *55*, 1104–1108.

Ryan, J. J.; Dupont, J. A. Identification and analysis of the major acids from fruit juices and wines. *J. Agric. Food Chem.* **1973**, *21*, 45–49.

Schols, H. A.; in't Veld, P. H.; van Deelen, W.; Voragen, A. G. J. The effect of the manufacturing method on the characteristics of apple juice. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 142–148.

Smock, R. M.; Neubert, A. M. *Apples and Apple Products*; Interscience Publishers: New York, 1950; pp 51–61.

Steenkamp, J.; Ferblanche, J. H.; de Villiers, O. T. The role of organic acids and nutrient elements in relation to bitter pit in Golden Delicious apples. *Acta Hortic.* **1983**, *138*, 35–42.

Tavernier, J.; Jacquin, P. Organic acid composition of apple juice. *C. R. Acad. Sci.* **1947**, *225*, 1373–1374.

Tavernier, J.; Jacquin, P. Studies on American dessert apple varieties from a Breton orchard. *Ann. Amelior. Plant.* **1952**, *2*, 231–251.

Ulrich, R. Organic acids. In *The Biochemistry of Fruits and their Products*; Hulme, A. C., Ed.; Academic Press: New York, 1970; Vol. 1, pp 89–118.

Whiting, G. C.; Coggins, R. A. Organic acid metabolism in cider and perry fermentations. II. Non-volatile organic acids of cider-apple juices and sulphited ciders. *J. Sci. Food Agric.* **1960**, *11*, 337–344.

Wills, R. B. H.; McGlasson, W. B. Changes in the organic acids of Jonathan apples during cool storage in relation to the development of breakdown. *Phytochemistry* **1968**, *7*, 733–739.

Wucherpfennig, K.; Bretthauer, G.; Huseh-err, C. Evaluation of chemical studies of apple juices of high commercial quality. *Fluess. Obst* **1977**, *44*, 229–232, 236–240.

Zubeckis, E. "Chemical composition of fruit grown at Vineland, Ontario"; Horticultural Experiment Station and Products Laboratory Report for 1962; Ontario Department of Agriculture, Toronto, ON, Canada, 1962; pp 97–116.

Zyren, J.; Elkins, E. R. Interlaboratory variability of methods used for detection of economic adulteration in apple juice. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 672–676.

Received for review August 12, 1994. Accepted December 28, 1994.[⊗] We gratefully acknowledge the financial support of the Agriculture and Food Research Fund of the Ontario Ministry of Agriculture, Food and Rural Affairs.

JF940466Z

[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1995.