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Processing Effects on the Composition of Sea Buckthorn Juice from *Hippophae rhamnoides* L. Cv. Indian Summer

T. BEVERIDGE,* J. E. HARRISON, AND J. DROVER

Food Research Program, Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia V0H 1Z0, Canada

Sea buckthorn juice is one product that can be derived from the sea buckthorn berry, a new alternative crop for the Canadian western provinces. Fresh pressed juice separates into three phases when allowed to stand overnight in the refrigerator: an upper cream phase, juice in the middle portion, and a sediment at the bottom. Enzymatic hydrolysis with commercial, broad spectrum carbohydrate hydrolyzing enzyme preparations reduced the juice viscosity, assisted juice separation, and provided an opalescent juice. Soluble solids averaged 10.2 °Brix, pH averaged 3.13, ascorbic acid averaged 174.2 mg/100 mL, and titratable acidity averaged 1.97% as malic acid all determined on centrifuged (10 000 rpm, 15 min) juice. Soluble sugars included glucose, fructose, and an unidentified component that was not sucrose or other common soluble monomeric or dimeric sugar. Quinic acid was quantitatively most important, while malic was next, and oxalic, citric, and tartaric acids were minor components. Washing berries by dipping reduced soluble solids (°Brix) in juice suggesting uptake of wash water.

KEYWORDS: Sea buckthorn; soluble sugars; acids; vitamin C; juice

INTRODUCTION

Sea buckthorn is a unique plant currently being domesticated in several places around the world, including Canada. The female plant sets fruit annually on second year wood. The fruit consists of an oval shaped berry with a fairly tough skin surrounding a juice enveloping cellular structure which in turn surrounds a single, sheathed seed. The juice derived from the fruit is high in vitamin C and β-carotene, which provides for the bright orange color (*1*). The pH of the juice is near 2.5, a pH lower than most common temperate fruits (*2*). Soluble solids average 11.4 °Brix, and glucose and fructose are the major monomeric sugars present in approximately equal amounts (*1*, *3*). The major organic acid reported is malic acid with minor quantities of citric and tartaric (*3*). Published procedures for processing suggest that treatment with enzyme systems designed to act on cell walls and middle lamella provide increased juice extraction, while berry washing prior to juice extraction has been recommended (*4*). The effects of these treatments have not been reported, and the organic acids contributing to the low pH need to be confirmed and identified. Quantitative data on the levels of glucose and fructose are lacking in the literature and requires further investigation. Present reports indicate that glucose represents 49.5−62.1% of total sugars, and fructose represents 37.3−50.4%. This publication addresses the measurement of organic acids and soluble sugars by HPLC and provides new data on the levels and identity of the constituents of sea buckthorn juice from the cultivar Indian Summer.

MATERIALS AND METHODS

Sea buckthorn berries were obtained from hedgerows near Estevan (bin A) and Summerberry (bin B), Saskatchewan, and juice was obtained by conventional rack and cloth pressing at pressures of 2500−3500 psi. The berries were picked in early September and were ripe as judged by hand manipulation and juiciness. Chlorine (155 ppm) and sodium dodecyl sulfate (SDS, 200 ppm)/chlorine washes, each as a separate treatment, were performed by dipping the berries for 30 s followed by at least 5 min of drainage. Enzyme treatments used commercially available Pectinex Ultra SP and Citrozym preparations from Novo Ferment Ltd. and were applied to the berry mash prepress for 4 h at 22 °C. Berries were broken by mashing throughout this 4 h period. Citrozym was considered to be especially useful for the preparation of cloudy juices and so was included in the tests. Individual treatments were performed in duplicate. Juices were centrifuged at 10 000 rpm (Sorval RC-5 Superspeed refrigerated centrifuge) for 15 min at 3 °C. Sea buckthorn juice separates into a pellet on the bottom, a floating solidified cream layer on top, and an opalescent aqueous layer in the middle. This middle layer was utilized for juice analysis by carefully inserting a pipet below the solid fat layer. The pretreatment cleanup procedure for HPLC was adapted from Beveridge et al. (*5*). The aqueous layer sample was passed through Bio-Rad AG1-X8 (100−200 mesh) ion-exchange resin in the acetate form to isolate the organic acids from the sugars. Neutral sugars passed through the resin and were washed off with double distilled water into a 10-mL volumetric flask. Organic acids were removed from the column with 15 mL of 1.5 N sulfuric acid into a second volumetric flask (25 mL). Both extracts were frozen for later analysis.

* To whom correspondence should be addressed (phone 250-494-6363; fax 250-494-0755), Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC. Contribution no. 2102.

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Table 1. Vitamin C, Titratable Acidity (TA, % Malic Acid) pH, and °Brix of Centrifuged Sea Buckthorn Juice (Cv. Indian Summer)[a]

treatment	bin	vitamin C mg/100 mL	TA % malic acid	pH	°Brix
control	A	174.7 ± 30.7	1.86 ± 0.23	3.2 ± 0.02	10.8 ± 0.46
	B	157.3 ± 22.6	1.97 ± 0.08	3.1 ± 0.00	9.8 ± 0.0
0.1% (w/w) Ultra SP	A	158.5 ± 23.4	1.91 ± 0.05	3.3 ± 0.07	10.3 ± 0.17
	B	181.1 ± 10.5	2.09 ± 0.01	3.0 ± 0.07	10.1 ± 0.26
0.02% (w/w) Ultra SP	A	158.4 ± 12.0	1.89 ± 0.28	3.2 ± 0.02	10.6 ± 0.14
	B	186.3 ± 3.8	2.02 ± 0.64	3.1 ± 0.00	9.7 ± 0.07
Citrozyme cloudy 100 L	A	185.0 ± 30.0	1.99 ± 0.78	3.2 ± 0.01	10.7 ± 0.15
(0.05%, w/w)	B	192.7 ± 39.2	2.04 ± 0.35	3.0 ± 0.07	9.9 ± 0.23
chlorine wash	A	136.9 ± 4.6	1.52 ± 0.16	3.2 ± 0.02	8.4 ± 1.3
	B	176.2 ± 8.3	1.80 ± 0.35	3.0 ± 0.00	8.7 ± 0.71
SDS/chlorine wash	A	153.9 ± 20.9	1.67 ± 0.49	3.1 ± 0.02	8.4 ± 1.1
	B	166.9 ± 14.5	1.78 ± 0.42	3.0 ± 0.00	8.4 ± 0.20

[a] ± values are standard deviations.

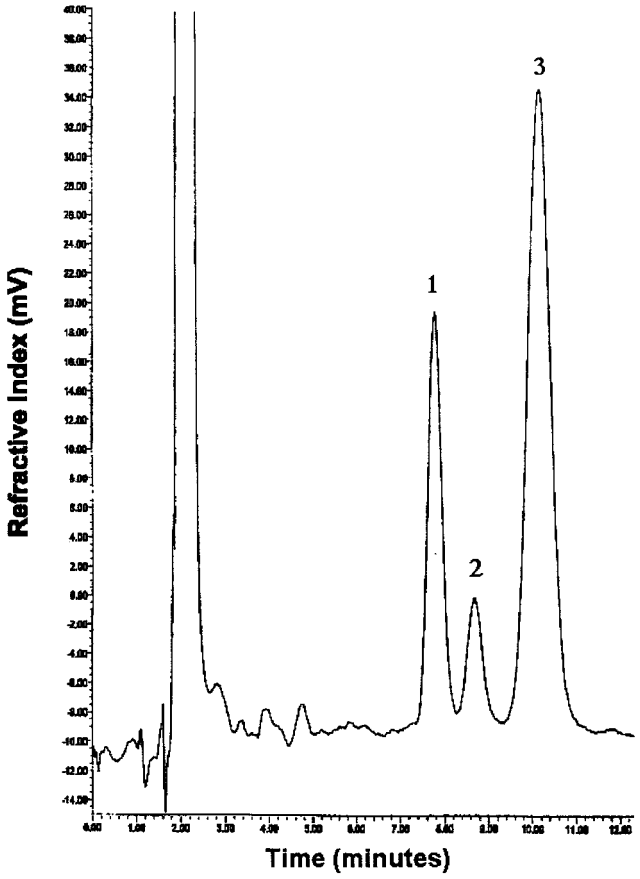

Figure 1. Chromatogram of the soluble sugar profile from centrifuged sea buckthorn juice. Peak identities: (1) fructose; (2) unknown; (3) glucose.

Organic acids were separated isocratically on a Bio-Rad HPX-87H, 300 × 7.8 mm column guarded by a Brownlee Polypore H 10 μ guard column 30 × 4.6 mm. Separations were done at 60 °C with 0.02 N sulfuric acid flowing at 0.4 mL/min. Injection volumes were 50 μL, and detection was with a Water's 410 refractive index detector (*5*). Acetic acid (from the Bio-Rad Column) retention time was 22 min. Chromatographic control and data collection and quantification was achieved with a Waters Millinium System.

Neutral, monomeric sugars were separated on a Water's Carbohydrate analysis column 300 × 3.9 mm guarded by a Water's μBondapak NH_2 20 × 3.9 mm guard column. Eluant was 85% acetonitrile/water flowing at 2.0 mL/min, injections were 25 μL and detection was with a Water's 410 refractive index detector.

Ascorbic acid was measured by an adaption of the HPLC method of Acar and Gokmen (*6*). The pressed juice samples were diluted with 0.05% KH_2PO_4 containing 0.1% dithiothreitol (DTT), to contain 40−110 mg/L ascorbic acid. The diluted sample was held at least 2 h in the dark to ensure reduction of dehydroacsorbic acid for detection purposes. The diluted samples were filtered through 0.2 μ nylon filter and 10 μL was injected. Separation was achieved on a 30 × 0.39 cm μBondapak C_{18} column (Waters PN 27324) preceded by a 3.0 × 0.46 cm Phenomenex Spherisorb 10μ, C_{18} guard column. Solvent was 0.5% KH_2PO_4 buffer (pH 4.4−4.7), flow was isocratic at 0.5 mL/min, and temperature was ambient (20−21 °C). Detection was by UV absorption at 254 nm utilizing a Waters 490 UV detector. Retention time for ascorbic acid was 7.5 min, and the DTT peak from reagent added to sample elutes at 17 min, requiring a run time of 20 min. Standard curves for ascorbic acid were linear to 110 mg/L.

Soluble solids were measured refractometrically as °Brix at 20 °C and titratable acidity by titration of 2 mL juice diluted to 10 mL, with 0.1 N sodium hydroxide to pH 8.2 (Metrahm 686 Titroprocessor with a 665 Dosimat).

RESULTS AND DISCUSSION

Juice obtained by conventional rack and cloth pressing was opalescent/opaque, bright orange in color, and generally visually attractive. The level of suspended solids was high enough that the juice was opaque rather than opalescent. Nonenzymed juice was obtained in yields ranging from 56 to 68% (wt/wt), and treatment of mashed berries with commercial enzyme preparations normally intended for apple juice extraction provided increases in yields to the 70−80% range.

Soluble solids of centrifuged juice averaged 9.7 °Brix (Table 1) and ranged between 8.4 and 10.7 °Brix, values in the lower ranges reported in the literature (*1*). Uncentrifuged juice gave higher values near 11.5 °Brix. This latter value is closer to the average of 11.4 °Brix reported for the Indian summer cultivar (*1*). Centrifugation reduces juice suspended solids and made the juice more easily handled by conventional measurement techniques. As can be seen from Table 1, the °Brix values for the washed berries appear lower than the values for the other juices prepared. In fact, there is a decrease in the average values of 10.2 ± 0.379 °Brix ($n = 23$) for juice from unwashed berries to 8.53 ± 0.593 °Brix ($n = 10$) for juice from dipped berries ($P \geq 0.05$; t test), suggesting the uptake of water during the washing process. It is likely this water uptake occurs because the stem end of the berries is susceptible to damage or skin rupture during picking.

Indian Summer juice gave an average pH of 3.13 and an average titratable acidity of 1.97% calculated as malic acid (Table 1). The titratable acidity values are in the lower ranges reported in the literature (*1*), while the pH is higher than the value of 2.7 reported by Bock et al. (*2*). Possible dilution by

Table 2. Soluble Sugar and Organic Acids in Sea Buckthorn Juice (Cv. Indian Summer) mg/mL of Centrifuged Juice[a]

		sugars			organic acids				
treatment	bin	fructose	unknown[b]	glucose	quinic	malic	citric	oxalic	tartaric
control	A	10.8 ± 1.17	4.52 ± 1.10	32.6 ± 2.56	26.5 ± 0.09	13.8 ± 0.41	2.21 ± 0.16	0.26 ± 0.09	0.81 ± 0.04
	B	11.1 ± 1.08	3.70 ± 0.60	30.3 ± 2.15	22.2 ± 0.79	15.1 ± 0.37	2.12 ± 0.16	0.19 ± 0.06	0.87 ± 0.11
0.1% Ultra SP	A	10.4 ± 0.88	4.94 ± 1.10	32.4 ± 1.77	25.5 ± 1.08	13.6 ± 0.70	1.99 ± 0.06	0.37 ± 0.10	3.12 ± 0.23
	B	11.2 ± 0.39	3.50 ± 0.42	30.1 ± 0.68	23.0 ± 0.39	15.5 ± 0.58	2.09 ± 0.09	0.41 ± 0.08	3.29 ± 0.16
0.02% Ultra SP	A	11.7 ± 0.74	5.26 ± 0.34	32.8 ± 1.03	26.1 ± 0.27	14.2 ± 0.72	2.02 ± 0.01	0.33 ± 0.11	1.40 ± 0.80
	B	11.8 ± 1.14	3.83 ± 1.24	30.9 ± 1.98	22.2 ± 0.74	15.4 ± 0.08	1.84 ± 0.40	0.26 ± 0.06	0.68 ± 0.38
Citrozym cloudy 100 L	A	11.0 ± 0.85	5.38 ± 0.93	35.4 ± 0.93	25.6 ± 1.38	13.6 ± 0.56	1.58 ± 0.34	0.50 ± 0.06	0.72 ± 0.93
	B	11.2 ± 1.02	3.12 ± 0.87	30.0 ± 0.87	22.2 ± 1.16	15.1 ± 0.47	2.05 ± 0.25	0.49 ± 0.05	0.74 ± 0.22
chlorine wash	A	9.33 ± 1.07	3.62 ± 0.56	26.9 ± 1.40	21.2 ± 1.67	11.4 ± 1.06	1.92 ± 0.09	0.14 ± 0.01	0.67 ± 0.07
	B	10.5 ± 0.56	2.69 ± 0.52	27.3 ± 1.28	20.3 ± 0.87	14.2 ± 0.29	2.10 ± 0.15	0.13 ± 0.01	0.84 ± 0.08
SDS/chlorine wash	A	7.91 ± 1.15	3.47 ± 1.12	24.5 ± 2.83	21.4 ± 1.83	11.8 ± 0.92	1.97 ± 0.12	0.20 ± 0.05	0.76 ± 0.15
	B	8.84 ± 0.51	2.79 ± 0.70	24.0 ± 2.09	19.6 ± 0.69	13.6 ± 0.53	1.97 ± 0.08	0.17 ± 0.07	0.69 ± 0.12
juice overall average		10.5 ± 1.19	3.90 ± 0.92	29.8 ± 3.48	23.0 ± 2.36	13.9 ± 1.31	1.99 ± 0.16	0.29 ± 0.13	1.22 ± 0.95

[a] ± values are standard deviations. [b] Unknown quantitated as fructose.

washwater was not readily detectable with these tests, although the two lowest values for titratable acidity were obtained on washed berries. Ascorbic acid levels (Table 1) averaged 173.3 mg/100 mL, a number calculated by omitting the values of the washed berries to avoid any possible dilution effects. These values are considerably lower than the values reported for Chinese sea buckthorn (*1*) but are higher than the 70 to 105 mg/100 mL reported by Bock et al. (*2*) in ultrafiltered juice. These values are still very high for fruit juices when compared to orange juice, which ranges from 35 to 56 mg/100 mL (*7*), and higher than virtually all other fruits and vegetables (*8*).

Sea buckthorn juice contains glucose and fructose as the major soluble sugars detected by HPLC (Figure 1). The unknown compound eluted between β-D-fructose and D-mannose in the region of 8.5−9.0 min in the present system and did not correspond to any of the known standard sugars (rhamnose, ribose, xylose, xylitol, sorbose, sorbitol, mannitol, sucrose, raffinose) examined. Since the preparation of samples for sugar analysis requires passage through an anion-exchange resin, the unknown component is either neutral or perhaps positively charged. From the retention time determinations, the unknown peak did not correspond to sorbitol, mannitol, xylose, or xylitol as might have been expected from the literature (*1*), nor did it correspond to any of the common hexose or pentose sugars commonly documented in lists compiled of HPLC retention times. This unknown awaits isolation and identification. Quantification of the sugar levels indicated that the juice sugars averaged 29.8 mg/mL (2.98%) for glucose, 10.5 mg/mL (1.05%) for fructose, and 4.03 mg/mL (0.403%) for the unknown compound quantitated as fructose (Table 2). Examination of this sugar data suggests that the levels detected in juice from washed berries was lower than in the other juices except for one analysis of the "B" bin. This pattern would be in agreement with the °Brix data provided earlier.

Soluble sugars as detected by HPLC averaged 4.43% (Table 2), and this level is incongruent with 8.4−10.8 °Brix observed (Table 1). However, the sum of glucose (2.98%), fructose (1.05%), unknown (0.39%), malic acid (1.39%), quinic acid (2.30%), citric acid (0.199%), and tartaric acid (0.122%) is 8.42%—a more congruent number. Also, if the titratable acidity reported at 1.97% malic acid (Table 1) is converted to quinic acid (see below) through multiplication by 2.86, the titratable acidity is 5.18%, giving a soluble solids estimate of 9.6 °Brix, also a more congruent number.

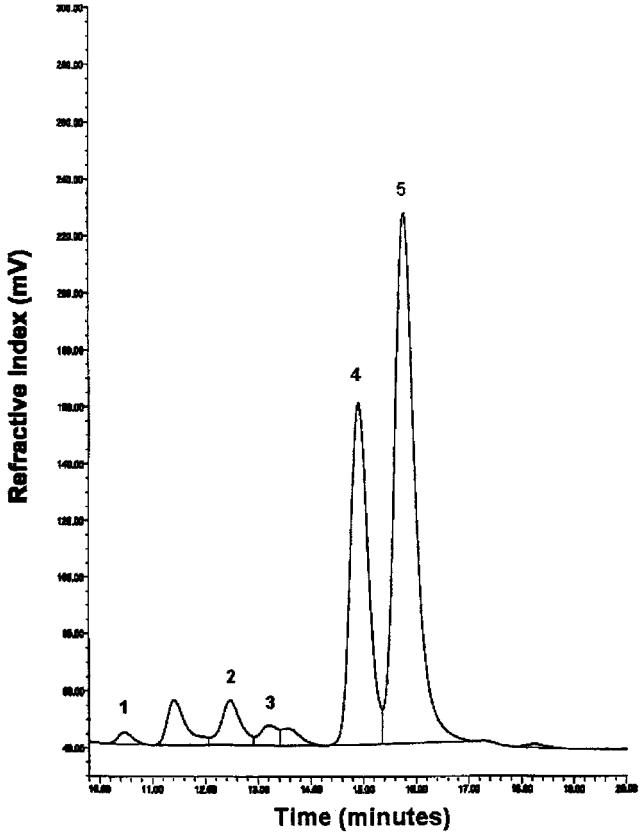

Figure 2. Chromatogram of organic acids in centrifuged sea buckthorn juice. Peak identities: (1) oxalic; (2) citric; (3) tartaric; (4) malic; (5) quinic.

Five components were detected in the organic acid extract obtained from the anion-exchange resin (Figure 2). These were identified as oxalic, citric, tartaric, malic, and quinic acids by comparison with the elution of authentic standards. Previous work by other authors (*1*) have identified the presence of malic acid as the major acid and citric and tartaric acids as minor components. The identification of quinic acid was, therefore, unexpected. Thin-layer chromatography on Whatman silica gel 250μ flourescent plates developed in 95% ethanol/water/concentrated ammonium hydroxide (78:9.5:12.5 by volume) gave a yellow spot with an R_f value identical to quinic acid detectable with bromcresol green after ammonium evaporation.

In the present HPLC ion exchange method, authentic quinic acid coeluted with the identified peak. Chromatography (HPLC) on a C_{18} reversed phase column and detection with a mass selective detector provided a peak with an R_f value identical to quinic acid containing a major component with an *m*/*e* of 191.0 expected from quinic acid. Oxalic acid was present in minor quantities. Overall, quinic acid averaged 23.0 mg/mL, malic 13.9 mg/mL, citric 1.99 mg/mL, tartaric 1.22 mg/mL, and oxalic 0.29 mg/mL (Table 2). The malic and citric acid levels were in good agreement with literature values, while tartaric acid levels were somewhat higher than previously reported (*1*). Quinic and oxalic acid levels have not been previously reported. On the basis of the results obtained here, titratable acidity in sea buckthorn juice should be reported in terms of quinic acid.

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