

International Multidimensional Authenticity Specification (IMAS) Algorithm for Detection of Commercial Pomegranate Juice Adulteration

YANJUN ZHANG,[†] DANA KRUEGER,[§] ROBERT DURST,[#] RUPO LEE,[†]
DAVID WANG,[†] NAVINDRA SEERAM,[†] AND DAVID HEBER^{*†}

Center for Human Nutrition, David Geffen School of Medicine, University of California, Los Angeles, California 90095; Krueger Food Laboratories, Inc., Billerica, Massachusetts 01821; and Food Science and Technology, Oregon State University, Corvallis, Oregon 97331

The pomegranate fruit (*Punica granatum*) has become an international high-value crop for the production of commercial pomegranate juice (PJ). The perceived consumer value of PJ is due in large part to its potential health benefits based on a significant body of medical research conducted with authentic PJ. To establish criteria for authenticating PJ, a new International Multidimensional Authenticity Specifications (IMAS) algorithm was developed through consideration of existing databases and comprehensive chemical characterization of 45 commercial juice samples from 23 different manufacturers in the United States. In addition to analysis of commercial juice samples obtained in the United States, data from other analyses of pomegranate juice and fruits including samples from Iran, Turkey, Azerbaijan, Syria, India, and China were considered in developing this protocol. There is universal agreement that the presence of a highly constant group of six anthocyanins together with punicalagins characterizes polyphenols in PJ. At a total sugar concentration of 16 °Brix, PJ contains characteristic sugars including mannitol at >0.3 g/100 mL. Ratios of glucose to mannitol of 4–15 and of glucose to fructose of 0.8–1.0 are also characteristic of PJ. In addition, no sucrose should be present because of isomerase activity during commercial processing. Stable isotope ratio mass spectrometry as > -25‰ assures that there is no added corn or cane sugar added to PJ. Sorbitol was present at <0.025 g/100 mL; maltose and tartaric acid were not detected. The presence of the amino acid proline at >25 mg/L is indicative of added grape products. Malic acid at >0.1 g/100 mL indicates adulteration with apple, pear, grape, cherry, plum, or aronia juice. Other adulteration methods include the addition of highly concentrated aronia, blueberry, or blackberry juices or natural grape pigments to poor-quality juices to imitate the color of pomegranate juice, which results in abnormal anthocyanin profiles. To adjust the astringent taste of poor-quality juice or peel extract, addition of nonpomegranate sugars is a commonly detected adulteration method. The profile generated from these analyses combined with information from existing databases and published literature has been integrated into a validated IMAS for PJ, which can be utilized to detect PJ adulteration. In this survey of commercial pomegranate juices, only 6 of 23 strictly met all of the IMAS criteria.

KEYWORDS: Pomegranate juice; *Punica granatum*; authenticity specification; adulteration

INTRODUCTION

Six anthocyanin pigments are responsible for the red-purple color of pomegranate juice (PJ). The anthocyanin profile determined by these six anthocyanins is the same in California, Spanish, Italian, Iranian, and Tunisian PJ (1–4). In addition,

pomegranate ellagitannins account for >90% of the antioxidant activity in PJ (5, 6). Among these ellagitannins, punicalagin is the one that is most characteristic and found almost exclusively in PJ (6). However, the presence of these ellagitannins alone is insufficient to establish the authenticity of PJ, because these substances can be obtained from peel extracts. Therefore, other chemical characteristics of PJ obtained from whole fruit were investigated to establish PJ authenticity.

Other studies have established that PJ has superior antioxidant activity compared to other popular refrigerated juices (7–9). Medical and nutritional research has catapulted pomegranate and PJ into a prominent position in international and domestic

* Address correspondence to this author at the UCLA Center for Human Nutrition, 900 Veteran Ave., Los Angeles, CA 90095 [telephone (310) 206-1987; fax (310) 206-5264; e-mail dheber@mednet.ucla.edu].

[†] University of California.

[§] Krueger Food Laboratories, Inc.

[#] Oregon State University.

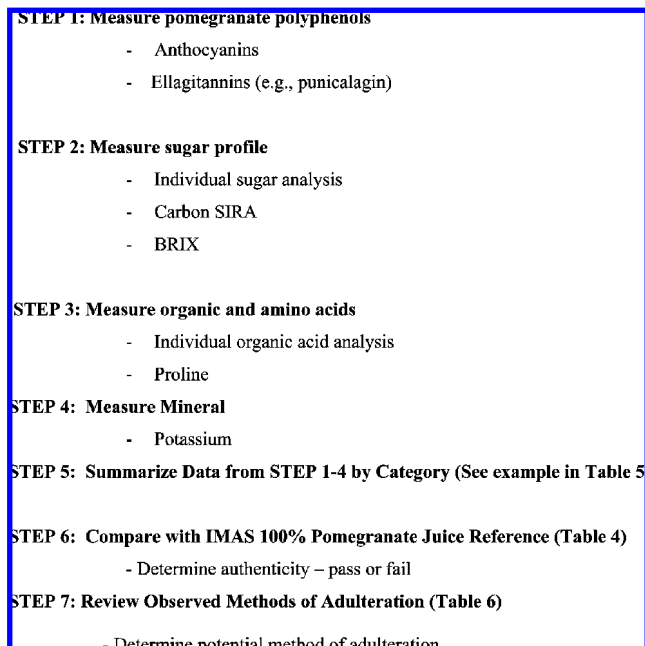


Figure 1. Algorithm for commercial pomegranate juice authentication.

Table 1. Anthocyanin Contents (Milligrams per Liter)^a

sample	Del-3.5	Cyn-3.5	Del-3	Pelar-3.5	Cyn-3	Pelar-3
A (n = 2)	3.7	5.5	2.3	1.5	1.7	0.5
B (n = 3)	2.4	3.5	2.1	0.4	2.9	0.2
C (n = 1)	81.3	95.5	42.8	3.7	74.7	4.8
D (n = 1)	4.1	7.1	0.7	0.4	2.2	0.1
E (n = 1)	2.8	3.6	1.4	1.3	1.0	0.3
F (n = 1)	3.0	6.5	0.7	0.4	1.4	0.2
G (n = 1)	0.1	0.3	nd	nd	nd	nd
H (n = 1)	1.8	2.8	0.5	0.7	0.7	0.5
I (n = 3)	0.7	1.9	0.1	0.2	0.7	nd
J (n = 3)	13.5	23.2	3.4	1.0	8.5	0.6
K (n = 1)	2.1	5.5	nd	0.4	1.6	0.4
L (n = 3)	0.5	1.3	0.4	0.1	0.5	0.1
M (n = 3)	2.1	1.6	0.7	1.0	0.7	nd
N (n = 2)	3.9	11.0	1.8	0.4	13.7	0.3
O (n = 6)	51.7	90.7	36.7	3.4	108.2	6.0
P (n = 4)	18.0	24.1	4.4	1.0	12.2	0.8
Q (n = 3)	0.4	0.9	0.2	nd	0.5	nd
R (n = 1)	7.3	10.5	5.0	0.4	9.3	0.4
S (n = 1)	34.1	58.2	10.7	2.8	36.3	4.2
T (n = 1)	0.5	1.1	0.1	1.0	0.7	0.1
U (n = 1)	0.2	7.7	nd	8.5	14.1	0.1
V (n = 1)	1.9	2.5	0.8	0.3	1.5	0.2
W (n = 1)	nd	nd	nd	nd	nd	nd

^a Anthocyanin abbreviations for delphinidin-3,5-diglucoside, delphinidin-3-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-glucoside, pelargonidin-3,5-diglucoside, and pelargonidin-3-glucoside, respectively. nd, not detected.

commerce, but the increased value of PJ compared to other juices has provided an economic incentive for the adulteration of pomegranate juice (10).

The observation by the U.S. Food and Drug Administration (11) that adulteration of juices is occurring commonly in the marketplace led to the present investigations designed to develop reliable analytical methods for detecting adulteration of pomegranate juice. Our previous research has demonstrated that the combination of polyphenols found in PJ after squeezing whole pomegranate fruit has greater biological activity than isolated polyphenols (12), and the scientific research on PJ demonstrating health benefits has largely been carried out with juice squeezed from whole fruit (13–15).

Adulteration of PJ is typically carried out for two reasons. Currently, PJ is in high demand and short supply, leading to

Table 2. Sugar Profile^a

sample	°Brix	mannitol	sorbitol	sucrose	carbon SIRA (–%)	glucose/fructose ratio
A (n = 2)	16.8	0.50	0.2	0.78	–26.6	0.95
B (n = 3)	13.5	0.37	0.08	0.08	–23.0	0.96
C (n = 1)	15.1	0.40	nd	nd	–26.6	0.94
D (n = 1)	15.7	1.09	0.38	0.84	–23.0	0.90
E (n = 1)	16.1	0.56	nd	nd	–26.9	0.97
F (n = 1)	13.1	0.57	0.13	nd	–21.7	0.87
G (n = 1)	15.4	0.30	nd	0.94	–20.2	1.00
H (n = 1)	17.0	1.12	0.92	0.20	–23.5	0.92
I (n = 3)	15.5	0.58	0.21	0.07	–26.8	0.87
J (n = 3)	16.4	0.76	nd	0.04	–27.5	0.89
K (n = 1)	16.2	0.44	nd	0.07	–25.2	0.89
L (n = 3)	13.6	0.42	nd	nd	–23.0	0.94
M (n = 3)	15.7	0.62	0.07	0.06	–27.0	0.87
N (n = 2)	16.9	0.62	nd	nd	–26.8	0.91
O (n = 6)	16.3	0.68	nd	nd	–27.5	0.95
P (n = 4)	16.3	0.50	nd	0.15	–25.6	1.03
Q (n = 3)	14.1	0.24	nd	0.12	–26.1	0.94
R (n = 1)	16.2	0.44	nd	nd	–27.1	0.99
S (n = 1)	15.5	0.37	nd	nd	–26.9	0.94
T (n = 1)	15.1	0.26	0.06	0.15	na	1.03
U (n = 1)	16.4	0.33	0.03	nd	–25.2	0.95
V (n = 1)	15.9	0.51	nd	nd	–27.8	0.94
W (n = 1)	12.2	0.37	1.06	nd	–18.0	1.03

^a For a single lot of juice sample (n = 1), the data are expressed as the average of two measurements; for multiple lots of juice samples (n = 2–6), the data are expressed as the average of multiple measurements. nd, not detected; na, analysis not performed.

Table 3. Organic and Amino Acids and Potassium^a

sample	malic acid (g/100 mL)	citric/isocitric ratio	proline (mg/L)	potassium (mg/L)
A (n = 2)	0.06	181.3	11.3	1720
B (n = 3)	0.09	292.7	30.0	963
C (n = 1)	0.05	181.1	2.0	1710
D (n = 1)	0.02	136.4	10.0	1240
E (n = 1)	0.08	176.0	6.0	2160
F (n = 1)	0.05	310.0	7.0	1060
G (n = 1)	0.02	248.0	17.0	1360
H (n = 1)	0.11	196.3	12.0	1360
I (n = 3)	0.04	232.7	27.7	1603
J (n = 3)	0.05	173.7	14.7	2086
K (n = 1)	0.01	241.0	9.0	1260
L (n = 3)	0.01	559.0	5.7	450
M (n = 3)	0.09	145.3	9.3	1463
N (n = 2)	0.05	338.0	8.0	1065
O (n = 6)	0.04	138.0	5.3	2100
P (n = 4)	0.05	196.3	59.5	2080
Q (n = 3)	0.01	344.7	6.0	460
R (n = 1)	0.05	261.9	6.0	1410
S (n = 1)	0.07	167.8	3.0	1550
T (n = 1)	0.05	na	na	630
U (n = 1)	0.03	248.0	10.0	1300
V (n = 1)	0.06	329.0	8.0	2570
W (n = 1)	0.03	523.1	8.0	860

^a For a single lot of juice sample (n = 1), the data are expressed as the average of two measurements; for multiple lots of juice samples (n = 2–6), the data are expressed as the average of multiple measurements. na, analysis not performed.

incentives to extend limited supplies by addition of other fruits or juices. Second, adulteration is used to mask the astringent taste and uncharacteristically pale color of low-quality juice. Poor color is due to the absence or reduced amounts of anthocyanins in PJ. Astringent taste is due to the presence of pomegranate tannins obtained from peels without the sweetness of the sugars found in whole pomegranate. Adulteration methods include adding sugars to mask the astringency of the tannins and adding small amounts of deeply colored fruit juices such as black currant or aronia juice to imitate the color of PJ.

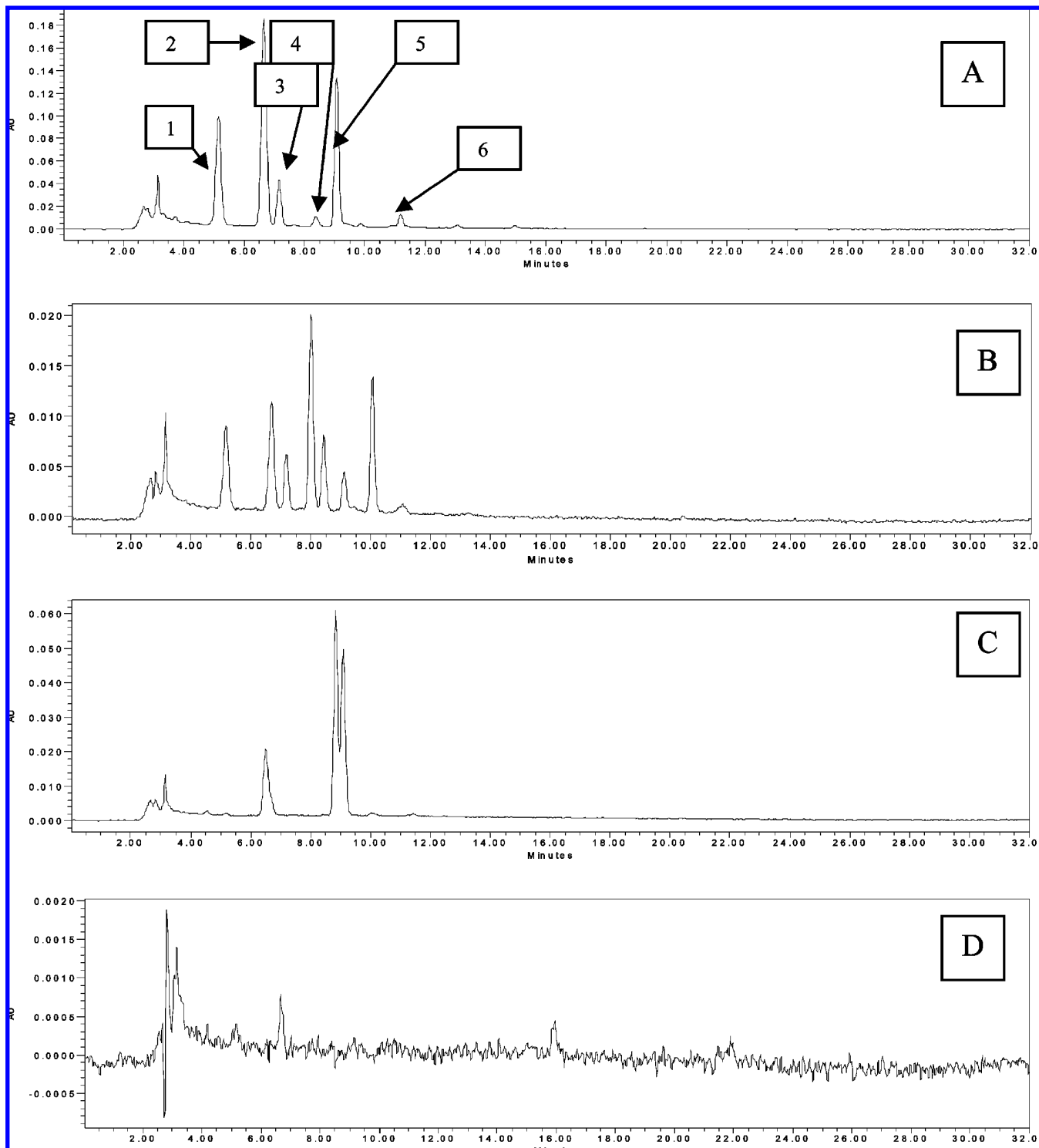


Figure 2. (A) Typical anthocyanin chromatogram illustrating the six anthocyanins found in authentic PJ (sample S, peak 1, delphinidin-3,5 diglucoside; peak 2, cyanidin-3,5-diglucoside; peak 3, delphinidin-3-glucoside; peak 4, pelargonidin-3,5-diglucoside; peak 5, cyanidin-3-glucoside; peak 6, pelargonidin-3-glucoside). (B) Atypical HPLC chromatogram from a juice illustrating the presence of PJ anthocyanins as well as anthocyanins not found in PJ (sample M). (C) Atypical HPLC chromatogram without any PJ anthocyanins but with other anthocyanins not found in PJ (sample G). (D) Atypical HPLC chromatogram from a juice with no detectable anthocyanins (sample W).

To establish a reference point for these studies, other databases from The European Fruit Juice Association (AIJN) (16) and a database from Krueger Food Laboratories (17) were used to establish initial parameters of authenticity. Then our analyses, reported in the present paper, developed new parameters for mannitol and tartaric content of PJ and stable isotope ratio analysis (SIRA) to detect adulteration with C4 sugars.

The present studies employed a panel of different analytical techniques to study pomegranate fruit purchased from the market and 23 different commercial juices from local markets claiming to be authentic pomegranate juice. Complete chemical profiles including pomegranate polyphenols, sugar profiles, organic acids, amino acids, and potassium were determined in all samples to develop a practical International

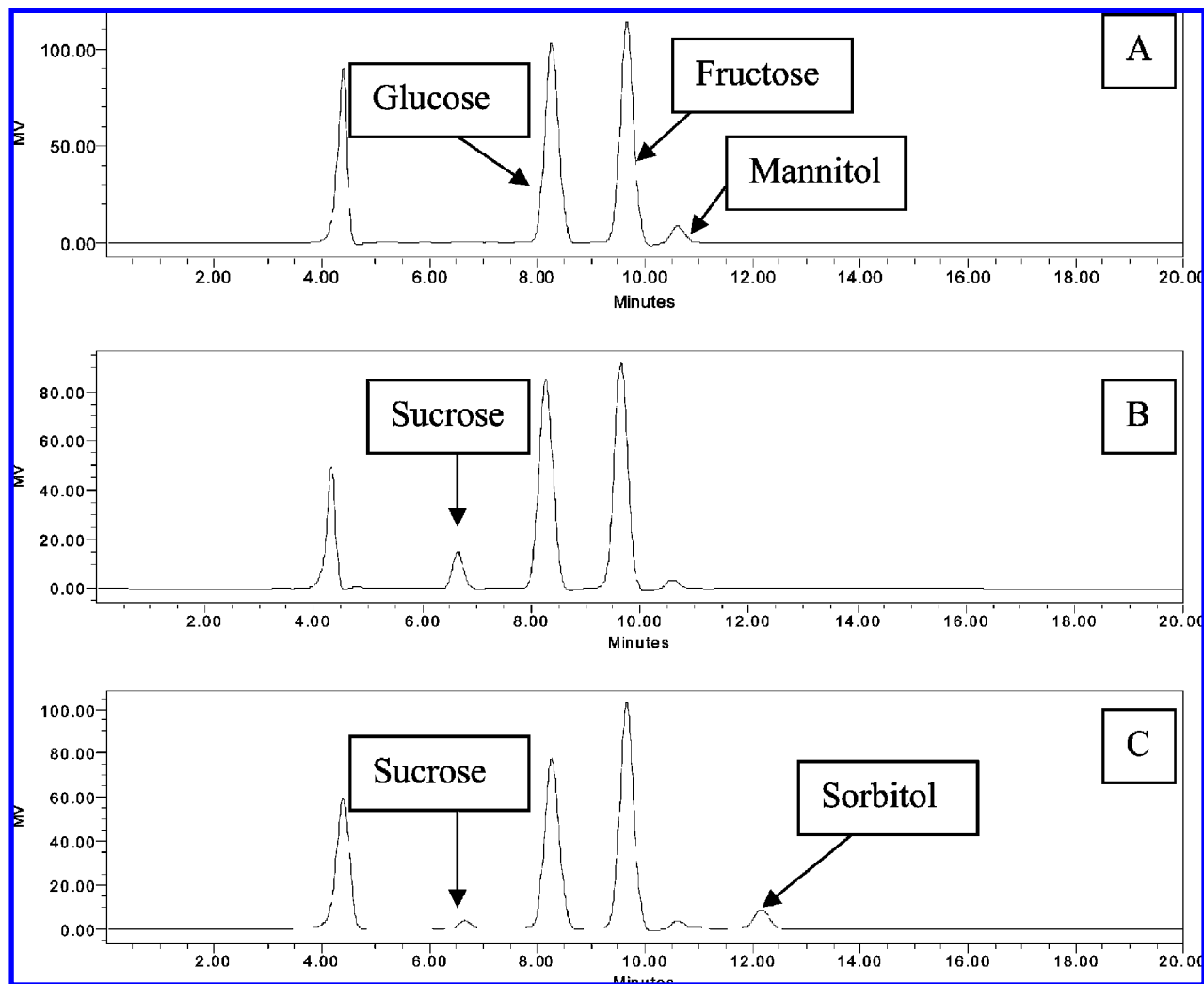


Figure 3. (A) Typical sugar HPLC chromatogram with Sugar Pak-II column separation illustrating glucose, fructose, and mannitol found in authentic PJ (sample O). (B) Atypical sugar HPLC chromatogram with Sugar Pak-II column separation (sample R, with detectable sucrose only). (C) Typical sugar HPLC chromatogram with Sugar Pak-II column separation (sample I, with sucrose and sorbitol).

Multidimensional Authenticity Specification (IMAS) algorithm for the determination of the authenticity of commercial pomegranate juice (Figure 1).

MATERIALS AND METHODS

Reagents. All solvents were of high-performance liquid chromatography (HPLC) grade and purchased from Fisher Scientific Co. (Tustin, CA). Cyanidin-3-glucoside and punicalagin A and B standards were purchased from Chromadex (Irvine, CA). Sucrose, glucose, fructose, mannitol, and sorbitol standards were purchased from Sigma-Aldrich. Ellagic acid, citric acid, D-malic acid, L-malic acid, oxalic acid, and tartaric acid were purchased from Sigma-Aldrich.

Pomegranate Juice Samples. All juice samples were purchased from local grocery stores. The numbers of lots of the same juice ranged from one to six on the basis of accessibility and market availability. All of the commercial pomegranate juices were labeled A–W. The juice samples were inventoried, shaken well, transferred into five 15-mL centrifuge tubes, and stored at $-20\text{ }^{\circ}\text{C}$ before testing. Frozen juices were thawed at $4\text{ }^{\circ}\text{C}$ in a refrigerator and then shaken by hand before sampling for analysis.

Pomegranate Polyphenol Analysis. Pomegranate polyphenols were quantitated using a Waters Alliance 2695 HPLC system coupled with a Waters 996 PDA detector with Empower 2 software. For anthocyanin analysis, the juice samples were applied to a Phenomenex Gemini-NX $5\text{ }\mu\text{m}$ C_{18} , $4.6 \times 250\text{ mm}$, column and detected at 520 nm . A linear gradient of two component solvents, acetonitrile (solvent A) and 1%

$\text{H}_3\text{PO}_4/10\%$ acetic acid in water (solvent B), at a flow rate of 0.75 mL/min was used for the elution of the components of juice. The initial gradient was 5% solvent A and 95% solvent B, with increases in solvent A to 15, 25, and 40% at 10, 30, and 40 min, respectively. For punicalagin A and B, punicalin, and ellagic acid level analysis, the juice samples were applied to an Agilent Zorbax SB C_{18} column ($5\text{ }\mu\text{m}$, $4.6 \times 250\text{ mm}$) with a guard column (C_{18} , $5\text{ }\mu\text{m}$, $3.9 \times 20\text{ mm}$) and detected at 360 nm . A linear gradient of two component solvents, acetonitrile (solvent A) and 0.4% H_3PO_4 in water (solvent B), at a flow rate of 0.75 mL/min was used for the elution of the components of juice. The gradient initially contained 5% solvent A and 95% solvent B, increasing solvent A to 15, 25, and 40% at 10, 30, and 40 min, respectively.

Sugar Analysis. A Waters Alliance 2695 HPLC system coupled with a Waters 2414 refractive index detector, an Alliance column heater, and a RI detector module controlled by Empower 2 software was applied for the sugar content analysis. For analysis of sucrose and maltose, the juice samples were separated on a Waters Spherisorb $5\text{ }\mu\text{m}$ NH_2 , $4.6 \times 150\text{ mm}$, column and detected by the Waters RI detector. An isocratic mix of two component solvents, acetonitrile (20%) and water (80%), at a flow rate of 0.5 mL/min was used for the elution of the components of juice. The column temperature was set at $35\text{ }^{\circ}\text{C}$. For analysis of glucose, fructose, mannitol, and sorbitol, the juice samples were separated on a Waters Sugar Pak I ($300 \times 6.5\text{ mm}$) column and detected by the Waters RI detector. Pure water at a flow

rate of 0.6 mL/min was used for the mobile phase. The column temperature was set at 80 °C.

For the detection of added cane sugar and high-fructose corn syrup, stable isotope ratio mass spectrometry was used to accurately determine carbon-13 enrichment. $^{13}\text{C}/^{12}\text{C}$ ratios were measured by mass spectrometry. In terrestrial plant tissue, the principal source of variations in $^{13}\text{C}/^{12}\text{C}$ ratios is the different photosynthetic pathways for CO_2 fixation. Plants fix CO_2 by one of two common pathways: the Calvin cycle pathway (C3) or the Hatch–Slack pathway (C4). C3 photosynthesis has a very large isotope effect associated with carbon dioxide fixation, whereas C4 photosynthesis involves only a small degree of carbon isotope fractionation. $^{13}\text{C}/^{12}\text{C}$ ratios for most plant tissues tend to be clustered in two ranges associated with the C3 and C4 pathways. The sample is combusted to CO_2 , and the ratio of the ions m/e 45 ($^{13}\text{C}^{16}\text{O}_2$) and m/e 44 ($^{12}\text{C}^{16}\text{O}_2$) is measured. This sample ratio is compared with that of a reference gas of known $^{13}\text{C}/^{12}\text{C}$ ratio. In this way, very small differences from the standard ratio may be precisely measured. $^{13}\text{C}/^{12}\text{C}$ ratios are reported as parts per thousand (per mil, ‰) difference between the sample ratio and the ratio of an arbitrary standard Pee Dee Belemnite limestone (PDB) according to the formula

$$\delta^{13}\text{C} = 1000 \times \left[\frac{R_{\text{sample}}}{R_{\text{PDB}}} - 1 \right]$$

where $R = ^{13}\text{C}/^{12}\text{C}$. The isotope ratio was measured with a VG 903 ratio mass spectrometer. The standard used was CO_2 prepared from acid hydrolysis of a marble secondary standard calibrated against NBS Solenhofen Limestone Standard Reference Material.

Organic Acid and Amino Acid Analysis. A Waters Alliance 2695 HPLC system coupled with a Waters 996 PDA detector and running Empower 2 software was applied for the organic acid content analysis. The juice samples were separated on a combination of Supelco Discovery (C_{18} , 5 μm , 4.6 \times 150 mm) and Agilent Zorbax SB (C_{18} , 5 μm , 4.6 \times 250 mm) columns with a guard column (C_{18} , 5 μm , 3.9 \times 20 mm) and detected at 214 nm. A linear gradient of two component solvents, acetonitrile (solvent A) and 0.4% H_3PO_4 in water (solvent B), at a flow rate of 0.6 mL/min was used for the elution of the components of juice. The gradient started from 0% of solvent A and 100% of solvent B, increasing solvent A to 15 and 50% at 30 and 40 min, respectively. Samples were analyzed for proline by photometric detection at 520 nm after reaction with ninhydrin (AOAC method 979.20).

Potassium. Potassium was measured with a flame photometer using a potassium filter. The PJ samples were diluted with water to below 10 ppm prior to analysis.

RESULTS

Chemical determinations of anthocyanin, ellagitannin, sugar profiles, organic and amino acids, and potassium contents were carried out in 45 commercial juice specimens from 23 different manufacturers purchased at local grocery stores (see **Tables 1–3**). Whereas concentrations of ellagitannins varied in commercial PJ samples tested, the characteristic ellagitannins were present in 45 samples from 23 different manufacturers, indicating the presence of some pomegranate juice or peel extract even in those juices having abnormal anthocyanin profiles. Characteristic HPLC anthocyanin profiles of an authentic juice and one that has an abnormal profile are shown in **Figure 2**. As shown, there are six anthocyanin peaks present in sample C, which is authentic, whereas there is an abnormal profile for sample G.

As indicated in federal guidelines (quality), PJ must have a °Brix level of 16 determined by the solids (sugar) content, if made from concentrate and without added flavors or other ingredients (29). This U.S. single criterion was designed as a quality specification to discourage overdilution of authentic juices, and other countries have designated various lower °Brix standards (e.g., 14–16). As shown in **Table 2**, juice

Table 4. International Multidimensional Authenticity Algorithm Specifications (IMAS) for Commercial Pomegranate Juice

attribute (method)	IMAS criterion
pomegranate polyphenols	characteristic anthocyanin profile present
anthocyanins (HPLC)	no atypical anthocyanins
four major peaks	
delphinidin-3,5-diglucoside	
delphinidin-3-glucoside	
cyanidin-3,5-diglucoside	
cyanidin-3-glucoside	
two minor peaks	
pelargonidin-3,5-diglucoside	
pelargonidin-3-glucoside	
ellagitannins (HPLC)	
punicalagin (marker for ellagitannins)	present
sugar profile	
glucose/fructose ratio (HPLC)	0.8–1.0
carbon SIRA ($^{13}\text{C}/^{12}\text{C}$ isotope ratio)	$\geq -25\%$
mannitol (HPLC-RI)	≥ 0.3 g/100 mL
sucrose (HPLC-RI)	not detectable
sorbitol (HPLC-RI)	≥ 0.03 g/100 mL
maltose (HPLC-RI)	not detectable
°Brix (refractometer)	U.S. = 16 ^a (international standards vary)
organic and amino acids	
citric/isocitric ratio (HPLC)	≤ 350
tartaric acid (HPLC)	not detectable
malic acid (HPLC)	≤ 0.1 g/100 mL ^b
malic acid, D-isomer (HPLC)	not detectable
proline (HPLC)	≤ 25 mg/L ^c
mineral	
potassium (flame photometer)	≤ 1800 mg/L ^d

^a If made from concentrate and without any added flavors or other ingredients.

The presence of added flavors or other gradients requires >16 ; for fresh squeezed juice, <16 is allowed. ^b Chinese varieties may have malic acid up to 0.2 g/100 mL.

^c Specifications according to AIJN (2008) and Fischer-Zorn, Krueger (2008), and Ara (2007). ^d Lower potassium-containing pomegranate varieties are known, but potassium <1300 mg/L is not found in commercial juices. Low potassium should be used to classify a juice as nonauthentic only when combined with other atypical criteria (AIJN, 2008).

samples from 19 of 23 manufacturers had a °Brix level of ≥ 14 . The mannitol, sorbitol, and sucrose levels and glucose to fructose ratios are also shown in **Table 2**. The ratio of glucose to fructose varied in a narrow range of 0.8–1.0 in the authentic PJ. In addition, sucrose was not detected in the authentic PJs, whereas sorbitol was present at ≤ 0.03 g/100 mL. However, there were a number of other commercial PJs in which sucrose and sorbitol were detected in significant amounts (see **Table 2**). As shown in **Figure 3** there are characteristic ratios of the glucose and fructose peaks demonstrated in the chromatogram of sample O (**Figure 3A**) and an abnormal ratio of these sugars together with the presence of sucrose in sample I (**Figure 3C**) and with the presence of sucrose in sample G (**Figure 3B**) on the HPLC profile.

Finally, stable isotope ratio mass spectrometry detected the presence of sugars from cane or corn (C4) sources rather than fruit sugars in multiple samples. Mannitol was detected at ≥ 4 mg/mL in samples judged to be authentic PJ by having a characteristic polyphenol profile.

Organic acids characteristic of authentic PJ were detected including malic acid at ≤ 0.1 g/100 mL. However, in several of the commercial juice samples, tartaric acid was detected. The authentic PJ samples contained >1800 mg of potassium/L. The organic acids and potassium detected are listed in **Table 3**.

DISCUSSION

An algorithm emerged from our consideration of the above analyses enabling the detection of major adulteration practices

Table 5. Summary of Authentication Results

sample	polyphenols	sugar profile (determinant)	organic and amino acids	potassium	conclusion
E (n = 1)	OK	OK	OK	OK	OK
O (n = 6)	OK	OK	OK	OK	OK
R (n = 1)	OK	OK	OK	OK	OK
S (n = 1)	OK	OK	OK	OK	OK
U (n = 1)	OK	OK	OK	OK	OK
V (n = 1)	OK	OK	OK	OK	OK
J (n = 3)	OK	OK (minimal sucrose)	OK	OK	OK ^a
N (n = 2)	OK	OK	OK	slightly low	OK ^a
C (n = 1)	OK	°Brix (slightly low)	OK	OK	OK ^a
A (n = 2)	OK	atypical (sucrose, sorbitol)	OK	OK	adulterated
B (n = 3)	OK	atypical (SIRA, sucrose, sorbitol)	atypical (proline)	atypical	adulterated
D (n = 1)	OK	atypical (SIRA, sucrose, sorbitol)	OK	atypical	adulterated
F (n = 1)	OK	atypical (SIRA, sorbitol)	OK	OK	adulterated
G (n = 1)	atypical	atypical (SIRA, sucrose, mannitol)	OK	OK	adulterated
H (n = 1)	OK	atypical (sucrose, sorbitol, SIRA)	OK	OK	adulterated
I (n = 3)	atypical	atypical (sorbitol, sucrose)	atypical (proline)	OK	adulterated
K (n = 1)	atypical	atypical (sucrose)	OK	atypical	adulterated
L (n = 3)	OK	atypical (SIRA)	atypical (Cit/Isocit ratio)	atypical	adulterated
M (n = 3)	atypical	atypical (sorbitol sucrose)	OK	OK	adulterated
P (n = 4)	OK	atypical (sucrose, Glu/Fru ratio)	atypical (proline)	OK	adulterated
Q (n = 3)	atypical	atypical (sucrose, mannitol)	OK	atypical	adulterated
T (n = 1)	OK	atypical (sucrose, sorbitol, mannitol, Glu/Fru ratio)	na	atypical	adulterated
W (n = 1)	atypical	atypical (sorbitol, SIRA, Glu/Fru ratio)	atypical (Cit/Isocit ratio)	atypical	adulterated

^a The °Brix of 15.1 (sample C) could simply be a dilutional issue if made from concentrate, and the very low potassium (sample N) is unlikely to be due to adulteration. The latter may be an analytical error, and more lots would be tested for IMAS specification in such a case.

Table 6. Observed Methods of Pomegranate Juice Adulteration

- addition of nonpomegranate anthocyanins from aronia, grape skin, elderberry, black currant, or black carrot as detected by atypical anthocyanin profile
- addition of cane sugar or corn sugar as detected by stable isotope ratio mass spectrometry, presence of sucrose, or presence of maltose
- addition of sorbitol-containing fruit juices such as apple, pear, cherry, or aronia as detected by the presence of nonpomegranate anthocyanins, elevated levels of sorbitol, malic acid, or sucrose
- addition of grape juice and grape skin color as detected by elevated levels of malic acid, proline, tartaric acid, grape anthocyanins, or other nonpomegranate anthocyanins
- addition of citric acid as detected by low isocitric acid and high citric/isocitric acid ratios

as outlined in **Table 4**. The IMAS was then configured to enable detection of each of these methods through measurement of polyphenols, sugar profile, organic and amino acids, and potassium by establishing the ranges that were characteristic of authentic and nonauthentic pomegranate juice samples (see **Table 5**).

We concluded that authentic commercial pomegranate juice made from whole fruit is characterized by consistent composition regardless of variety or geographic origin. There are always six characteristic anthocyanins present, but the concentrations may vary (see **Figure 1**). Punicalagins are always present, but do not prove authenticity, necessitating further chemical characterization. There is a characteristic multidimensional sugar profile based on measurement of mannitol, glucose, and fructose. Characteristic organic acid concentrations and ratios and an absence of uncharacteristic organic acids further enable detection of common adulteration methods. Finally, both °Brix and potassium are in characteristic ranges of concentration.

The establishment of the IMAS for PJ based on the analyses in the present study is a model that can be used for other examples of fruit juice adulteration. The current federal guideline for PJ authenticity is based only on the minimum quality specification of 16 °Brix value for juice made from concentrate. This criterion assumes that the starting concentrate is authentic PJ and merely establishes a degree of dilution that is permissible.

In today's competitive health food marketplace, considerable economic incentives exist to sell diluted and adulterated juices (11). The high cost of the fruit along with the potential for high earnings motivates some manufacturers to adulterate pomegranate juice. In some cases, the juice concentrate, especially when imported from abroad, can be adulterated prior to shipment to the domestic juice manufacturer. The practice of obtaining lower cost juice concentrates that have been adulterated allows some companies to easily gain an economic advantage over those companies selling 100% pure pomegranate juice made from concentrate.

Detection of the fraudulent methods being used is important not only to those who manufacture the authentic product but also to the economic health of the farmers who grow the pomegranates and to consumers who purchase this product for its health value but are cheated through substitution of non-pomegranate juice ingredients. Of the commercial PJs tested in this research only about 35% qualified as authentic pomegranate juice (see **Table 5**).

Sucrose, invert sugars, and high-fructose syrups such as high-fructose corn syrup can be detected by using SIRA. The photosynthetic process in fruits is different than that in cane sugar or corn, resulting in their having a different preference for carbon-13 compared to carbon-12 in the process of photosynthesis. Corn and sugar cane belong to the less common C4 photosynthetic category, whereas most other commercially important plants belong to the C3 category (18). This observation was subsequently developed into a practical method for detecting cane sugar and corn syrup in orange juice (19, 20) and apple juice (21). This procedure has since been collaboratively evaluated and incorporated as an AOAC official method (22). Subsequent work has extended these findings to evaluations of grape juice (23), cranberry juice (24), raspberry juice (25), pineapple juice (26), and other fruits (27), including pomegranate (28). SIRA can accurately detect adulteration with cane or corn-derived sugars.

Both sugar profile and organic acids can be used to detect adulteration with grape or apple sugars. The total level of acids strongly depends on the pomegranate variety and the degree of

ripeness. The time of the harvest significantly influences the sugar/acid ($^{\circ}$ Brix/acid) ratio (29, 30). Total titratable acid concentrations of 10.6–13.5 g/L were found. Additionally, malic acid at <0.1 g/100 mL was detected, whereas D-malic acid, an artificial additive, should not be found in authentic PJ. Contrary to Melgarejo et al. (31) and Poyrazoglu et al. (32), who reported tartaric acid contents of up to 0.014 g/100 g in Spanish pomegranates and 0.28–2.83 g/L in Turkish pomegranates, respectively, we did not detect tartaric acid in U.S. California pomegranate fresh fruit or juice. A close examination of the methodologies in the above papers indicates that they are likely detecting other organic acids such as L-malic acid normally found in pomegranate. We conclude that pomegranates do not contain tartaric acid, which is most commonly found in grapes. The detection of tartaric acid indicates adulteration with grape products. Sucrose, sorbitol, and maltose were not found in authentic PJ in our laboratory, and their presence indicates adulteration. Previous studies of 40 Spanish pomegranate cultivars detected low sucrose concentration levels of 0.07 g/100 g in fresh fruit (31). In several other studies, no sucrose was detected in fresh pomegranate fruit (33). The detection of sucrose indicates that adulteration with cane sugar is likely. Other common sources of sugars for adulteration are high-fructose syrups and invert sugars. Beet or cane total invert sugars are produced from refined beet or cane sucrose by either acid or enzymatic hydrolysis, resulting in a finished product containing a 1:1 ratio of glucose/fructose and low levels of sucrose. High-fructose syrups are produced by the enzymatic hydrolysis of corn, potato, rice, or wheat starch. The resulting glucose syrup is partially converted to fructose by enzymatic treatment.

The sugar alcohol sorbitol is not found in pomegranate juice at concentrations of >0.03 g/100 mL but occurs in apple juice, and its detection at higher concentrations suggests adulteration with apple juice or other sorbitol-containing juices (such as aronia). D-Sorbitol indicates addition of synthetic sweetener and should not be detected in PJ. Generally, investigators find zero to trace levels of sorbitol in the pomegranate (34). D-Sorbitol may also be an indicator of spoilage as microorganisms are capable of reducing the carbonyl group of fructose to form sorbitol (35). Mannitol is found in significant amounts in pomegranate but is not balanced by sorbitol as is commonly found in apple juice. Therefore, most of the adulteration tactics can be detected by examining the sugar profile.

Ultimately, adulteration can result in dilution of components other than sugar including potassium. Pomegranate juice is a rich source of potassium containing >2000 mg/L. The finding of <1300 mg/L is consistent with the dilution of PJ with other juices. Potassium typically is the main mineral in pomegranate juice. Like most other fruit varieties, fresh pomegranates contain only traces of sodium by nature.

The history of juice adulteration for profit is extensive, and this latest example seeks to capitalize on millions of dollars of research conducted with genuine PJ while denying the public the very phytochemicals that account for the unique health benefits of PJ. Furthermore, use of nonauthentic PJ in research studies could lead to false-negative results, undermining the progress made in research to date. The IMAS established in our studies could potentially be used by government inspectors to deter manufacturers who may be importing inferior juices from international sources or purposely using inferior materials from domestic sources. Labeling adulterated juices as 100% PJ is misleading and a disservice to consumers. It also carries a potential safety risk.

LITERATURE CITED

- (1) Gil, M. I.; Garcia-Viguera, C.; Artes, F.; Tomas-Barberan, F. A. Changes in pomegranate juice pigmentation during ripening. *J. Sci. Food Agric.* **1995**, *68*, 76–81.
- (2) Hernandez, F.; Melgarejo, P.; Tomas-Barberan, F. A.; Artes, F. Evolution of juice anthocyanins during ripening of new selected pomegranate (*Punica granatum*) clones. *Eur. Food Res. Technol.* **1999**, *210*, 39–42.
- (3) Miguel, G.; Fontes, C.; Antunes, D.; Neves, A.; Martins, D. Anthocyanin concentration of “Assaria” pomegranate fruits during different cold storage conditions. *J. Biomed. Biotechnol.* **2004**, 338–342.
- (4) Alighourchi, H.; Barzegar, M.; Abbasi, S. Anthocyanins characterization of 15 Iranian pomegranate (*Punica granatum* L.) varieties and their variation after cold storage and pasteurization. *Eur. Food Res. Technol.* **2008**, *227*, 881–887.
- (5) Gil, M. I.; Tomas-Barberan, F. A.; Hess-Pierce, B.; Holcroft, D. M.; Kader, A. A. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem.* **2000**, *48*, 4581–4589.
- (6) Tzulker, R.; Glazer, I.; Bar-Ilan, I.; Holland, D.; Aviram, M.; Amir, R. Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *J. Agric. Food Chem.* **2007**, *55*, 9559–9570.
- (7) Seeram, N. P.; Aviram, M.; Zhang, Y.; Henning, S. M.; Feng, L.; Dreher, M.; Heber, D. Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *J. Agric. Food Chem.* **2008**, *56*, 1415–1422.
- (8) Guo, C.; Wei, J.; Yang, J.; Xu, J.; Pang, W.; Jiang, Y. Pomegranate juice is potentially better than apple juice in improving antioxidant function in elderly subjects. *Nutr. Res. (N.Y.)* **2008**, *28*, 72–77.
- (9) Heber, D.; Seeram, N. P.; Wyatt, H.; Henning, S. M.; Zhang, Y.; Ogden, L. G.; Dreher, M.; Hill, J. O. Safety and antioxidant activity of a pomegranate ellagitannin-enriched polyphenol dietary supplement in overweight individuals with increased waist size. *J. Agric. Food Chem.* **2007**, *55*, 10050–10054.
- (10) Jimenez, D. M.; Ramazanov, A.; Sikorski, S.; Ramazanov, Z.; Chkhikvishvili, I. A new method of standardization of health-promoting pomegranate fruit (*Punica granatum*) extract. *Georgian Med. News* **2006**, 70–77.
- (11) U.S. Food and Drug Administration Guide to Inspection of Foods Section 10, Orange and other juices; available at http://www.fda.gov/ora/Inspect_ref/igs/foodspa.html.
- (12) Seeram, N. P.; Adams, L. S.; Henning, S. M.; Niu, Y.; Zhang, Y.; Nair, M. G.; Heber, D. In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *J. Nutr. Biochem.* **2005**, *16*, 360–367.
- (13) Pantuck, A. J.; Leppert, J. T.; Zomorodian, N.; Aronson, W.; Hong, J.; Barnard, R. J.; Seeram, N.; Liker, H.; Wang, H.; Elashoff, R.; Heber, D.; Aviram, M.; Ignarro, L.; Belldegrun, A. Phase II study of pomegranate juice for men with rising prostate-specific antigen following surgery or radiation for prostate cancer. *Clin. Cancer Res.* **2006**, *12*, 4018–4026.
- (14) Sumner, M. D.; Elliott-Eller, M.; Weidner, G.; Daubenmier, J. J.; Chew, M. H.; Marlin, R.; Raisin, C. J.; Ornish, D. Effects of pomegranate Juice consumption on myocardial perfusion in patients with coronary heart disease. *Am. J. Cardiol.* **2005**, *96*, 810–814.
- (15) Hartman, R. E.; Shah, A.; Fagan, A. M.; Schwetye, K. E.; Parsadanian, M.; Schulman, R. N.; Finn, M. B.; Holtzman, D. M. Pomegranate juice decreases amyloid load and improves behavior in a mouse model of Alzheimer’s disease. *Neurobiol. Dis.* **2006**, *24*, 506–515.
- (16) Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (AIJN). Reference Guideline for Pomegranate Juice. Code of Practice for Evaluation of Fruit and Vegetable Juices, 6.x.

- (17) Krueger, D. A. Composition of commercial pomegranate juice. Abstract, 122nd International Meeting of the Association of Official Analytical Chemists, 2008.
- (18) Smith, B. N.; Epstein, S. Two categories of $^{13}\text{C}/^{12}\text{C}$ ratios for higher plants. *Plant Physiol.* **1971**, *47*, 380–384.
- (19) Nissenbaum, A.; Lifshitz, A.; Stepek, Y. Detection of citrus juice adulteration using the distribution of natural stable isotopes. *Lebensm.-Wiss. Technol.* **1974**, 152–154.
- (20) Doner, L. W.; Bills, D. D. Stable carbon isotope ratios in orange juice. *J. Agric. Food Chem.* **1981**, *29*, 803–804.
- (21) Doner, L. W.; Krueger, H. W.; Reesman, R. H. Isotopic composition of carbon in apple juice. *J. Agric. Food Chem.* **1980**, *28*, 362–364.
- (22) *Association of Official Analytical Chemists Official Methods of Analysis*, 14th ed.; AOAC: Arlington, VA, 1984; pp 427–428, 594–595, 597.
- (23) Krueger, H. W.; Reesman, R. H. Carbon isotope analyses in food technology. *Mass Spectrom. Rev.* **1982**, *1*, 205–236.
- (24) Hong, V.; Wrolstad, R. E. Cranberry juice composition. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 199–207.
- (25) Durst, R. W.; Wrolstad, R. E.; Krueger, D. A. Sugar, nonvolatile acid, $^{13}\text{C}/^{12}\text{C}$ ratio, and mineral analysis for determination of the authenticity and quality of red raspberry juice composition. *J. Assoc. Off. Anal. Chem.* **1995**, *78*, 1195–204.
- (26) Gonzalez, J.; Remaud, G.; Jamin, E.; Naulet, N.; Martin, G. G. Specific natural isotope profile studied by isotope ratio mass spectrometry (SNIP-IRMS): $^{13}\text{C}/^{12}\text{C}$ ratios of fructose, glucose, and sucrose for improved detection of sugar addition to pineapple juices and concentrates. *J. Agric. Food Chem.* **1999**, *47*, 2316–2321.
- (27) Parker, P. L. The chemical basis of the use of carbon-13/carbon-12 ratios to detect the addition of sweeteners to fruit juice concentrates. *Fluess. Obst* **1982**, *49*, 692–694.
- (28) Krueger, D. A.; Krueger, R. G.; Krueger, H. W. Carbon isotope ratios of various fruits. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 1035–1036.
- (29) U.S. Food and Drug Administration. Percentage juice declaration for foods purporting to be beverages containing fruit or vegetable juice. Code of Federal Regulations 21: Chapter 1. Section 101.3.
- (30) Miguel, G.; Fontes, C.; Martins, D.; Neves, A.; Antunes, D. Effects of post-harvest treatment and storage time on the organic acid content in Assaria and Mollar pomegranate (*Punica granatum* L.) fruit. *Ital. J. Food Sci.* **2006**, *18*, 317–322.
- (31) Melgarejo, P.; Salazar, D. M.; Artes, F. Organic acids and sugars composition of harvested pomegranate fruits. *Eur. Food Res. Technol.* **2000**, *211*, 185–190.
- (32) Poyrazoglu, E.; Goekmen, V.; Artik, Nt. Organic acids and phenolic compounds in pomegranates (*Punica granatum* L.) grown in Turkey. *J. Food Compos. Anal.* **2002**, *15*, 567–575.
- (33) Fischer-Zorn, M.; Ara, V. Pomegranate juice—chemical compositions and adulteration potential. *Fluess. Obst* **2007**, *74*, 386–393.
- (34) Velioglu, S.; Unal, C.; Cemeroglu, B. Chemical characterization of pomegranate juice. *Fruit Process.* **1997**, *7*, 307–310.
- (35) Jonas, R.; Silveira, M. M. Sorbitol can be produced not only chemically but also biotechnologically. *Appl. Biochem. Biotechnol.* **2004**, *118*, 321–336.

Received for review October 10, 2008. Revised manuscript received December 23, 2008. Accepted February 10, 2009.

JF803172E