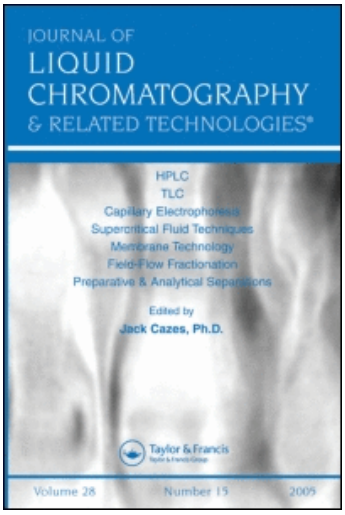

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QUANTITATIVE STUDY OF FREE SUGARS AND MYO-INOSITOL IN CITRUS JUICES BY HPLC AND A LITERATURE COMPILATION

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ABSTRACT

Three hundred and six (306) commercial samples from 19 processing plants in Florida, composed of frozen concentrated orange juice, orange juice from concentrate, and pasteurized orange juice, were analyzed for their sugars content by HPLC. In addition, the sugar profile of fresh-squeezed juice from 9 different cultivars of sweet oranges, 2 tangors, and 4 tangelos grown in Florida are included. Sucrose, fructose, glucose, total sugars, glucose/fructose ratio, and myo-inositol contents are presented.

Mean ± standard deviation sugar levels for 100 mL of frozen concentrated orange juice were 4.1 ± 0.5 g of sucrose, 2.1 ± 0.2 g of fructose, 1.9 ± 0.2 g of glucose, and 0.1 ± 0.1 g of myo-inositol, from juices processed in Florida. A review of the methodology for the detection of sugars by HPLC in citrus products is presented; most results having been acquired by HPLC analysis utilizing NH_2 column and acetonitrile/water. A compilation of literature furnishing information on free sugar contents in orange and grapefruit juices by cultivar, and in commercial samples, is also presented.

INTRODUCTION

Sugars are the major components of citrus juice soluble solids and the sweetness of orange juice is intrinsic to its sugar composition. The main portion of carbohydrates in citrus fruits are the three simple sugars: sucrose, glucose, and fructose. Together, they represent about 80% of the total soluble solids of orange juice, and the ratios of sucrose:glucose:fructose are generally about 2:1:1.[1] An earlier study[2] indicated that sucrose and invert sugars (fructose and glucose) are the principal sugars in orange juice. Besides free sugars, galactose, arabinose, xylose, mannose, and fucose have been reported as sugar units of soluble polysaccharides in orange juice.[3]

Based on agricultural statistics,[4] Florida is the major citrus producing area in the United States. Most of the orange production (ca. 95%) in Florida is utilized for juice processing. Due to an increase in demand for orange juice throughout the world and previous severe freezes in Florida, there has been an increase in the marketing of products purporting to be orange juices. Orange juice is easily adulterated by blending corn syrup or sugar syrup with orange juice concentrate and other cheaper ingredients.[5] Thus, considerable information on orange juice composition, which can be used in establishing grades and standards, and in determining the authenticity of orange juice, has been required for regulatory compliance. Furthermore, sugar analysis needs to be performed to address the economic interest as well. In Japan, for example, the tax rate of imported citrus juice depends on the sugar content.

Sugar profile is one of the most important properties to consider for orange juice and relatively little information is available in the literature on the sugar composition of citrus juice. In the 34th Annual Citrus Processor's Meeting held at the Citrus Research and Education Center, Lake Alfred, FL, several types of HPLC sugar analyses, and use of these techniques to detect sugar adulteration in orange juice, were discussed.[6] Analysis of soluble polysaccharides has also been suggested as an additional criterion for orange juice evaluation.[3] In previous work,[7-11] information on the analysis of sugars and sugar contents and their structures in various foods has been presented.

The purpose of this work is to provide, as a guide, a variety of HPLC methods for the analysis of free sugars (glucose, fructose, sucrose) and sugar alcohol (myo-inositol) in citrus products. Also, a discussion of the values and ranges of free sugars and sugar alcohol in commercially prepared citrus juices from Florida processing plants, in the literature and in fresh-squeezed juices from citrus grown in Florida, is presented for use in the determination of the authenticity of the juices and concentrates, as well as, to address the economic interest of sugar content in citrus products.

Measurements of Sugars by Liquid Chromatography in Citrus Products

Normal phase mode with amino-bonded silica column, and ion exchange mode using cation and anion ion exchange resins are the most commonly used approaches for sugar analysis in citrus products.

Normal Phase Mode

In the HPLC analysis of sugars in citrus juices, the most common system involves chemically bonded amino columns[12,13] or amine-modified silica columns[6,14] in a normal phase mode using very polar eluents, and a refractive index detector. The chemically bonded amino ($-NH_2$) columns such as the μ-Bondapack carbohydrate column and the Waters carbohydrate column are available in the prepacked form from Waters, and amine-modified silica columns are available from other major suppliers (Mac-Mod Analytical, Supelco, Brownlee, YMC, etc.).

Amine-modified silica columns are becoming more widely used for separation of sugars in foods. The amino column is operated with a mixture of acetonitrile and water; 80% acetonitrile,[15]; 83%;[16] 76%;[17] 75%;[6,14] and the system is predominantly used for analysis of sugars in citrus products. Since the amino-bonded column operates under normal phase elution conditions, water, which is more polar than acetonitrile, is the stronger solvent.[18] However, selection of mobile phase composition depends on the resolution, as well as molecular weight range of sugars eluting. Addition of small amounts of ethanol into the aqueous acetonitrile solution also has been attempted to improve the separation in orange (*Rutaceae*) from which identification of maltose was possible.[13]

This normal phase mode is best suited for low molecular weight sugars such as mono- and disaccharides in citrus products. However, with traditional monomeric amino-bonded columns, retention times shorten over time due to Schiff's base formation between carbonyl groups and the amino groups on the column, as well as loss of amino functional groups often leading to poor resolution of sugars. The loss of resolution between fructose and glucose is particularly noticeable.[10]

Recently, a polyamine column has been developed which incorporates primary amine functionalities into the polymer resin coating, providing a selectivity that is identical to the conventional propylamine-bonded column, but has improved stability and longer column life.[18] The polyamine column is stable over time allowing for reproducible retention times and analyses. It can also be reconditioned to remove acidic impurities, which can irreversibly bind and contaminate the column, and break Schiff's base formation which causes reduced retention of sugars.

Figure 1 shows the characteristic separation of sugars in orange juices using the polyamine column conducted in the author's lab. The elution order is fructose, glucose, sucrose, and myo-inositol. Myo-inositol (I-inositol or hexahydroxycyclohexane) is a cyclic sugar alcohol and has been reported in citrus.[1] Glucose, fructose, and sucrose were purchased from Fisher Scientific (Pittsburgh, PA) and myo-inositol and rhamnose from Sigma Chemical Co. (St. Louis, MO). Deionized water was purified through a Milli-Q (Millipore Corp., Bedford, MA) water filtration system. HPLC grade acetonitrile (Fisher Scientific) was used for the mobile phase, which consisted of CH_3CN and H_2O (75:25, v/v), filtered through a 0.45 μm Durapore filter (Millipore Corp.) and degassed in an ultrasonic bath prior to use. The system consisted of a Waters (Milford, MA) model 600E pump, a Waters 717+ autosampler with chiller, a model 1037A refractive index detector (Hewlett Packard, Palo Alto, CA), and Waters Millennium Chromatography Manager software.

A YMC, Inc. (Milford, MA) YMC-Pack PA column (250 x 4.6 mm, i.d.) was used to separate the sugars. Flow rate was 1.0 mL/min. Injection was 10 μL. Retention time for rhamnose (internal standard) was 6.9 min, fructose-9.9 min, glucose-11.6 min, sucrose-16.1 min and myo-inositol-18.7 min.

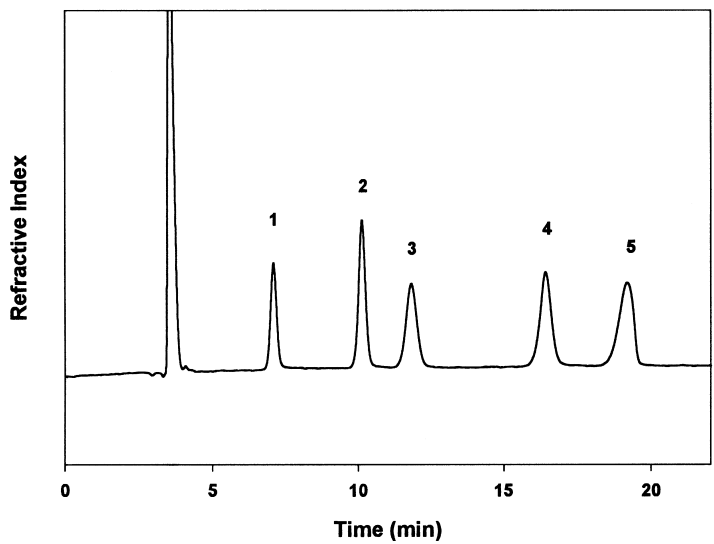

Figure 1. HPLC analysis of std. sugars by NH_2 column with aqueous acetonitrile, YMC-Pack PA column (4.6 x 250 mm), 75% ACN/H_20, RI 16x, flow rate 1 mL/min, 10 μL injection, 25°C. 1.) Rhamnose (10 mg/mL), 2.) Fructose (10 mg/mL), 3.) Glucose (10 mg/mL), 4.) Sucrose (10 mg/mL), 5.) myo-Inositol (10 mg/mL).

Coefficient of variation of retention time and response factor for free sugars and myo-inositol were less than 3.0%. Percent recoveries of these compounds were determined by comparing peak areas of standard sugars to peak areas of the same solution subjected to the C18 Sep-Pak cartridge clean-up procedure. Average recoveries of these compounds were over 97% of the tested levels.

Cation Exchange Mode

This mode uses sulfonated styrene divinylbenzene resins in lead (Pb^{2+}) form or calcium (Ca^{2+}) form and employs water or water with organic modifier (CaEDTA) as the mobile phase at elevated temperatures (80-90°C). This mode provides selectivity to separate mono- and disaccharides as well as sugar alcohols and low molecular weight sugar alcohols by hydroxyl coordination to the metal cations.[19] The principal separation mechanism is steric exclusion, but ligand formation and partitioning effects may play some role resulting in separation of a number of compounds with similar or identical molecular weights. Elution order is that the higher oligosaccharides elute first; the smaller di-and monosaccharides elute later.

An ion-exchange type column that has been cross-linked with calcium such as the Sugar-pak I from Waters,[20] the Shodex S-8801/S from Showa Denko,[21] and the SCR-101N column from Shimadzu[22] have been used for sugar analysis in citrus products. The mobile phase is usually water and the elution order of the ion-exchange type column is reversed from the amine-modified silica type column; sucrose is eluted first, then glucose, and fructose. The advantage of the ion-exchange type column is it can be used to detect the simultaneous presence of polyols such as sorbitol and mannitol, but no such sugar alcohols have been reported in citrus. However, for proper separation, the ion-exchange column cross-linked with calcium must be operated at elevated temperatures (80-90°C), while the chemically bonded amine column is operated at ambient temperature.

Anion Exchange Separation

Sugars are very weak acids with dissociation constants (pKa's) in the range of 12-13, so at high pH they can be separated as anions. Under basic conditions (pH greater than 12), anion exchange columns[23] are used to separate a wide range of sugars from monosaccharides to complex carbohydrates in the order of increasing molecular weight. For monosaccharide and myo-inositol, isocratic elution with sodium hydroxide can successfully separate myo-inositol, glucose, fructose, sucrose by elution order, but gradient elution using changes in the sodium hydroxide concentrations can be applied to improve separation or to accelerate the elution of late-eluting components.[24] However, a slight pH change in gradient elution can generate a slight baseline shift.

The advent of pulsed amperometric detection (PAD) and anion exchange columns have made HPLC the clear choice for analysis of sugars, especially for oligosaccharides. HPLC-PAD chromatography takes advantage of the weakly acidic nature of sugars to give highly selective separations at high pH using a strong anion exchange stationary phase.[24] The analysis of sugars and sugar alcohols through the use of amperometric detection[25] utilizes anion-exchange separation with a Dionex (Sunnyvale, CA) CarboPac PA1 column (4 x 250 mm) at ambient temperature. A guard column was not used to avoid band spreading. The mobile phase consisted of sodium hydroxide (50% w/w) diluted to 0.14 M (pH 13) with filtered (0.45 μm) HPLC grade carbonate-free water and pumped at a flow rate of 1.0 mL/min. through stainless steel tubing and fittings at a run time of 10 min.

The detector, equipped with a single gold electrode, was operated in the pulse mode at potentials (vs. Ag:AgCl): E_1 = +50 mV (167 ms), E_2 = +650 mV (167 ms), and E_3 = -950 mV (500 ms). Current was sampled during E_1 with sensitivity set at 100 μA full-scale. Sugars were eluted under less than 10 min of analysis time but elution order between glucose and fructose was reversed compared to the normal phase HPLC with amine column. Resolution depends largely on the pH of the eluent and control of temperature in the 20-45°C range.[17] The increase in mobile phase strength (OH^-) causes a decrease in analyte retention, but the higher pH increases the degree of dissociation and increases sample retention.

Simple sugars (glucose, fructose, & sucrose) in Valencia orange were also analyzed using an HPLC system incorporating a Waters (Milford, MA) model 464 metal-free electrochemical detector set at 100 μA[26] as a function of fruit maturation. The columns consisted of an ATC-1 anion trap, a CarboPac PA1 guard column, and two CarboPac PA100 analytical columns (Dionex Corp., Sunnyvale, CA). Sugars were eluted with a mobile phase of 0.3 N NaOH with a run time of 40 min. using an injection of 1 μL of solution.[26]

Others

A rapid analysis of sugars in citrus products can be performed with capillary electrophoresis. Using a basic buffer together with a bare fused-silica capillary, sucrose and other sugars in orange juice can be analyzed within 17 minutes. Sample preparation was simple, consisting of only dilution and ultrafiltration for protein removal. Capillary electrophoresis with indirect UV-detection provides comparable sensitivity to HPLC with reflective-index detection at short over all run times, based on recent technical brochure from Hewlett-Packard.[27] For sugar analysis with CE: orange juice (1:20 dilution with water), capillary (Fused –silica, 80.5 cm, 50 μm, i.d.), basic anion buffer, –25 kV, 15°C, injection (6s at 50 mbar), and detection (signal 350/20 nm, reference 275/10 nm) were utilized. Elution followed the order: fructose, glucose, and sucrose.

Detection

Since simple sugars do not possess chromophores or fluorophores, the reflex index (RI) detector is by far the most commonly used in citrus sugar analysis. RI detectors do not respond uniformly to all sugars but RI detectors that are currently available are more sensitive than UV detectors (10). RI detection does not offer selectivity for sugars over other compounds in fruit juice. However, since sugars exhibit only weak absorbence in the UV region, using a UV detector for sugars analyses in orange juice required more elaborate sample cleanup than RI detection.[28] An alternative approach is to use fluorescence after pre-column derivatization with DNS-hydrazine.[29] The sensitivity for reducing sugars could increase but non-reducing sugars such as sucrose do not react with DNS-hydrazine reagent.

Another potential means of increasing the sensitivity for mono-, di-, oligosaccharides and sugar alcohols is using an electrochemical detector with a gold electrode. The electrochemical detector has high specificity and sensitivity for sugars. Sugars are detected by measuring the electrical current generated by their oxidation at the surface of a gold electrode. Electrochemical detection systems are available from Dionex Corp., BAS, and E. G. & G. Princeton Applied Research Corp., etc. Pulsed Amperometric Detector (PAD) with a gold electrode increases the detection sensitivity for sugars as much as 100 times that of the refractive index detector, and limits of detection were estimated to be 45 ng for glucose, 60 ng for fructose, and 300 ng for sucrose.[17] HPLC-PAD has been applied for sugars in citrus products.[17,25,26]

Sample Preparation

Simple dilution with water and filtering before injection is effective and easily done for sugar analysis by HPLC, but chemical clean up of the sample is advisable to extend the life of the column. Substances such as pigments, proteins, lipids, polysaccharides, salts, and acids could contaminate your column. Citrus is a complex matrix; HPLC sugar analysis for citrus products often requires sample preparation with pretreatment. Johnson and Harris[30] reported various results from 7 different membranes and 2 cartridges for sample filtration prior to HPLC sugar analysis. Also, Nomura and co-workers[31] compared the recoveries of sugars from different solid phase extraction systems such as Sep-Pak C18 and Alumina A (Waters), Bond Elute PSA (Analytichem International) and Chrom-Prep mixed resin (Hamilton). There were no significant differences in the recovery of tested sugars among solid phase extractions, but mixed-bed ion exchange resin removed all apparent ionic interference, including the organic acids, resulting in the cleanest chromatogram both under RI and UV (195 nm) detections.

Analysis of sugars from citrus fruit requires blending the sliced fruit with ethanol, concentration, redissolving with water, and purification through a C18 cartridge[13] or, blending with methanol/water/acetic acid (79:20:1) instead of ethanol solution.[12] With juice, reflux the juice with ethanol concentrate, extract with ethyl ether to remove colorants, and then purify with ion-exchange resin.[20] With the use of solid-phase extraction, sample preparation for citrus juice sugar analysis by HPLC could be done more simply with Sep-Pak C18 cartridges[6] and C18 cartridge and Chrom-prep mixed resin.[14]

For HPLC-Amperometric detection (PAD) for simple sugars, no sample preparation was applied other than dilution and filtration. Standards and juice samples were diluted 100 times with HPLC grade water and filtered through a 0.2 μm nylon syringe filter prior to injection.[25] However, sample preparation for the analysis of both mono- and disaccharides by HPLC-PAD may require the dilution of samples and pre-treatments to prevent column overloading.[26] Juice sample was centrifuged at 10,000 x g for 15 min and supernatant passed successively through a Dowex AG50W-X8 (100-200 mesh, H^+ form) resin, Dowex AG 1-X4 (100-200 mesh, formate form) resin from Bio-Rad (Richmond, CA), a Sep-Pak C18 cartridge (Waters Corp., Milford, MA), and filtered through a 13 mm, 0.45 μm nylon Acrodisc (Gelman Sciences, Inc., Ann Arbor, MI).

In our laboratory, for sugar analysis using normal phase mode with a mobile phase consisting of acetonitrile/water (75:25) at a flow rate of 1.0 mL/min on a YMC-Pack polyamine column (4.6 x 250 mm, 5 μm), as described in the description of the normal phase mode (Figure 2), single strength orange juice (10 mL) was centrifuged for 10 minutes at 10,000 rpm. Approximately 5 mL of the juice was passed through a Waters C18 Sep-Pak cartridge that had been conditioned by rinsing with 3 mL of methanol, followed by rinsing with 5 mL of purified, deionized water. The first 2 mL of juice passed through the cartridge were discarded and the remainder, approximately 3 mL, collected. A 1 mL aliquot of sample and 1 mL of internal standard rhamnose (1.0 g/100 mL) were mixed, filtered through a 1.2 μm Acrodisc (Gelman Sciences, Ann Arbor, MI) filter, and passed through a 0.45 μm Acrodisc filter before injection. Analysis was carried out in duplicate.

Sugars and Myo-Inositol Contents in Citrus Juices

Orange Juices

In a recent study conducted in our laboratory, commercially processed orange juices were obtained from 19 plants in Florida. The samples were of the following breakdown: 39 pasteurized orange juice (POJ), 104 reconstituted orange juice from concentrate (OJFC), and 163 frozen concentrated orange juice (FCOJ). Other orange cultivar samples were freshly prepared at the Citrus

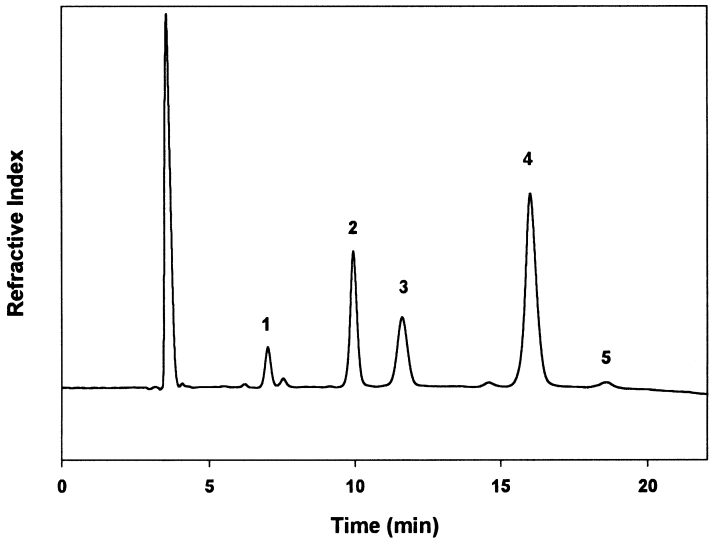

Figure 2. HPLC analysis of sugars in orange juice by NH_2 column with aqueous acetonitrile, YMC-Pack PA column (4.6 x 250 mm), 75% ACN/H_20, RI 16x, flow rate 1 mL/min, 10 µL injection, 25 °C. 1.) Rhamnose (internal std.), 2.) Fructose, 3.) Glucose, 4.) Sucrose, 5.) myo-Inositol.

Research & Education Center, Lake Alfred, FL. Results are normalized to an 11.8 °Brix (measurement of soluble solids of juice) and reported as g/100 mL juice. Table 1 lists the quantitative results for sugars in three different Florida processed orange juice products. The overall mean, range, standard deviation, and percent coefficient of variance (% CV) are reported.

Sucrose is present in the largest amounts for all orange juice samples, accounting for about 50% (40.4-59.0%) of orange juice's total sugar content (Table 1). Orange juice contained both reducing and nonreducing sugars in about equal amounts. Mean value of total sugar content was fairly constant for each type of juice product; 8.0 g/100 mL for POJ, 8.1 g/100 mL for FCOJ and 8.1 g/100 mL for OJFC. The overall mean value of glucose/fructose ratio is 0.9 each. There is less variation (6.0-6.8% CV) in the glucose/fructose ratio for the orange juice samples than for the glucose and fructose content. Thus, this value may be a useful index in adulteration investigations. In most fruits, glucose exceeds the fructose concentration,[32] and in apple and pears, fructose exceeds glucose by an order of three times.[33] But orange fruit contains glucose and fructose in nearly equal quantities or fructose is present in a slightly greater amount. If a sample purported to be orange juice shows no such pattern, it cannot be

Table 1

Free Sugars and Myo-Inositol Contents in Commercial Florida Processed Orange Juice Products

	Fructose		Glucose			Sucrose		TS[b]	Myo-Inositol	
	g/100 mL	%TS	g/100 mL	%TS	G/F[a]	g/100 mL	%TS	g/100 mL	g/100 mL	%TSI[c]
FCOJ (n=163)										
Mean	2.1	25.5	1.9	23.9	0.9	4.1	50.6	8.1	0.1	1.5
Min.	1.7	21.5	1.5	19.7	0.8	2.9	42.5	6.3	0.1	0.8
Max.	3.0	29.1	2.9	28.3	1.1	5.2	58.8	10.3	0.3	3.5
S.D.	0.2	1.6	0.2	1.7	0.1	0.5	3.1	0.8	<0.1	0.4
%CV	10.6	6.4	12.4	7.3	6.0	11.1	6.0	9.2	29.1	27.7
OJFC (n=104)										
Mean	2.1	25.9	2.0	24.2	0.9	4.1	49.9	8.1	0.1	1.6
Min.	1.5	21.5	1.4	20.5	0.7	3.0	40.4	6.3	0.1	0.8
Max.	2.8	34.3	2.9	28.7	1.1	5.9	58.0	10.6	0.3	2.9
S.D.	0.2	2.0	0.2	2.1	0.1	0.6	3.7	0.8	<0.1	0.7
%CV	9.5	7.9	11.9	8.5	6.3	14.8	7.5	10.4	29.6	46.4
POJ (n=39)										
Mean	2.0	24.4	1.8	22.4	0.9	4.3	53.1	8.0	0.1	1.4
Min.	1.5	21.0	1.4	19.8	0.8	3.0	44.9	6.8	<0.1	0.5
Max.	2.8	29.7	2.4	27.0	1.1	5.4	59.0	10.4	0.2	2.4
S.D.	0.3	2.1	0.3	1.5	0.1	0.6	3.3	0.9	<0.1	0.4
%CV	14.6	8.6	13.7	6.4	6.8	13.0	6.1	11.6	33.1	30.1

G/F[a]= ratio of glucose to fructose. TS[b] = the total sugar; sum of fructose, glucose, and sucrose. %TSI[c] = percent of total sugar sugar plus myo-Inositol.

100% orange juice and adulteration with other fruit juice concentrate should be suspected.

Orange juice also contains a substantial amount of myo-inositol. Mean values for POJ, FCOJ, and OJFC were 0.1 g/100 mL each (Table 1). These values fall within the range, 0.10-0.18 g/100 mL, which was previously reported from canned orange juices by microbiological assay,[34] and close to the mean value of 0.14 g/100 mL found from 59 commercial Florida orange juices by HPLC.[35] Myo-inositol is not a carbohydrate, but a cyclic polyol and has been reported in juices of orange, tangerine, grapefruit, lemon,[25,34,36,37] and from Moro blood oranges grown in California and Florida.[38] Presence of this sugar alcohol is rarely identified in other fruits, except apricot.[39] Other straight chain sugar alcohols such as sorbitol, mannitol, and xylitol have been reported in many other fruits,[40,41] and sorbitol has been suggested as an index of adulteration in some fruit juices,[40] but sorbitol has not been found in oranges.[42]

Also, in our previous work with Sugar-pak I column (Waters, Milford, MA) with 0.1 mM aqueous EDTA solution at 80°C, no sorbitol, mannitol, and xylitol were detected (detection limits were 0.01 mg/mL each) from the 14 juice samples of sweet orange cultivars.[37] The % CV for myo-inositol content is very high, between 29.1 and 33.1, compared to other sugars (Table 1). This might be due to analytical error as it has a low concentration compared to other sugars, and most probably is due to its natural variation in total solids. Myo-inositol accounts for below 2% of total sugars; the percentage of myo-inositol in orange juice is expressed as a percentage of total sugars plus myo-inositol.

In a study of red colored Moro blood orange juice,[38] juices grown in California and Florida were determined to have a similarly characteristic invert sugar pattern to sweet orange juices, sucrose:fructose:glucose (2:1:1). Substantial amounts of myo-inositol were also found in Moro blood orange juice, 0.2 g/100 mL in juices from California and Florida. The nutritional significance of myo-inositol has not been determined, but it is evident that blood orange juice is a good source of this cyclic alcohol.

Since considerable amounts of Brazilian and other non-Florida orange juice are currently used for blending in Florida, the sugar data in Table 1 may not be an exact representation of oranges grown in Florida, but could represent values found in current blended products in Florida processing operations.

Sugar profiles listed in Table 2 were collected from 9 different cultivars of sweet oranges (*C. sinensis*), 2 of tangor, and 4 of tangelos grown in Florida. The total sugars of fresh-squeezed sweet orange juices ranged from a low of 3.9 to a high of 10.0 g/100 mL with 15.8% CV. Total sugar contents are somewhat lower than the values from commercially prepared orange juice samples in Table 1, but the overall mean value (8.2 g/100 mL) still falls in the range of

reported total sugars values from sweet oranges, 5.4-10.3%.[43] The sugar content in Valencia, which is one of the major varieties used for juice in Florida, was agreeable to the previous data; 23.7% glucose, 25.8% fructose, and 50.5% sucrose based on total sugars present from 86 Florida Valencia orange juices.[43]

Mean value for myo-inositol of fresh-squeezed sweet orange juices was 0.2 g/100 mL with standard deviation of 0.1. The % CV for sugars in fresh-squeezed sweet orange juice (Table 2) is higher than the % CV for commercial orange juices which are presented in Table 1, which might be due to the fact that commercial orange juices are well blended to produce a constant °Brix/acid ratio. Also, other factors such as maturity, varietal differences, and sample size should be considered. Although the % CVs are high, sweet oranges, produced in Florida have a characteristic 2:1:1 sugar pattern, distinguishable from 1:1:1 sugar pattern produced in citrus hybrid.[44]

Sugar data from tangor and tangelos grown in Florida (Table 2) could be useful for processors since both are tangerine hybrids of which up to 10% of these juices can be added to orange juice for color enhancement. Tangors are orange x tangerine hybrids, the sugar in tangor is primarily sucrose, comprising about 50% (51.8-56.0%) of total sugars (Table 2). The glucose/fructose ratio in tangors, 0.9 for murcott and 0.9 for temple, is identical to the mean values found in sweet oranges (Table 2). Tangelos are either tangerine x grapefruit or tangerine x pummelo hybrids.[45] Interestingly, the reducing sugar contents (sum of glucose and fructose) in Sampson and Seminole tangelos are significantly higher than the content of the nonreducing sugar, sucrose (Table 2). It is a sugar pattern similar to that found in less sweet citrus cultivars such as lemons or limes,[43] and grapefruit juices.[6] Sampson has especially been known as a grapefruit-like tangelo due to the presence of naringin and neohesperidin, which are not present in sweet oranges.[45]

Table 2 also presents the sugar contents of several varieties of orange[1,25,43,46,47] juices with results from some commercial juices[12] compiled from the literature. A study of seasonal changes in citrus juice varieties[46] provided ranges of sugar contents for a growing season. Total sugars in early and mid season oranges (Hamlin and Pineapple) increased rapidly due to an accumulation of sucrose as the temperature decreased.[46]

Grapefruit Juices

The sugar contents of grapefruit juice cultivars[25,43,46] and commercially packaged grapefruit juices[6,12,47] from literature are presented in Table 3. The sugar profiles in grapefruit juices appear to have little difference in terms of sucrose content. Sucrose comprises a considerably smaller proportion of the total sugars, about 36.6%,[6] but still the glucose/fructose ratio from 149 com-

Table 2

Free Sugars and Myo-Inositol in Sweet Oranges, Tangor, and Tangelo Juices Grown in Florida, and From the Literature

	Fructose		Glucose			Sucrose		TS[b]	Myo-Inositol	
Cultivar	g/100 mL	%TS	g/100 mL	%TS	G/F[a]	g/100 mL	%TS	g/100 mL	g/100 mL	%TSI[c]
Sweet Oranges										
Bahianinha Navel	2.2	21.8	2.2	22.1	1.0	5.5	56.0	9.9	0.3	2.6
Hamlin	1.8	23.6	1.6	21.0	0.9	4.3	55.4	7.7	0.1	1.2
MediterraneanBlood	1.5	21.5	1.3	18.6	0.9	4.3	59.9	7.1	0.2	2.3
Parson Brown	1.6	20.5	1.3	17.0	0.8	4.8	62.5	7.8	0.1	1.5
Pineapple	1.5	22.1	1.4	21.1	1.0	3.8	56.8	6.7	0.1	1.5
Ruby Blood	2.0	25.1	1.8	22.5	0.9	4.2	52.4	7.9	0.2	2.3
Shamouti	1.2	22.6	1.1	19.3	0.9	3.2	58.1	5.4	0.1	1.6
Valencia	1.9	25.8	1.8	24.6	1.0	3.6	49.6	7.2	0.1	1.2
Viciedo	2.1	23.2	2.1	23.3	1.0	4.9	53.5	9.1	0.2	2.2
Hamlin[1]	2.6-3.0*	---	---	---	---	2.0-3.9	---	4.6-6.7	---	---
Hamlin[2]	2.1	25.9	1.0	12.3	0.5	4.6	56.8	8.1	---	---
Hamlin[3]	2.2	22.7	1.9	19.6	0.9	5.6	57.7	9.7	---	---
Pineapple[1]	2.4-3.9*	---	---	---	---	1.5-3.1	---	3.9-6.4	---	---
Pineapple[2]	2.7	27.0	1.5	15.0	0.6	5.8	58.0	10.0	---	---
All Varieties[4]	2.5	26.9	2.0	21.5	0.8	4.8	51.6	9.3	---	---
All Varieties[5]	2.4	28.2	2.2	25.9	0.9	3.9	45.9	8.5	---	---
Commercial OJ[6]	4.6	53.5	4.0	46.5	0.9	<0.5	<5.8	8.6	--	---

(continued)

Table 2 Continued

Tangor										
Murcott	2.3	25.1	2.1	23.1	0.9	4.8	51.8	9.2	0.1	1.3
Temple	1.7	23.0	11.6	21.0	0.9	4.2	56.0	7.6	0.1	1.2
Tangelo										
Minneola	2.0	22.2	1.7	19.1	0.9	5.2	58.7	8.9	0.1	1.1
Orlando	1.6	22.6	1.4	19.4	0.9	4.1	58.0	7.0	0.1	1.1
Sampson	2.4	31.0	2.2	28.4	0.9	3.1	40.6	7.6	0.1	1.4
Seminole	2.7	31.3	2.8	32.5	1.0	3.2	36.4	8.7	0.2	1.8

G/F[a]= ratio of glucose to fructose. TS[b] = the total sugar; sum of fructose, glucose, and sucrose. %TSI[c] = percent of total sugar sugar plus myo-Inositol. * = Sum of glucose and fructose. [1] From ref. 43. [2] From ref. 46. [3] From ref. 25. [4] From ref. 1. [5] From ref. 47. [6] From ref. 12.

Table 3

Literature Compilation of Sugar Contents in Grapefruit Juices

Grapefruit Juices	°Bx	Sample Size	Free Sugar Contents in Grapefruit Juice (%) Fructose	Glucose	Sucrose	Total Sugar	Methods	References
Duncan	n/a	n/a	3.0-5.1*	---	2.4-4.0	5.0-8.3	n/a	43
Duncan	n/a	n/a	2.1	2.1	3.4	7.6	HPLC	25
Star Ruby	n/a	n/a	1.5	1.4	3.0	5.9	HPLC	25
Marsh	n/a	n/a	2.3-4.8*	---	2.6-3.1	5.1-7.8	n/a	43
Marsh	n/a	n/a	1.8	1.7	2.6	6.0	n/a	46
Marsh	n/a	n/a	1.9	1.8	1.9	5.6	HPLC	25
Comm. GJ	10	n/a	0.8-2.4	2.1-2.8	1.7-4.6	n/a	n/a	47
Comm. GJ	10	n/a	2.0-5.0	2.0-5.0	0.5-4.0	n/a	n/a	47
Comm. GJ	10	n/a	2.3	2.1	2.4	6.8	n/a	47
Comm. GJ	n/a	n/a	3.1	3.0	1.3	7.3	HPLC	12
Canned SSGJ	n/a	149	2.4	2.2	2.7	7.5	HPLC	6

* = sum of glucose and fructose.

mercial Florida grapefruit juices is 0.9, almost identical to the values from commercial Florida orange juices used in this study.

CONCLUSIONS

In summary, a review of methodologies for the analysis of sugars and results of studies for the analysis of citrus juices and fruit are presented. The present study conducted in our laboratory provides a considerable amount of information on the sugar profile of citrus juices produced in Florida.

Typically, orange juices originating in Florida have the characteristic 2:1:1 ratio previously discussed. Commercial orange juices analyzed in our lab showed no unusual changes in sugar quantities or ratios occurred due to processing and were consistent with expectations of blended orange juices produced in Florida citrus processing plants.

The important features of these sugar data are that the fructose is present in a slightly greater amount than glucose, but glucose/fructose ratio is almost a constant ratio of about 1:1, sucrose accounts for about 50% of the total sugars, and there is a substantial amount of myo-inositol present. The range for myo-inositol is considerable, however, myo-inositol content deserves special attention since its presence in low quantities may indicate dilution if it is not seen from commercial orange juices.

This data can be used to address the regulatory compliance as well as the economic interest of sugar contents in citrus juices.

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