Rapid Determination of Sugars, Nonvolatile Acids, and Ascorbic Acid in Strawberry and Other Fruits

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An analytical procedure to determine major sugars and organic acids, including vitamin C, in fruits was developed using a C_{18} Sep-Pak cleanup process and an ion exclusion HPLC column. Dual UV monitoring and refractive index were performed for detection. To attain optimal separation and quantitation, 0.0085N H_2SO_4 was used as the mobile phase and the column temperature was maintained at 23 °C. This procedure was compared to others for the individual quantitation of sugars, organic acids, and vitamin C. Recovery and reproducibility of this analytical procedure were quite acceptable for strawberry and four other common fruits, allowing the analysis of all the components using a single-injection HPLC analysis in <22 min.

Keywords: *Simultaneous analysis; high-performance liquid chromatography; sugars; organic acids; vitamin C; strawberry; fruits*

INTRODUCTION

Knowledge of the exact qualitative and quantitative distribution of the characteristic sugars and organic acids in fruits or fruits products is of capital importance to evaluate quality, either as a powerful tool to detect adulteration in juices and other fruit products or as indices to control changes in the production and storage based on their relative stability. Sugars and organic acids of strawberries have been investigated from two different points of view, as an index of fruit development and ripening and as components of fruit flavor. Research conducted with commercial strawberry varieties has detected variations for these constituents due to stage of ripeness, production year, and cultivar (Sweeney et al., 1970; Sistrunk and Cash, 1973; Wrolstad and Shallenberger, 1981; Reyes et al., 1982; Forney and Breen, 1986), so that advanced strawberry selections, among other traits, tend to balance sugars and organic acids. On the other hand, organic acids are also implicated indirectly in color quality, as was demonstrated by Wrolstad et al. (1970) in strawberries.

Fresh fruits and vegetables present the highest content of ascorbic acid, although it varies among species and varieties. This compound, better known as vitamin C, is associated with health by most consumers so that it is an attractive index of product quality for them. Although *Citrus* fruits have the greatest reputation for a high content of vitamin C, there are some other fruits having higher contents, as is the case for strawberries. However, this characteristic is not well indicated in the strawberry sale points. Because of its important nutritional implications, vitamin C is considered very important lately and its content measurements are included among the quality parameters evaluated for fruits and fruit products (Blom and Skrede, 1984; Romero-Rodríguez et al., 1992; Cano et al., 1994; Gensler et al., 1995). Contrary to other organic acids and sugars, vitamin C is quite unstable, mainly due to the activity of ascorbic acid oxidase and the reaction with oxygen in the presence of heavy metal ions and light (Bode et al., 1990; Angberg et al., 1993), and thus it is taken as an indication of fruit freshness and retention of other components.

Most of the determinations of sugars and organic acids from various sources have been conducted by the GLC technique and detected as methyl, acetyl, silyl, oxime, or oxime-silyl derivatives (Churms, 1990; Robards and Whitelaw, 1986; Chapman and Horvat, 1989) and by the HPLC technique with different detections (Churms, 1990; Falqué-López and Fernández-Gómez, 1996; Cano et al., 1994; Doyon et al., 1991). This last technique seems to provide the best results for the individual quantitation of these compounds. In this sense, numerous procedures describing ascorbic acid analysis are based on different techniques, including titration (AOAC, 1980), colorimetry (Bajaj and Kaur, 1981), electrochemistry (Lau et al., 1989), and enzymatic analysis (Uchiyama et al., 1991), but HPLC is the technique with higher specificity and sensitivity (Kissinger and Pachla, 1987; Grahan and Annette, 1992; Kishida et al., 1992; Nísperos-Carriedo et al., 1992; Romero-Rodríguez et al., 1992).

Due to our current interest in strawberry physiology and quality, and the lack of information on postharvest changes of sugars and organic acids, including ascorbic acid, the purpose of this work was to establish a simple and rapid analytical procedure for the quantitation of these constituents in this fruit. An additional objective was to investigate the possible application of this analytical procedure for the quality evaluation of other fruits.

EXPERIMENTAL PROCEDURES

Plant Material. Strawberry (*Fragaria* × *ananassa)* fruits, cultivar Oso Grande, were grown in Torreagro fields (Cartaya, Huelva, Spain) and sampled at commercial maturity stage (>75% red color). Fruits of peach (*Prunus persica*) cultivar Frederica, apple (*Malus domestica*) cultivar Golden Delicious, kiwi (*Actinidia deliciosa*) cultivar Hayward, and banana (*Musa acuminata*) cultivar Cavendish were purchased from a local whole market.

Standards. To determine retention times and calibration curves, standard solutions were prepared individually in the

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appropiate solvent depending on the analytical method from analytical reagent grade main sugars and organic acids (Sigma Chemical Co., St. Louis, MO) found in fruits.

Preparation of Fruit Extracts. For the ethanolic extraction of sugars and organic acids, all fruits were peeled, except for strawberries, and symmetrically cut in eight pieces. Eight pieces from eight different fruits were blended in the dark with 95% ethanol for 3−5 min, depending on tissue softness, at maximum speed with an Omni-mixer (Sorvall, Newtown, PA). The homogenate was vacuum-filtered through Whatman No. 1 filter paper and the residue washed twice with 80% ethanol. The filtrates were combined and adjusted to 5 mL/g of fresh weight (FW) with 80% ethanol. This is considered the ethanolic extract henceforth.

For the comparative study with the method of Picón et al. (1993) for ascorbic acid quantitation, strawberries were sliced and blended as described above but with 10 volumes of 0.2 N H_2SO_4 containing 0.05% disodium ethylenediaminetetraacetate (EDTA) (Merck, Darmstadt, Germany). The homogenate was centrifugued at 27000*g* for 10 min and the supernatant vacuum-filtered through Whatman No. 1 filter paper.

Fractionation of Fruit Extracts for HPLC Analysis. To compare two different methods (C_{18} Sep-Pak cleanup and QAE-A-25 fractionation) for sugars and organic acids separation from the ethanolic extract, 10 mL of this extract was evaporated in the dark to dryness at 50 °C. The dry residue was redissolved in 1 mL of 0.2 N H_2SO_4 and 0.05% EDTA, loaded onto a C_{18} Sep-Pak cartridge (Lida, Kenosha, WI), and eluted with up to 4 mL of the same solution. Total sugars and organic acids were contained in the eluate. For the QAE-A-25 fractionation, 10 mL of the ethanolic extract was evaporated as above and the dry residue was redissolved with 1 mL of deionized H_2O and loaded onto a 2 mL QAE-A-25 resin (Pharmacia, Uppsala, Sweden) glass column according to the procedure described by Redgwell (1980). Total sugars were eluted from the column with 5 mL of deionized H_2O, and total organic acids were obtained by eluting the column with 10 mL of 0.4% formic acid. These extracts containing sugars and/or organic acids and the ascorbic acid extract obtained according to the method of Picón et al. (1993) were filtered through 0.45 μm nylon filters before HPLC analysis.

HPLC Conditions. Sugars and organic acids were analyzed in a Hewlett-Packard 1090 liquid chromatograph equipped with a photodiode array detector and a Waters 410 differential refractometer (Millipore) connected in series. Data were processed by means of a Hewlett-Packard 85-B computing system and a Beckman Analogue Interface Module 406 and a Gold V.711 software, respectively. Isocratic separations of the compounds were made on a stainless steel Ion-300 (300 mm × 7.8 mm, 10 μm) column, containing a cation-exchange polymer in the ionic hydrogen form, with an IonGuard GC-801 guard column (Interaction, San Jose, CA), and thermostated at 23 °C. The mobile phase utilized for the elution consisted of a filtered (0.22 μm nylon) and degassed solution of 0.0085 N H_2SO_4 and a flow rate of 0.4 mL/min. UV detection was selected at 195 and 245 nm, the refractive index detector was used at sensitivity 16×, and the injection volume was 20 μL.

Ascorbic acid prepared according to the method of Picón et al. (1993) was analyzed in the Hewlett-Packard liquid chromatograph described above using an ODS (250 mm × 2 mm, 5 μm) column (Beckman, San Ramon, CA) maintained at 30 °C. The mobile phase consisted of a filtered and degassed solution of 0.2 M $NaHCO_3$.

RESULTS AND DISCUSSION

Sugars and organic acids of strawberry fruits have been widely studied both as components of fruit flavor and as indices of fruit development and ripening. They are routinely assessed for fruit quality by determining total soluble solids (TSS) and titrable acidity (TA): for the former, by means of a refractometer, and for the latter, an acid−base titration. These parameters are taken as total content of sugars and organic acids,

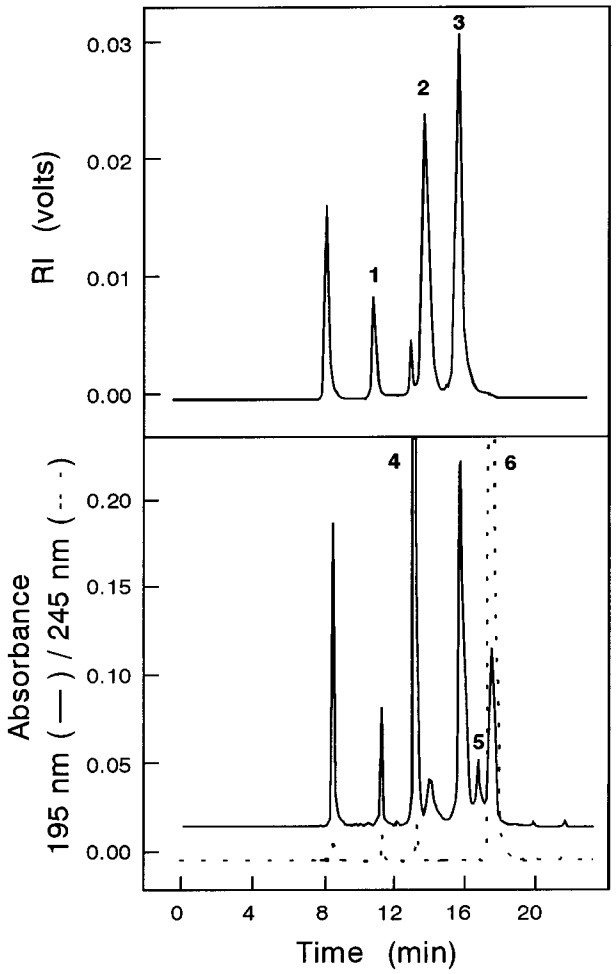

Figure 1. Chromatogram of a strawberry extract according to the described analytical methodology. Peaks: 1, sucrose; 2, glucose; 3, fructose; 4, citric acid; 5, malic acid; 6, ascorbic acid.

respectively, due to the demonstrated correlation between these measurements and component contents in some fruits. However, we have found a very poor correlation value for TSS and total sugars ($r = 0.8$), and TA and total organic acids ($r = 0.3$), which means that these parameters are not good enough for the evaluation of strawberry quality. This had been previously observed by Shaw (1988), especially for total sugars and TSS. Use of TSS and TA should be limited to comparative studies in which variations by genotype and environment are low. Our interest in looking for an objective and rapid method to determine strawberry, and other fruit, quality led to the development of a simple, rapid, and reliable procedure to determine this quality, as is described under Experimental Procedures.

Figure 1 shows a typical HPLC chromatogram of a strawberry extract. A total run time of 22 min is enough to elute all of the components of interest in the extract. Different HPLC mobile phases, H_2SO_4 concentrations ranging from 5 to 10 mN, and HPLC column temperatures ranging from 20 to 80 °C were studied in order to resolve and quantify main sugars and organic acids in strawberry. Among them, the chromatographic conditions described under Experimental Procedures were shown to be the best for this purpose. By using two detectors in series, a photodiode array detector (UV) set at 195 and 245 nm and a refractive index detector (RI), it is possible to identify and quantify main sugars and organic acids of importance for strawberry quality either from a flavor, nutritional, or physiological point

Table 1. Retention Times (R_t), Detection, Range of Concentrations, Correlation Coefficients of Linearity (r), and Limits of Detection (LOD) for the Calibration Standards Curves

compd	R_t (min)	detector	range (mg/mL)	r	LOD (mg/mL)
oxalic acid	8.75	UV−195 nm	0.004−0.300	0.9996	0.00379
sucrose	10.95	RI	0.001−34.261	0.9998	0.00074
citric acid	12.76	UV−195 nm	0.020−7.170	0.9995	0.01860
glucose	13.56	RI	0.002−27.644	0.9996	0.00151
fructose	15.25	RI	0.010−37.060	0.9999	0.00656
malic acid	15.87	UV−195 nm	0.030−3.576	0.9997	0.02870
quinic acid	16.47	UV−195 nm	0.150−4.480	0.9997	0.12080
ascorbic acid	16.54	UV−245 nm	0.010−0.477	0.9994	0.00829
succinic acid	21.43	UV−195 nm	0.150−0.600	0.997	0.12330

Table 2. Recovery Yield of Main Sugars and Organic Acids in Strawberry Extraction and HPLC Analysis

	content (mg/g of FW)				
compd	strawberry	added	calcd	found	recovery (%)
sucrose	5.060	6.058	11.118	10.701	96.25
glucose	16.886	9.730	26.616	28.489	107.04
fructose	18.854	12.668	31.522	33.271	105.55
citric acid	5.524	9.749	15.273	14.537	95.18
malic acid	1.045	2.027	3.072	3.068	99.87
ascorbic acid	0.203	0.454	0.657	0.697	106.09

Table 3. Repeatability (n = 10) of the HPLC Analysis for a Strawberry Extract

compd	content of (mg/g of FW)	CV[a] (%)
sucrose	5.15	3.10
glucose	17.77	0.61
fructose	19.40	2.62
citric acid	3.21	4.48
malic acid	1.11	4.42
ascorbic acid	0.19	4.10

[a] CV % coefficient of variance.

of view. This triple detection allows the quantitation, for instance, of compounds such as fructose and ascorbic acid, the elutions of which are partially masked by malic acid. This acid almost does not absorb at 245 nm, thus not interferring with the ascorbic acid quantitation, in the concentration range found in most frutis, while fructose is quantified by RI.

Standard solutions containing each of the main sugars and organic acids present in fruit were prepared to determine retention times and to obtain calibration curves in concentrations typical for most fruits. Regression equations were obtained with correlation coefficients ranging between 0.9994 and 0.9999 for most of these compounds in the concentration intervals shown in Table 1, with the exception of succinic acid which showed a correlation coefficient of 0.997 and a low sensibility for the UV detection.

To evaluate the yield recovery for sugars and organic acids, strawberries were spiked with a mixture of a known amount of each compound and the extraction, C_{18} Sep-Pak cleanup, and HPLC analysis procedures were carried out. Data of the analyses are shown in Table 2. A good recovery yield was obtained for each compound tested, with an average ranging from 95.18 to 107.04%.

Control of the repeatability was evaluated by analyzing a strawberry extract 10 times. Table 3 shows the average amount found for each compound and the coefficients of variance (CV). These data show repeatability CV to range between 0.61 and 4.48%. On the other hand, reproducibility during the HPLC analysis was assessed with a strawberry extract that had been stored at 4 °C for a period of 4 days (Table 4).

Table 4. Reproducibility of the HPLC Analysis of a Strawberry Extract Stored at 4 °C over 4 Days

	content (mg/g of FW)				
compd	day 1	day 2	day 3	day 4	mean (CV%)[a]
sucrose	5.090	5.084	4.428	4.733	4.833 (6.57)
glucose	17.430	17.255	16.558	16.632	16.968 (2.58)
fructose	20.301	19.894	21.721	20.503	20.604 (3.81)
citric acid	3.201	3.040	3.104	3.168	3.128 (2.27)
malic acid	1.150	1.092	1.115	1.138	1.123 (2.28)
ascorbic acid	0.178	0.165	0.154	0.154	0.164 (6.14)

[a] CV%, coefficient of variance %.

Reproducibility seems to be very good after 2 days from the preparation of the strawberry extract. However, sucrose and ascorbic acid seem to suffer some alterations during the last 2 days, increasing the CV up to 6.57 and 6.14%, respectively. After these results, all HPLC analyses were carried out the same day of the strawberry extraction or the day after at the latest.

Sugars and organic acids from fruits and vegetables have been classically analyzed from alcoholic extracts after fractionation with cation- and anion-exchange resins into the two groups of compounds (Redgwell, 1980; Reyes et al., 1982; Shaw, 1988; Calull et al., 1992). More recently, C_{18} Sep-Pak cartridges have been used as a cleanup step before analysis (Calull et al., 1992; Cano et al., 1994). We have compared the C_{18} Sep-Pak cleanup of a strawberry ethanolic extract spiked with all sugars and organic acids to the fractionation with QAE-A-25 resin as described under Experimental Procedures. The latter also showed a good recovery efficiency, 91.8−108.6% for all compounds. However, as the ethanolic extract is separated into two different extracts, one containing sugars and the other organic acids, two different HPLC analyses are necessary for their quantitation. Besides, the QAE-A-25 resin fractionation has the limitation of sample dilutions because it needed 5 and 10 mL of H_2O and 4% formic acid to completely recover sugars and organic acids, respectively. This is critical especially for the organic acids detection and quantitation. All of the fruit sample extracts were deeply pigmented and would thus severely reduce analytical column life if they were injected directly into the system. Therefore, the combination of C_{18} Sep-Pak cleanup of the ethanolic extract and the HPLC analysis on a cation-exchange polymer Ion-300 column in the selected conditions allowed a suitable separation and quantitation of main sugars and organic acids present in strawberries in a single injection without producing much damage to the column. Tables 3 and 4 show sugars and organic acids contents we have found with this analytical methodology in strawberries. Results are in good agreement with those reported by Molnár-Perl and Morvai (1992) and Reyes et al. (1982), although Richmond et al. (1981) found higher contents for sugars in this fruit. Wrolstad and Shallenberger (1981) found considerable variation in the free sugar composition reported in the literature for strawberries.

Recently, Picón et al. (1993) developed a simple extraction and analysis of ascorbic acid from strawberries based on the method established by Ashoor et al. (1984). This nutrient is considered one of the main parameters to be measured to evaluate fruit and vegetable quality. Ascorbic acid seems to be the compound most affected by processing of fruits and vegetables. This instability of ascorbic acid is mainly due to its tendency to react with oxygen, forming dehydroascorbic acid and further degradation products (Bode et al., 1990; Angberg et al., 1993). Shaw (1988) also

Table 5. Content of Main Sugars and Organic Acids in Different Fruits

	content (mg/100 g)			
compd	peach	apple	kiwi	banana
sucrose	6203.3	1532.2	1468.2	6848.4
glucose	576.3	1384.0	3153.0	3499.3
fructose	570.9	5022.6	3059.2	3105.7
oxalic acid	tz[a]	1.3	0.8	6.7
citric acid	197.2	tz	985.0	359.0
malic acid	282.4	412.2	190.8	289.4
ascorbic acid	nd[b]	nd	46.2	nd
quinic acid	154.1	nd	585.1	97.3
succinic acid	nd	tz	tz	113.9

[a] tz, traces, concentration below the limit of detection. [b] nd, not detected.

reported this instability of ascorbic acid during storage once extracted from strawberries. On the basis of the Ashoor et al. (1984) method for this acid extraction, the C_{18} Sep-Pak cleanup step of the methodology described in this work was carried out with 0.05% EDTA in 0.2 N H_2SO_4, preserving ascorbic acid from degradation as shown in the reproducibility studies (Table 4). Quantitation of ascorbic acid, simultaneously with other organic acids and sugars, by our methodology presented a very good recovery value (106.09%). Ascorbic acid content found in strawberries was compared to those obtained using the method by Picón et al. (1993) that exclusively quantifies this acid, as described under Experimental Procedures. This method showed a high recovery yield, close to 100%, for ascorbic acid, and the comparative data with both methods were in good agreement (data not shown). Ascorbic acid contents found in this experience showed to be also in consonance with data found by Picón et al. (1993) for Chandler and Douglas strawberries. However, higher values for this acid were obtained by Hudson et al. (1985), due possibly to the cultivar investigated or the use of more mature fruits.

The methodology evolved from this work to quantify in a single injection most important sugars and organic acids in strawberries was investigated for other fruits. This analytical methodology seems to be quite suitable for the quality evaluation of these fruits. Table 5 shows the mean values found among them. Thus, content of main sugars and organic acids present in peach seems to be in good agreement with those reported by Chapman and Horvat (1989) except for a lower content in glucose and fructose. However, contents in apple were apparently quite similar, except for the low amount of succinic, citric, and quinic acids, reported as very low in that work, that we were unable to quantify or detect. Sugar contents in this fruit were lower in general compared to those reported by Molnár-Perl and Morvai (1992), but the amount of oxalic acid we have quantified was 2−3 times higher. Richmond et al. (1981) published somewhat different contents for sugars in banana, higher values for sucrose and lower values for glucose and fructose. On the other hand, Cano et al. (1994) found high values for ascorbic acid in this fruit, whereas we could not detect any. Interestingly, Quán de Serrano et al. (1993) detected only dehydroascorbic acid in this fruit. For the other acids, we have found higher contents in citric, quinic, and succinic acids and quite similar results in oxalic and malic acids. Sugar contents found by Richmond et al. (1981) were of the same order of magnitude as our results in banana, except for a much higher sucrose content. Organic acids from kiwi found in this study (Table 5) are also consistent with those published by Cano et al. (1994), although while we found oxalic acid to be 0.8 mg/100 g, they reported values around 25 mg/100 g in three kiwi cultivars and only traces in Abbot kiwi. Contents in citric acid were also different. Our analysis showed around 3 times more citric acid than they have found in the four kiwi cultivars investigated. Quinic acid contents obtained by our methodology were quantified indirectly because it is partially masked in the HPLC analysis by the high concentration of ascorbic acid in this fruit. The calculation was carried out by considering that ascorbic acid shows a UV_{195}/UV_{245} absorbance ratio equal to 0.311. Sugars and organic acids from grape were also investigated, but an interference between glucose and tartaric acid peaks was noticed. As the sugar is quantified by RI detection and tartaric acid by UV_{195}, the content of this acid could also be calculated indirectly, although we have found a higher error than the case of quinic acid with ascorbic acid. Small changes in the HPLC temperature analysis could overcome this problem. Disagreement between some of the results we have found and others in the literature could be explained on a varietal, phenotipic, or maturity basis.

Data resulting from this work show that the analytical procedure developed is fairly simple and rapid and allows for the detection and quantitation of main sugars and organic acids found in fruits, in general, and in strawberry particularly. The HPLC column utilized was found to be reliable and operated well under continual use. This procedure is allowing us to conduct more precise studies on strawberry quality changes after different postharvest treatments of the fruit.

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