

Determination of citric acid in fruit juices using HPLC

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Abstract

A naturally occurring acid found in many fruit juices, citric acid was quantified using external standards and high-performance liquid chromatography (HPLC). Eight orange juice samples were analyzed ranging in concentrations from 36.0 to 427 mg/oz of citric acid. The California navel orange had the highest citric acid concentration, followed by the 100% orange juices, the orange flavored juices, and with the lowest concentration, Nestle's Juicy Juice, the 100% mixed fruit juice.

Introduction

Citric acid is a naturally occurring weak organic acid. It is small, very soluble, and easily manufactured. Although it is naturally occurring it is used as an additive to many drinks to enhance flavor and increase stability in soft drinks and syrups. It is also used to prevent color change by oxidation.¹ Because they are so easily modified, citrus juices are subject to alteration by addition of citric acid, a cheap ingredient, used to improve taste and increase shelf life.² It is difficult to determine which juices have additional synthetic citric acid, which involves the analysis of isotopic forms of the acid,³ and these higher concentrations can have unwanted effects on tooth enamel. These effects may be worse than soft drinks or sugar because the acid affects the whole mouth equally and the effects cannot be felt like sugar coating the teeth.⁴ A 2009 study published in the Journal of Dentistry showed that high levels of citric acid in orange juice reduced the hardness of enamel by 84%.⁵

According to literature sources, ready-to-drink orange juices contain around 16 g/L of total citric acid, while juice from actual oranges contains less, around 9 g/L.⁶ Factors that contribute to differences in citric acid concentrations in oranges include fruit variety, degree of maturity, geographical location, climate zone, and soil conditions.³ Citric acid dependence in orange juices is more difficult to define because the manufacturer can add additional citric acid. Other factors include the percentage of fresh orange juice, orange juice concentrate, and the addition of other fruit juices such as pear, grape, or apple.

The most popular method for quantifying citric acid concentrations in juices is reverse-phase high-performance liquid chromatography (HPLC).^{7,8} With all organic acids, including citric acid, phase collapse may be an issue because these polar compounds separate best in purely aqueous mobile phases.⁹ If the surfaces of the alkyl chains dewet it becomes harder for the polar compounds to adsorb onto the stationary phase, when this happens the phase is said to have collapsed. Indicators of this phenomenon are erratic chromatograms with ultimate retention loss.⁹ To overcome this issue, the column can be regenerated by rinsing with high concentrations of organic solvent, but collapse will likely reoccur. Another solution is to obtain an Ultra-Aqueous or Water-Compatible Allure™ column.¹⁰ These columns are manufactured to preserve retention in up to 100% aqueous

mobile phases. Organic acids can also be determined by ion chromatography (IC) or capillary electrophoresis (CE).¹¹

Experimental

Preparation of Mobile Phase

A 50-mM phosphate buffer solution to use as the mobile phase was prepared by dissolving 6.82 g of potassium phosphate monobasic (Fisher) in deionized water. The pH of the buffer was then adjusted to 2.80 by adding concentrated phosphoric acid drop-wise while monitoring with a calibrated pH meter. The solution was then diluted to 1.00 L with deionized water. To obtain an HPLC grade mobile phase the solution was filtered through a 0.22- μm nylon membrane by vacuum filtration. HPLC grade methanol was used before and after the experiment to prevent phase collapse.

Standard Preparation

Citric acid standards of 100, 200, 300, 500, and 1000 ppm were prepared from granular citric acid (Fisher) and deionized water. These were then transferred to autosampler vials for HPLC analysis.

Preparation of Samples

Eight orange juices were purchased at local supermarkets. Four were 100% orange juice: Kemps, Langers, Minute Maid, and Tropicana. Two were orange flavored drinks: Sunny D and Tang, and one was 100% mixed fruit juice: Juicy Juice. A navel orange from California was also used.

From each bottled juice a 10.00-mL aliquot was transferred to a 50-mL volumetric flask. The Tang, which had to be dissolved, was prepared by dissolving 1.01 g into a 50-mL volumetric flask. This mass was equivalent to a 10-mL sample of Tang prepared according to the instructions on the label. The navel orange sample was prepared by juicing an orange and then pipetting 10 mL of the liquid into a 50-mL volumetric flask. All 50-mL flasks were diluted to volume with deionized water. All dilute samples were filtered through a 0.45- μm filter membrane before HPLC analysis.

After an initial analysis by HPLC, all of the juices were further diluted by transferring 10 mL of the once diluted samples into new 50-mL volumetric flasks and diluting to volume with deionized water. This provided peaks that would be more accurately analyzed than the more highly concentrated samples except for Juicy Juice, which was kept at the higher concentration level for final analysis.

Instrument Preparation

An isocratic elution was carried out on a Varian ProStar HPLC system with a 50-mm \times 4.6-mm Kinetex XB-C18 column (Phenomenex) with a 2.6- μm particle size and 100- \AA pore size. The mobile phase was 100% 50-mM phosphate buffer at pH 2.80. The HPLC system operated at a flow rate of 0.5 mL/min, the injection volume was 5 μL , and the

external temperature control column oven was set at 35°C. The detection wavelength was 214 nm.

Results and discussion

Standard Calibration

Figure 1 displays the overlaid standard chromatograms of citric acid, which eluted from the column at 2.16 min. Based on the integrated peak areas from the HPLC chromatogram, a standard calibration plot was created for citric acid as seen in Fig. 2.

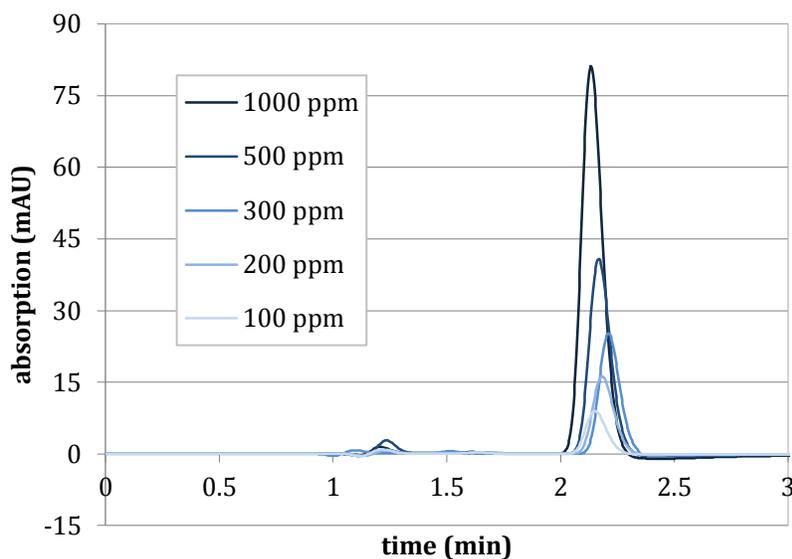


Figure 1. Chromatogram produced from citric acid standards: 100, 200, 300, 500, and 1000 ppm.

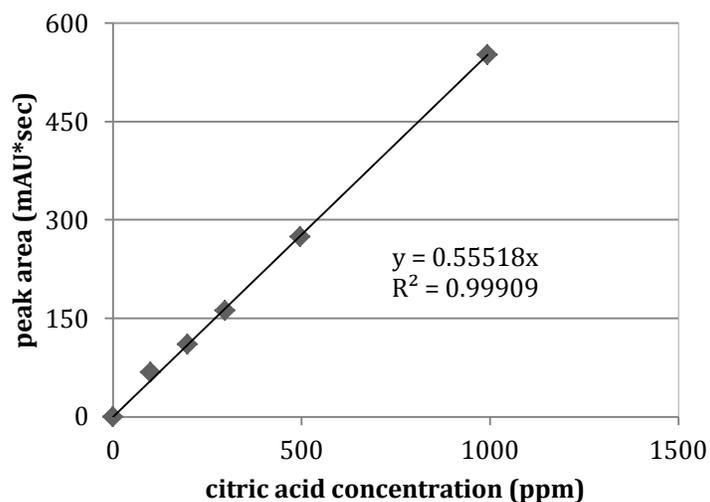


Figure 2. Calibration plot for citric acid.

Sample Analysis

Figure 2 shows a typical juice chromatogram, the peak at 2.16 min is citric acid. The concentration of citric acid was determined in each sample by integrating the citric acid peak and comparing that value to values in the linear regression curve in Fig. 2. Calculations were then made to determine the original citric acid concentration in the undiluted sample as seen in Table 1 and Fig. 4. The calculations were different for Juicy Juice because of its differing dilution factor. From these citric acid concentrations the juices could be compared with literature values for citric acid levels in orange juices.

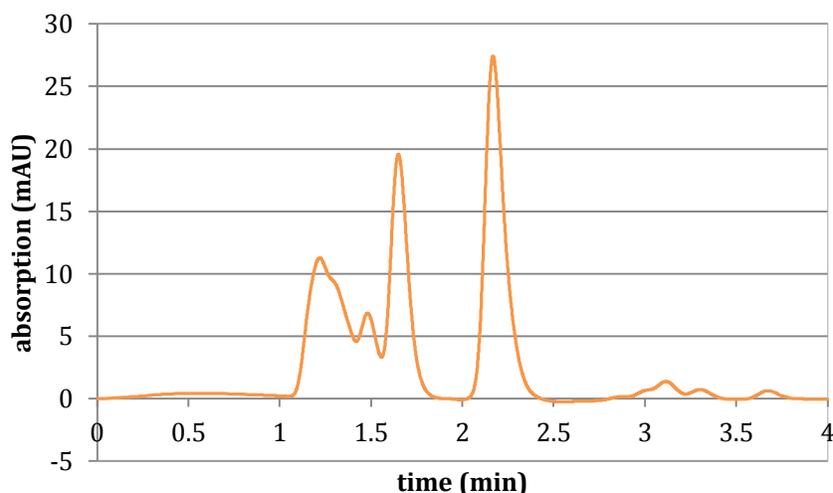


Figure 3. Chromatogram of Tropicana orange juice.

The literature reports citric acid concentrations of 499.8 mg/oz for bottled orange juices and 269.1 mg/oz for juiced oranges.⁵ Although in that particular experiment many juices were analyzed, only two different orange juices were sampled. The juices in this experiment ranged from 241.4 to 340.9 mg/oz for 100% orange juice drinks, 186.0 to 198.7 mg/oz for orange flavored drinks. The navel orange contained 426.8 mg/oz of citric acid, and the mixed fruit juice contained 36.01 mg/oz. Possible explanations for the variance in concentrations include the processing of the juice. The juices varied in actual juice percentage as well. Sunny D claimed 5% juice and there was no actual juice in Tang. The other juices claimed to be 100% juice, some being from concentrate. The navel orange had the highest citric acid concentration, but per serving it is actually much lower in comparison to the juices because the average orange contains approximately 2 oz. of juice, much less than the 6 to 12 oz. fruit juice servings. The Juicy Juice was expected to have a much lower concentration because it is not 100% orange juice, it also includes apple, pear, grape, and tangerine juices with orange juice listed fourth on the ingredients label. It was not possible to determine how much citric acid was natural verses synthetic because isotopically different compounds cannot be separated in HPLC. According to the ingredients labels on the juices only Juicy Juice, Sunny D, and Tang contain added citric acid. This is likely due to the lower percentages of actual orange juice in these samples. By adding citric acid to the juices the manufacturers are enhancing the flavor and improving the shelf life.

Table 1. Citric acid concentrations in juice samples.

Sample	n	mean (mg/oz)	std dev	% RSD
Juicy Juice	1	36	-	-
Tang	2	186	1.60	9.86
Sunny D	2	198.6	0.85	0.43
Minute Maid	3	240	10.2	4.27
Kemps	2	270	26.6	9.86
Tropicana	2	277	3.11	1.12
Langers	2	341	1.60	0.86
navel orange (CA)	2	427	6.87	1.61

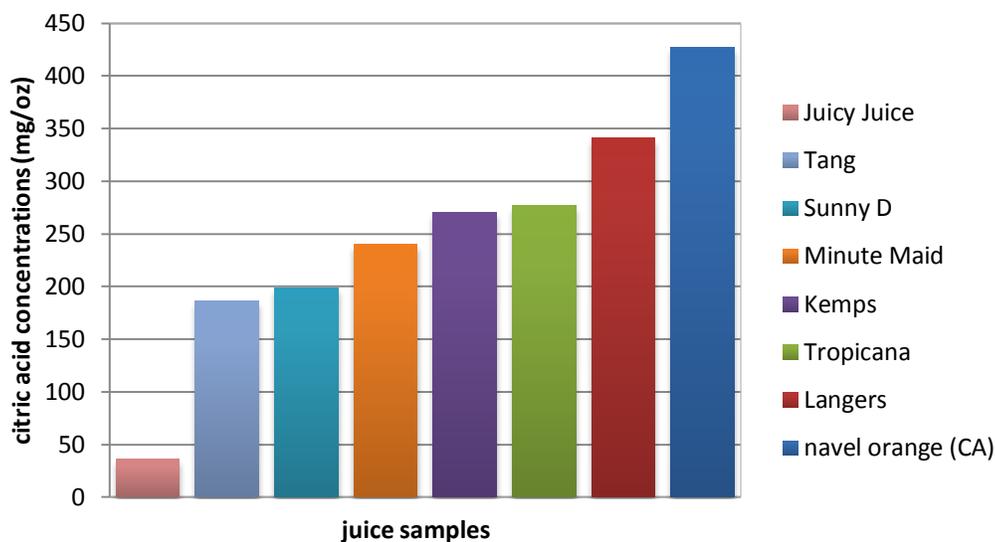


Figure 4. Concentration of citric acid for each juice sample.

Conclusions

This experiment utilized external standards and high-performance liquid chromatography to determine the amount of citric acid in various orange juices, orange flavored drinks, and a navel orange. The navel orange had the highest citric acid concentration, followed by the 100% orange juices, the orange flavored juices, and then the 100% mixed fruit juice. Although the total citric acid concentration was determined, differentiating between natural and added manufactured citric acid was not possible. Phase collapse was not an issue until the third analysis of the juices at which point the experiment was stopped to rewet the column. To determine multiple organic acids simultaneously it is

recommended to obtain a column designed for highly soluble aqueous mobile phases and samples.

References

- 1) European Citric Acid Manufacturers Association. Citric Acid Applications: Soft Drinks and Beverages. http://www.ecama.org/level_2/applic/softdrinks1.htm (accessed April 14, 2012).
- 2) Sass-Kiss, A.; Toth-Markus, M.; Sass, M. Chemical Composition of Citrus Fruits (Orange, Lemon, and Grapefruit) with Respect to Quality Control of Juice Products. *Nutraceutical Beverages*, **2004**, 871, 24-34.
- 3) Schmidt, H.; Weber, D.; Rossmann, A.; Werner, R. The Potential of Intermolecular and Intramolecular Isotopic Correlations for Authenticity Control. In *Flavor Chemistry: 30 Years of Progress*; Teranishi, R.; Wick, E.; Hornstein, I. Kluwer Academic/Plenum: New York, 1999; pp. 55-57.
- 4) Park, M. Juices, Tea and Energy Drinks Erode Teeth. *CNN*, July 2009, http://articles.cnn.com/2009-07-23/health/teeth.erosion.drinks_1_energy-drinks-carbonated-sports-drinks?_s=PM:HEALTH (accessed April 14, 2012).
- 5) Ren, Y.-F.; Amin, A.; Malmstrom, H. Effects of Tooth Whitening and Orange Juice on Surface Properties of Dental Enamel. *J. Dent.* **2009**, 37, 424-431
- 6) Penniston, K. L.; Nakada, S. Y.; Holmes, R. P.; Assimos, D. G. Quantitative Assessment of Citric Acid in Lemon Juice, Lime Juice, and Commercially-Available Fruit Juice Products. *J. Endourol.* **2008**, 22, 567-570
- 7) Shui, G.; Leong, L.P. Separation and Determination of Organic Acids and Phenolic Compounds in Fruit Juices and Drinks by High-Performance Liquid Chromatography. *J. Chromatogr. A.* **2002**, 977, 89-96.
- 8) Nour, V.; Trandafir, I.; Ionica, M. E. HPLC Organic Acid Analysis in Different Citrus Juices under Reversed Phase Conditions. *Not. Bot. Hort. Agrobot. Cluj.* **2010**, 38, 44-48.
- 9) Przybyciel, M.; Majors, R. E. Phase Collapse in Reversed-Phased LC. *LCCG North America*, **2002**, 20, 516-523
- 10) Kowalski, J.; Wittring, B. Simple, Reliable HPLC Analyses of Organic Acids. Restek. http://www.restek.com/Technical-Resources/Technical-Library/Foods-Flavors-Fragrances/fff_A011 (accessed April 10, 2012).
- 11) Peres, R.; Moraes, E.; Micke, G.; Tonin, F.; Tavares, M.; Rodriguez-Amaya, D. Rapid Method for the Determination of Organic Acids in Wine by Capillary Electrophoresis with Indirect UV Detection. *Food Control.* **2009**, 20, 548-552.