Capillary Electrophoresis for Evaluating Orange Juice Authenticity: a Study on Spanish Oranges

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Fruit juices have very distinct organic acid profiles that can be used as fingerprints for establishing possible adulteration. Recently, our group developed and validated a capillary electrophoresis method using UV detection for determining citric, isocitric, tartaric, and malic acids in natural and commercial orange juices. Sample treatment consisted of only dilution and centrifugation or filtration. This method has been applied to evaluate these acids and their ratios in 63 samples of Navelina, the most common variety of Spanish oranges, over a three month period. This evaluation has been conducted to establish ranges of acid concentrations and to compare them with those found in commercial juices. The more reliable parameter, because of the lower variability in fresh samples, was found to be the citrate/isocitrate ratio with a value of 113 (RSD = 10%). Only one of nine ramdonly selected commercial juices presented values within the range of those of the population of just-pressed Navelina orange juice. Moreover, three of them had measurable tartrate values, which is not a natural component of orange juice, showing mixtures with cheaper fruits.

Keywords: *Orange juice; adulteration; organic acids; capillary electrophoresis*

INTRODUCTION

The economic importance of identifying fraudulence related to orange juice adulteration is enormous, because of the large quantities consumed in the world. It is apparent that the development of methods to easily detect these adulterations must be a continuing process. Much work has been done, but unscrupulous formulators use adulteration methods that are just as sophisticated as the methods the analytical chemist uses to detect adulteration, and new parameters must be evaluated.

The most frequent types of adulteration have been identified by Royo (1969):

1. The addition of substances which are natural constituents of orange juice but that come from other sources. The simplest type of adulteration is likely to be the addition to the pure juice of a solution of sugar and citric acid.

2. The addition of extraneous constituents (colorants, tartaric acid, etc.).

3. The addition of cheaper juices coming from other fruits, mainly grapefruit.

Moreover, when duty-free laws are enacted to aid the economic development of countries, methods for determining the geographical origin of an orange juice are necessary.

Because of the diversity in adulteration techniques, a matrix of text methods may be necessary (Robards and Antolovich, 1995; Navarro and Izquierdo, 1988) and, among them, organic acids measurement is always considered (Lee, 1993; Saccani et al., 1995). Fruit juices each have very distinct organic acid profiles that can be used as fingerprints for establishing authenticity. HPLC methods that compare ratios of the major acid

components have been developed (Antolín Maté and Lugar Abril, 1984; Lee, 1993; Shaw and Wilson, 1983; Coppola, 1984); however, this is of limited value in most cases because synthetic organic acids can be added so that the ratios are consistent with those found in a pure natural juice. Minor acids analysis is a more powerful technique (Tisza and Molnár-Perl, 1994), because it is not economically feasible for adulterators to adjust the levels of all acid components, many of which are expensive (Henshall, 1998).

Grapefruit added to orange juice can be detected by tartaric acid. Isocitrate, existing in natural orange juices in small but constant amounts, has been used as an adulteration indicator (Park, 1983; Robards and Antolovich, 1995) and the use of ratios in quality control may be a more sensitive means of detecting additives.

Several HPLC methods (Schwarzenbach, 1982; Saccani et al., 1995) have substituted previous gas chromatography methods which needed extraction and derivatization steps. But generally they do not completely achieve the intended separations (Lee, 1993).

Capillary electrophoresis (CE) has proved to be a good choice for investigation of samples in aqueous media. Recently, our work group has developed and validated a capillary electrophoresis method for determining citric, isocitric, tartaric, and malic acids in natural and commercial orange juices by capillary electrophoresis with direct UV detection. Sample preparation required only dilution and filtration (Saavedra et al., 2000).

The aim of the present work was to apply this method to evaluate a number of natural orange juices with a population large enough to evaluate these acids and to establish their ratios and ranges. These values would be compared with those found in commercial juices. These data could be used to measure adulteration markers in Spanish orange juices.

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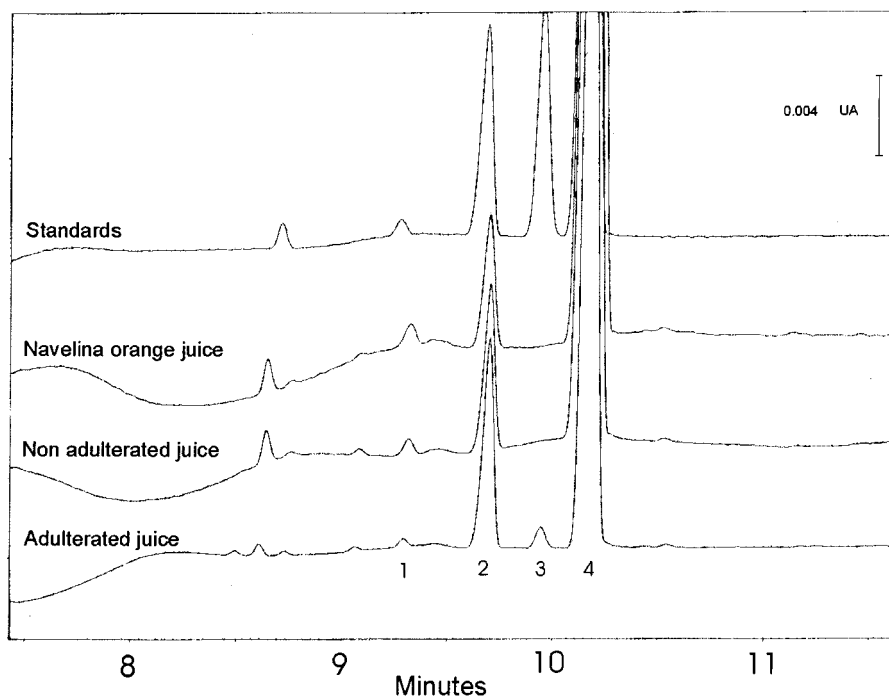
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Figure 1. From top to bottom, electropherograms: a standard solution; just-pressed Navelina oranges, which provide the typical orange juice organic acids profile; commercial orange juice non adulterated and adulterated, respectively. Adulteration can be seen by mean of the drop of isocitric amount and the presence of tartaric acid. Peaks assignment: 1, isocitrate; 2, malate; 3, tartrate; and 4, citrate.

MATERIALS AND METHODS

Instrumentation. The separation was performed on a capillary electrophoresis P/ACE (Beckman, Palo Alto, CA) with UV detection at 200 ± 10 nm. The injection was by pressure for 5 s (20 psi). The neutral capillary was polyacrilamide coated (Beckman, Madrid, Spain), 57 cm long and 50 μm i.d., and was operated at -14 kV potential. The electrolyte used was phosphate buffer 200 mM, pH 7.50, prepared from phosphoric acid and NaOH. Between runs the capillary was rinsed with the separation electrolyte for four minutes.

Chemicals. Standards were obtained from Sigma (St. Louis, MO). Phosphoric acid 85% was from Merck (Darmstadt, Germany), and sodium hydroxide was from Panreac (Madrid, Spain).

Samples. Fresh-pressed orange juices were obtained from Navelina variety, which accounts for approximately 70% of the total orange production in Spain (Ministerio de agricultura pesca y alimentación, 1999). The oranges were purchased from different origins, but mainly Valencia (Spain), from November through February. Oranges were maintained at room temperature and measured before one week. Sixteen 2-kg samples were pressed. Four aliquots of 10 mL from each sample were centrifuged at 2000g for 20 min at 20 °C, diluted 1:1, and measured. Centrifugation was performed prior to filtration in samples containing suspended pulp, mainly fresh-pressed samples. Centrifugation considerably facilitates filtration, and during the validation of the method it was demonstrated that it does not change the results.

Commercial samples used for testing were obtained from the many brands available in the market, and they were named with letters. Three of them were purchased over four different months to study their variability. Samples were diluted as 1:1 ratio with milli-Q purified water (Millipore Ibérica, Madrid, Spain) before its filtration for the measurement. Filtration was made with acetate filters (MSI, Minnetonka, MN) of 0.45-μm pore size.

Statistics. Every statistical analysis has been performed with the statistical software SPSS 9.0 (SPSS Inc., Chicago, IL).
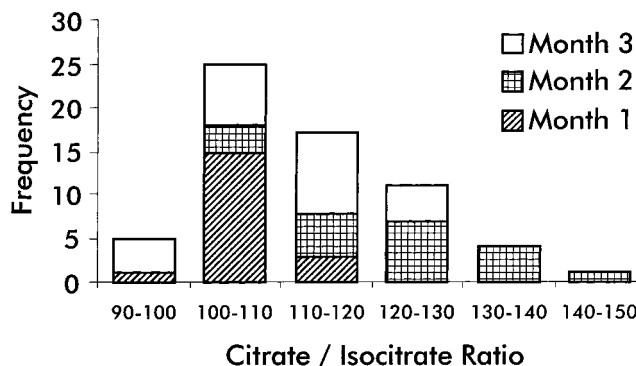

Figure 2. Frequencies distribution plot, cut by months, for Navelina studied population. Results follow a normal distribution (Shapiro-Wilk's test, p value $= 0.13$ for $\alpha = 0.1$ as significance level).

RESULTS AND DISCUSSION

The electropherograms in Figure 1 (from top to bottom) show the characteristic profiles for isocitric, malic, tartaric, and citric acids in a standard mixture, fresh-pressed orange juice, and two mass market orange juices, respectively. The last electropherogram includes tartaric acid, which is not present in natural orange juice and a lower isocitric acid. It can be seen that, despite the complexity of the matrixes and considering the imbalance between the different anions, the technique allows the measurement of the four acids with acceptable validation parameters of the method (Saavedra et al., 2000).

Results for isocitric, malic, tartaric, and citric acids in the assayed samples are shown in Table 1 for individual acids and in Table 2 for their ratios. Table 3 shows the values reported in the bibliography for oranges coming from different countries. Values found with the capillary electrophoresis technique for Na-

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Table 1. Individual Values for Isocitric, Malic, Tartaric, and Citric Acids in Different Juices

		isocitrate (g/L)	malate (g/L)	tartrate (g/L)	citrate (g/L)
navelina (n = 63)	mean	0.11	1.31	ND	11.72
	standard deviation	0.03	0.29		2.30
	RSD	25.9	21.7		19.6
orange juice D (n = 4)	mean	0.05	1.98	0.06	8.1
	standard deviation	0.02	0.34	0.03	2.25
	RSD	41.0	19.2	52.0	27.7
orange juice H (n = 4)	mean	0.08	1.42	ND	10.56
	standard deviation	0.006	0.27		0.50
	RSD	6.8	19.5		4.8
orange juice I (n = 4)	mean	0.04	1.97	ND	7.62
	standard deviation	0.002	0.03		0.15
	RSD	4.9	1.3		1.9
orange juice A (n = 1)		0.04	1.62	0.13	7.05
orange juice B (n = 1)		0.04	1.86	ND	8.25
orange juice C (n = 1)		0.05	1.60	ND	8.53
orange juice E (n = 1)		0.05	1.26	0.01	7.43
orange juice F (n = 1)		0.04	0.85	ND	6.49
orange juice G (n = 1)		0.05	2.09	ND	7.69

Table 2. Ratio Between Acids for All Analyzed Samples

		citrate/isocitrate ratio	citrate/malate ratio	malate/isocitrate ratio
navelina oranges (n = 63)	mean	113.1	9.5	13.8
	standard deviation	11.3	3.4	6.1
	RSD	10	36	44
orange juice D (n = 4)	mean	161.3	4.6	36.5
	standard deviation	22.9	0.8	10.9
	RSD	14.2	17.8	29.7
orange juice H (n = 4)	mean	124.1	7.7	16.8
	standard deviation	3.5	1.7	3.6
	RSD	2.8	21.9	22.7
orange juice I (n = 4)	mean	175.7	3.9	45.4
	standard deviation	5.8	0.1	2.4
	RSD	3.3	2.3	5.3
orange juice A (n = 1)		156.6	4.4	35.8
orange juice B (n = 1)		192.0	4.3	44.5
orange juice C (n = 1)		156.3	5.3	29.4
orange juice E (n = 1)		161.9	5.7	28.4
orange juice F (n = 1)		165.8	8.1	20.4
orange juice G (n = 1)		164.9	3.7	45.0

Table 3. Mean Values ± Confidence Intervals Described in the Bibliography for Different Samples of Different Origins

origin	isocitrate (g/L)	malate (g/L)	citrate (g/L)	reference
Spain	0.1 ± 0.02	1.8 ± 0.3	12.6 ± 2	Bergner-Lang, 1977
Spain	0.115 ± 0.005		10.9 ± 0.5	Aristoy et al., 1989
California	0.077	0.9	9.6	Park et al., 1983
Mediterranean basin	0.16 ± 0.03	1.2 ± 0.3	11.5 ± 1.1	Saccani et al., 1995
USA and Cuba	0.09 ± 0.02	1.6 ± 0.2	8.4 ± 1.2	Saccani et al., 1995
others South America	0.11 ± 0.02	1.7 ± 0.1	11.4 ± 1.5	Saccani et al., 1995
Spain	0.111 ± 0.003			Navarro and Izquierdo, 1988
Israel	0.129 ± 0.003	1.1 ± 0.04		Cohen, 1988

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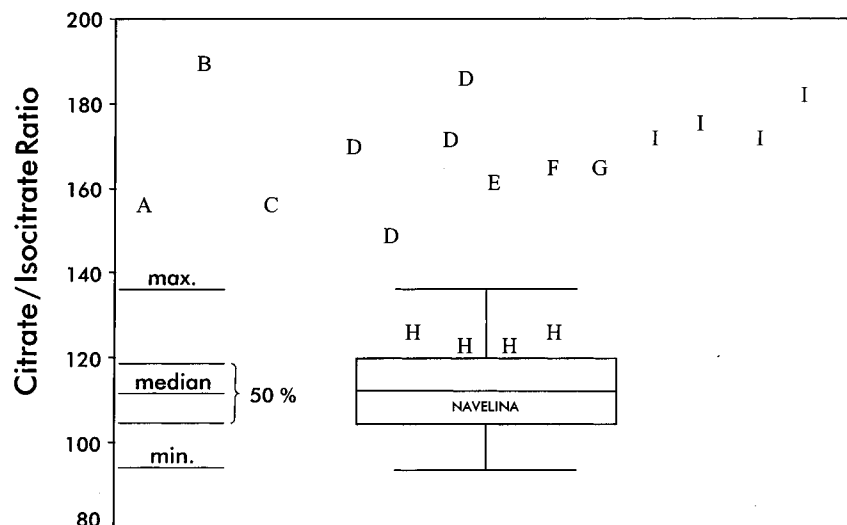
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Figure 3. Box-and-whisker plot for Navelina studied population. The box contains the 50% of the population, and whiskers mark both highest and lowest non influentialvalues. The superposed letters correspond to the values of several commercial orange juices as described in text.

velina oranges are within the ranges described. Furthermore, it must be considered that the values of RSDs (or CVs) are not due to the method but to the natural variability of samples, and it was one of the parameters to be evaluated. As can be seen, the citrate/isocitrate ratio has the lowest RSD, which makes that parameter more reliable to establish regulations.

Frequencies of the citrate/isocitrate ratio for Navelina oranges during three months were studied to evaluate normality of data displayed in Figure 2. Among the many tests developed for this purpose, the most commonly used is the Shapiro Wilk's test (Shapiro and Wilk, 1965). With this test, hypothesis of normal distribution cannot be discarded with a 90% level of confidence. In practice, that indicates that the usual tests for comparison of means that suppose normal distribution are applicable (p value = 0.13).

On the other hand, Figure 3 shows the box-and-whisker plot for that population and over it the values obtained for commercial orange juices, indicated with letters. This histogram-like method of displaying data was proposed by J. Tukey (1977). It consists of a box with ends at the quartiles Q_1 and Q_3, with the median drawn as a horizontal line inside. The "whiskers" extend to the farthest data. For every data point that is more than $3/2$ times the interquartile range from the end of a box, a dot is drawn on the corresponding top or bottom of the whisker. If two dots have the same value, they are plotted side by side.

Only one of juices presents citrate/isocitrate values within the range of the population of fresh-pressed Navelina orange juice. That could be in agreement with their publicity which says that the juice is prepared just by pressing, and is not from concentrate. All the others have higher values than Navelina oranges for this ratio and some of them present different amounts of tartaric acid, which is not natural in oranges.

The simplest statistical approach in fruit juice quality control is to determine the acceptable range for each attribute with its limits, and to compare a tested specimen with standards. Therefore, to be able to detect adulterations, it is first necessary to have a detailed insight into the composition of authentic juices and their variations.

CONCLUSION

Capillary electrophoresis is a helpful tool for investigating the organic acid profile in orange juices to use as an adulteration index. The lack of specific and nonvarying properties has forced investigators to base purity control of orange juices on the analysis of many characteristics. Nevertheless, the time consumed in the analysis is a strong limitation, and only improved instrumentation will allow the establishment of purity control norms.

Present research has been focused on the simple and quick acquisition of data on several organic acids, but many possibilities remain to be explored on the applicability of capillary electrophoresis in this area.

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