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HPLC DETERMINATION OF ORGANIC ACIDS IN MOROCCAN APRICOT

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Abstract

Organic acids content of Moroccan apricot (*Prunus armeniaca L.*) *var. Canino* were separated and measured by HPLC. Good chromatograms were obtained by coupling three RP18 columns of 25 cm of length. The mobile phase was adjusted at an optimum pH of 2.15. The absorbance at 210 nm, measured with a diode array UV detector, was used for quantification. The method is quantitative, with recoveries in the range 95.7-103.5%. Study of the repeatability gives coefficients of variation lower than 3.2 %. The proposed procedure can be considered to be a rapid method for organic acids determination in apricot with satisfactory repeatability.

Key words: Organic acids, apricot, HPLC, reversed phase

Resumen

Se separaron y cuantificaron los ácidos orgánicos de albaricoque de Marruecos (*Prunus armeniaca L.*) *var. Canino* por HPLC. Se obtuvieron buenos cromatogramas acoplando 3 columnas RP18 de 25 cm de longitud. La fase móvil se ajustó a un pH óptimo de 2,15. La absorbancia a 210 nm, medida con un detector de diodos UV, se usó para la cuantificación. El método es cuantitativo, con recuperaciones en el intervalo 95,7-103,5%. El estudio de reproducibilidad dio coeficientes de variación menores de 3,2 %. El procedimiento propuesto se puede considerar un método rápido para la determinación de ácidos orgánicos en albaricoque con una satisfactoria reproducibilidad.

Palabras clave: Ácidos orgánicos, albaricoque, HPLC, fase reversa

Resumo

Separáronse e cuantificáronse os ácidos orgánicos de albaricoque de Marrocos (*Prunus armeniaca L.*) *var. Canino* por HPLC. Obtivéronse bos cromatogramas acolando 3 columnas RP18 de 25 cm de lonxitude. A fase móbil axustouse á unha pH óptima de 2,15. A absorbencia a 210 nm, medida cun detector de díodos UV, usouse para a cuantificación. O método é cuantitativo, con recuperacións nun intervalo de 95,7-103,5%. O estudio de reproducibilidade dou coeficientes de variación menores de 3,2 %. O procedemento proposto pódese considerar un método rápido para a determinación de ácidos orgánicos en albaricoque cunha satisfactoria reproducibilidade.

Palabras clave: Ácidos orgánicos, albaricoque, HPLC, fase reversa

INTRODUCTION

The identification of quantitative analysis of major organic acids in fruit is considered very important for food and beverage technology and quality evaluation. These acids often influence flavor, stability, and keeping quality and have been proposed as an index of maturity, ripeness, or spoilage in some foods (Gregory 1988; Jezek and Suhaj 2001; Jian 1996; Saavedra *et al*., 2000; Trifiro *et al*., 1997).

Besides analytical methods involving colorimetric reaction and enzyme assays, chromatographic techniques allow simultaneous analysis of most of the organic acids. In this field, high performance liquid chromatography (HPLC) is one of the more promising and more used techniques, either by direct determination or by the analysis of derivatized products. Most of procedures developed until now for food and beverage analysis utilize either reverse phase partition chromatography (Czajkowska and Jaroniec 1997; Fransson and Ragnarsson 1998; Goiffon *et al.*, 1985) or ion exchange chromatography (Casella and Gatt 2001; Guillèn *et al*., 1998, Linget *et al.*, 1998; Morales *et al.*, 1998; Toofan *et al.*, 1997; Trifiro *et al.*, 1997) with refractive index (RI), UV spectrophotometric, conductimetric or electrochemical detection (Buchberger, 2000).

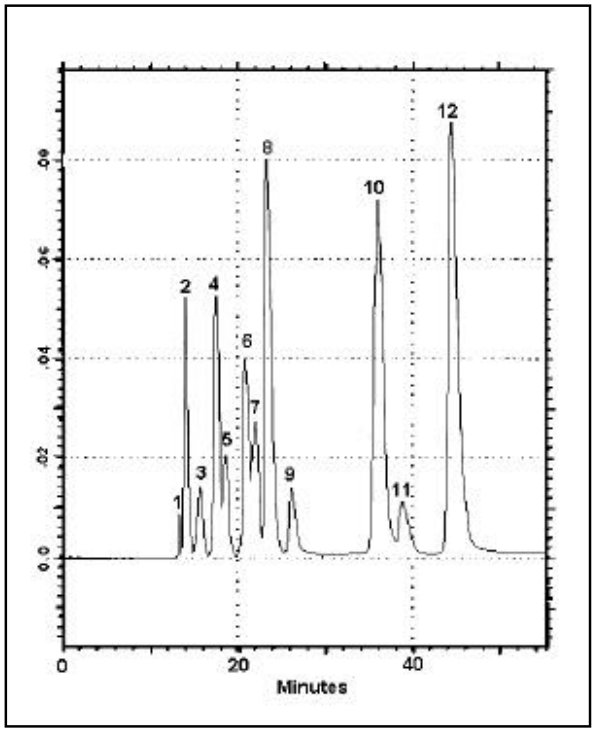

Figure 1. Chromatogram of organic acids standard mixture: Three 25 cm RP18 columns connected. Mobile phase: Water + H_3PO_4 at pH 2,15 and debit: 0,5 ml/minute. Detector : UV at 210 nm. Temperature 25°C. 1: Unretined compounds, 2 : Oxalic (50,6 mg/L), 3 : Galacturonic (580 mg/L), 4 : Tartaric (500 mg/L), 5 : Quinic (550 mg/L), 6 : Malic (1000 mg/L), 7 : Malonic (520 mg/L), 8 : Shikimic (35 mg/L), 9 : Acetic (570 mg/L), 10: Citric (2100 mg/L), 11 : Succinic (550 mg/L), 12 : Fumaric (22 mg/L)

This work is a contribution to the development of a rapid and precise HPLC procedure for quantitative determination of organic acids of Moroccan apricot using a reverse phase column and UV absorption detector.

MATERIAL AND METHODS

Sample preparation

The organic acids of Moroccan apricot (*Prunus armeniaca L.*) *var. Canino* were extracted by distilled water (1:10, w/v), clarified by centrifuging at 3150 x g for 10 minutes and the supernatant was membrane filtered (0.45 µm) before injection.

Analysis by HPLC

The organic acids extracted from apricot were separated using a reverse phase column. The optimum pH obtained was 2.15 at 25°C. Under these conditions, the best approach for complete resolution of all peaks would be an increase in column length, thus increasing the number of theoretical plates. Good chromatograms were obtained by coupling three RP18 columns of 25 cm of length.

For analysis, 10 µL of the prepared sample or standard solution were injected into a GILSON liquid chromatograph equipped with a diode array UV detector (CRYSTAL 240) and analyzed using three coupled Lichrosorb RP18 columns (25 x 0.46 cm). The columns were operated at a temperature of 25°C. The mobile phase (flow rate 0.5 mL/min) was consisted of acidified distilled water adjusted to pH 2.17 with phosphoric acid (SDS, 85% analytical grade), filtered and degassed before use. Eluting compounds were detected at 210 nm.

RESULTS AND DISCUSSION

Figure 1 shows the separation of 11 organic acids in a standard mixture solution. Table 1 lists retention times (RT) of various separated acids.

Linearity of the method was evaluated, with area response. Concentration ranges of lineal response for standard organic acids are shown in Table 2. The

Table 1.- Retention times (RT) values of various organic acids.

	Organic acids	RT (min)
1	Unretined compounds	13.30
2	Oxalic	14.41
3	Galacturonic	15.80
4	Tartaric	17.67
5	Quinic	18.60
6	Malic	20.90
7	Malonic	22.09
8	Shikimic	23.49
9	Acetic	26.28
10	Citric	36.28
11	Succinic	39.07
12	Fumaric	44.65

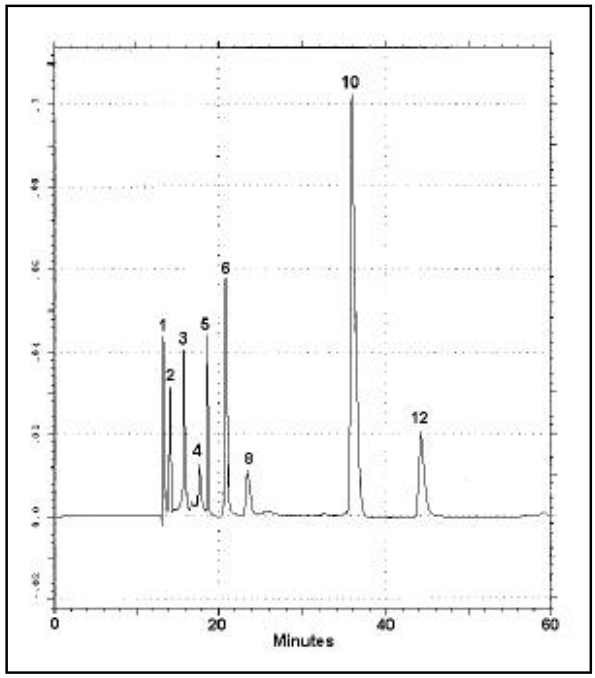

Figure 2. Chromatogram of apricot extract (1:10) (w/v).Three 25-cm RP18 columns connected. Mobile phase: Water + H_3PO_4 in pH 2,15 and debit: 0,5 ml/min. Detector: UV at 210 nm. Temperature 25°C. 1: Unretined compounds, 2 : Oxalic acid, 3 : Galacturonic acid, 4 : Tartaric acid, 5 : Quinic acid, 6 : Malic acid, 8 : Shikimic acid, 10: Citric acid, 12 : Fumaric acid.

Table 4.- Recovery of organic acids from apricot extract.

Organic acids	Added (mg/L)	Found (mg/L)	Recovery (%)
Malic acid	0.0	585.2	
Malic acid	91.4	692.6	102.4
Malic acid	250.0	863.2	103.4
Shikimic acid	0.0	2.4	
Shikimic acid	3.3	5.6	97.0
Shikimic acid	34.4	37.2	101.0
Citric acid	0.0	1644.5	
Citric acid	223.2	1892.5	101.3
Citric acid	500.4	2162.8	100.8
Fumaric acid	0.0	4.1	
Fumaric acid	3.1	7.2	100.1
Fumaric acid	10.2	14.6	102.1
Oxalic acid	0	4.8	
Oxalic acid	5.0	9.5	96.7
Oxalic acid	10.1	14.7	98.6
Galacturonic acid	0.0	691.0	
Galacturonic acid	100.3	773.3	97.7
Galacturonic acid	251.4	924.4	98.1
Tartaric acid	0.0	550.0	
Tartaric acid	102.3	662.3	101.5
Tartaric acid	254.2	832.2	103.5
Quinic acid	0.0	330.0	
Quinic acid	98.6	436.3	101.8
Quinic acid	250.2	555.2	95.7

Table 2.- Concentration ranges of lineal response for standard organic acids.

Acids	Concentration range (mg/L)	Response factor	Correlation coefficient
Oxalic	5 -100	1.5E-03	0.9997
Galacturonic	115 -1150	1.5E-05	0.9992
Tartaric	13 - 650	6.2E-05	0.9996
Quinic	100 - 550	2.1E-05	0.9995
Malic	50 -1000	3.2E-05	0.9997
Shikimic	6.9 - 69	2.0E-03	1.0000
Citric	508 -10155	4.1E-05	0.9994
Fumaric	5.5 -110	4.2E-03	0.9997

calibration graphs were linear over these concentration ranges with correlation coefficients higher than 0.999. Although the response factor was different from acid to acid. Hence, the quantitative analysis of the major acids was carried out with the external standard method.

The used method was applied to various samples of the variety of apricot the most cultivated in Morocco. These samples are from different areas in the central Moroccan region (*Tadla*). Under the described conditions, the major organic acids of apricot elute within 50 minutes.

Figure 2 illustrates the chromatographic separation of organic acids extracted from apricot.

Table 3.- Repeatability of organic acids concentrations in Moroccan apricot. % C.V.: % Coefficient of variation; % C.I.: % Confidence interval.

	Oxalic (mg/Kg)	Galacturonic (g/Kg)	Tartaric (g/Kg)	Quinic (g/Kg)	Malic (g/Kg)	Shikimic (mg/Kg)	Citric (g/Kg)	Fumaric (mg/Kg)
1	8.33	10.14	0.33	8.61	7.45	31.68	17.01	21.38
2	8.47	10.59	0.34	8.15	7.70	32.29	16.82	21.51
3	8.19	10.72	0.35	8.20	7.55	33.71	17.32	22.36
4	7.84	10.62	0.34	7.87	7.47	31.47	16.37	21.3
5	7.92	10.18	0.33	8.13	7.79	31.38	16.67	20.78
6	8.13	10.89	0.35	7.92	7.34	32.04	16.89	21.76
Average	8.15	10.52	0.34	8.15	7.39	31.86	16.95	21.57
% C.V.	2.93	2.85	3.18	3.24	2.27	2.71	1.89	2.43
% I.C.	2.34	2.28	2.55	2.59	1.82	2.17	1.51	1.95

Table 5.- Organic acids content of Canino variety of Moroccan apricot.

	Oxalic (mg/Kg)	Galacturonic (g/Kg)	Tartaric (g/Kg)	Quinic (g/Kg)	Malic (g/Kg)	Shikimic (mg/Kg)	Citric (g/Kg)	Fumaric (mg/Kg)
1	30.05	7.21	0.42	2.43	5.17	20.28	15.13	21.46
2	8.15	10.52	0.34	8.15	7.39	31.86	16.95	21.57
3	40.91	6.01	0.51	4.32	7.54	22.75	16.95	31.07
4	18.13	9.52	0.31	6.14	6.23	18.60	15.94	26.03
5	28.22	6.62	0.45	4.56	6.52	27.50	17.05	24.62
6	15.68	8.52	0.34	5.61	6.74	21.80	15.19	25.70
7	28.17	7.25	0.36	3.21	5.76	24.50	15.25	21.07
8	28.35	6.35	0.41	4.56	6.35	25.85	16.52	25.08
9	32.07	7.26	0.34	2.34	7.01	25.75	16.85	22.14
10	28.11	6.58	0.39	4.56	6.96	22.64	16.21	19.15
11	47.91	6.91	0.55	3.30	5.85	24.15	16.44	40.69
12	32.07	6.21	0.46	3.58	5.70	19.68	15.19	20.64
Average	28.15	7.41	0.41	4.58	6.44	23.78	16.14	24.94
Ecartype	10.63	1.40	0.07	1.61	0.73	3.69	0.77	5.92
%CV	37.8	18.9	18.4	35.1	11.3	15.5	4.8	23.8

These acids are identified by comparison of their times of retention and absorption spectra with those of the standards. Oxalic, galacturonic, tartaric, quinic, malic, shikimic, citric and fumaric acids have been determined simultaneously. The absorption spectra of compounds with RT 25.75, 32.87 and 59.5 showed two maxima at 215 and 280 nm identical to that of phenolic compound, especially the flavan-3-ol.

Precision was tested on six replicated analyses of independent preparations of an apricot extract (Table 3). The coefficients of variation (%C.V.) obtained by the method ranged from 1.89 to 3.24% indicating that the method is precise with a high degree of repeatability, especially for malic, citric and fumaric acid (<2%). The confidence intervals (%C. I.) for repeated injections did not exceed 2.6%. The recovery of organic acids from apricot extract at two different levels ranged from 95.7 to 103.5% (Table 4) reflecting the precision and accuracy of the method.

The amounts of each organic acid found in apricot and their coefficient of variation are shown in Table 5. Coefficients of variation were between 4.8 and 37.8%. Citric acid (16.14 g/Kg) appeared to be the major organic acid in apricot pulp, followed by galacturonic acid (7.41 g/Kg), malic acid (6.56 g/Kg), quinic acid (4.58 g/Kg) and tartaric acid (0.41 g/Kg). Oxalic, shikimic and fumaric acid were present in apricot pulp at minor quantities averaged 28.15, 23.78 and 24.94 mg/Kg respectively. These acids may vary with cultivar, and differences in horticulture practices.

CONCLUSION

Organic acids in Moroccan apricot were separated and measured by HPLC with satisfactory repeatability. Oxalic, galacturonic, tartaric, quinic, malic, shikimic, citric acids have been determined simultaneously and eluted within 60 minutes. Considering how little sample preparation is required, the proposed procedure can be considered to be a precise and rapid method for organic acids determination.

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