

Determination of Organic Acids in Olive Fruit by HPLC

PELIN GÜNÇ ERGÖNÜL¹ and CEVDET NERGİZ¹

¹Department of Food Engineering, Engineering Faculty, Celal Bayar University, Muradiye Campus, Manisa, Turkey; ²Department of Chemistry, Faculty of Arts and Sciences, Fatih University, Büyükçekmece, Istanbul, Turkey

Abstract

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Organic acids (oxalic, citric, malic, and succinic) contents of Domat, Memecik and Uslu varieties of olives grown in Turkey were investigated using HPLC method. Organic acids were extracted from olives with water-methanol mixture solution 75:25 (v/v) and were analysed through KC-118 ion-exchange column using UV absorbance detector at 214 nm. The mobile phase was phosphoric acid (0.1%, w/v). The recovery values of the organic acids added into olive fruit samples were 92.8%, 98.75%, 110%, and 86% for oxalic, citric, malic, and succinic acids, respectively.

Keywords: organic acids; olive fruit; HPLC

Organic acids are naturally found in vegetables and fruits and may be formed during processes like fermentation or may be added into food during the manufacturing process. Organic acids can be separated from food by water or solvent extraction, vapour distillation, or precipitation with lead or other elements (JOSLYN 1970). Today, the most common way used for the extraction of organic acids present in fruits and vegetables is the solvent extraction.

The maturation stage and geographical origin affect the amounts and types of organic acids found in different kinds of fruits (CÁMARA *et al.* 1994). Organic acids are one of the minor components of olive fruit and their amount is 1.5% of the fleshy part. Organic acids that play an important role in metabolic activity are the products formed during the formation and degradation of the other components in olive fruit like carbohydrates (CUNHA *et al.* 2001). Malic and citric acids which affect the colour of the olive are the major organic acids

found in it. They also have an important role in olive processing through affecting the buffering activity of olive tissue. It has also been reported that organic acids influence the protection of stability, quality, and aroma of the olive fruit (JOSLYN 1970).

Recently, there have been a great deal of research into the determination of organic acids in several fruits and fruit juices by HPLC method (CHARLES *et al.* 1982; EISELE & HEUSER 1990; CASTALDO *et al.* 1992; WANG *et al.* 1993; CÁMARA *et al.* 1994; POYRAZOĞLU *et al.* 2002; SHUI & LEONG 2002; SOYER *et al.* 2003; KARADENİZ 2004; WU *et al.* 2005). For example, SHUI and LEONG (2002) used HPLC with a diode-array detector at 215 nm for the determination of organic acids in fruit juices. KARADENİZ (2004) determined organic acids in citrus juices using UV-VIS detector at 214 nm, the mobile phase having been phosphoric acid. In general, ethanol and water were used for the extraction of carboxylic acids from the sample matrix by

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the several researchers. The detection wavelength varied from 210 nm to 215 nm. CUNHA *et al.* (2001) also used ethanol for the extraction of carboxylic acids from table olive and brine and determined them using an HPLC method after a derivatisation process at 265 nm. The aim of this study was to determine the amounts of organic acids found in olive fruit by using direct HPLC method.

MATERIALS AND METHODS

Materials. Memecik, Domat, and Akhisar Uslu varieties of olive fruits which are widely cultivated in Egean Region of Turkey were used. Olive fruits were harvested manually from the trees in the collection garden of Bornova Zeytincilik Araştırma Enstitüsü (Institute of Olive Researches) and were kept frozen (−18°C) until the analyses.

Chemicals. Oxalic, malic, and citric acids were purchased from Fluka. Succinic acid was obtained from Sigma Chemical Corporation (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). HPLC grade water was provided by Merck Chemicals (Merck KGaA, Darmstadt, Germany), methanol was obtained from Riedel (Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany) as HPLC grade. The other reagents used were of analytical grade.

Standard solutions. Stock solutions (1000 ppm) of oxalic, citric, malic, and succinic acids were prepared in the mobile phase consisting of 0.1% (w/v) phosphoric acid solution. Standard solutions of each organic acid were made in the mobile phase by appropriate dilutions.

Extraction of organic acids from olive fruit. Twenty grams of the olive fruit sample was thawed at ambient temperature, then mashed well in a mortar. 10 ml water-methanol mixture solution (75:25 v/v) was added and the mixture was centrifuged at 25°C and 3500 rpm/min for 30 minutes (Hettich Universal 32 R, Tuttlingen). The upper phase was filtered through filter paper (Whatmann No. 2, blue stripe). The centrifugation was repeated three times and the filtrates were collected. The combined filtrates were filtered again through Supelco Discovery DSC-18 filter.

HPLC separation and quantification. A Perkin Elmer Series 200 Model HPLC apparatus was used equipped with a UV absorbance detector set at 214 nm. Chromatographic separation was performed on a Shodex RSpak KC-118 model ion-exchange organic acid column (300 × 8 mm i.d.). The mobile

phase was 0.1% (w/v) phosphoric acid in distilled water (HPLC grade) with a flow rate of 0.8 ml/min.

50 µl aliquots of the individual standards were injected onto the column and their retention times were determined. For obtaining the calibration curves, mixtures of the standards of selected concentrations were injected into HPLC and their chromatograms were obtained. After the injection of the samples, chromatographic peaks were identified by comparing the retention times of the samples with those of the known standards. The quantities of organic acids were estimated from the peak areas, injecting known amounts of the standards. In the calculation of the amounts of organic acids, the dilution ratios were taken into consideration.

Recovery studies. A standard addition technique was employed in order to determine the percent recoveries of the organic acids in olive fruit samples for the verification of the effectiveness of the extraction step and the accuracy of the method used.

The recovery experiments were carried out with all organic acids standards conducting the entire procedure applied for the samples. The percent recovery was calculated as follows:

$$\% \text{ Recovery} = [C/(A + B)] \times 100$$

where:

A – amount of organic acid found in the original sample (µg)

B – amount of organic acid added into the sample (µg)

C – amount of organic acid found in the standard added sample (µg)

RESULTS AND DISCUSSION

Oxalic, citric, malic, and succinic acids, which are the major organic acids found in the olive fruit,

Table 1. Retention times and recovery values of organic acids

Organic acid	Retention time (min)	Recovery (%)
Succinic acid	9.36 ± 0.17	86 ± 2.30
Citric acid	7.02 ± 0.04	110 ± 2.83
Malic acid	7.92 ± 0.07	98.75 ± 1.77
Oxalic acid	5.82 ± 0.03	92.80 ± 1.13

Values are expressed as mean ± standard deviation of duplicate analysis

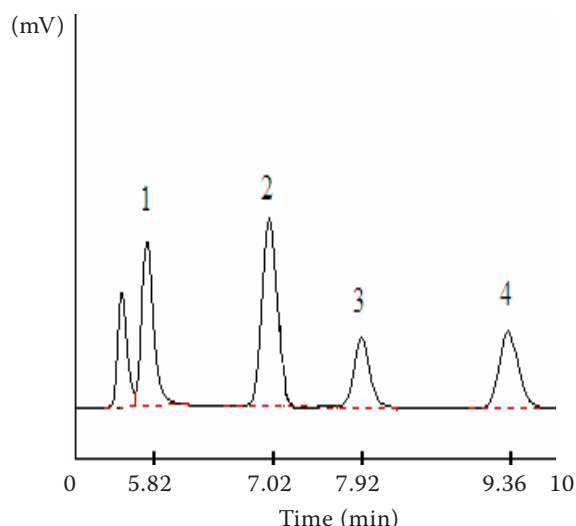


Figure 1. A standard chromatogram for organic acids: (1) oxalic acid, (2) citric acid, (3) malic acid, (4) succinic acid unidentified peak is the peak of solvent (0.1% H_3PO_4)

were separated and analysed by means of a new technique of HPLC.

An example of the chromatogram of the organic acids standards is given in Figure 1. All organic acids could be separated in less than 10 minutes (Table 1). The detector response for the peak areas was linear ($R^2 = 0.99$) in the range of 0.15–0.50 μg

Table 2. Amount of organic acids found in different varieties of olive fruit harvested in September

Olive type	Organic acid	Amount (mg/100 g)
Domat	succinic acid	614 \pm 3.54
	malic acid	764.50 \pm 4.95
	citric acid	702.50 \pm 17.68
	oxalic acid	75.70 \pm 2.40
	total	2156.20 \pm 21.50
Memecik	succinic acid	539 \pm 4.24
	malic acid	1476 \pm 5.66
	citric acid	1064 \pm 19.80
	oxalic acid	67.50 \pm 0.00
	total	3146.50 \pm 29.70
Uslu	succinic acid	518 \pm 7.07
	malic acid	2056.50 \pm 14.85
	citric acid	2030 \pm 11.31
	oxalic acid	66.60 \pm 0.68
	total	4671.10 \pm 32.55

per injection under the chromatographic conditions. The results of the recovery analysis are given in Table 1. These data indicate good accuracy of the HPLC method. The recovery was found to be 110%, 86%, 98.75%, and 92.80% for the added malic, succinic, citric, and oxalic acids, respectively. The three olive varieties were analysed in duplicates to obtain the analytical precision and the results showed a RSD lower than 3% for all organic acids. Similar recovery results were obtained for succinic and citric acids by CUNHA *et al.* (2001).

The concentrations of organic acids in different olive varieties are presented in Table 2. The concentrations vary depending upon the variety. Among the organic acids, malic acid showed the highest value followed by citric, succinic, and oxalic acids in all samples. The level of total organic acids in Uslu variety was found to be higher than in the others (Table 2). The individual and total levels of organic acids in olive fruits may change in relation to the maturation and variety. In our study, the effect of maturation could be ignored since the harvesting times of the olive fruit samples were the same. The analysis of variance indicated a significant ($P < 0.05$) difference in organic acids for the variety of olive fruit.

In conclusion, this method is suitable for the determination of organic acids in olive fruits. They can be determined in low concentrations with a great sensitivity. The procedure of organic acids extraction is simple and rapid. Other organic acids can be easily determined by this method as well.

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Corresponding author:

MSc. PELİN GÜNÇ ERGÖNÜL, Celal Bayar Üniversitesi, Muhendislik Fakültesi, Gıda Muhendisliği Bölümü,
45140 Muradiye Kampusu, Manisa, Turkey
tel.: + 90 236 241 21 44 (281), e-mail: pelin.ergonul@bayar.edu.tr
