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High-performance liquid chromatographic determination with photodiode array detection of ellagic acid in fresh and processed fruits

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Abstract

A high-performance liquid chromatographic (HPLC) procedure based on an isocratic elution with photodiode array detection has been developed for a simple and rapid determination of ellagic acid (EA) in fresh and processed fruits. The homogenized sample was refluxed with methanol and then the extract was refined using a solid-phase cartridge before HPLC. We analyzed EA in 40 kinds of fresh fruits and 11 kinds of processed fruits by the developed method. EA was found in several berries, fueijoa, pineapple and pomegranate. This is the first occurrence of the detection of EA in bayberry, fueijoa and pineapple.

Keywords: Food; Food analysis; Ellagic acid

1. Introduction

Polyphenolic compounds are widely distributed in the vegetable kingdom and are often encountered in our daily lives, being contained in tea, red wine, fruits, beverages and various medicinal plants [1–6]. Among them, ellagic acid (EA) as shown in Fig. 1, a dimeric derivative of gallic acid, mainly exists in high plants including fruits, nuts, etc. [7–14], combined with its precursor, hexahydroxydiphenic acid or bound as ellagitannins. EA has been found to have antimutagenic, antiviral, whitening of the skin and antioxidative properties [15–18]. Additionally, EA has now been allowed for use as a food additive in Japan, functioning as an antioxidant [19]. Thus, the development of a simple method for its determination in foodstuffs is required and is important for health and food hygiene.

Although many analytical methods for EA that involved liquid–liquid extraction and gradient elution have been reported [9–14,20–27], they are complicated and required much time and labor to complete. Furthermore, isocratic elution using the reported HPLC conditions of EA tended to cause tailing and broadening of the peak. In the present study, our aim was to develop a simple and rapid method for the identification and determination of EA by HPLC based on an isocratic elution with photodiode array detection using a solid-phase extraction, as well as its good chromatographic resolution. For that, we tested with several fruits under various extraction conditions, such as the ratio of solvents, acid concentration and the solid-phase cartridge, as well as the chromatographic column and

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solvent system. Finally, the optimized method was used to analyze EA in fresh and processed fruits.

2. Experimental

2.1. Samples, chemicals and reagents

Fresh and processed fruits were purchased from grocery stores, and some of the fresh fruits were donated by the Kobe quarantine station in Japan. Ellagic acid dihydrate was obtained from Wako (Osaka, Japan). Methanol and acetonitrile used were of HPLC-grade from Wako. The Sep-Pak Plus tC_{18} cartridge (900 mg) used for refinement was from Waters (USA).

2.2. Apparatus

The HPLC analysis was carried out on a Shimadzu class LC-VP HPLC system with class LC-VP software, a pump (LC-10Advp), an autosampler (SIL-10AD) and a diode-array detector (SPD-M10Avp) (Shimadzu, Kyoto, Japan). An L-column ODS (5 μm, 250×4.6 mm I.D., Chemicals Inspection and Institute, Japan) was used for the analysis.

2.3. Analytical conditions

The chromatographic conditions were as follows: flow-rate, 1.0 ml/min; volume injected, 10 μl; temperature, 40°C; detection, 360 nm. The mobile phase composition was 5 m*M* potassium dihydrogenphosphate solution (pH 2.5)–acetonitrile (41:9, v/v). The UV spectrum was recorded between 200 and 400 nm.

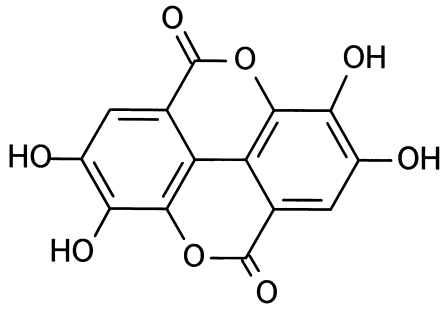

Fig. 1. Structure of ellagic acid.

The retention time and UV spectrum of the peak compared to the standard were used to identify the compound and to check the purity. An ambiguous peak in agreement with the standard on HPLC chromatogram was distinguished by photodiode array detection. Quantitative determination was carried out using calibration graphs obtained from standard solutions of EA diluted with methanol in the concentration range 0.1–100 μg/ml.

2.4. Sample preparation

Fresh or processed fruits (10 g) were weighed and homogenized in methanol (30 ml), and then the homogenate was refluxed for 1 h. After refluxing, the refluxed sample was filtered in vacuo. The filtrate with 10 ml of water added was evaporated to ca. 10 ml and then 0.1 *M* HCl solution (100 μl) was added. The extract was directly loaded on to the Sep-Pak Plus tC_{18} cartridge, previously conditioned with 10 ml of methanol, followed by 10 ml of distilled water and washed with 10 ml of distilled water. EA on the cartridge was eluted with 10 ml of methanol. The eluate was collected in a flask and then evaporated to dryness under reduced pressure below 40°C using a rotary evaporator. The residue was dissolved in 5 ml of methanol and the sample solution was filtered through a 0.5-μm filter before HPLC.

3. Results and discussion

The HPLC analysis of EA by elution using mobile phases and columns of several systems has already been reported [9–14,20–27]. However, isocratic elution using them tended to cause tailing and broadening of the peak as shown in Fig. 2b–d. For example, the HPLC analysis of EA by isocratic elution using a LiChrospher 100 RP-18 with a mobile phase of the water–methanol–phosphoric acid system [10] caused broadening of the peak (Fig. 2d), and using the column in this condition with a phosphate buffer–acetonitrile system caused tailing of the peak (Fig. 2c). On the other hand, the chromatogram using an L-column ODS with a

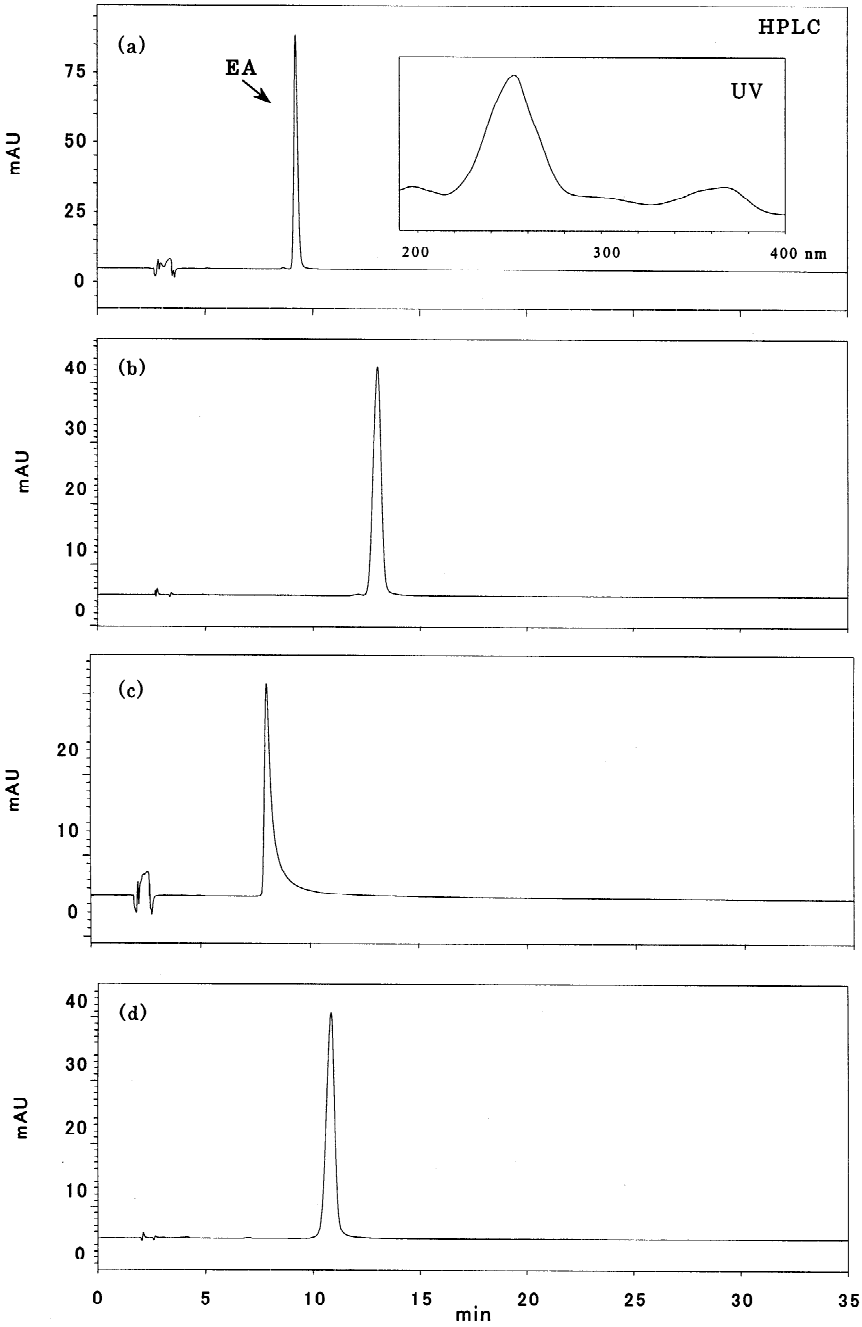

Fig. 2. Comparison of HPLC chromatograms of EA (each 50 μg/ml, injected 10 μl) and the UV spectrum from EA [illustrated in frame (a)]. Conditions: A flow-rate of 1.0 ml/min, temperature of 40°C and detection at 360 nm were used in all cases. Columns and mobile phases were as follows: (a) L-column ODS (250 mm×4.6 mm I.D., 5 μm) column using 5 mmol/l potassium dihydrogenphosphate solution (pH 2.5)–acetonitrile (41:9, v/v) (the proposed method); (b) L-column ODS (250 mm×4.6 mm I.D., 5 μm) column using water–methanol–phosphoric acid (62.40:37.45:0.15, v/v); (c) LiChrospher 100 RP-18 (250 mm×4.0 mm I.D., 5 μm, Merck) column using 5 mmol/l potassium dihydrogenphosphate solution (pH 2.5)–acetonitrile (41:9, v/v); (d) LiChrospher 100 RP-18 (250 mm×4.0 mm I.D., 5 μm) column using water–methanol–phosphoric acid (62.40:37.45:0.15, v/v).

water–methanol–phosphoric acid system caused also broadening as shown in Fig. 2b. We attempted to obtain the optimum conditions for HPLC with good resolution of EA using isocratic elution. As a consequence, the sample solution was chromatographed on an L-column ODS with a mobile phase of 5 m*M* potassium dihydrogenphosphate solution (pH 2.5)–acetonitrile (41:9) at a flow-rate of 1.0 ml/min. Under these HPLC conditions, a well resolved HPLC chromatogram was obtained. A chromatogram of EA obtained by this method is shown in Fig. 2a.

For sample clean-up, a Sep-Pak Plus tC_{18} cartridge was employed. The pH of the sample solution for the recoveries of EA from the cartridge should be ca. 2 to ensure stable recoveries of EA. Consequently, 100 μl of 0.1 *M* HCl was added to the sample solution in order to maintain a pH of ca. 2.

Fig. 3 shows the relationship between volume (or concentration) of methanol in the eluting solution and the recoveries of EA from the Sep-Pak Plus tC_{18} cartridge. According to this, namely, EA could be almost eluted with 100 % (v/v) methanol as shown in Fig. 3a. On the other hand, most of the EA was eluted with ca. 5 ml of methanol, but its complete elution should be done with 10 ml (Fig. 3b).

Table 1
Recoveries of ellagic acid from fresh and processed fruits

Sample	Added (μg/g)	Found (μg/g)	Recovery[a] (% mean±SD)
Strawberry	0	14.7	
	25	39.3	98.0±1.0
	50	63.9	98.3±2.5
Pineapple	0	1.2	
	25	24.1	91.5±2.3
	50	47.2	91.0±1.3
Raspberry	0	4.0	
	25	26.5	90.1±2.5
	50	52.9	97.9±0.9
Strawberry jam	0	20.1	
	50	49.4	97.0±1.5
Blueberry jam	0	ND[b]	
	50	48.4	96.8±2.8
Raspberry jam	0	22.5	
	50	70.9	96.9±5.0

[a] The values are means of triplicate determinations.
[b] ND=Not determined (<0.1 μg/g).

Consequently, the tC_{18} cartridge was directly loaded with the sample solution and washed with 10 ml of distilled water. After washing, it was eluted with 10 ml of methanol. These eluates were evaporated under

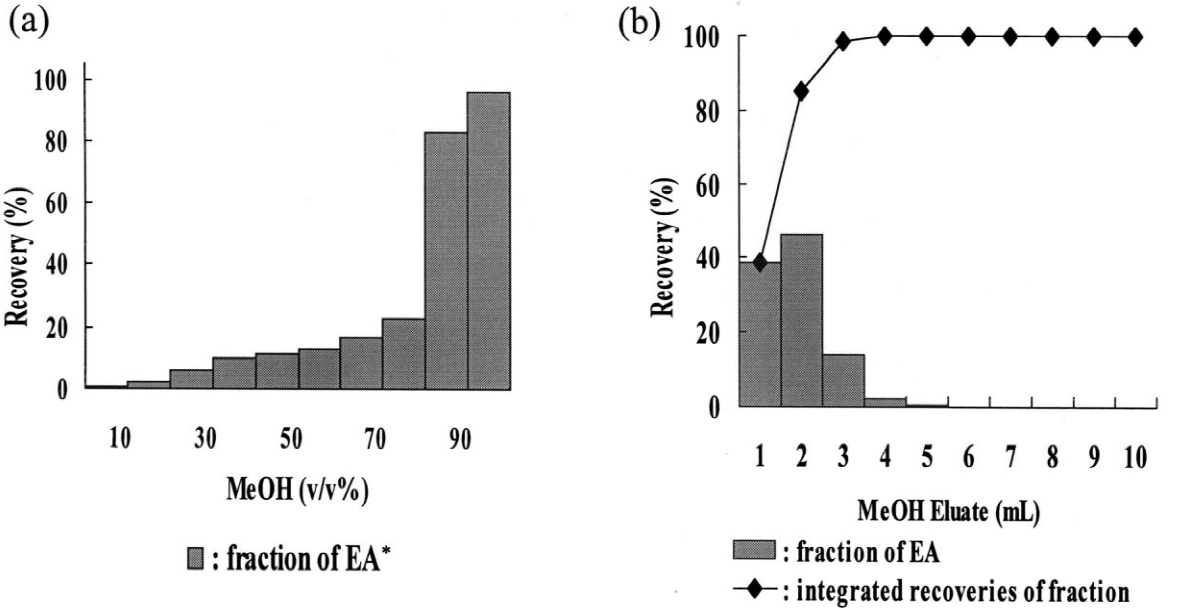

Fig. 3. Relationship between concentration (a) [or volume (b)] of methanol in elution solution and recoveries of ellagic acid (EA) from Sep-Pak Plus tC_{18}. * Eluted with 10 ml of each solution.

vacuum, and it was dissolved in methanol before the HPLC as a sample solution.

In a recovery test, the proposed method was applied to six kinds of fresh and processed fruits spiked with EA at levels of 25 and 50 μg/g. The recoveries were measured by carrying out three identical analyses and the results are given in Table 1. The recoveries of EA from the samples were found to be 90.1~98.3% (SD 0.9~5.0%). The detection limit was 0.015 μg/g, and the limits of quantification were 0.05 μg/g for EA.

We have also analyzed 40 fresh and 11 processed fruits by this proposed method, and this was repeated in triplicate for each sample. In this analytical result,

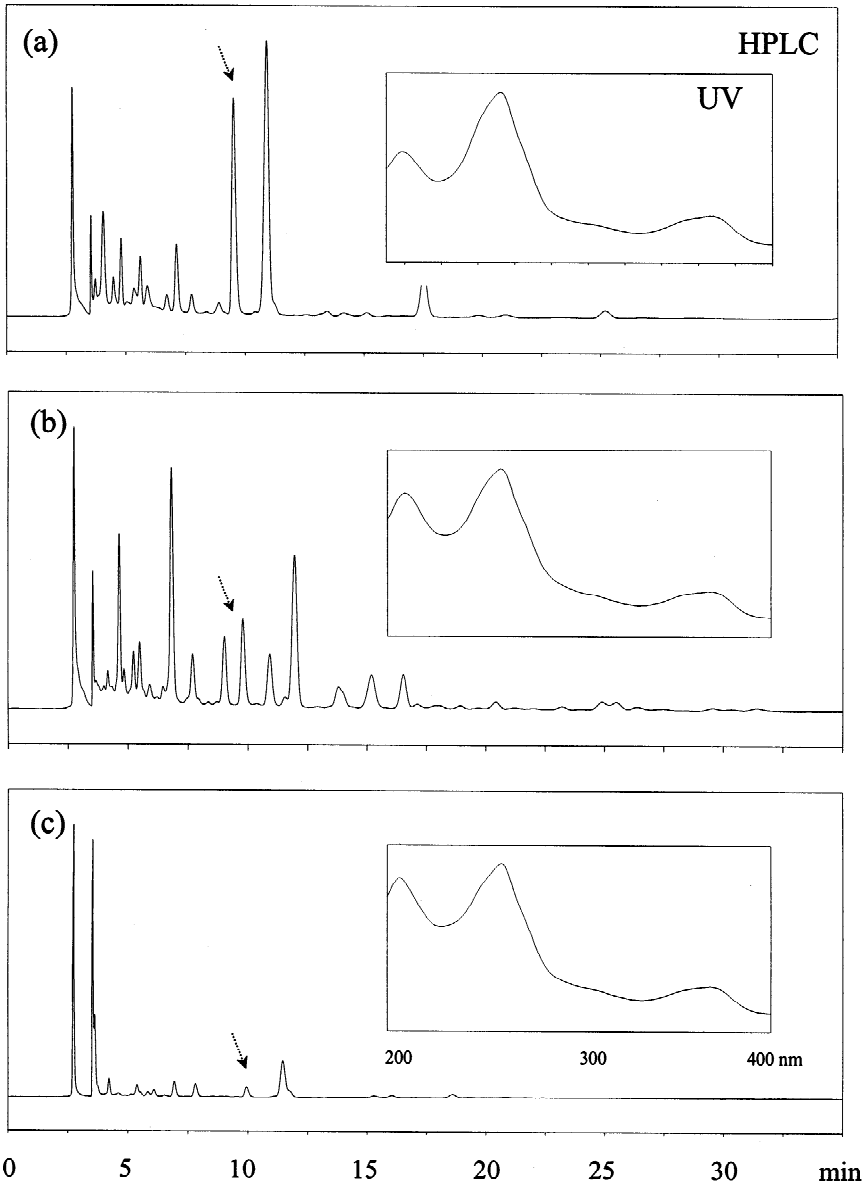

Fig. 4. HPLC chromatograms of (a) strawberry, (b) fueijoa and (c) raspberry samples and UV spectra of the peaks at 360 nm in the chromatograms.

EA was found in many berries [blackberry (87.66 μg/g), strawberry (17.66 μg/g), raspberry (5.84 μg/g) and bayberry (1.82 μg/g)], pomegranate (17.31 μg/g), fueijoa (10.57 μg/g) and pineapple (0.80 μg/g), and to the best of our knowledge, the presence of EA in bayberry, fueijoa and pineapple was elucidated for the first time.

Figs. 4 and 5 illustrate the chromatograms of strawberry, fueijoa, raspberry, blueberry, lemon and lime at 360 nm UV. An ambiguous peak in agreement with EA on the HPLC chromatogram could be distinguished by photodiode array detection. That is to say, the assigned peak on the HPLC chromatograms of blueberry, lemon and lime, illustrated in

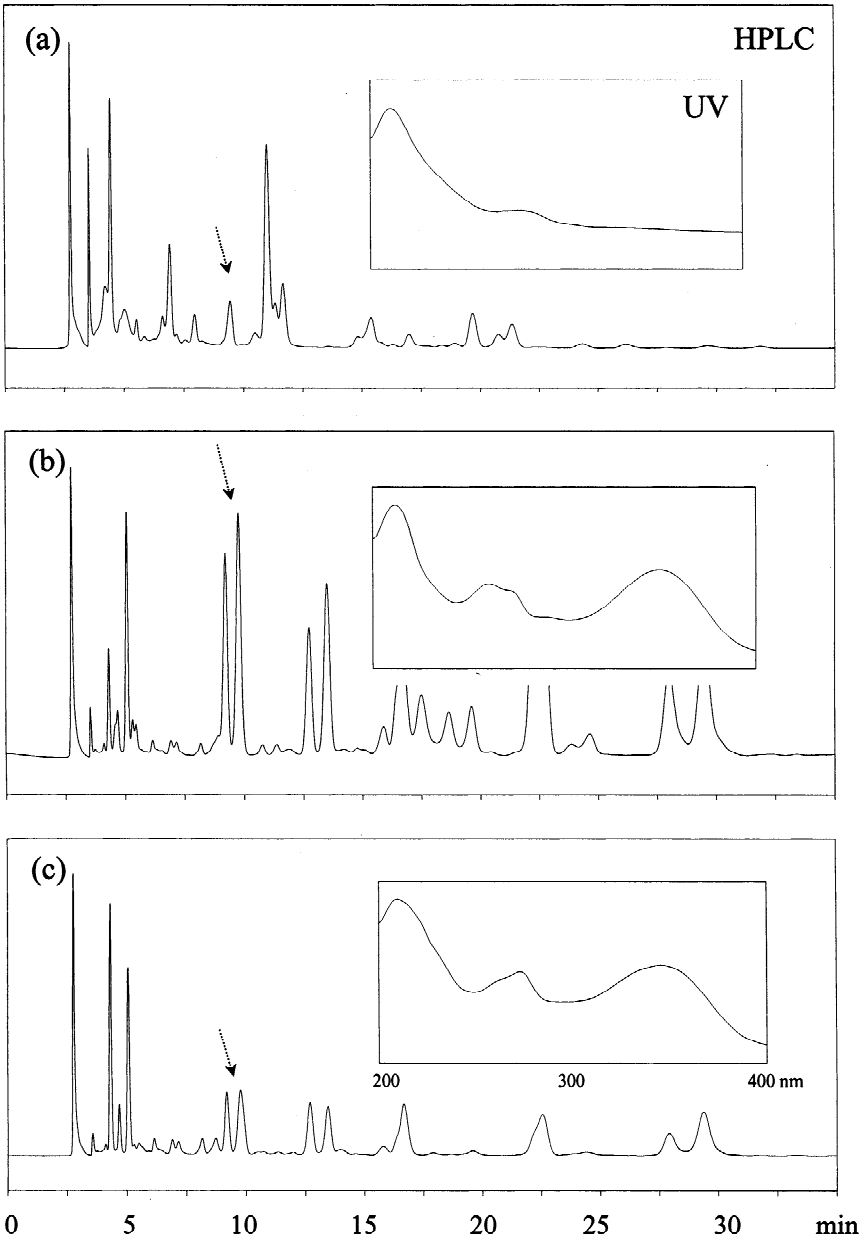

Fig. 5. HPLC chromatograms of (a) blueberry, (b) lemon and (c) lime samples and UV spectra of the peaks at 360 nm in the chromatograms.

Fig. 5, indicated a retention time in agreement with EA, but the UV pattern by photodiode array detection was different. Accordingly, we concluded that the peak from blueberry, lemon and lime was from another compound. The results of this show that the identification of the UV pattern by photodiode array detection are essential for the analysis of EA.

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