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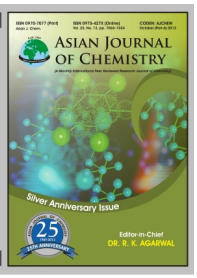

Determination of Phenolic Compounds in Litchi Juices by Solid-Phase Extraction and High Performance Liquid Chromatography

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A simple analytical method for the quantitative determination of phenolic compounds in litchi juice was developed. The phenolic compounds in litchi juice were extracted by solid-phase extraction using Bound Elut C_{18} cartridges and the extract was analyzed by HPLC coupled with UV detection. Solid-phase extraction conditions were as follows: samples were adjusted to pH 3.0, cartridges firstly were washed by 5 mL 0.01 M HCl then 5 mL distilled water, at last phenolic compounds on C_{18} cartridge were eluted by 7 mL methanol. Elution were blowed to 1 mL by pressure blowing concentrator. Phenolic compounds separation effect was good and extraction rate was high in this solid-phase extraction procedure. This paper gave a method to extract and determine fastly phenolic compounds present in Litchi juice.

Key Words: Solid-phase extraction, High performance liquid chromatography, Litchi juices, Phenolic compounds.

INTRODUCTION

Litchi (*Litchi chinesis* Sonn.), which belongs to family of Sapindaceae, is cultivated in tropical and sub-tropical countries and areas as mainland China, India, Taiwan, Thailand, Vietnam, Pakistan, Madagascar, Mauritius, Australia and Israel[1]. Litchi contain a variety of vitamins, organic acid, a large number of free arginine and serine and some phenolic compounds, the nutritional value is extremely rich[2]. The chemical property of phenolic compounds is not stable. They are easy to be decomposed and be oxidated at the environment of light, heat, oxygen, which will affect the accuracy of determination results[3]. Currently, there are several studies about juice to determine phenolic compounds by chromatographic conditions using HPLC method, but few reports about studies of juice sample pretreatment methods. The report is mainly for reference of pretreatment methods of organic solvent extraction process developed by Wu *et al.*[4]. In recent years, as a sample pretreatment technique, solid phase extraction has been developed rapidly, which can remove interference of the complex samples matrix and improve sample purity and instrument detection sensitivity[5]. Detering wine polyphenols, solid-phase extraction technology has been widely applied to make the wine polyphenols and organic acid, sugar and other components be separated[6-8]. C_{18} cartridges were widely used in phenolic compounds extraction[9]. This paper provides a reference for the extraction and determination of phenolic compounds in fruit juice, through establishing a solid phase extraction conditions based on treating litchi juice with solid phase extraction technology and determining litchi juice phenolic compounds content by high performance liquid chromatography.

EXPERIMENTAL

Heli litchi juice were purchased from a local food factory in Guangxi (China) and were frozen at -18 ºC until use.

(-)-Epicatechin, caffeic acid, chlorogenic acid, (+)-catechins, gallic acid, rutin were purchased from Sigmar (American). Methanol and acetonitrile were of HPLC grade which were purchased from Oceanpak (Sweden). Hydrochloric acid and methanoic acid were of analytical grade. TSQ Quantum Access MAX liquid chromatograph/mass spectrometer (LC/MS)(automated variable-wavelength UV-VIS detector Finnigan Svrveyor Pda plus Detector and Xcalibur chromatography workstation) (American Thermo Fisher company), Bound Elut C_{18} (500 mg, 6 mL) solid phase-extraction cartridge (Agilent Technologies), solid-phase extraction vacuum device which allowed 12 samples to be handled simultaneously (Agilent Technologies), PGC-11D pressure blowing concentrator.

Chromatographic condition: Chromatographic column: Thermo Hypersil GOLD C_{18} (150 mm × 4.6 mm, 3 μm). Mobile phase: binary gradient, 100 % acetonitrile (A) and 0.1 % methanoic acid solution (B). Flow rate: 0.2 mL/min. Injection

volume: 3 μL. Detector: UV detector, 280 nm wavelegth[10]. Column temperature: 30 ºC. The gradient elution program were as follows Table-1.

TABLE-1
GRADIENT ELUTION PROGRAM OF PHENOLIC COMPOUNDS

Elution gradient (min)	A (%)	B (%)
0.00	95	5
10.00	92	8
20.00	88	12
30.00	85	15
35.00	80	20
38.00	80	20
38.01	95	5
41.00	95	5

Solid-phase extraction condition of litchi juice sample: The litchi juice samples were centrifuged (5000 rp, 5 min) and 10 mL supernatant was collected separately. 0.01 M HCl was added to the supernatant which was adjusted to pH 3. The Bound Elut C_{18} cartridges were preconditioned by using 10 mL of methanol and then 10 mL water when methanol would run off. The supernatant was passed through by means of a solid-phase extraction vacuum device from supelco, which allowed 12 samples to be handled simultaneously. The flow velocity was 1 mL/min when preconditioning and extracting. After the supernatant which pH was 3 passed through preconditioned C_{18} cartridges, the cartridges was washed with 5 mL of 0.01 M HCl firstly and then 5 mL distilled water to remove sugar, organic acids and other water-soluble constituents. Leacheate was discarded. The hydrophobic phenolic compounds were adsorbed on the cartridge and not eluted by water. Following this step 7 mL methanol was used to elute phenolic compounds from C_{18} cartridges. The elution solution was concentrated to 1 mL by pressure blowing concentrator in 35 ºC temperature. The concentration solution was filtered by 0.45 μm organic membrane then analysised by HPLC.

Standard solution preparation: Accurately weight (+)-catechins, (-)-epicatechin, chlorogenic acid, caffeic acid, gallic acid, rutin 1 mg, respectively. Then 1 mg each of standard reagents was dissolved in 1 mL of acetonitrile and the volume was made up to 10 mL with acetonitrile. These solution was as stock solution. When using various stock solution was removed in different volume, then was diluted by mobile phase [V(acetonitrile) : V(0.1 % methanoic acid) = 5:95] to different concentration standard solution.

RESULTS AND DISCUSSION

HPLC chromatogram of phenolic compounds standard substance: A typical HPLC chromatograms of phenolic standard substance was shown in Fig. 1. According to the Fig. 1, we could see that the selected six phenolic standard substances' chromatograms peak shape was symmetrical, separation effect was good. Fig. 1 illustrated that the HPLC condition what we choose could determine phenolic variety and content in licthi juice.

HPLC chromatogram of phenolic compounds in litchi juice sample: The HPLC chromatogram of litchi juice sample which was extraced by Bound Elut C_{18} was shown in Fig. 2. According to Fig. 2, there were two kinds of phenolic

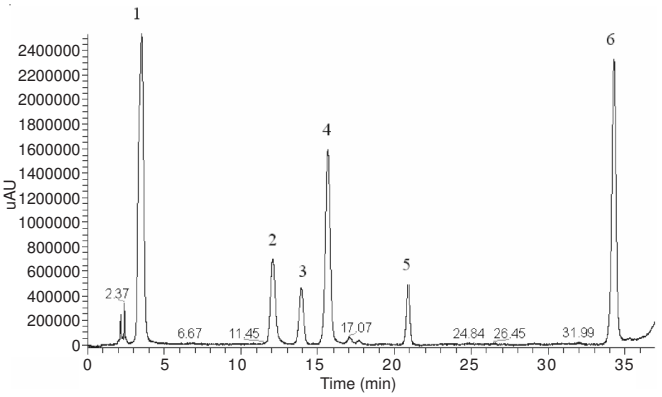

Fig. 1. HPLC chromatograms of phenolic standard substance: (1) gallic acid; (2) chlorogenic acid; (3) (+)-catechins; (4) caffeic acid; (5) (-)-epicatechin; (6) rutin

TABLE-2
SOLID PHASE EXTRACTION CONDITION

Serial number	Sample pH	Leacheate	Eluent volume (mL)
A	2	5 mL HCl+5 mL distilled water	7
B	3	5 mL HCl+5 mL distilled water	5
C	4	5 mL HCl+5 mL distilled water	7
D	3	10 mL distilled water	7
E	3	10 mL HCl	7
F	3	5 mL HCl+5 mL distilled water	10
G	3	5 mL HCl+5 mL distilled water	7

compounds in lichi juice sample. They were (-)-epicatechin and rutin. Zhong Hui-zhen *etc.*, also detected (-)-epicatechin and rutin when determinating phenolic compounds in litchi pulp[2].

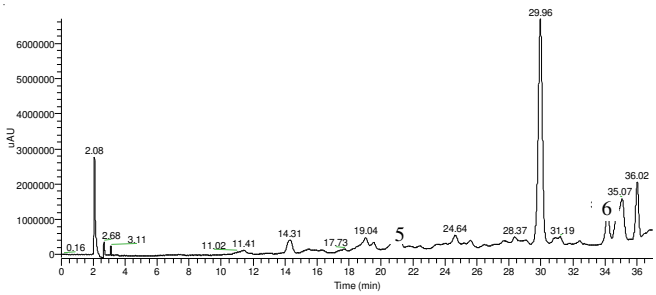

Fig. 2. HPLC chromatogram of phenolic compounds in litchi juice sample: (5) (-)-epicatechin; (6) rutin

Optimizing condition of solid-phase extraction: adjust sample pH value: According to the characteristics of that, the phenolic substances in acid condition (pH = 2.0-3.5) exist as the neutral molecules[11]. Their hydrophobicity is strong. They would reserve in reversed phase column The litchi juice samples were adjusted to pH 2.0, 3.0 and 4.0, respectively by using 0.01 M HCl. According to the "solid-phase extraction condition of litchi juice sample" method, the litchi juice samples were extraced and detected by HPLC. The measured phenolic substance concentration of litchi juice samples which had different pH value were shown in Fig. 3. Fig. 3 showed that the concentration of (-)-epicatechin and rutine were highest when the litchi juice samples pH value were 3.0. (-)-Epicatechin belong to flavanol. Rutine belong to flavonol. The stronger acid condition pronoted the phenolic hydroxyl group ionizing. Forming positive ions would increase solubility of (-)-epicatechin and rutine.

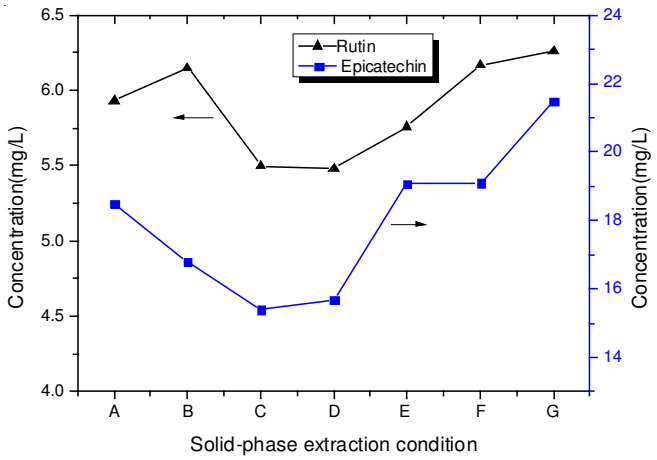

Fig. 3. Concentration of (-)-epicatechin and rutin under all kinds of solid-phase extraction condition. Remark: solid-phase extraction conditions are shown in Table-2

Selecting leacheate: There were three kinds of leacheate, 10 mL HCl, 10 mL distilled water, 5 mL HCl and 5 mL distilled water, respectively. Althought impurities on C_{18} cartridge were washed off well by three kinds of eluent. The concentration of litchi juice sample's phenol was hightest and impurities were less, when C_{18} cartridge was washed by 5 mL HCl firstly then 5 mL distilled water than by 10 mL HCl or 10 mL distilled water. The increasing of phenolic concentration was due to phenol would be reserved well when HCl was added to the cartridge to acidify the matrix[12]. Distilled water washed off impurities well.

Changing eluent volume: Methyl alcohol was choosed as eluent to elute phenolic compounds on C_{18} cartridge usually. Eluting effct was good. So that this paper also choosed methyl alcohol being eluent. The eluetn volume were 5, 7, 10 mL. As shown in Fig. 3, when litchi juice sample pH value was 3, the C_{18} cartridge was washed by 5 mL HCl firstly and then 5 mL distilled water. Eluent which was methyl alcohol volume was 7 mL. The HPLC measuring concentration of (-)-epicatechin and rutine in litchi juice were highest.

Conclusion

This paper established a litchi juice samples preliminary treatment method through choosing and optimizing solid-phase extraction leacheate, sample pH valume, eluent volume when phenolic compounds in litchi juice samples were measured by HPLC. Using this solid-phase extraction condition to extract phenolic compound in juice, there were a lot of advantage, for example loss of phenolic compound were little, extration rate was high, separated effect was good and less interference substances. In summary, solid-phase extraction-HPLC is a simple and precise analytical method for determining phenolic compounds in juices and has a significant meaning with regard to controling quality of juice.

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