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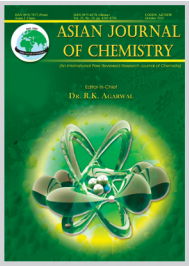

Fast Determination of Phenolic Compounds in Fatty Food Simulant with HPLC

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The phenolic and other related compounds such as vanillin, cinnamaldehyde, thymol, eugenol and isoeugenol in olive oil, a fatty food simulant and their stabilities during thermal treatment were examined. The olive oil samples were diluted with the solution of isopropanol and chloroform (85:15 v/v). Chromatographic separation was performed on high performance liquid chromatograph (HPLC) with detection at 230 nm and 280 nm at 40 ºC. The samples were eluted gradiently using acetonitrile and water. The average recoveries were 98.9-102.1 % with standard deviation below 2 %. The proposed analytical methods for phenolic compounds are sample, rapid and less expensive. The method could be used for stability testing and migration studies of the similarly structured additives in olive oil.

Key Words: Phenolic compounds, Olive oil, HPLC analysis, Release, Migration.

INTRODUCTION

As a new kind of packaging material, active packaging material[1-3] is produced by adding the active substances into the packaging material to control the microbiological/oxidation decay of perishable food products. Because foodstuffs are complex and diverse, water, 3 % (w/v) acetic acid, 10 % (v/v) ethanol and olive oil appointed by legislation[4,5] as food simulants often take place of the aqueous/acidic/alcoholic/fatty foods to investigate the migration of active compounds in packaging materials. Gemili *et al.*[6-9] investigated the release of lysozyme, thymol or essential oils from new active packaging materials into the water food simulants. However, these methods were found to be unsuitable for the determination of compounds in olive oil samples due to the fact that oil is thermo-unstable, nonvolatile and easy to pollute the column. So these sample pretreatments often were adopted and were able to provide the phenolic profile in olive oil, but the analytical techniques often needed one or more separation steps involving methods like liquid-liquid extraction[10-13] and solid phase micro extraction (SPE/SPME)[14-16]. These are time consuming methods and they can not satisfy the needs of rapid detection of chemical compounds in release and migrant study. Direct injection of an olive oil solution is probably the most optimal method. The preliminary study on direct injection for high performance liquid chromatograph (HPLC) has been reported[11,17,18]. As far as is known, there are no published methods for phenolic additives in olive oil. In this study, a mixture of dichloromethane and isopropanol was used to dilute olive oil containing the selected phenolic compounds. And then the samples were directly analyzed by HPLC-UV with a good result. The method is useful to investigate heat stability and migration of the selected phenolic antimicrobial from packaging films into olive oil at different time-temperature conditions.

EXPERIMENTAL

Olive oil (rectified olive oil, Taiwan, China) was purchased from Jian Kang Hao Li You. Vanillin, cinnamaldehyde, eugenol, isoengenol, thymol standards were purchased from Sigma (St. Louis, MO, USA). Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Dichloromethane and isopropanol (HPLC grade) were purchased from Tedia (Ohio, USA). Ultrapure water was prepared using an EPED purification system (Nanjin, China).

Waters 2695 HPLC system with Empower software (Waters, Milford, MA, USA) was equipped with a 2996 diode array detector and a 2487 UV-VIS detector. Standards and samples were weighed using AL204 model electronic balance (Mettler Toledo, China).

Preparation of the standard solution: Quantification was based on the external standard method. A stock solution of phenolic compounds standard (500 mg/L) was prepared by dissolving phenolic compounds in a mixture of isopropanol and chloroform (85:15 v/v). The working standard solutions for linear calibration were prepared by diluting the stock solution to produce a series of concentrations of 0.1, 0.5, 1, 3, 5, 8, 10, 50, 80, 100, 150, 200 mg/L.

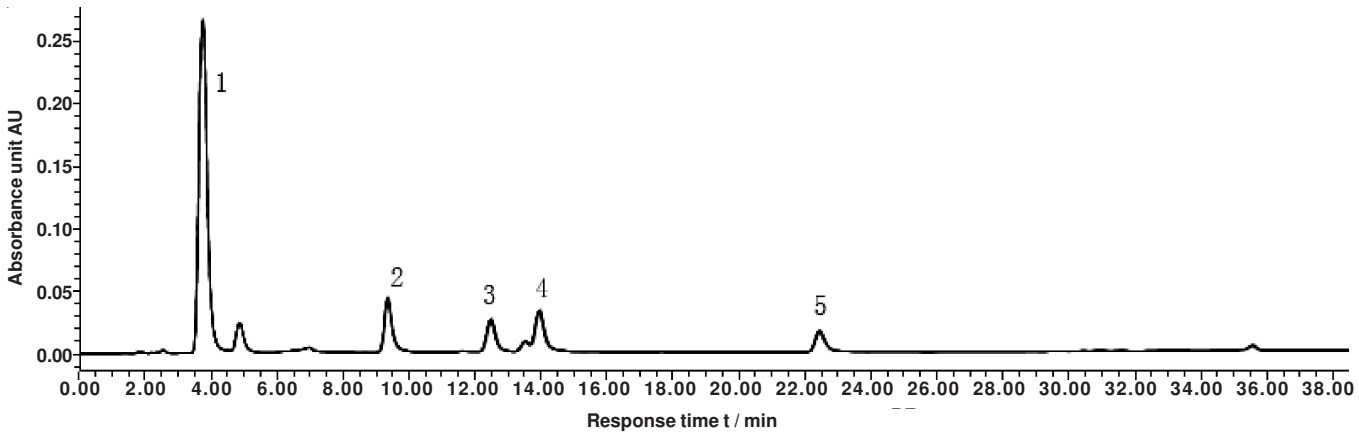

Fig. 1. HPLC-UV chromatograms of phenolic compounds in olive oil

Chromatographic conditions: A X-Terra RP-18 reverse phase column (4.6 mm ID × 150 mm, 5 µm) was protected by a 5 µm × 20 mm C_{18} guard column. The detection wavelength was set at 230 nm and 280 nm, respectively. The flow rate was set at 1.0 mL/min and the injection volume was 20 µL. The column temperature was held at 40 ºC. Samples were injected into the above HPLC system with a UV-VIS detector and were eluted according to the linear gradient conditions (0 min, 25 % acetonitrile and 75 % water; 0.5 h, 50 % acetonitrile and 50 % water; 35 min, 100 % acetonitrile; 40 min, 100 % acetonitrile; 45 min, 25 % acetonitrile and 75 % water; 50 min, 25 % acetonitrile and 75 % water).

HPLC analyses: An aliquot of 1 mL of olive oil was diluted with the solution of isopropanol and chloroform (85:15 v/v). The sample was filtered through a polytetrafluoroethylene (PTFE) 0.45 µm filter using a disposable syringe. The filtrate was analyzed according to the above method.

RESULTS AND DISCUSSION

Analysis method: A direct injection technique of oil sample was used for HPLC, which is easy to pollute the column. In order to avoid the column life shortened, olive oil was diluted with the solution of isopropanol and chloroform and the column temperature was held at 40 ºC. The mobile phase containing acetonitrile and water was selected which gave satisfactory resolution and a stable baseline. To improve selectivity and efficiency, different compositions of mobile phase were investigated. Finally, a mobile phase containing 25 % acetonitrile was chosen for the determination of phenolic compounds in olive oil. HPLC-UV chromatograms of phenolic compounds samples were presented in Fig. 1. The peaks 1-5 represented vanillin, cinnamaldyhyde, eugenol, isoeugenol and thymol, respectively. Their corresponding retention times were 4.0, 9.5, 12.8, 14.3, 22.3 min, respectively. A 2996 diode array detector was used to obtain the spectra of the analytes in order to optimize the detection wavelengths for the analysis. The ultraviolet spectrum of phenolic compounds showed obvious absorbance values at around 200 nm and 230 nm with the exception of 280 nm for cinnamaldyhyde. A strong absorption of olive oil was found at around 200 nm. Thus 230 nm and 280 nm were selected in order to avoid the interference of the olive oil components.

Stability assays: The legislation allows the use of food simulants in control time/temperature (usually 5, 20, 40 and 60 ºC) conditions to simplify migration tests. Since phenolic compounds are volatile samples, a stability test should be carried out at 5, 20, 40 and 60 ºC. The stability of phenolic compounds was assessed by comparison of the results of repeated HPLC analysis of the samples at different times. The concentration changes of thymol in olive oil at different times at 40 ºC are shown in Fig. 2. The other stability data are summarized in Table-1. Experimental results have shown that, during 10 days, there are no significant differences in the phenolic compounds concentration. It indicated that phenolic compounds in olive oils are quite stable at the temperature below 60 ºC.

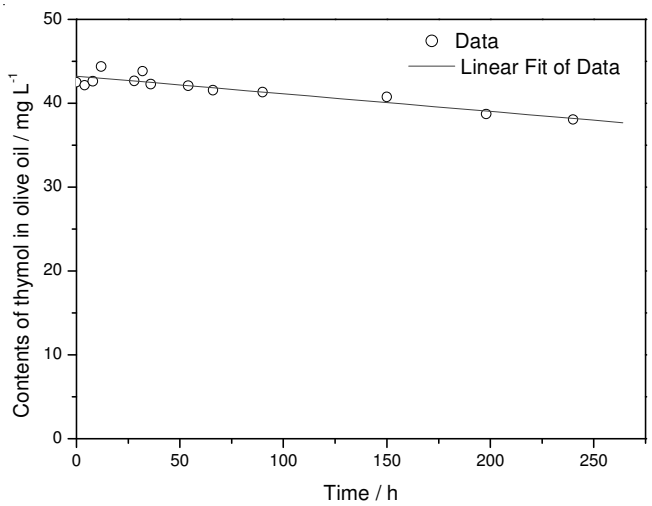

Fig. 2. Stability of thymol in olive oil with different time at 40 ºC

TABLE-1
LOSS OF ESSENTIAL OILS IN OLIVE OIL STORED AT 5, 20, 40 AND 60 ºCFOR 10 DAYS

Temp. (ºC)	Vanillin	Cinnamaldehyde	Eugenol	Isoeugenol	Thymol
60	4.4	6.8	3.6	7.6	12.0
40	3.1	3.9	3.3	6.2	10.4
20	2.3	2.5	3.1	5.4	9.7
5	1.3	2.0	2.7	4.0	9.0

And there was no new peak in the chromatogram of phenolic compounds obtained by HPLC-UV after 30 days of storage.

TABLE-2
PERFORMANCE CHARACTERISTICS OF THE ANALYTICAL METHODS PROPOSED

Label	Compounds	Detection wavelength (nm)	Linear relationship	Correlation coefficient	Linear range (mg/L)
1	Vanillin	230	y = -0.0772 + 1.0191E-5*x	0.9998	0.5-100
2	Cinnamaldehyde	280	y = -0.4399 + 5.0010E-6*x	0.9993	0.5-80
3	Eugenol	230	y = -0.0512 + 2.2127E-5*x	0.9999	1-100
4	Isoeugenol	230	y = 0.58601 + 2.1222E-5*x	0.9998	1-100
5	Thymol	230	y = 0.23770 + 4.0585E-5*x	0.9999	2-100

Thus the phenolic compounds losses might be due to opening the cap frequently during the process of sampling instead of the chemical instability of phenolic compounds. The losses could be avoided as far as possible if the sampling was prompt and effective.

Linearity of calibration curve and limit of detection of the method: The linearity of calibration curve was calculated using various concentrations of phenolic compounds. The linear relationship from calibration plots for phenolic compounds could be seen in Table-2, where y and x were concentration of the standard solution and the peak area, respectively. Linear regression showed good linearity with a correlation coefficient of more than 0.9993.

Based on signal-to-noise ratios of 3, the limit of detection (LOD) was determined using standard solutions of phenolic compounds subjected to HPLC. The limit of detection values for vanillin, cinnamaldehyde, thymol, eugenol and isoeugenol were 0.08, 0.04, 0.18, 0.17, 0.33 mg/L, respectively.

Recovery, accuracy and precision: Recovery was examined by adding a known amount of phenolic compounds standard to olive oil samples (seen in Table-3). The mean recoveries were found at range from 98.9 % to 102.1 %. It could be seen that the five objects had the high recoveries. This was mostly due to direct injection without extraction.

TABLE-3
RECOVERY AND ACCURACY TEST OF THE METHOD

Compound	Recruitment (mg/L)	Observed value (mg/L)	RSD[a] of Repeatability (n=5) (%)	Recovery (%)
Vanillin	5	4.8	96.0	98.9
	10	10.2	102.0	
	50	49.3	98.6	
Cinnamaldehyde	5	5.2	104.0	101.4
	10	10.4	104.0	
	50	48.1	96.2	
Eugenol	5	5.3	106.0	102.1
	10	10.1	101.0	
	50	49.6	99.2	
Isoeugenol	5	5.1	102.0	101.8
	10	10.3	103.0	
	50	50.2	100.4	
Thymol	5	4.9	98.0	100.8
	10	10.4	104.0	
	50	50.2	100.4	

The peak areas of five repeated measurements of the samples with a known concentration were obtained by the above mentioned HPLC methods. The relative standard deviations (RSDs) of analysis varied from 1.00 % to 1.81 % and were presented in Table-3. Thus the method was proved to be precise.

Release data of phenolic additives into olive oil: To assess the efficiency of the method, it was applied to the analysis of phenolic compounds released at 40 ºC from edible soy protein isolate films. After release experiments, 1 mL homogenized olive oil sample was diluted to 10 mL with mixed solvent. Subsequently, it was analyzed by direct injection into HPLC system. The details were summarized in Fig. 3. The results showed that the analytical methods proposed could be used to determine the release of antimicrobial from active packaging materials.

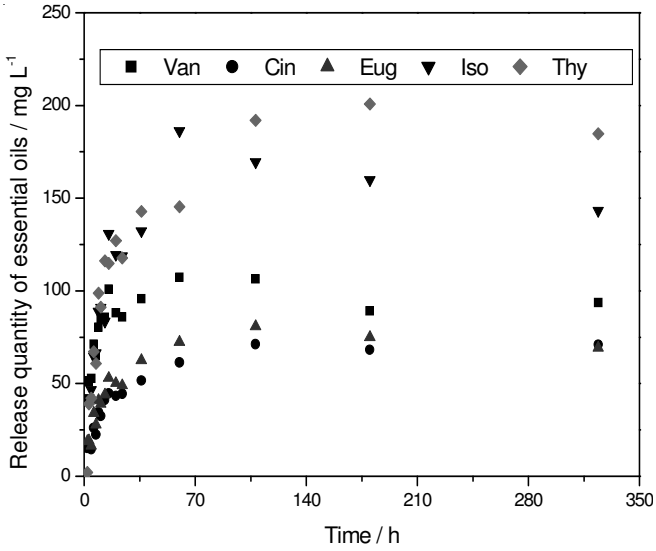

Fig. 3. Release of phenolic compounds from SPI films into olive oil samples at different time

Conclusion

In this study, a HPLC method is described for the analyses of phenolic compounds in olive oil samples. The determination of the five selected phenolic additives in olive oil was performed in a short time. The analysis results proved that the thermal treatment did not affect their stability. The detection limits for the selected phenolic compounds in olive oil were satisfactory. The methods are recommended for determination of the release of phenolic/aldehydes antioxidant and antimicrobial from packaging materials into olive oil, since they are stable in olive oil and there is no need to use substitute stimulants.

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