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DETERMINATION OF THE CONTENT OF PHENOLIC COMPOUNDS IN YOGURT EXTRACT (USING THE HPLC METHOD)

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Abstract
In this study, a high-performance liquid chromatography (HPLC) method based on the HPLC technique was developed for the determination of phenolic compounds (salicylic acid, quercetin, apigenin, rutin, gallic acid, and kaempferol) in yogurt extract. Standard solutions were prepared in 96% ethanol using an ultrasonic bath, while the sample was extracted with 96% ethanol at 60°C for 20 minutes, followed by centrifugation and filtration through a 0.45 µm membrane filter. The analysis was performed on a Shimadzu LC-40 Nexera Lite system using a Shim-pack GIST C18 column (150 × 4.6 mm; 5 µm) under gradient elution conditions with acetonitrile and 0.5% acetic acid. The injection volume was set at 10 µL, the flow rate at 0.5 mL/min, the column temperature at 40°C, and detection was carried out at 300 nm.

The results indicated that only salicylic acid (11.188 mg/100 g) and quercetin (50.235 mg/100 g) were detected in the sample, while the concentrations of other compounds were below the detection limit. Given its high reproducibility and sensitivity, this method is recommended for the determination of polyphenols in food matrices.

Keywords: Yogurt extract; phenolic compounds; HPLC method; high-performance liquid chromatography; quercetin; salicylic acid.

Introduction

Reagents and Equipment. Gallic acid was obtained from Macklin (China), salicylic acid from Rhydburg Pharmaceuticals (Germany), while quercetin, apigenin, and kaempferol were purchased from Regal (China). Rutin was isolated from natural sources through extraction and column chromatography methods. High-purity HPLC-grade water, acetonitrile, glacial acetic acid, and sodium hydroxide were used as reagents.
The quantification of polyphenolic compounds was performed using a high-performance liquid chromatography (HPLC) system, LC-40 Nexera Lite, manufactured by Shimadzu Corporation (Japan).

Preparation of Standard Solutions. Gallic acid (5.2 mg), salicylic acid (5.2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg), and kaempferol (5 mg) were each dissolved in 96% ethanol

using ultrasonic treatment for 20 minutes and then transferred to a 50 mL volumetric flask, adjusting to the mark with ethanol. From each stock solution, 200 µL was taken and mixed to prepare a composite solution. Subsequent dilutions were performed to obtain four different concentrations. All prepared solutions were stored in vials and used for HPLC analysis.

Preparation of Plant Extract. For the extraction of phenolic compounds, 1.0 g of the sample was accurately weighed using an NV222 precision balance (OHAUS, USA) with an accuracy of 0.01 g and placed into a 50 mL conical flask. Then, 25 mL of 96% ethanol was added. The mixture was subjected to ultrasonic extraction in a GT SONIC-D3 ultrasonic bath (China) at 60°C for 20 minutes. After extraction, the mixture was cooled, filtered, and the volume was adjusted to 25 mL with ethanol in a volumetric flask. An aliquot of 1.5 mL of the extract was centrifuged at 7000 rpm using a Mini-7 centrifuge (BIOBASE, China) and subsequently filtered through a 0.45 µm syringe filter before being used for HPLC analysis.

Chromatographic Conditions

Determination of Phenolic Compounds. The standard solutions and sample extracts were analyzed using a reversed-phase Shim-pack GIST C18 column (150 × 4.6 mm; 5 µm, Shimadzu, Japan). The mobile phase consisted of a gradient elution system using acetonitrile (solvent A) and a 0.5% aqueous solution of acetic acid (solvent B), as described in Table 1. The injection volume was set at 10 µL, the flow rate at 0.5 mL/min, and the column thermostat temperature was maintained at 40°C. The analytical signals for the phenolic compounds (peak areas) were recorded at a detection wavelength of 300 nm (Figure 1).

Table 1. Mobile Phase Gradient Program

Time	Acetonitrile (A), %	0.5% acetic acid (B), %
0	5	95
5	5	95
17	40	60
22	40	60
22,1	5	95
40	Finish	

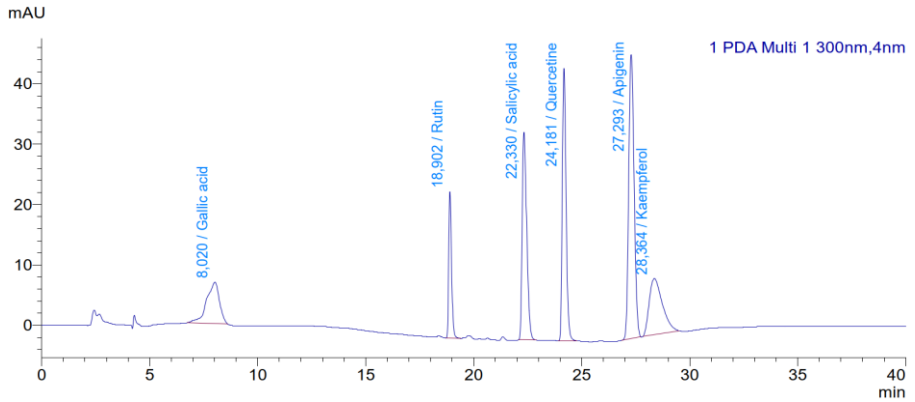

Figure 1. Chromatogram of standards at 300 nm.

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Results

Determination of the amount of phenolic compounds in the sample extract. A chromatogram of a sample extract weighing 1 g was obtained (Figure 2), and based on the results, the amount of phenolic compounds in 100 g of the sample was calculated using the following formula and presented in Table 3.

$$X = \frac{C_{phen} \cdot V_{extract}}{m_{sample}} \cdot 100\ g$$

Here,

X – the amount of phenolic compounds in 100 grams of the fruit sample, expressed in mg;

C_{phen}– the concentration of the phenolic compound in the extract determined by the HPLC method, expressed in mg/L;

$V_{extract}$ – the volume of the sample extract, expressed in liters (L);

m_{sample} – the mass of the sample taken for extraction, expressed in grams (g).

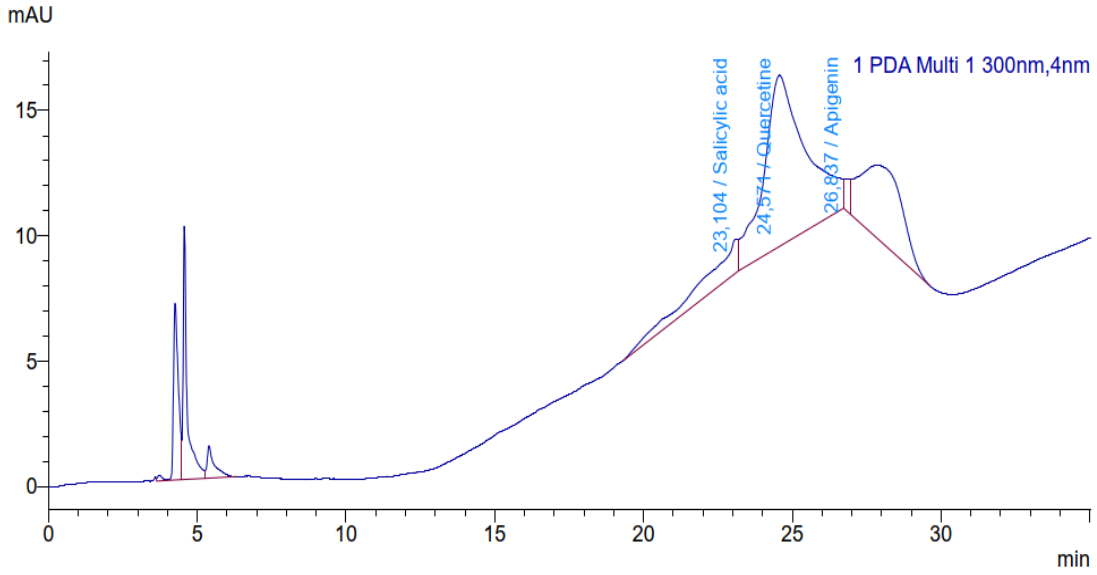

Figure 2. Chromatogram of polyphenols in the sample extract.

Table 2. Amount of polyphenols in the extract and retention times.

Phenol compound name	Capture time, sec	Concentration, mg/l	Amount in 100 g of sample, mg
Gallic acid	Not specified	0	0.000
Routine	Not specified	0	0.000
Salicylic acid	23.104	4.475	11.188
Quercetin	24.571	20.094	50.235
Apigenin	26.837	0.379	0.948
Kaempferol	Not specified	0	0.000

Conclusions

In this study, a high-performance liquid chromatography (HPLC) method was successfully developed and applied for the determination of phenolic compounds in yogurt extract. The method involved extraction using 96% ethanol, ultrasonic-assisted extraction, centrifugation, and filtration, followed by chromatographic separation on a reversed-phase C18 column under gradient elution conditions. Detection at 300 nm enabled the precise quantification of phenolic compounds.

The results revealed that among the analyzed compounds, only salicylic acid (11.188 mg/100 g) and quercetin (50.235 mg/100 g) were detected, while the concentrations of other phenolic compounds were below the detection limits. The method demonstrated high reproducibility and sensitivity, suggesting its applicability for the analysis of polyphenols in food matrices.

Moreover, the successful identification of bioactive phenolic compounds in yogurt extracts highlights the potential of such products as functional foods with added health benefits. Future studies are recommended to optimize extraction conditions further, validate the method across different food matrices, and investigate the bioavailability and stability of the detected compounds during storage and processing.

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