

DETERMINATION OF PHENOLIC COMPOUNDS CONTENT IN PÂTÉ EXTRACT USING THE HPLC METHOD

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Abstract

For the extraction of phenolic compounds, 1 g of the test sample was accurately weighed using an NV222 analytical balance (OHAUS Corporation, USA) with a precision of 0.01 g and transferred into a 50 mL conical flask. Subsequently, 25 mL of 96% ethanol was added. The chromatogram of the extract obtained from the 1 g sample was recorded, and based on the analytical results, the contents of phenolic compounds in 100 g of the sample were determined.

Keywords: pâté; reproduction; recipe formulation; resource; extraction; chromatogram; phenolic compounds

Introduction

Pâté is a food product prepared from the meat and offal of domestic animals. In Uzbekistan, the consumption of pâté is not yet widespread. However, pâtés are rich in calories and considered a nutritious food that can be easily prepared at home.

Moreover, liver pâtés are classified as dietary foods, serving to aid in the treatment of anemia and to improve the functioning of the nervous system.

Popularizing the consumption of pâté products among the population could help meet part of the daily nutritional and caloric needs.

The primary objective of this research is to emphasize the importance of pâtés in the food sector and to promote their consumption among the public.

Materials and Equipment

Gallic acid was obtained from *Macklin* (China), salicylic acid from *Rhydburg Pharmaceuticals* (Germany), and quercetin, apigenin, and kaempferol from *Regal* (China). Rutin was extracted and isolated from natural sources using extraction and column chromatography techniques. HPLC-grade reagents, including water, acetonitrile, glacial acetic acid, and sodium hydroxide, were used throughout the analysis. The quantification of phenolic compounds was performed using the LC-40 Nexera Lite high-performance liquid chromatography (HPLC) system manufactured by Shimadzu Corporation (Japan).

Preparation of Standard Solutions

Standard solutions were prepared by dissolving 5.2 mg of gallic acid, 5.2 mg of salicylic acid, 5 mg of rutin, 5 mg of quercetin, 5 mg of apigenin, and 5 mg of kaempferol in 96% ethanol using an ultrasonic bath for 20 minutes. The resulting solutions were transferred to 50

mL volumetric flasks and brought to volume with ethanol. From each standard solution, 200 μ L was taken and mixed, and four different dilutions were prepared by serial dilution. Each solution was placed into vials and used for chromatographic analysis.

Preparation of Sample Extract

For the extraction of phenolic compounds, 1.0 g of the pâté sample was accurately weighed using an NV222 analytical balance (OHAUS Corporation, USA) with a precision of 0.01 g.

The sample was transferred into a 50 mL conical flask, and 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 using a Mini-7 centrifuge (BIOBASE, China) at 7000 rpm, then filtered through a 0.45 μ m syringe filter and 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 \times 4.6 mm; 5 μ m, Shimadzu, Japan). A gradient mobile phase was employed, consisting of acetonitrile (solvent A) and 0.5% aqueous acetic acid solution (solvent B), as detailed in Table 1. The injection volume was set at 10 μ L, the flow rate at 0.5 mL/min, and the column oven temperature was maintained at 40°C. The analytical signals (peak areas) of the phenolic compounds were recorded at a wavelength of 300 nm (Figure 1).

Table 1. Gradient program of the mobile phase used for the determination of phenolic compounds.

| Time, min. | 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 | |

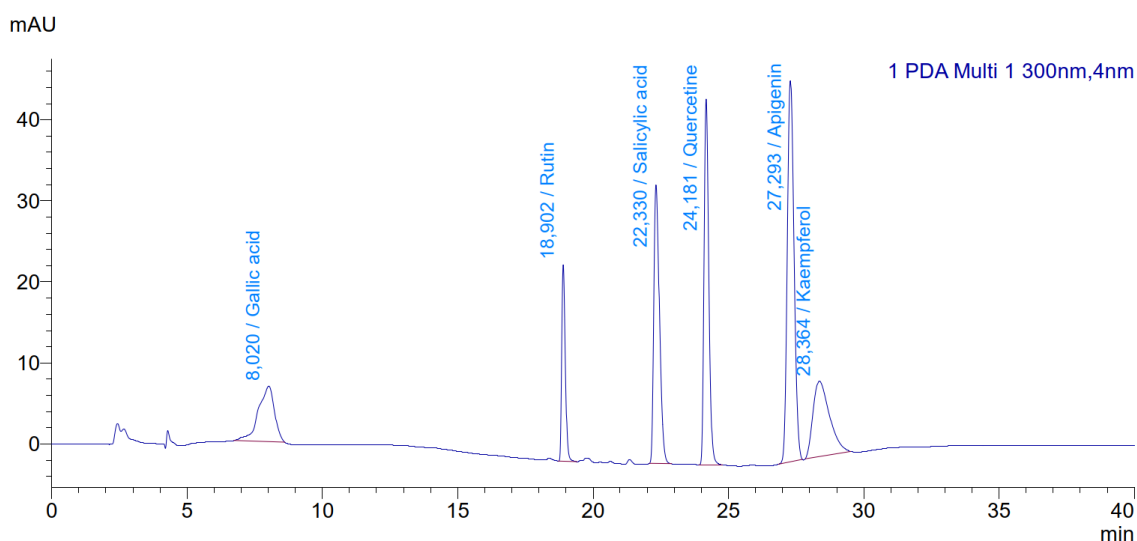


Figure 1. Chromatogram of standards at 300 nm.

Results

Determination of Phenolic Compounds in the Sample Extract. The chromatogram of the sample extract obtained from a 1 g pâté sample was recorded (Figure 2). Based on the chromatographic data, the content of phenolic compounds per 100 g of the sample was calculated using the following formula, and the results are presented in Tables 3.

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

Here,

X – the content of phenolic compounds in 100 grams of the 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 used for extraction, expressed in grams (g).

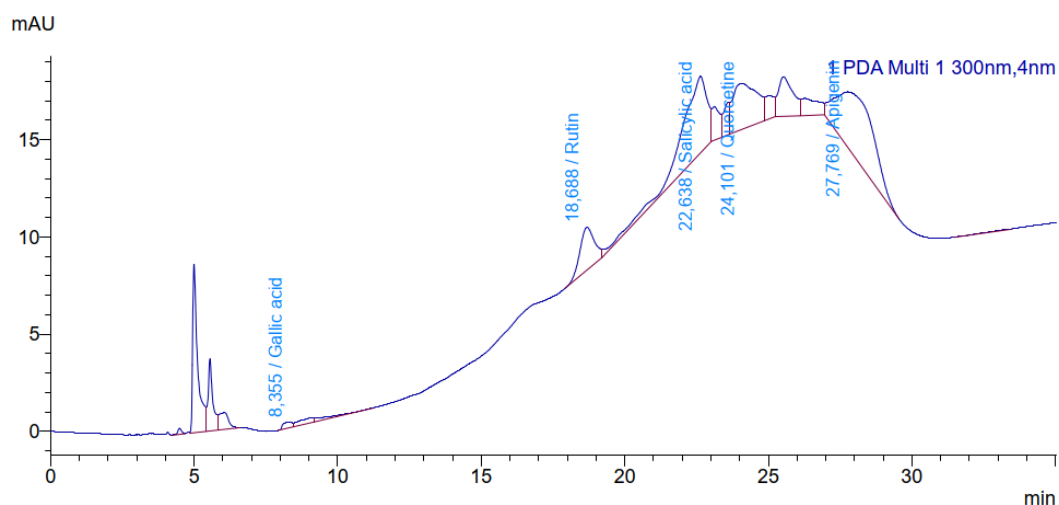


Figure 2. Chromatogram of polyphenols in the sample extract.

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

| Phenol compound name | Holding time, sec | Concentration, mg/l | Amount in 100 g of sample, mg |
|----------------------|-------------------|---------------------|-------------------------------|
| Gallic acid | 8,355 | 0,32 | 0,800 |
| Rutin | 18,688 | 5,852 | 14,630 |
| Salicylic acid | 22,638 | 9,024 | 22,560 |
| Quercetine | 24,101 | 4,039 | 10,098 |
| Apigenin | 27,769 | 6,173 | 15,433 |
| Kaempferol | Not specified | 0 | 0,000 |

Conclusions

In this study, a high-performance liquid chromatography (HPLC) method was successfully applied for the determination of phenolic compounds in pâté extract.

The chromatographic analysis confirmed the presence of phenolic compounds in the extract, and their quantitative content per 100 grams of sample was calculated. The developed method demonstrated high sensitivity, reproducibility, and precision in detecting phenolic compounds in complex food matrices.

The obtained results indicate that pâté products can serve not only as a valuable source of calories but also as a potential source of biologically active compounds that contribute to human health. The findings suggest that incorporating pâté into the diet could help enrich daily nutritional intake with important antioxidants derived from phenolic compounds. Further research is recommended to study the stability of these compounds during processing and storage, as well as their biological activity after consumption.

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