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DETERMINATION OF WATER-SOLUBLE VITAMINS CONTENT IN PASTRY USING THE HPLC METHOD

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Abstract
For the extraction of water-soluble vitamins, 1 g of the sample was accurately weighed and placed into a 50 mL conical flask, followed by the addition of 25 mL of 0.1 N hydrochloric acid (HCl) solution. The mixture was subjected to ultrasonic extraction using a GT SONIC-D3 ultrasonic bath (China) at 60°C for 20 minutes. The standard solutions and sample extract were analyzed using a high-performance liquid chromatography (HPLC) system comprising an LC-40D pump, SIL-40 autosampler, and SPD-M40 photodiode array (PDA) detector, operated with LabSolutions software version 6.92, based on the LC-40 Nexera Lite platform.

Keywords: pâté; vitamin B_{12}; vitamin C; vitamin content; sample extract; calorie; vitamins.

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. In addition, liver pâtés are regarded as dietary foods and are known to assist in the treatment of anemia and improve the functioning of the nervous system [1-3]. Promoting the consumption of pâté products among the population could help partially meet the nutritional and caloric needs of individuals. The main objective of the present study is to highlight the significance of pâté in the food sector and to promote its wider consumption [4-7].

Materials and Equipment Used

Vitamin B_{12} was obtained from *Rhydburg Pharmaceuticals* (Germany), vitamin C from *Carl Roth GmbH* (Germany), vitamin B_9 from *DSM Nutritional Products GmbX* (Germany), and vitamins B_1, B_2, B_3, B_6, and PP from *BLDPharm* (China). HPLC-grade water, acetonitrile, glacial acetic acid, and analytical-grade sodium hydroxide were used as reagents [8-13].

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The determination of water-soluble vitamin contents was performed using a high-performance liquid chromatography (HPLC) system, LC-40 Nexera Lite, manufactured by Shimadzu Corporation (Japan) [1].

Preparation of Standard Solutions

Standard solutions of vitamins C (CAS 50-81-7), B_1 (CAS 59-43-8), B_6 (CAS 58-56-0), B_3 (CAS 59-67-6), B_{12} (CAS 68-19-9), and PP (CAS 98-92-0) were prepared by dissolving 5 mg of each vitamin in 50 mL of 0.1 N hydrochloric acid (HCl) to achieve a concentration of 100 mg/L. Standard solutions of vitamins B_2 (CAS 83-88-5) and B_9 (CAS 59-30-3) were prepared by dissolving 5 mg of each in 50 mL of 0.025% sodium hydroxide (NaOH) solution. Subsequently, 200 µL aliquots of the B_1, B_6, B_3, B_{12}, and PP vitamin standard solutions were mixed to prepare a combined solution with a concentration of 14.286 mg/L for each vitamin. Further dilutions were prepared to obtain standard solutions of 7.143, 3.571, and 1.786 mg/L.

Similarly, standard solutions of vitamin C were prepared at concentrations of 286, 143, 71.5, and 57.2 mg/L. Distilled water was used for the preparation of the 0 mg/L blank sample for calibration curve construction [14-19].

Preparation of Sample Extract

For the extraction of water-soluble vitamins, 1.0 g of the test sample was weighed and placed into a 50 mL conical flask, followed by the addition of 25 mL of 0.1 N HCl solution. The mixture was subjected to ultrasonic-assisted extraction using a GT SONIC-D3 ultrasonic bath (China) at 60°C for 20 minutes. After extraction, the mixture was cooled to room temperature, filtered, and the final volume was adjusted to 25 mL with distilled water in a volumetric flask. An aliquot of 1.5 mL of the extract was filtered through a 0.22 µm syringe filter into a vial and used for subsequent HPLC analysis [20-23].

Chromatographic Conditions

Determination of Vitamins. The standard solutions and sample extracts were analyzed using a high-performance liquid chromatography (HPLC) system comprising an LC-40D pump, SIL-40 autosampler, and SPD-M40 photodiode array (PDA) detector, operated with LabSolutions software version 6.92, based on the LC-40 Nexera Lite platform (Shimadzu Corporation, Japan) [24-26].

Separation was achieved using a reversed-phase Shim-pack GIST C18 column (150 × 4.6 mm; 5 µm, Shimadzu, Japan) with a gradient mobile phase composed of acetonitrile (solvent A) and 0.25% aqueous acetic acid solution (solvent B), as described in Table 1 [27-29].

The injection volume was set at 10 µL, the flow rate at 0.6 mL/min, and the column oven temperature was maintained at 40°C. Analytical signals (peak areas) for each vitamin were recorded at three different detection wavelengths: 265 nm, 291 nm, and 550 nm (Figures 1–3) [28].

For the determination of vitamin C, a specific 15-minute gradient program was applied (Table 2), and the analytical signal was monitored at 265 nm [30].

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Table 1. Gradient Program Used for Vitamin Determination

Time, min.	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Finish	

Table 2. Mobile phase gradient program for vitamin C quantification.

Time, min.	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Finish	

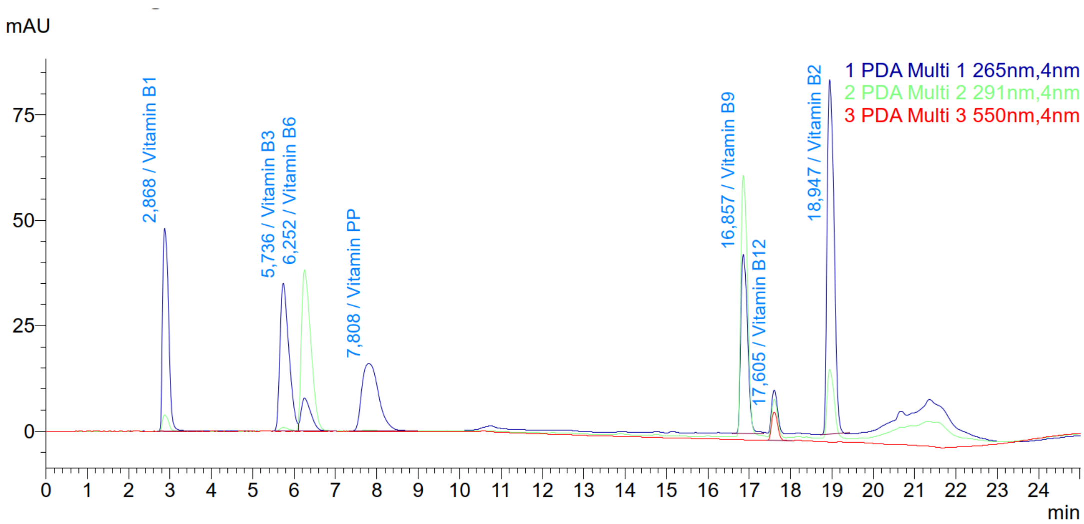

Figure 1. Chromatogram of a standard solution of vitamins.

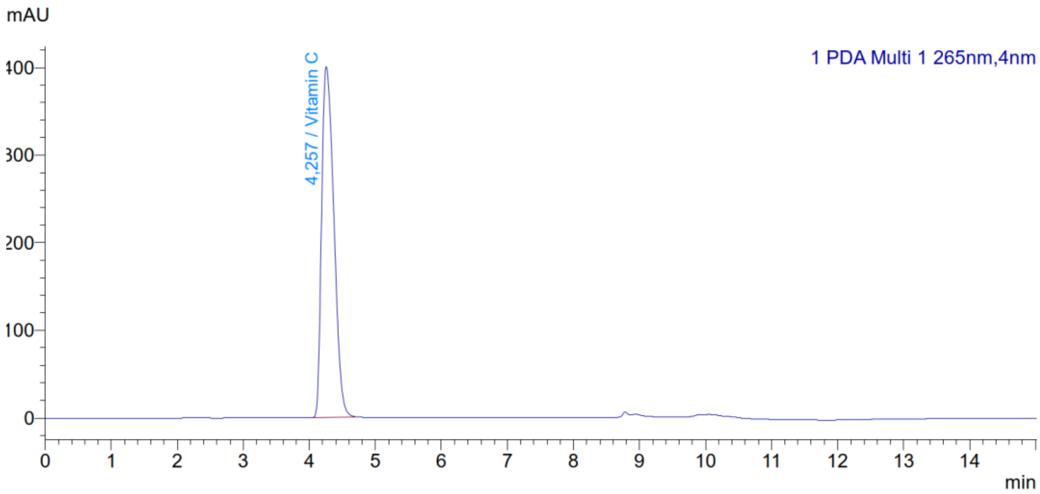

Figure 2. Chromatogram of a vitamin C standard solution

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Results

Determination of vitamins in the sample extract. A chromatogram of the sample extract (Figures 3-4) was obtained and based on the results, the amounts of vitamins in 100 g of the sample were calculated using the following formula and are presented in Table 3.

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

Here,

X – the amount of vitamins in 100 grams of the sample, expressed in mg; C_{vit} – the concentration of the vitamin in the extract determined by the HPLC method, expressed in mg/L;

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

$m_{samplem}$ – the mass of the sample taken for extraction, expressed in grams

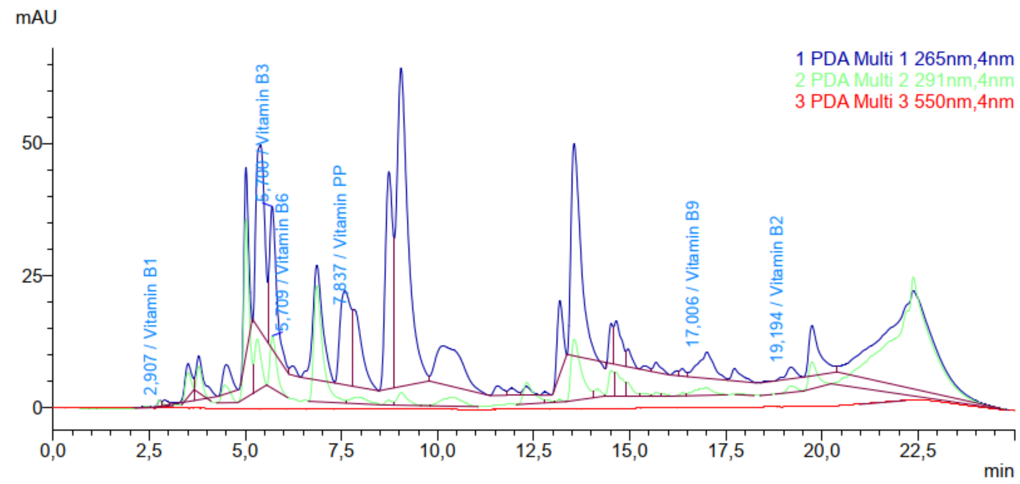

Figure 3. Chromatogram of the determination of vitamins in the sample extract.

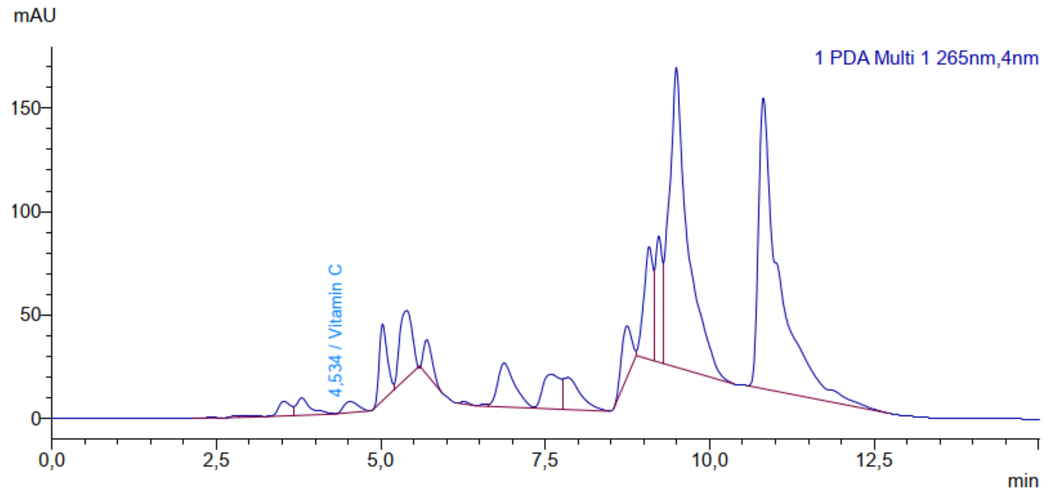

Figure 4. Chromatogram of vitamin C in the sample extract.

Table 3. Amount of vitamins in the extract and retention times.

Vitamin	Capture time, sec	Concentration, mg/l	Amount in 100 g of sample, mg
Vitamin B_1	2,907	0,343	0,858
Vitamin B_3	5,7	9,251	23,128
Vitamin PP	7,837	7,403	18,508
Vitamin B_9	17,006	4,186	10,465
Vitamin B_2	19,194	0,839	2,098
Vitamin B_6	5,709	2,752	6,880
Vitamin B_{12}	Not specified	0	0,000
Vitamin C	4,534	4,271	10,678

Conclusions

In this study, a high-performance liquid chromatography (HPLC) method was successfully developed and applied for the determination of water-soluble vitamins in pâté samples. The ultrasonic-assisted extraction with 0.1 N hydrochloric acid at 60°C, combined with optimized chromatographic conditions using a reversed-phase C18 column, allowed for the accurate identification and quantification of vitamins B_1, B_2, B_3, B_6, B_9, B_{12}, PP, and C.

The analysis demonstrated that certain vitamins, particularly vitamin B_6 and vitamin C, were present in notable concentrations, while vitamin B_{12} was either undetected or found at trace levels. The method exhibited high sensitivity, repeatability, and precision, making it a reliable approach for the assessment of water-soluble vitamins in complex food matrices such as pâté.

Given the nutritional significance of these vitamins, promoting the consumption of pâté products enriched with essential micronutrients could contribute to improving the dietary intake and overall health of the population. Future research should focus on validating the method across different types of pâté formulations and studying the stability of water-soluble vitamins during storage and processing.

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