American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

The Amount of Water-Soluble Vitamins Was Determined by High-Performance Liquid Chromatography

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Received: 2024, 15, Mar
Accepted: 2025, 21, Apr
Published: 2025, 13, May

Annotation: A high-performance liquid chromatography (HPLC) method was developed and validated for the simultaneous quantification of water-soluble vitamins (C, B_1, B_2, B_3, B_6, B_7, B_9, B_{12}) in food samples. Extraction was performed on 5-10 g of sample using 40 % ethanol: boiling with magnetic stirring for 1 h, standing at 20 °C for 2 h, two successive extractions with 25 mL each, combining filtrates and diluting to 100 mL, then centrifuging at 7000 rpm for 10 min. Working standards (1 mg/mL) were prepared by dissolving 50 mg of each vitamin in 40 % ethanol to 50 mL. The mobile phase comprised an acetate buffer-acetonitrile system, providing sharp separation and excellent reproducibility. This method is suitable for routine monitoring of water-soluble vitamin content in food and pharmaceutical matrices.

Keywords: water-soluble vitamins; HPLC; 40 % ethanol extraction; acetate buffer; acetonitrile; quantitative analysis.

INTRODUCTION

Water-soluble vitamins constitute a group of organic compounds that readily dissolve in water and perform essential metabolic functions in the body. These include ascorbic acid (vitamin C) and the B-complex vitamins – thiamine (B_1), riboflavin (B_2), niacin (B_3), pyridoxine (B_6), biotin (B_7), folate (B_9), and cobalamin (B_{12}). Because they are not stored in significant amounts and are excreted in urine, regular dietary intake is required. Their primary roles encompass energy metabolism, DNA synthesis, immune support, and antioxidant defense. Vitamin C is renowned for its antioxidant activity, its role in collagen biosynthesis, and its modulation of immune function. B-vitamins are crucial metabolic cofactors: thiamine participates in carbohydrate metabolism; riboflavin and niacin serve in redox reactions; and folate and cobalamin are vital for cell division and hematopoiesis. Despite their importance, deficiencies still occur, especially in regions with limited dietary diversity. Vitamin C deficiency causes scurvy – characterized by connective-tissue breakdown and gum bleeding – while thiamine deficiency leads to beriberi with neurological and cardiovascular manifestations. Folate and B_{12} deficiencies result in megaloblastic anemia and neurological complications. Diets rich in fresh fruits, vegetables, whole grains, dairy, meat, and eggs ensure adequate intake and prevent avitaminosis, supporting normal physiology and healthy longevity [1, 2].

In this study, water-soluble vitamins were quantified by high-performance liquid chromatography (HPLC). A 5-10 g sample was weighed on analytical scales and placed in a 300 mL flat-bottom flask. Fifty milliliters of 40 % ethanol were added. The mixture – equipped with a magnetic stirrer and reflux condenser – was boiled with vigorous stirring for 1 hour, then stirred at room temperature for 2 hours. After settling, the extract was filtered. The remaining residue underwent two further extractions with 25 mL portions of 40 % ethanol. The combined filtrates were transferred to a 100 mL volumetric flask and brought to volume with 40 % ethanol (5-10 % sample). The resulting solution was centrifuged at 7 000 rpm for 10 minutes, and the supernatant was taken for analysis.

MATERIALS AND METHODS

Working solutions of the water-soluble vitamins at a concentration of 1 mg/mL were prepared. For this purpose, 50.0 mg of each vitamin standard was accurately weighed on an analytical balance and dissolved in 40% ethanol in a 50 mL volumetric flask. The flask was then filled to the calibration mark.

In the literature, phosphate and acetate buffer systems and acetonitrile have been used as eluents for HPLC determination of water-soluble vitamins. In this study, an acetate buffer system and acetonitrile were used.

Chromatographic conditions:

1. HPLC system: Agilent 1200 (equipped with an autosampler)
2. Column: Eclipse XDB-C18 (reversed-phase), 5 μm, 4.6 × 250 mm
3. Detector: diode array detector (DAD) set at 250 nm
4. Flow rate: 0.8 mL/min

Mobile phase (eluent): acetate buffer : acetonitrile

- 0-5 min: 96:4
- 6-8 min: 90:10
- 9-15 min: 80:20
- 15-17 min: 96:4
- Column oven temperature: 25 °C
- Injection volume: 5 μL

Initially, the working standard solutions were injected into the HPLC system, followed by the prepared working solutions.

RESULTS AND DISCUSSION

Experimental drying trials of garlic were carried out in the laboratory of Tashkent State Technical University. Eight dried garlic samples – prepared using three different drying methods – were analyzed for their water-soluble vitamin profiles. Table 1 shows the results of these laboratory determinations of water-soluble vitamin content in garlic [3, 4].

Table 1. Water-soluble vitamins were detected in dried garlic

Vitamins	Sample Number							
	№1	№2	№3	№4	№5	№6	№7	№8
	Concentration (mg/g)							
B-2	3,13	4,21	9,82	6,84	5,33	6,89	2,34	7,44
B-6	2,98	3,05	8,71	4,09	7,15	7,24	1,22	8,16
B-9	5,04	4,51	6,92	5,43	5,57	4,69	2,40	4,21
B-3	1,74	2,04	3,11	3,08	2,44	3,02	0,10	1,97
C	1,73	1,09	2,11	1,64	1,36	1,97	0,69	1,09

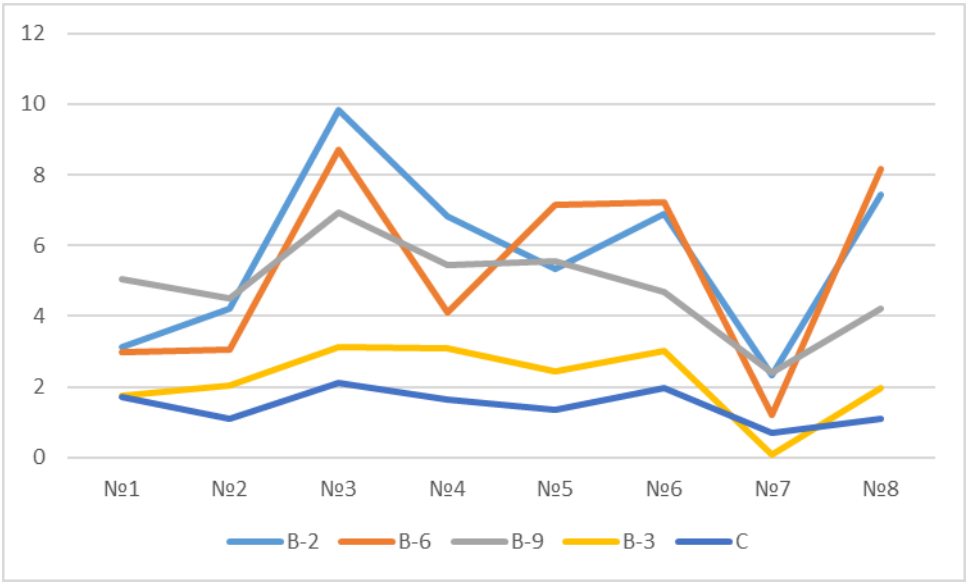

Fig. 1 Vitamins

CONCLUSION

Among the eight samples prepared using three garlic-drying methods, Sample No. 3 – dried in the vibro-convective drying unit (the author's proposed method) – exhibited the following water-soluble vitamin concentrations: vitamin B_2 – 9.82 mg/g; vitamin B_6 – 8.71 mg/g; vitamin B_9 – 6.92 mg/g; vitamin B_3 – 3.11 mg/g; vitamin C – 2.11 mg/g [5-7].

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