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DETERMINATION OF VITAMIN CONTENT IN THE "ASSHAN" FOOD SUPPLEMENT USING THE HPLC METHOD

Nodira I. Ikromova
Andijan State University, PhD student in the Department of Chemistry
https://orcid.org/0009-0000-5944-3832
E-mail: Nodina94@mail.ru

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Annotation

The content of water-soluble vitamins in the "Asshan" food supplement, which is a composition based on orange and grapefruit peels, was determined using high-performance liquid chromatography (HPLC). It was established that vitamin C has the highest concentration in the new supplement — 239.175 mg/100 g, followed by B-group vitamins: B2 (3.16 mg/100 g), B1 (1.095 mg/100 g), and B9 (0.74 mg/100 g). Analysis of the obtained results indicates that the "Asshan" food supplement is a promising product for use in modern medicine and traditional practice for the prevention and treatment of cardiovascular and bone diseases, anemia, disorders of the nervous and muscular systems, as well as inflammatory conditions of internal organs.

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Introduction. Orange (*Citrus sinensis* L. Osb.) and grapefruit (*Citrus paradisi*) are among the most widely consumed citrus fruits across all age groups worldwide. Their sweet taste contributes to increased consumption, which simultaneously leads to growing volumes of peel waste. Several studies [1] have shown that orange and grapefruit peels contain significantly higher levels of biologically active compounds — flavonoids, polyphenols, vitamins, alkaloids, and mineral substances — compared to the fruit pulp, making them a valuable raw material for the food, pharmaceutical, and cosmetic industries.

Vitamins are organic substances that play essential roles in numerous cellular processes, particularly in energy metabolism and anabolic pathways. They are necessary for the normal physical and intellectual functioning of the human body. Vitamins are classified into water-soluble and fat-soluble groups. Fat-soluble vitamins (A, D, E, K) can be stored in fatty tissues and the liver for extended periods, whereas water-soluble vitamins are easily excreted from the body. Since vitamins are practically not synthesized in the human body, their intake is mainly ensured through fruits and food products. Several studies have confirmed that citrus peels contain more vitamins than many other fruits [2].

In our previous studies, the antioxidant [3] and antiradical activity of the peels of various orange and grapefruit varieties was investigated. According to the obtained results, a mixture of Iranian orange and red grapefruit peels at a 3:1 ratio exhibited strong antiradical activity, and this mixture was tentatively named "Asshan." The present article presents the results of the study aimed at determining the vitamin content of the "Asshan" food supplement.

Materials and Methods

Reagents and equipment. Standard samples of vitamins B_1, B_2, B_3, B_6, and PP (BLDPharm, China), vitamin B_{12} (Rydburg Pharmaceuticals, Germany), vitamin C (Carl Roth GmbH, Germany), and vitamin

B_9 (DSM Nutritional Products GmbH, Germany) were used. HPLC-grade purified water, acetonitrile, acetic acid, hydrochloric acid, and sodium hydroxide were used for the chromatographic analysis [4].

Preparation of standard solutions. Five milligrams of each vitamin (C, B_1, B_6, B_3, and PP) were dissolved in 50 ml of 0.1 M hydrochloric acid. Similarly, 5 mg of vitamins B_2 and B_9 were dissolved in 50 ml of 0.025% sodium hydroxide solution. From each prepared solution, 150 µl was collected and combined to obtain a mixed standard solution with a vitamin concentration of 12.5 mg/L. Serial dilutions were then performed to obtain a set of standards with lower concentrations [4].

Extraction procedure. For the analysis, 1.00 g of the "Asshan" sample was placed into a 50-ml conical flask and mixed with 25 ml of 0.1 M HCl. Extraction was carried out in a GT SONIC-D3 ultrasonic bath at 60 °C for 20 minutes. After cooling, the solution was transferred to a 25-ml volumetric flask and brought up to volume with distilled water. Then, 1.5 ml of the extract was centrifuged at 7000 rpm using a Bibse Mini-7 centrifuge, filtered through a 0.22-µm syringe filter, and used for analysis.

HPLC analysis. Standard solutions and the sample extract were analyzed using an LC-40 Nexera Lite HPLC system equipped with the LabSolutions ver. 6.92 software. Separation was performed on a Shim-pack GIST C18 column (150 × 4.6 mm; 5 µm, Shimadzu) with a gradient mobile phase consisting of acetonitrile (A) and 0.25% aqueous acetic acid (B) [4]. The injection volume was 10 µl, the flow rate was set to 0.6 ml/min, and the column temperature was maintained at 40 °C. Analytical signals were recorded at 265, 291, and 550 nm. A separate 15-minute gradient was used for vitamin C detection [4].

Results and Discussion

Chromatograms of the B-group vitamins and vitamin C in the "Asshan" extract are presented in Figures 1 and 2. Calibration curves were constructed for each vitamin based on the relationship between peak area and concentration. Regression equations and coefficients of determination (R^2) were calculated. High calibration accuracy was confirmed by R^2 values that deviated from 1.000 by no more than 0.002 [4].

Table 1 presents the results of determining the vitamin content in the "Asshan" food supplement.

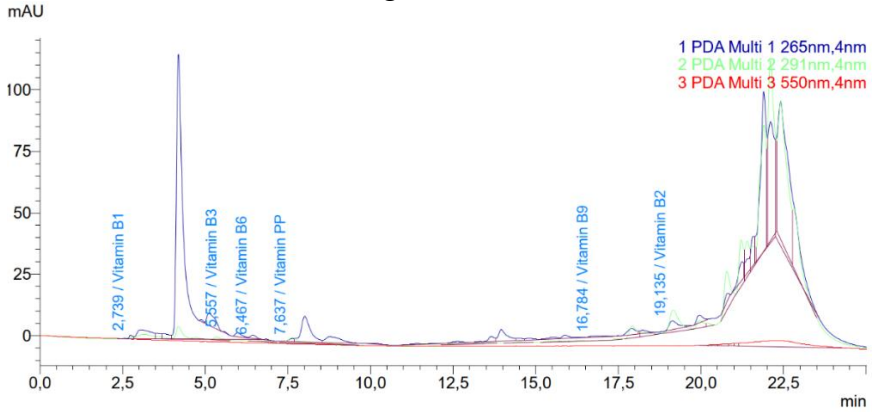


Figure 1. Chromatogram of vitamins group B determination in the "Asshan" extract.

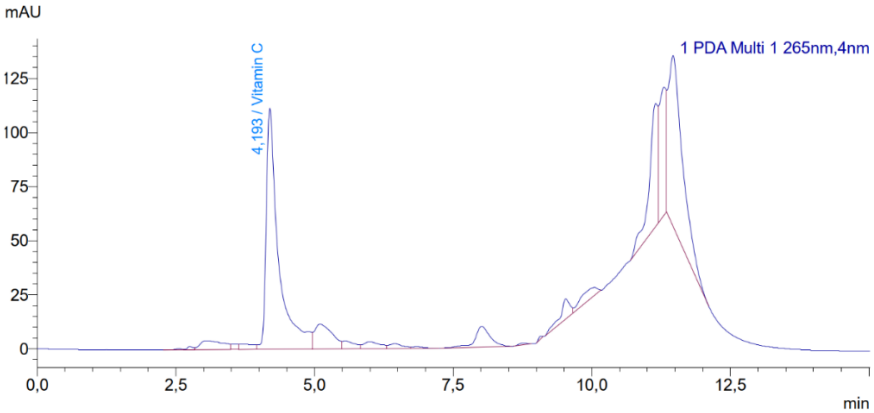


Figure 2. Chromatogram of vitamin C determination in the "Asshan" extract.

Table 1. HPLC results of vitamin determination in the extract of the “Asshan” food supplement.

Vitamin		Retention time, min	Concentration in extract, mg/L	Amount in 100 g FS, mg
B_1	Thiamine	2,74	0,44	1,10
B_3	Nicotinic acid	5,56	0,02	0,05
PP	Niacin	7,64	0,47	1,17
B_9	Folic acid	16,78	0,30	0,75
B_2	Riboflavin	19,14	1,26	3,16
B_6	Pyridoxine	6,47	0,32	0,80
B_{12}	Cyanocobalamin	*Aniqlanmadi*		
C	Ascorbic acid	4,19	95,67	239,18

The chromatograms and the differences in retention times demonstrate that the selected chromatographic conditions ensure good separation of hydrophilic vitamins. Vitamin B_{12} was not detected in the “Asshan” food supplement.

The analysis indicates that the supplement is rich in vitamins, particularly vitamin C, whose content significantly exceeds that of other vitamins. Vitamin C (ascorbic acid), being water-soluble, functions as a coenzyme in cellular processes and regulates redox reactions. It also plays crucial roles in collagen synthesis, iron absorption, immune protection and defense against free radicals [5]. Deficiency of vitamin C leads to scurvy, characterized by gum bleeding, tooth loss and impaired immunity [6].

C vitamin content in orange and grapefruit peels, investigated by various researchers using multiple solvents [9] and analytical methods [8], ranged between 53.8 and 232.98 mg/100 g. In our “Asshan” food supplement, a similar value of 239.18 mg/100 g was obtained. This exceeds daily requirements by more than threefold, as shown in Table 2.

Table 2. Daily requirement coverage (for adult men) by 100 g of “Asshan” food supplement.

Vitamin	Daily requirement, mg	Amount in 100 g FS, mg	Relative to requirement, %
B_1	1.1-1.5	1,095	99.5-73
B_2	1.3	3,160	243.1
B_9	0.3-0.4	0,745	248-186
C	75-90	239,175	319-266

These data show that, in addition to vitamin C, the levels of vitamins B_1, B_2 and B_9 in 100 g of the supplement significantly exceed daily requirements. B-group vitamins are water-soluble compounds that act as coenzymes in numerous enzymatic systems. They participate in DNA synthesis, cell division, nerve impulse transmission, hematopoiesis and many physiological processes. Recent studies confirm their role in modulating immune responses, reducing oxidative stress and maintaining genetic stability [2, 5].

Vitamin B_1 deficiency results in beriberi, characterized by muscle weakness, reduced reflexes, edema, fatigue, irritability and the Wernicke–Korsakoff syndrome [5]. Riboflavin (B_2) has been shown to reduce migraine attacks and regulate oxidative stress (Gropper & Smith) [10]. Deficiency of vitamin B_9 leads to megaloblastic anemia and neural tube defects, such as spina bifida and anencephaly [5].

Oloye et al. [9] studied B-vitamin levels in pectin extracted from orange and grapefruit peels dried at 30°C for 3 days. B_2 was reported as 0.150 mg/g in orange and 0.110 mg/g in grapefruit [8]. Differences from our results are due to variations in drying conditions (35°C, 7 days with air circulation), analytical method (HPLC), and solvent (0.1 N HCl).

Thus, inclusion of “Asshan” in the daily diet may help prevent deficiencies of these vitamins. In particular, the high vitamin C content suggests that the supplement may be effective in preventing and treating inflammation, diabetes and tumor development.

Conclusions.

HPLC analysis of eight hydrophilic vitamins in the “Asshan” food supplement revealed that four vitamins were quantified with high accuracy. Among them, vitamin C showed the highest concentration (239.175 mg/100 g), followed by B_2, B_1 and B_9. The findings confirm that the newly developed “Asshan” supplement is a promising candidate for use in modern medicine and traditional healing for prevention and treatment of cardiovascular, skeletal, hematologic, neuromuscular and inflammatory conditions associated with vitamin deficiencies.

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