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DISCUSSION OF THE RESULTS OF THE INVESTIGATION OF WATER-SOLUBLE VITAMINS IN THE BIOMATERIAL

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Annotation. This study focuses on developing a technology for producing nutritionally valuable granules for livestock and poultry from cottonseed meal with a reduced gossypol content through biomaterial-based processing, as well as re-evaluating the analytical results. During the study, four types of samples were subjected to laboratory analyses, and the results obtained were evaluated.

Keywords: cottonseed meal, bioproduct, vitamins, chromatography.

How to Cite

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Regardless of whether a product is intended for human consumption, non-food applications, or animal feed, its macroelement, microelement, vitamin, and protein contents are of considerable importance [1]. Deficiencies of macro- and microelements in consumed products can contribute to the development of various diseases and physiological disorders. According to data from the Republican Scientific and Practical Medical Center of Endocrinology, iodine deficiency affects 40.2% of the population of Uzbekistan. The significance of addressing iodine deficiency at the national level is reflected in the adoption of Law No. O‘RQ-907, dated May 3, 2007, entitled “On the Prevention of Iodine Deficiency Disorders” [2].

Deficiencies of macroelements, microelements, and vitamins in the body can be addressed through the daily intake of food and animal feed. Based on the physiological requirements of animals, previous studies have recommended the following concentrations per kilogram of dry matter in daily feed rations: 3–12 mg of Cu, 20–60 mg of Zn, 25–60 mg of Mg, 0.06–1.2 mg of iodine (I), 0.3–1.0 mg of Co, and 0.5 mg of Se [3, pp. 44–45].

Several studies have demonstrated that deficiencies of essential elements in animal feed can lead to infertility, changes in the biochemical composition of milk, increased aggression, growth retardation, impaired hormone synthesis, altered activity of certain enzymes, deterioration of liver function, irregular reproductive cycles in female animals, and abortion [4,5,6].

In addition to the importance of macro- and microelements in animal diets, vitamins also play a crucial role in maintaining physiological functions and productivity.

Vitamins are low-molecular-weight organic compounds of diverse chemical structures that regulate biological processes and act as catalysts in living organisms [1]. Insufficient vitamin intake in animal diets can result in avitaminosis, increased susceptibility to disease, tooth loss, hair loss, and other clinical manifestations.

The higher the productivity of an animal, the greater its vitamin requirements. Consequently, partial vitamin deficiencies, known as hypovitaminosis, are frequently observed in highly productive animals. Prolonged hypovitaminosis in productive livestock can eventually lead to avitaminosis. Conversely, excessive vitamin intake through daily feed rations may cause hypervitaminosis. Vitamin D deficiency, in particular, can develop into a chronic condition in poultry. Factors such as keeping chicks indoors and limited exposure to sunlight may reduce vitamin D synthesis and utilization. This deficiency disrupts calcium–phosphorus metabolism and impairs bone tissue development. The condition is particularly common in chicks aged 3–5 months [8].

Initially, affected chicks may exhibit weakness, ruffled feathers, drooping wing feathers, and reduced or altered appetite. In some cases, crop enlargement, atony of the muscular stomach, and diarrhea may also occur. As the condition progresses, weakness of the legs, lameness, inability to stand, and impaired motor coordination may develop. Affected chicks often remain recumbent and experience difficulty rising [9].

Natural feedstuffs consumed by animals, particularly green forage, are rich in vitamins. Furthermore, vitamin C can be synthesized in animal tissues, while microorganisms in the rumen of ruminants synthesize B-group vitamins and phylloquinone (vitamin K_1). These factors may suggest that animals can meet their vitamin requirements through natural processes. Nevertheless, periodic monitoring of vitamin status, together with the observation and documentation of physiological changes, remains advisable [10].

Indonesian researchers S. Sadarman and colleagues investigated the effects of dietary zinc (Zn) supplementation in broiler chickens. Their research examined improvements in metabolism and the role of zinc in protein, carbohydrate, and lipid metabolism [11].

J. Lisman, S. Sadarman, A. Efendi, and colleagues investigated the beneficial effects of *Phaleria macrocarpa* on broiler chick development. In addition to growth-related outcomes, they examined its potential effects on antioxidant activity, resistance to external stressors, and several other physiological parameters [12].

Eid Y., Ebeid T., and Younis H. reported that the inclusion of vitamin E in poultry feed formulations reduced stress levels in the animals [13].

According to the widely used classification of vitamins based on solubility, they can be divided into two major groups: water-soluble and fat-soluble vitamins. The following table presents the classification of vitamins according to their solubility.

Table 1. Classification of Vitamins

№	Water-Soluble Vitamins	Fat-Soluble Vitamins
1	Vitamin B_1 (thiamine)	Vitamin A (retinol)
2	Vitamin B_2 (riboflavin)	Carotene (provitamin A)
3	Vitamin B_3 (pantothenic acid)	Vitamin D_1 (calciferol)
4	Vitamin B_4 (choline)	Vitamin D_2
5	Vitamin B_5 or PP (nicotinamide)	Vitamin D_3
6	Vitamin B_6 (pyridoxine)	Vitamin E (α-, β-, and γ-tocopherols)
7	Vitamin B_{12} (cyanocobalamin)	Vitamin K_1 (phylloquinone)

8	Vitamin BC (folic acid)	
9	Vitamin H (biotin)	

The table above presents the classification of vitamins according to their solubility. The inclusion of these vitamins in compound feed formulations at appropriate concentrations helps prevent various diseases in productive animals and improve their productivity.

In the present study, cottonseed meal was subjected to biotechnological treatment to reduce the concentration of gossypol, a toxic compound, increase its protein content, and improve the digestibility of carbohydrates present in the meal. The laboratory apparatus used for producing the biomaterial from cottonseed meal is presented below.

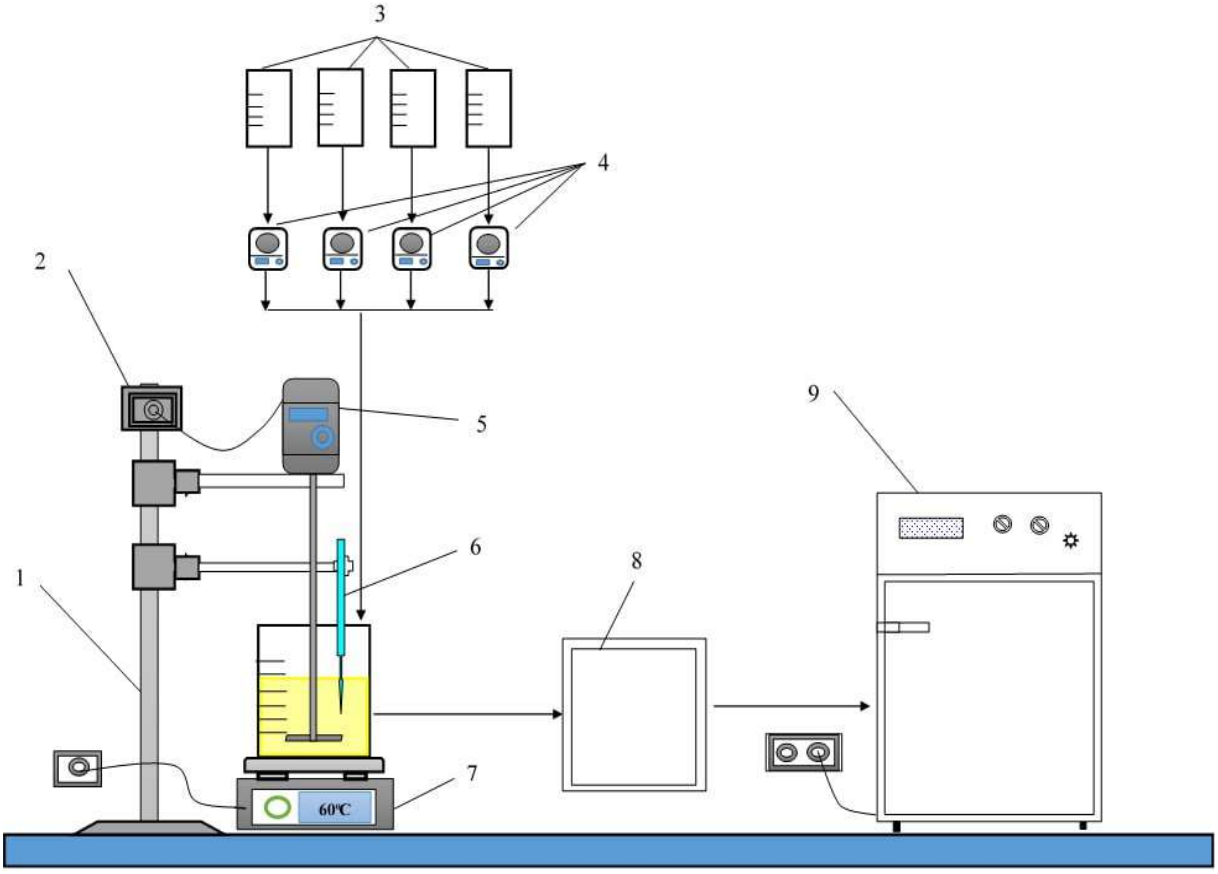


Figure 1. Laboratory Apparatus for Biomaterial Production

1. Stand, 2. Mixer, 3. Sample container, 4. Electronic balance for samples, 5. Mixer, 6. Thermometer, 7. Magnetic stirrer, 8. Raw material tray, 9. Dryer

The laboratory apparatus shown above was used to produce biomaterial from toasted cottonseed meal through biotechnological treatment. Initially, 1,000 g of cottonseed meal, approximately 300–400 mL of water, 3–4 g of microorganisms, and 100–150 g of carbohydrates were weighed using an electronic balance (4), with the samples placed in a sample container (3). The mixture was then transferred into a 2,000 mL beaker and placed on a magnetic stirrer (7). The temperature was raised to 30–35 °C to ensure optimal microbial activity. The resulting biomaterial, which contained 60–65% moisture, was dried in a drying cabinet at 50–60 °C for 30 min to reduce its moisture content to 16–18%, the level required for granulation. The required mass of the dried biomaterial was then weighed for the granulation process.

Methodology

Reagents and Equipment. Vitamin B_{12} was obtained from Rhydburg Pharmaceuticals (Germany), vitamin C from Carl Roth GmbH (Germany), and vitamin B_9 from DSM Nutritional Products GmbH

(Germany). Vitamins B_1, B_2, B_3, B_6, and PP were obtained from BLDPharm (China). HPLC-grade water and acetonitrile, as well as analytical-grade acetic acid and sodium hydroxide, were used in the study.

The concentrations of water-soluble vitamins in the plant-derived samples were determined using an LC-40 Nexera Lite high-performance liquid chromatograph (HPLC) manufactured by Shimadzu, Japan [1].

Preparation of standard solutions. Standard solutions (100 mg/L) of vitamins C (CAS No. 50-81-7), B_1 (CAS No. 59-43-8), B_6 (CAS No. 58-56-0), B_3 (CAS No. 59-67-6), B_{12} (CAS No. 68-19-9), and PP (CAS No. 98-92-0) were prepared by dissolving 5 mg of each vitamin in 50 mL of 0.1 N hydrochloric acid (HCl). Standard solutions of vitamins B_2 (CAS No. 83-88-5) and B_9 (CAS No. 59-30-3) were prepared by dissolving 5 mg of each vitamin in 50 mL of 0.025% sodium hydroxide solution.

Subsequently, 200 µL aliquots of the initial standard solutions of vitamins B_1, B_6, B_3, B_{12}, and PP were combined to prepare a mixed standard solution containing each vitamin at a concentration of 14.286 mg/L. Further standard solutions with concentrations of 7.143, 3.571, and 1.786 mg/L were prepared by serial dilution. Standard solutions of vitamin C were also prepared at concentrations of 286, 143, 71.5, and 57.2 mg/L. Pure water was used as the blank for the calibration curve corresponding to a concentration of 0 mg/L.

Preparation of sample extracts. To extract water-soluble vitamins, 1 g of the sample was weighed and transferred into a 50 mL conical flask, followed by the addition of 25 mL of 0.1 N HCl. The mixture was extracted in an ultrasonic bath (GT SONIC-D3, China) at 60 °C for 20 min. The mixture was then cooled and filtered, and the filtrate was made up to a final volume of 25 mL with water in a volumetric flask. An aliquot of 1.5 mL of the extract was passed through a 0.22 µm syringe filter into an HPLC vial for subsequent analysis.

Chromatographic conditions. Determination of vitamins. Standard solutions and sample extracts were analyzed using an LC-40 Nexera Lite high-performance liquid chromatograph equipped with an LC-40 pump, an SIL-40 autosampler, and an SPD-M40 photodiode array (PDA) detector. Data acquisition and processing were performed using LabSolutions software, version 6.92.

A Shim-pack GIST C18 reversed-phase column (150 × 4.6 mm, 5 µm; Shimadzu, Japan) was used. Gradient elution was performed using acetonitrile (mobile phase A) and a 0.25% aqueous acetic acid solution (mobile phase B), according to the gradient program presented in Table 1. The injection volume was 10 µL, the flow rate was 0.6 mL/min, and the column temperature was maintained at 40 °C. The analytical signals (peak areas) of the vitamins were recorded at wavelengths of 265, 291, and 550 nm, as illustrated in Figures 1–3. For vitamin C determination, a 15-minute gradient program was used (Table 2), and the analytical signal was measured at a wavelength of 265 nm.

Results and Discussion

Table 1. Mobile Phase Gradient Program 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	End	

Table 2. Mobile phase gradient program for vitamin C determination

Time (min)	Acetonitrile (A), %	0.5% Acetic Acid (B), %
0	0	100
2	0	100

6	50	50
6,01	0	100
15	End	

Results obtained

Determination of Vitamins in the Sample Extract. Chromatograms of the sample extracts (Figures 3–6) were obtained. Based on the chromatographic results, the vitamin contents per 100 g of sample were calculated using the following formula and are presented in Tables 3 and 4.

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

Here, X represents the amount of vitamins in 100 g of fruit, expressed in mg.

C_{vit} – the concentration of the vitamin in the extract, determined using the HPLC method, expressed in mg/L;

$V_{extract}$ – the volume of the sample extract, L;

M_{sample} – the mass of the sample weighed for extract preparation, g;

Vitamin	Retention Time (s)	Concentration (mg/L)	Amount per 100 g of Sample (mg)
Vitamin B_1	2,964	1,524	3,810
Vitamin B_3	5,786	2,442	6,105
Vitamin PP	7,658	0,135	0,338
Vitamin B_9	16,709	0,207	0,518
Vitamin B_2	18,826	0,318	0,795
Vitamin B_6	6,393	0,317	0,793
Vitamin B_{12}	Not detected	0	0,000
Vitamin C	4,453	0,017	0,043

During this study, water-soluble B-group vitamins and vitamin C in the obtained biomaterial were determined using high-performance liquid chromatography (HPLC). The following figures present the chromatograms of the vitamins detected in the biomaterial and the aqueous fraction of the biomaterial.

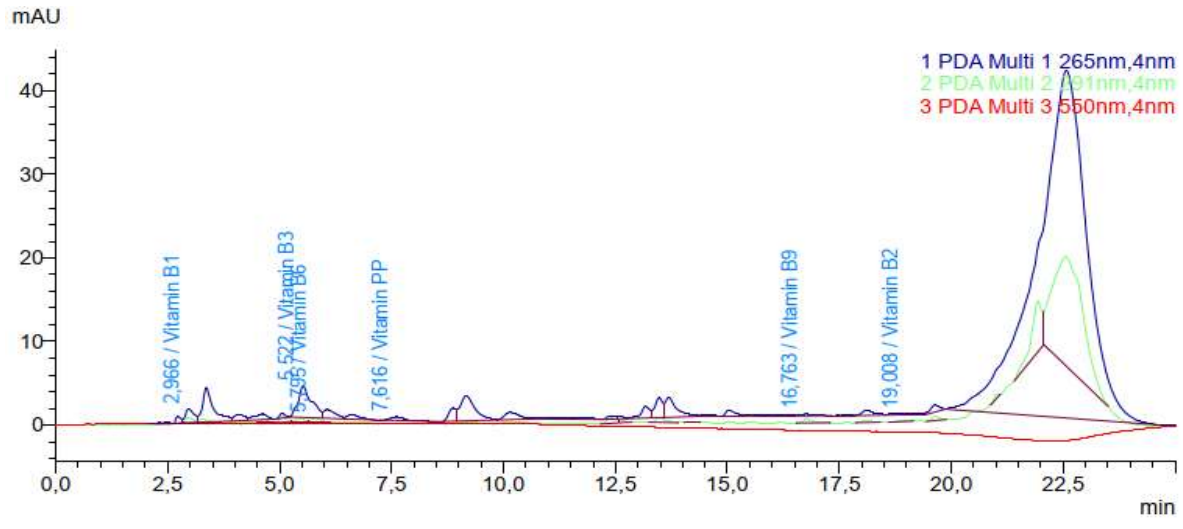


Figure 2. Chromatogram of water-soluble B-group vitamins in the biomaterial

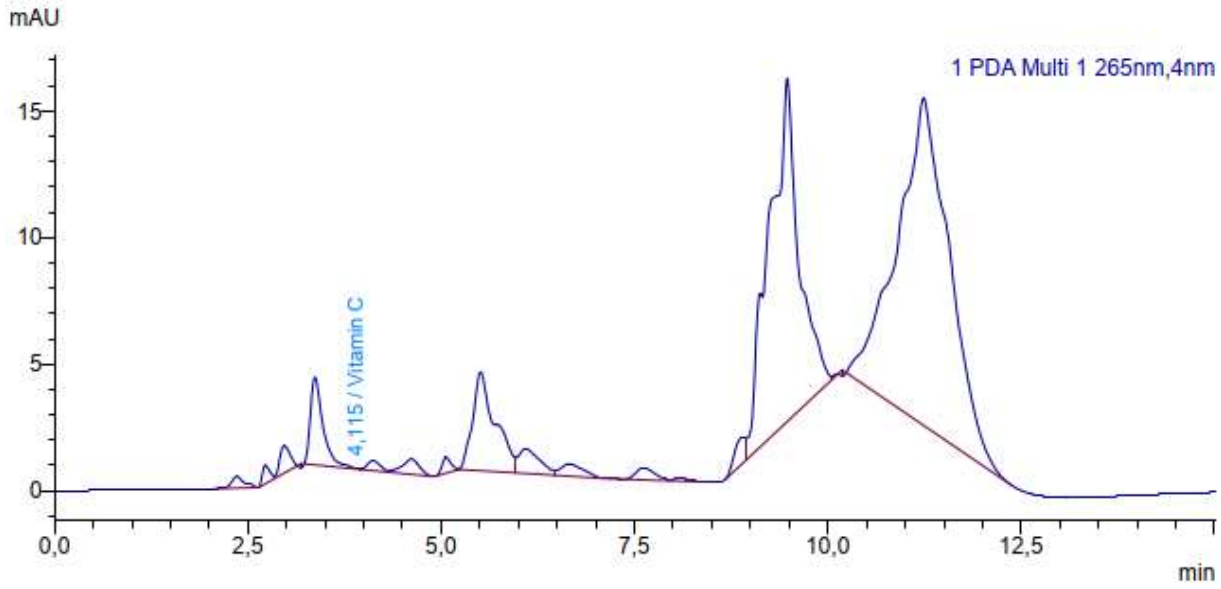


Figure 3. Chromatogram of vitamin C in the biomaterial

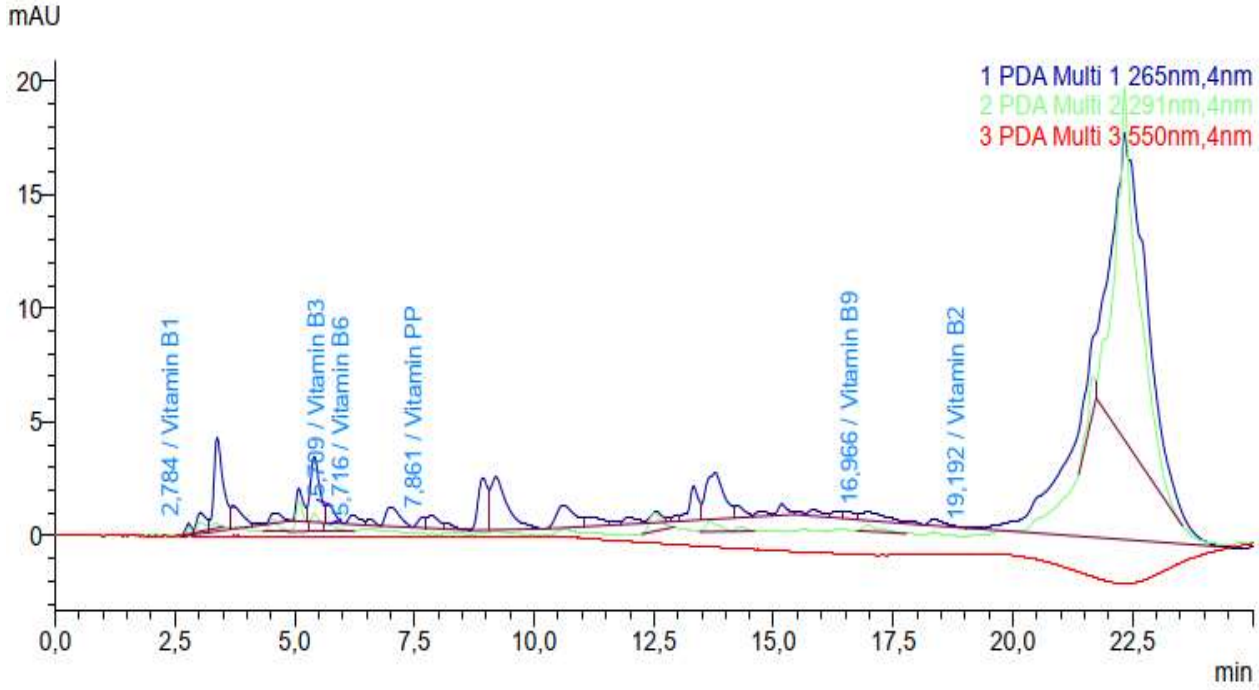


Figure 4. Chromatogram of water-soluble B-group vitamins in the biomaterial aqueous fraction

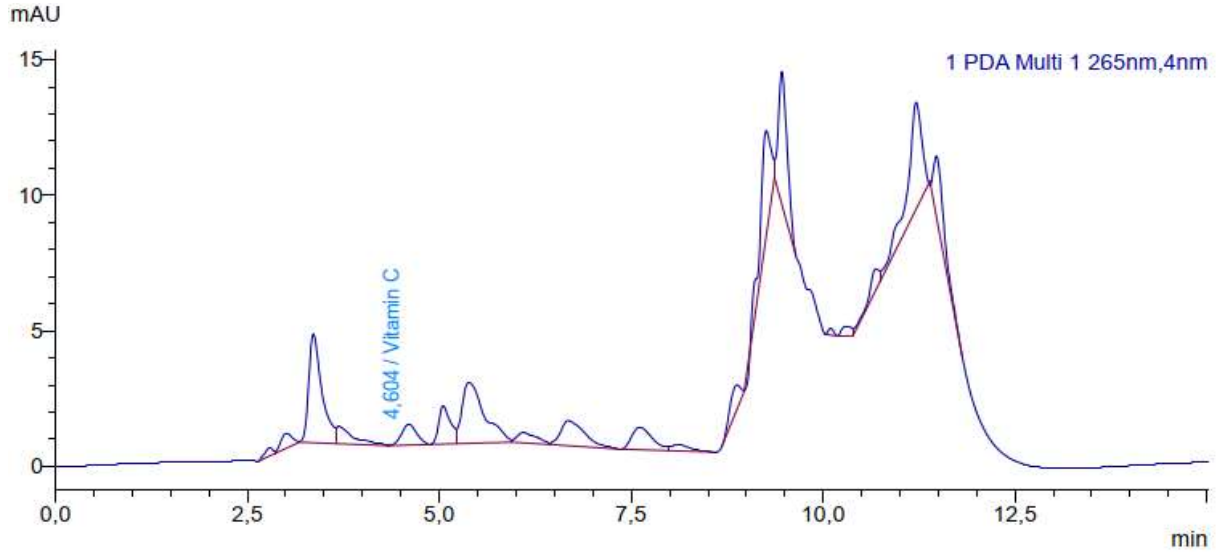


Figure 5. Chromatogram of vitamin C in the biomaterial aqueous fraction

Based on the results of the investigation, the vitamin contents of the biomaterial and its aqueous fraction were calculated per 100 g of sample and are presented in the following table.

Table 2. Vitamin content of the biomaterial and its aqueous fraction

	Vitamins	Retention time (s)	Concentration (mg/L)	Amount per 100 g of sample (mg)
Biomaterial				
1.	Vitamins	2,966	0,737	1,843
2.	Vitamin B_1	5,522	2,108	5,270
3.	Vitamin B_3	7,616	0,286	0,715
4.	Vitamin PP	16,763	0,15	0,375
5.	Vitamin B_9	19,008	0,219	0,728
6.	Vitamin B_2	5,795	0,178	0,445
8.	Vitamin B_6	4,115	0,05	0,125
Biomaterial aqueous fraction (juice)				
1	Vitamin B_1	2,784	0,214	0,535
2	Vitamin B_3	5,709	0,227	0,568
3	Vitamin PP	7,861	0,311	0,778
4	Vitamin B_9	16,966	0,419	1,048
5	Vitamin B_2	19,192	0,284	0,710
6	Vitamin B_6	5,716	0,146	0,365
7	Vitamin C	4,604	0,213	0,533

Note: Vitamin B_{12} was not detected in the samples.

As shown in the table above, the contents of vitamins B_1, B_3, PP, B_9, B_2, B_6, and C are expressed in milligrams per 100 g of sample. These vitamins were detected in both the biomaterial and its aqueous fraction, with substantial differences observed between the two. For example, the vitamin B_1 content was 1.843 mg/100 g in the biomaterial, whereas the corresponding value in the aqueous fraction was 0.535 mg/100 g. In contrast, laboratory analyses revealed that the vitamin B_9 content was 0.375 mg/100 g in the biomaterial and 1.048 mg/100 g in its aqueous fraction.

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