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#### DEVELOPMENT OF A FUNCTIONAL COMPOSITION OF PLANT RAW MATERIALS

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#### Abstract

Designing compositions of plant materials intended for enriching food products is a rather laborious process; creating a prescription formula may take a long time, which is due to many different options that cannot be measured with a finite number. Therefore, when developing a functional composition of plant raw materials, the authors used the method for planning experiments to compose mixtures. Using the Designs for Constrained Surfaces and Mixtures procedure of the Design of Experiments module of the STATISTICA 10 software suite, a design of the experiments has been made, studies have been performed for determining the quantitative content of functional ingredients in the test samples, the results have been mathematically processed, a scientifically substantiated optimal component composition has been obtained for two variants of plant raw materials composition with the following component ratio, % wt.: variant 1: leaves of stinging nettle - 15, herb of echinacea purpurea — 15, inflorescences of red clover — 40, and rosehips — 30; variant 2: leaves of stinging nettle — 25, herb of echinacea purpurea — 25, inflorescences of red clover — 30, and rosehips — 20. It has been experimentally found that in the composition of the first variant, the content of vitamin E is 17.769 mg of tochopherol equivalent/100 g, which is 1.2 times higher than the recommended daily consumption, and in the second variant, the content of calcium is 640.36 mg/100 g or 53.4 % of the recommended daily consumption, which allows attributing the developed composition variants of plant material to functional products.

#### Keywords

Analysis of Variance - Biologically active substances - Calcium - Design of experiments

#### Para Citar este Artículo:

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#### Introduction

Human nutrition is one of the main factors that have a significant effect on health, performance, and resistance of the human organism to the environmentally harmful factors of production and living environment.

According to<sup>1</sup>, today the world's population is facing a deficiency of vital vitamins, minerals, and minor components, which is associated with an increased share of refined food products as a result of society urbanization.

One of the ways to solve this problem is the systematic use of foods with specific chemical composition achieved through enrichment. The effectiveness of this method in preventing nutritional diseases has been evidenced by the results of many Russian and foreign studies<sup>2</sup>.

Compositions of the medicinal plant material containing a complex of biologically active substances with various pharmacological activities may be used as the enriching additive.

Based on the data of scientific and scientific-technical literature<sup>3</sup> and the results of the authors' previous studies of the chemical composition of many types of plant material growing in the North Caucasus<sup>4</sup>, the authors assume that the functional composition of plant raw materials intended for compensating for the deficiency of functional ingredients in the diet, such as vitamin E and calcium, should contain leaves of stinging nettle, herb of *echinacea purpurea*, inflorescences of red clover, and rosehips.

<sup>&</sup>lt;sup>1</sup> A. S. Prasad, "Discovery of Human Zinc Deficiency: Its Impact on Human Health and Disease", Advances in Nutrition Vol: 4 num 2 (2013): 176-190 y D. D. Mille y R. M. Welch, "Food system strategies for preventing micronutrient malnutrition", Food Policy num 42 (2013): 115-128.

<sup>&</sup>lt;sup>2</sup> O. G. Pozdnyakova; G. A. Belavina; A. N. Avstrievskikh y V. M. Poznyakovskiy, "Development of a specialized product for therapeutic purposes based on plant materials", Achievements of science and agricultural technology Vol: 32 num 12 (2018): 94-97; A. Godard; P. De Caro; E. Vedrenne; Z. Mouloungui y S. Thiebaud-Roux, "From crops to products for crops: preserving the ecosystem through the use of bio-based molecules", OCL – oilseeds and fats, crops, and lipids Vol: 23 num 5 (2016): D510 y M. Silagadze; E. Gamkrelidze; S. Gachechiladze; M. Khurtsidze y G. Pkhakadze, Development of new generation "live" foods with rational use of raw materials from Georgian resourses. In: Scientific enquiry in the contemporary world: theoretical basics and innovative approach (San Fransisco: B&M Publishing, 2016).

<sup>&</sup>lt;sup>3</sup>O. G. Pozdnyakova; G. A. Belavina; A. N. Avstrievskikh y V. M. Poznyakovskiy, "Development of a specialized product...; A. Godard; P. De Caro; E. Vedrenne; Z. Mouloungui y S. Thiebaud-Roux, "From crops to products...; M. Silagadze; E. Gamkrelidze; S. Gachechiladze y M. Khurtsidze, G. Pkhakadze, Development of new generation "live" foods...; S. Đurović; B. Pavlić; S. Šorgić; S. Popov; S. R. Savic y M. Petronijević, "Chemical composition of stinging nettle leaves obtained by different analytical approaches", Journal of Functional Foods num 32 (2017): 18-26; G. Menendez-Baceta; L. Aceituno-Mata; J. Tardío; V. Reyes-García y M. Pardo-de-Santayana, "Wild edible plants traditionally gathered in Gorbeialdea (Biscay, Basque Country)", Genetic Resources and Crop Evolution num 59 (2012): 1329-1347 y A. V. Zaushintsena; I. S. Milentyeva; O. O. Babich; S. Yu. Noskova; T. F. Kiseleva y D. G. Popova, "Quantitative and qualitative profile of biologically active substances extracted from purple echinacea (Echinacea Purpurea L.) growing in the Kemerovo region: functional foods application", Foods and Raw Materials Vol: 7 num 1 (2019): 84-92.

<sup>&</sup>lt;sup>4</sup> Certificate of state registration of the database No. 2018621175. Biologically active substances of wild-growing vegetative raw materials of the North Caucasus region. August 3, 2018.

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It is known that the pharmacological properties of the above plants are determined by their chemical composition. For instance, echinacea purpurea<sup>5</sup> has pronounced immunostimulating, antiviral, and antiallergic effects, leaves of nettle<sup>6</sup> have a rich multivitamin composition, and the chlorophyll contained in them in significant amounts has a stimulating and tonic effect, enhances the basal metabolism, improves the functioning of the cardiovascular system, inflorescences of red clover contain substances with antiinflammatory and antisclerotic effects, and can remove toxins, rosehips help increase immunity, improve metabolism, relieve fatigue; the use of rosehips for preventive purposes gives the organism the strength to resist cancer and many chronic diseases.

According to the data of the authors<sup>7</sup>, vitamin E and calcium are found in the leaves of stinging nettle, herb of echinacea purpurea, inflorescences of red clover, and rosehips in sufficient quantities.

In this regard, the selected plants may be used in the prescription formula of a general strengthening and immunostimulating composition of plant materials.

To confirm the hypothesis put forward, studies were performed for determining the optimal composition of plant materials and the quantitative content of functional ingredients in it.

Creating a prescription formula for the composition "manually" would take long, which was due to a large number of variants. Therefore, when developing a functional composition of plant raw materials, the authors used the Design of Experiments method to compose mixtures.

Works are known<sup>8</sup>, where a variety of statistical modeling methods were used for predicting the optimal combination of the components in a blend of wine products using the parameters of the blends that allowed instrumental measurement, but there are almost no works devoted to developing the optimal composition of functional raw materials using the methods of data analysis implemented in the STATISTICA software suite.

The work is aimed at development of scientifically substantiated functional multicomponent composition of plant raw materials. Novelty. With the use of the data analysis methods implemented in the STATISTICA software suite, the optimal component shares were determined in two variants of a composition based on well-known medicinal plants of the Republic of Advgea, in which the most important are functional ingredients (vitamin E and calcium).

#### Materials and methods

At various stages of the research study, the objects were medicinal plants growing

<sup>&</sup>lt;sup>5</sup> O. G. Pozdnyakova; G. A. Belavina; A. N. Avstrievskikh y V. M. Poznyakovskiy, "Development of a specialized product... y A. V. Zaushintsena; I. S. Milentyeva; O. O. Babich; S. Yu. Noskova; T. F. Kiseleva y D. G. Popova, "Quantitative and qualitative profile... <sup>6</sup> S. Đurović; B. Pavlić; S. Šorgić; S. Popov; S. R. Savic y M. Petronijević, "Chemical composition...

<sup>&</sup>lt;sup>7</sup> Certificate of state registration of the database No. 2018621175...

<sup>&</sup>lt;sup>8</sup> P. Vismara; R. Coletta y G. Trombettoni, "Constrained global optimization for wine blending", Constraints Vol: 21 num 4 (2016): 597-615 y S. Yin; L. Liu y J. Hou, "A multivariate statistical combination forecasting method for product quality evaluation", Information Sciences Vol: 355-356 num 10 (2016): 229-236.

on the territory of the Republic of Adygea: stinging nettle (lat. *Urtica dioica L.*, leaves), *echinacea purpurea* (lat. *Echinacea purpúrea*, herb), red clover (lat. *Trifolium pratense L.*, inflorescences), briar (lat. *Rósa majális*, rosehips), and experimental compositions from the abovementioned plant materials.

This work was performed under the aegis of the research laboratory of the FSBEI HE Maikop State Technological University.

The composition of plant raw materials was developed using the *Designs for Constrained Surfaces and Mixtures* procedure of the *Design of Experiments* module of the *STATISTICA 10* software suite.<sup>9</sup>

In the settings of the *Building a design* tab in the initial window of the module, the number of factors (components of the mixture, i.e., plant materials) is four. Design of the experiment was built based on the determined limitations.

According to the plan, the compositions were made and experimental studies were performed for determining the content of vitamin E and calcium in them using the generally adopted standard methods, following the regulatory documents currently in force.

The content of vitamin E was determined according to<sup>10</sup> in the extract obtained from the analyzed sample by separating tocopherols using the method of high-performance liquid chromatography on a JASCO 875-UV liquid chromatograph, followed by photometric detection.

Processing and presentation of the results:

The mass fraction of  $\alpha$ -,  $\beta$ -,  $\lambda$ -, and  $\delta$ -tocopherols,  $\alpha$ -tocopherol acetate X, ppm was calculated using the calibration curve according to the following formula (1):

$$X = \frac{Kgr \cdot Srev \cdot V}{m},\tag{1}$$

where kgr - was the coefficient of the calibration graph according to 8.4 GOST R 54634-2011.

 $S_{\text{rev}}$  - was the arithmetic mean of the results of measuring the peak area of the analyzed component for two parallel chromatographic analyzes of the test solution, mAU·s or AU·s,

V- was the dilution volume, cm<sup>3</sup>, and

m - was the weight of the analyzed sample, g.

In analyzing each sample, two parallel determinations were made, starting from taking weighed portions of the test sample.

<sup>&</sup>lt;sup>9</sup> A. A. Khalafyan, Statistica 6. Mathematical statistics with the elements of the probability theory (Moscow: Binom, 2010) y A. A. Khalafyan, Industrial statistics: Quality control, process analysis, DESIGN of Experiments in the STATISTICA software suite (Moscow: Librocom book house, 2013). <sup>10</sup> GOST R 54634-2011. Functional food products. Method of vitamin E determination. 2013

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The result of determining the content of vitamin E was according to the following formula (2):

$$X_{\text{mean}}\pm\Delta$$
, ppm, P = (2) 95 %,

where  $X_{\mbox{\scriptsize mean}}$  - was the arithmetic mean of the results of two parallel determinations, ppm,

 $\Delta$  - was the value of the absolute determination error limit, ppm, calculated according to the following formula (3):

$$\Delta = \frac{\delta X \text{mean}}{100},$$
 (3)

The mass concentration of calcium in the experimental compositions was determined according to<sup>11</sup> with the use of the Kapel 105 M capillary electrophoresis device.

A weighed sample of dry test material m = 1.0 g was poured with distilled water (water duty 1:10, water temperature = 36°C). The sample was kept for 24 hours with periodic stirring every eight hours. After that, the solution was filtered through an ash-free filter. The resulting solution was diluted 10 times with distilled water and centrifuged at 6,000 rpm in Eppendorf tubes for 3 - 4 minutes. After that, the ready sample was placed in the Kapel 105 M capillary electrophoresis device for performing the analysis according to a previously built calibration curve.

#### **Results and discussion**

An analysis of the scientific literature<sup>12</sup> showed that scientists around the world had a genuine interest in medicinal plants and the possibility of using them both in preventing diseases and in primary health care.

With that, it should be noted that in each individual work, the emphasis was made on the chemical composition and on the benefit of plants in a particular growing region. However, none of the studies contained evidence of developing a composition based on this plant material, which would be used for enriching mass consumption products.

In contrast to these studies, the authors searched for a possibility to create

<sup>&</sup>lt;sup>11</sup> StP00668034-23-14-2009. Materials of plant origin. The method for determining the mass concentration of ammonium, potassium, sodium, magnesium, and calcium cations using capillary electrophoresis. Certification: SSI North Caucasus Federal Scientific Center for Horticulture, Viticulture, and Winemaking.

<sup>&</sup>lt;sup>12</sup> K. Nag y Z.-U. Hasan, "Uses of Wild Medicinal Herbs and Ecology of Gardens of District Bhopal, Madhya Pradesh (India)", Biological Forum — An International Journal Vol: 3 num 1 (2011): 29-31; S. Singh; A. Gupta; A. Kumari y R. Verma, "Antimicrobial and Antioxidant Potential of Hibiscus Rosa-Sinensis L. in Western Himalaya", Biological Forum – An International Journal Vol: 11 num 1 (2019): 35-40; M. Muradashvili; N. Jabnidze; L. Koiava; R. Dumbadze; K. Memarne y L. Gorgiladze, Antibacterial and Antifungal Activity of Stevia rebaudiana (Asteraceae) Leaf Extract in vitro Condition. Biological Forum – An International Journal Vol: 11 num 1 (2019): 212-216 y A. Singh; R. Rani y M. Sharma, "Medicinal Herbs of Punjab (India)", Biological Forum – An International Journal Vol: 10 num 2 (2018): 10-27.

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a prescription formula for a phytocomposition with a certain functional purpose using the modern methods of data analysis.

When designing the composition, possible ranges of the component composition were set in percent. The main criteria for determining the functional purpose (quality) of the composition are indicators of the vitamin E and calcium content (concentration). Vitamin E has strong antioxidant properties: it strengthens the immune system, helping the organism fight harmful bacteria and viruses, etc. Calcium is involved in nerve tissues excitability, muscle contractility, and in the processes of blood clotting; it is part of the cell nucleus and membranes, cellular and tissue fluids; it has an antiallergic and anti-inflammatory effect; it prevents acidosis and activates enzymes and hormones.

The contents of vitamin E and calcium are determined from the composition. With that, it was necessary to find such an optimal formulation of the composition when the limitations to the content of the components would be satisfied with the maximum concentration of these biologically active substances.

Table 1 shows the determined formulation of the obtained composition based on the analysis of the experimental data about the chemical composition of individual components of the mixture<sup>13</sup>, the possible ranges of the shares of all components are shown.

Fractional composition	Formulation, %	Boundary conditions %		
		from	to	
Stinging nettle (lat. Urtica dioica L., leaves)	20	15	25	
Echinacea purpurea (lat. Echinacea purpúrea, herb)	20	15	25	
Red clover (lat. <i>Trifolium pratense L.</i> , inflorescences)	35	30	40	
Briar (lat. Rósa majális, rosehips)	25	20	30	

According to the determined formulation, the total content of vitamin E is 10 mg of tochopherol equivalent/100 g; the total content of calcium is 630 mg/100 g.

Table 1

The determined formulation of the composition of functional plant materials

The design of experiments built using the *Designs for Constrained Surfaces and Mixtures* procedure of the *Design of Experiments* module<sup>14</sup> with the number of factors (components) equal to four and limitations (Table 1) is shown in Table 2. The last columns show the contents of vitamin E and calcium in the experimental compositions according to the results of the studies.

<sup>&</sup>lt;sup>13</sup> GOST R 54634-2011...

<sup>&</sup>lt;sup>14</sup> A. A. Khalafyan, Statistica 6... y A. A. Khalafyan, Industrial statistics: Quality control, process analysis...

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Vertex (V)	Four-factor	mixture with lin	mitations ([No	active dataset])		
Centroid						
(C)	No. of initial	limitations for	the mixture:			
. ,	Stinging	Echinacea	Red clover	Briar	Vitamin E	Calcium
	nettle	purpurea				
1 V	25.00000	15.00000	30.00000	30.00000	17.19	613.62
2 V	15.00000	25.00000	30.00000	30.00000	16.89	565.16
3 V	25.00000	25.00000	30.00000	20.00000	16.41	652.53
4 V	15.00000	15.00000	40.00000	30.00000	17.85	548.86
5 V	25.00000	15.00000	40.00000	20.00000	17.81	636.13
6 V	15.00000	25.00000	40.00000	20.00000	17.11	587.67
7 C(1)	15.00000	25.00000	35.00000	25.00000	17.00	576.42
8 C(1)	15.00000	20.00000	40.00000	25.00000	17.48	568.22
9 C(1)	15.00000	20.00000	35.00000	30.00000	17.37	556.96
10 C(1)	25.00000	15.00000	35.00000	25.00000	17.26	624.87
11 C(1)	25.00000	20.00000	30.00000	25.00000	16.88	633.08
12 C(1)	25.00000	20.00000	35.00000	20.00000	16.89	644.33
13 C(1)	20.00000	15.00000	40.00000	25.00000	17.61	592.45
14 C(1)	20.00000	15.00000	35.00000	30.00000	17.50	581.19
15 C(1)	20.00000	25.00000	30.00000	25.00000	17.22	608.85
16 C(1)	20.00000	25.00000	35.00000	20.00000	17.33	624.58
17 C(1)	20.00000	20.00000	30.00000	30.00000	17.02	589.39
18 C(1)	20.00000	20.00000	40.00000	20.00000	17.24	611.90
19 C(2)	15.00000	21.66667	36.66667	26.66667	17.283	570.19
20 C(2)	25.00000	18.33333	33.33333	23.33333	16.326	508.42
21 C(2)	21.66667	15.00000	36.66667	26.66667	18.026	602.50
22 C(2)	18.33333	25.00000	33.33333	23.33333	16.803	601.79
23 C(2)	21.66667	21.66667	30.00000	26.66667	16.816	610.43
24 C(2)	18.33333	18.33333	40.00000	23.33333	17.444	590.86
25 C(2)	21.66667	21.66667	36.66667	20.00000	16.963	632.92
26 C(2)	18.33333	18.33333	33.33333	30.00000	16.356	575.85

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Table 2

#### Design of experiments

Table 3 shows the results of the designed composition variance analysis for vitamin E; one can see that the linear model is statistically significant since the significance level of the Fisher's criterion (F)p = 0.00 takes a value that is less than the accepted critical significance level for statistical hypotheses (0.05). The quadratic model is not statistically significant since the significance level of the Fisher's criterion (F)p = 0.465 takes a value that is greater than the critical significance level.

Model	Analysis of variance; Variable: Vitamin E (26 experiments) Four-factor design for mix; total value of mix. = 100, 26 experiments Last fitting of the models of incresing complexity						
	SS effect	cc effect	MS effect	SS Error	F	р	R <sup>2</sup>
Linear	2.40610 6	3	0.80203 5	2.313336	7.62741 8	0.00112 6	0.509829
Quadratic	0.62532 5	6	0.10422 1	1.688011	0.98786 9	0.46558 1	0.642328

Table 3

The results of plant materials composition analysis of variance, vitamin E

The  $R^2 = 0.51$  value means that the model explains only 51 % of the response variability from the mean value. However, since the quadratic model is statistically insignificant, the dependence between the response of Vitamin E and the components of the composition is approximated by a linear dependence.

In accordance with the letter designations of the predictors in Table 4, and denoting the Vitamin E response by character Z, the linear regression equation takes the following form (4):

$$Z = 0.145A + 0.121B + (4)$$
  
0.215C + 0.173D.

The limitations on the model predictors may be represented as a system of linear inequalities (5):

$$15 \le A \le 25 
15 \le B \le 25 
30 \le C \le 40 . (5) 
20 \le D \le 30 
A + B + C + D = 100$$

Equation (4) with conditions (5) represents the mathematical formulation of the linear programming problem, and, since  $R^2 = 0.51$ , it is not an adequate model of the dependence response (the level of vitamin E on the mixture component fractions). This means that some response values corresponding to the 26 experiments may be located not near the four-dimensional response surface, i.e., large residues are possible (Table 4).

Factor	•	Coeff. (initial comp.); Variable: Vitamin E; R <sup>2</sup> . = 0.5098; Corrected 0.443 (26 experiments)					
		,	nix; total valu	e of mix. = 10	0, 26 experim	nents	
				idual SS = 0.1			
	Coeff.	of St. Er.	t(22)	р	-95,% Confidence limit	+95,% Confidence limit	
(A) Stinging nettle (leaves)	0.144769	0.013655	10.60209	0.000000	0.116450	0.173087	
(B) Echinacea purpurea (herb)	0.120687	0.013655	8.83847	0.000000	0.092369	0.149005	
(C) Red clover (inflorescences)	0.214962	0.011042	19.46760	0.000000	0.192062	0.237861	
(D) Briar (rosehips)	0.172962	0.012843	13.46738	0.000000	0.146327	0.199596	

Table 4

The coefficients of the regression equation for Vitamin E

This means that some response values corresponding to the 26 experiments may be located insufficiently close to the four-dimensional response surface — the hyperplane, i.e., there may be considerable residues — the differences between the experimental values and the values calculated with the use of the linear model. Using the Pareto curve in Figure 1, the following fractions of the mixture components were found: stinging nettle (leaves) =

24.459, *echinacea purpurea* (herb) = 23.757, red clover (inflorescences) = 26.504, and briar (rosehips) = 25.28.



The Pareto curve for the designed composition (Vitamin E)

The calculated predicted values satisfy the conditions (5). The predicted line in Table 5 shows the approximate optimal value of Vitamin E content predicted by the application's calculator, which is 16.478 with a 95 % confidence interval (16.138; 16.818). It is easy to see that the found value of Vitamin E content (16.478) exceeds the set value (10).

Factor	Predicted value; Variable: Vitamin E; R <sup>2</sup> = 0.50983; Corrected 0.44299 (26 experiments)						
		Dependent variable: Vitamin E; Residual SS = 0.1051516					
	Coeff.						
(A) Stinging nettle	16.78528	0.472950	7.93860	24.45900			
(B) Echinacea purpurea	16.30365	0.437850	7.13855	23.75700			
(C) Red clover	18.18915	-0.174800	-3.17946	26.50400			
(D) Briar	17.34915	0.264000	4.58018	25.28000			
predicted			16.47787				
-95, % Conf.			16.13773				
+95, % Conf.			16.81800				

#### Table 5

The predicted value of Vitamin E content in the Pareto curve

Let us try to improve the result using the *Profiles of the Predicted Values and Desirability Function* chart in Figure 2.



Figure 2 Profiles of the predicted values and desirability function for the designed composition of plant raw materials (Vitamin E).

The optimal shares of the components with which the response reached a maximum value of 17.769 are marked at the base of the desirability curves: stinging nettle (leaves) = 15, *echinacea purpurea* (herb) = 15, red clover (inflorescences) = 40, and briar (rosehips) = 30. The calculations made by the calculator of the module are shown in Table 6.

Predicted value; Variable: Vitamin E; $R^2 = 0.50983$ ; Corrected				
Dependent val		Sidual SS = 0.10		
Coeff.	Pseudocomp.	Coeff. * Val.	Init. comp.	
16.78528	0.000000	0.00000	15.00000	
16.30365	0.000000	0.00000	15.00000	
18.18915	0.500000	9.09457	40.00000	
17.34915	0.500000	8.67457	30.00000	
		17.76915		
		17.43764		
		18.10066		
	0.44299 (26 ex Dependent var Coeff. 16.78528 16.30365 18.18915	0.44299 (26 experiments)           Dependent variable: Vitamin E; Res           Coeff.         Pseudocomp.           16.78528         0.000000           16.30365         0.000000           18.18915         0.500000	0.44299 (26 experiments)         Dependent variable: Vitamin E; Residual SS = 0.10         Coeff.       Pseudocomp.         16.78528       0.000000         16.30365       0.000000         18.18915       0.500000         9.09457         17.34915       0.500000         8.67457         17.43764	

#### Table 6

The predicted value of Vitamin E content according to the profile curve

It should be noted that an approximately optimal value was achieved at the boundaries of the component shares variation ranges (corresponds to experiment v4). With that, the experimental value of 17.85 is slightly greater than the one calculated by the linear model (17.769). Table 7 shows the differences between the experimental response value and the response value predicted by the model.

The observed	The observed and t	he predicted values and resid	dues (26 experiments)					
design	Four-factor design f	Four-factor design for mix; total value of mix. = 100, 26 experiments						
-	Dependent variable: Vitamin E; R <sup>2</sup> = 0.5098; Corrected 0.443							
	Observed	Predicted	Residues					
1	17.19000	17.06722	0.122783					
2	16.89000	16.82640	0.063601					
3	16.41000	16.54447	-0.134467					
4	17.85000	17.76915	0.080851					
5	17.81000	17.48722	0.322783					
6	17.11000	17.24640	-0.136399					
7	17.00000	17.03640	-0.036399					
8	17.48000	17.50777	-0.027774					
9	17.37000	17.29777	0.072226					
10	17.26000	17.27722	-0.017217					
11	16.88000	16.80584	0.074158					
12	16.89000	17.01584	-0.125842					
13	17.61000	17.62818	-0.018183					
14	17.50000	17.41818	0.081817					
15	17.22000	16.68543	0.534567					
16	17.33000	16.89543	0.434567					
17	17.02000	16.94681	0.073192					
18	17.24000	17.36681	-0.126808					
19	17.28300	17.28065	0.002351					
20	16.32600	17.03297	-0.706967					
21	18.02600	17.44119	0.584806					
22	16.80300	16.87242	-0.069421					
23	16.81600	16.81269	0.003306					
24	17.44400	17.50092	-0.056921					
25	16.96300	17.09269	-0.129694					
26	16.35600	17.22092	-0.864921					

Table 7

The differences between the experimental response value and the response value predicted by the model

Thus, the recommended composition (by vitamin E) in accordance with the built linear dependence is the following: stinging nettle (lat. *Urtica dioica L.*, leaves) = 15, *echinacea purpurea* (lat. *Echinacea purpúrea*) = 15, red clover (lat. *Trifolium pratense L.*, inflorescences) = 40, and briar (lat. *Rósa majális*, rosehips) = 30.

The results of the analysis of variance of the designed composition according to the second composition functionality criterion (calcium) are shown in Table 8.

Model	Analysis of variance; Variable.: Calcium (26 experiments) Four-factor design for mix; total value of mix. = 100, 26 experiments Last fitting of the models of increasing complexity						
	SS effect	cc effect	MS effect	SS Error	F	р	R <sup>2</sup>
Linear	13,137.45	3	4,379.151	14,364.81	6.706759	0.002203	0.477686
Quadratic	2,200.80	6	366.800	12,164.01	0.482472	0.811800	0.557709

Table 8

The results of the analysis of variance of the designed composition for calcium

Table 8 shows that the linear model is statistically significant since the significance level of the Fisher's criterion (F)p = 0.00 takes a value less than the accepted critical significance level of statistical hypotheses (0.05). The quadratic model is not statistically significant since the significance level of the Fisher's criterion (F)p = 0.482 takes a value greater than the critical significance level.

The  $R^2 = 0.478$  value means that the model explains only about 48 % of the response variability from the mean value. However, since the quadratic model is statistically insignificant, the dependence between the calcium response and the components of the mixture is approximated by the linear function.

In accordance with the letter designations of the predictors in Table 4, and denoting the calcium response by character Z, the linear regression equation takes the following form:

 $Z = 10.019 \cdot A + 6.933 \cdot B + (6)$ 5.290 \cdot C + 2.891 \cdot D

The limitations on the model predictors may be represented as a system of linear inequalities (5).

Equation (6) with conditions (5) represents the mathematical formulation of the linear programming problem, and since  $R^2 = 0.478$  (less than 0.5), it is not an adequate model of the dependence response (the level of calcium in the mixture component fractions). This means that some response values corresponding to the 26 experiments may be located not near the four-dimensional response surface — the hyperplane, i.e., large residues are possible.

Factor	Coeff. (initial comp.); Variable: Calcium; $R^2 = 0.4777$ ; Corrected 0.4065 (26 experiments) 4 factors design for mix; total value of mix. = 100, 26 experiments Dependent variable: Calcium; Residual SS = 652.9459					
	Coeff.	St. Er.	t(22)	р	-95,% Confidence limit	+95,% Confidence limit
(A) Stinging nettle	10.01952	1.076002	9.311802	0.000000	7.788026	12.25101
(B) Echinacea purpurea	6.93327	1.076002	6.443545	0.000002	4.701776	9.16476
(C) Red clover	5.29039	0.870120	6.080072	0.000004	3.485874	7.09491
(D) Briar	2.89148	1.012039		0.009163	0.792643	4.99032

Table 9

The coefficients of the regression equation for calcium

Using the Pareto curve in Figure 1, the following fractions of the mixture components were found: stinging nettle (leaves) = 28.1, *echinacea purpurea* (herb) = 25.5, red clover (inflorescences) = 24.2, and briar (rosehips) = 22.2.

The calculated predicted values do not satisfy the conditions (5). The *predicted* line in Table 10 shows the approximate optimal value of calcium content calculated by the application's calculator, which is 650.565 with a 95 % confidence interval (613.502; 687.629). It is easy to see that the found value of calcium content (650.565) exceeds the set value (630).





Figure 3 The Pareto curve for the designed composition (calcium)

Factor	Predicted value; Variable: Calcium; R <sup>2</sup> . = 0.47769; Corrected 0.40646 (26 experiments) Dependent variable: Calcium; Residual SS = 652.9459						
	Coeff.						
(A) Stinging nettle	671.2236	0.655000	439.651	28.10000			
(B) Echinacea purpurea	609.4986	0.525000	319.987	25.50000			
(C) Red clover	576.6411	-0.290000	-167.226	24.20000			
(D) Briar	528.6629	0.110000	58.153	22.20000			
predicted			650.565				
-95, % Conf.			613.502				
+95, % Conf.			687.629				
Table 10							

Table 10

The predicted value of calcium content in the Pareto curve

Let us try to improve the result using the *Profiles of the Predicted Values and Desirability Function* chart in Figure 4.





The optimal shares of the components with which the response reached a maximum value of 640.36 are marked at the base of the desirability curves: stinging nettle (leaves) = 25, *echinacea purpurea* (herb) = 25, red clover (inflorescences) = 30, and briar (rosehips) = 20. The calculations made by the calculator of the module are shown in Table 11.

	Predicted va	lue; Variable:	Calcium; R <sup>2</sup>	= 0.47769;
Factor		0646 (26 experi		
	Dependent va	riable: Calcium;	Residual SS =	652.9459
	Coeff.	Pseudocomp.	Coeff. * Val.	Init. comp.
(A) Stinging nettle	671.2236	0.500000	335.6118	25.00000
(B) Echinacea purpurea	609.4986	0.500000	304.7493	25.00000
(C) Red clover	576.6411	0.000000	0.0000	30.00000
(D) Briar	528.6629	0.000000	0.0000	20.00000
predicted			640.3611	
-95, % Conf.			614.2377	
+95, % Conf.			666.4845	

#### Table 11

The predicted value of calcium content according to the profile curve

It should be noted that an approximately optimal value was achieved at the boundaries of the component shares variation ranges (corresponds to experiment v3). With that, the experimental value of 652.53 is slightly greater than the one calculated by the linear model (640.36).

Table 12 shows the differences between the experimental response value and the response value predicted by the model.

Observed	The observed and predicted values and residues (26 experiments)		
design	4 factors design for mix; total value of mix. = 100, 26 experiments Dependent variable: Calcium; $R^2 = 0.4777$ ; Corrected 0.4065		
	1	613.6200	599.9432
2	565.1600	569.0807	-3.921
3	652.5300	640.3611	12.169
4	548.8600	552.6520	-3.792
5	636.1300	623.9323	12.198
6	587.6700	593.0698	-5.400
7	576.4200	581.0753	-4.655
8	568.2200	572.8609	-4.641
9	556.9600	560.8664	-3.906
10	624.8700	611.9378	12.932
11	633.0800	620.1522	12.928
12	644.3300	632.1467	12.183
13	592.4500	588.2922	4.158
14	581.1900	576.2976	4.892
15	608.8500	604.7209	4.129
16	624.5800	616.7155	7.865
17	589.3900	584.5120	4.878
18	611.9000	608.5011	3.399
19	570.1900	571.6009	-1.411
20	508.4200	621.4122	-112.992
21	602.5000	592.1759	10.324
22	601.7900	600.8372	0.953
23	610.4300	603.1284	7.302
24	590.8600	589.8847	0.975
25	632.9200	619.1211	13.799
26	575.8500	573.8920	1.958

Table 12

The differences between the experimental response value and the response value predicted by the model

#### Conclusion

As a result of the studies performed with the use of the data analysis methods implemented in the STATISTICA software suite, a qualitative and quantitative composition of two variants of plant raw materials composition with a general strengthening and immunostimulating effect has been developed.

In the first variant of the composition, the criterion of functionality was the content of vitamin E, in the second variant — the content of calcium.

The optimal shares of the components in the first variant of the composition are the following: stinging nettle (lat. *Urtica dioica L.*, leaves) = 15, *echinacea purpurea* (lat. *Echinacea purpúrea*) = 15, red clover (lat. *Trifolium pratense L.*, inflorescences) = 40, and briar (lat. *Rósa majális*, rosehips) = 30.

The optimal shares of the components in the second variant of the composition are the following: stinging nettle (lat. Urtica dioica L., leaves) = 25, *echinacea purpurea* (lat. *Echinacea purpúrea*) = 25, red clover (lat. *Trifolium pratense L.*, inflorescences) = 30, and briar (lat. *Rósa majális*, rosehips) = 20.

It has been experimentally found that in the composition of the first variant, the content of vitamin E is 17.769 mg of tochopherol equivalent/100 g, which is 1.2 times higher than the recommended daily consumption, and in the second variant, the content of calcium is 640.36 mg/100 g or 53.4 % of the recommended daily consumption, which allows attributing the developed composition variants of plant material to functional products.

The use of the computer analysis methods opens up broad perspectives for creating new compositions of plant materials of various functional purpose, since various instrumentally measured parameters can be used as the criteria of functionality, and the ability to generate an experimental design and mathematical processing of experimental results reduce the time required for finding the optimal prescription formula of the composition.

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#### Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

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