ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Development of an antioxidant phytocomposite mixture using modern data analysis methods

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Abstract.
The article describes the use of experiment planning method for preparing mixtures in the development of the content of a phytocomposition from functional vegetable raw materials. Using procedures 'Plans for surfaces and mixes with module limitations' and' Planning experiments' of the STATISTICA 10 software suite, a plan of experiments has been generated, the results of the experiments performed according to the plan have been analyzed, and an optimal component composition has been obtained for the antioxidant phytocomposition mixture that is ensured by natural phenolic antioxidants (flavonoids) contained in medicinal plants of the North Caucasus region.
Keywords: medicinal plants, flavonoids, phytocomposition mixture, plan of the experiment, analysis of variance, linear regression equation.

INTRODUCTION

In the perception of modern man, medicinal herbs are associated with safety and benefits to the health, as they contain significant concentrations of biologically active substances necessary for the organism. These substances belong to classes of chemical compounds, among which a special place is occupied by flavonoids – polyphenolic substances of plant origin.

Numerous studies in the recent years have shown [1–3] that flavonoids are powerful antioxidants that inhibit the development of the oxidative stress in the cells where metabolism is disrupted as a result of the action of toxic prooxidants, UV radiation and other damaging factors. Antioxidant properties of flavonoids are determined both by these molecules' ability to capture free radicals, and the ability to chelate cations of metals of variable valence involved in the oxidation processes. It is noteworthy that upon formation of complexes with metals, antioxidant properties of flavonoids are improved. Thus, metal complexes of flavonoids exhibit superoxide-dismutase activity, which free flavonoids do not have. In addition, the lipophilicity of flavonoids changes upon interaction with metals. In the presence of small quantities of metals, their compounds with flavonoids are lipophilic, and can be immersed into the lipid bilayer, contributing to the protection of biological membranes. In the conditions of excess of metals, the formed compounds, on the contrary, have increased solubility in water, and are able to interact with soluble products of oxidation.

Antioxidant action of flavonoids is not limited to the direct influence of these substances on the processes of peroxide oxidation. The flavonoids' ability to activate natural mechanisms of cell protection against oxidative stress is more efficient.

This circumstance determines the necessity to use widely medicinal plant raw materials with a high content of polyphenolic compounds in antioxidant food production.

With that, mixtures of several types of plant materials are of special interest for the food industry as an enriching component. The interaction between the biologically active compounds of various plants can significantly affect the pharmacological activity of the mixture, providing a multifaceted effect on the human organism.

One of the key tasks in the development of a phytocomposite mixture is scientifically based selection of the physiologically functional ingredients of vegetable raw materials with targeted therapeutic properties. For solving this problem in an optimal way, modern data analysis technologies are widely used today, including statistical packages that allow to efficiently solve the problem of choosing optimal compositions of the mixtures.

Purpose of the work is the development through statistical modeling of a scientifically substantiated component composition of antioxidant phytocompositional mixture ensured by natural phenolic antioxidants (flavonoids) and its use in the technology of functional beverages with direct effect.

OBJECTS AND METHODS

The object of the study was an air-dry raw material obtained from the above-ground parts of medicinal plants harvested in the territory of the Republic Adygea in 2017:

common origanum (lat. Oríganum vulgáre), black currant (lat. *Ríbes nígrum*, leaves), little duckweed (lat. Lémna mínor, leaves), bilberry (lat. Vaccinium myrtillus L., leaves), common thyme (thyme) (lat. Thimus serpyllum L).

By safety characteristics, the raw materials complied with the requirements of TR CU 021/2011.

To determine the content of polyphenolic compounds (flavonoids), the authors used a capillary electrophoresis system equipped with a UV photometric detector operating at the wavelength of 254 nm, or a spectrophotometric detector with adjustable wavelength of 200 to 400 nm, a quartz capillary not less than 0.5 m long to the detector, with the inner diameter between 50 and 100 μm, a high voltage positive polarity source with adjustable voltage from 1 to 25 kV, and a personal PC with appropriate software for collecting and processing the information. The components were separated using a solution of sodium tetraborate [4-6].

The phytocomposition mixture was developed with the use of procedure *Plans for surfaces and mixtures with limitations* of the *Planning experiments* module from the *STATISTICA 10* package [7]. Plans of experiments, all tables and diagrams were generated in the program.

RESULTS AND DISCUSSION

To meet the requirements to antioxidant properties, possible ranges of the component composition in percent were set in the development of the phytocomposition mixture. The criterion of the antioxidant action (quality) of the composition was the total content (concentration) of flavonoids. According to the known composition of the mixture, the content of flavonoids was determined. With that, it was necessary to find such an optimum formulation of the mixture that would allow the maximum flavonoids' concentration when limitations to the composition of the mixture were applied.

Table 1 shows the composition of the mixture – common origanum (lat. Oríganum vulgáre), black currant (lat. Ríbes nígrum, leaves), little duckweed (lat. Lémna mínor, leaves), bilberry (lat. Vaccinium myrtillus L., leaves), and common thyme (thyme) (lat. Thimus serpyllum L); it also shows the formula obtained based on analysis of the data obtained during the experimental studies of the chemical composition of individual components in the mixture (hereinafter referred to as "set formulation"), and possible ranges of shares of each component that have been determined. According to the established formulation, the total content of flavonoids is 3,500 mg/100 g.

Table 1 - Fractional composition of the developed antioxidant phytocomposition mixture

Fractional composition	Formulation, %	Boundary conditions %	
		from	to
Common origanum (Oríganum vulgáre)	20	10	30
Black currant (Ríbes nígrum, leaves)	20	10	30
Little duckweed (Lémna mínor, leaves)	15	10	30
Bilberry (*Vaccinium myrtillus L.*, leaves)	30	20	60
Common thyme Thimus serpyllum L).	15	10	30

Table 2 - Experiment schedule for the developed antioxidant phytocomposition mixture

Vertex (V) Centroid (C)	5 actual mixtures with limitations. N of user-defined limitations: 0 N of initial limitations for the mixture:					
	Common origanum (Oríganum vulgáre)	Black currant (*Ríbes nígrum,* leaves)	Little duckweed (Lémna mínor, leaves)	Bilberry (Vaccinium myrtillus L., leaves)	Common thyme Thimus serpyllum L).	Flavonoids
1 V	30.00	10.00	10.00	20.00	30.00	3,013.84
2 V	10.00	30.00	10.00	20.00	30.00	3,105.80
3 V	30.00	30.00	10.00	20.00	10.00	3,353.68
4 V	10.00	10.00	30.00	20.00	30.00	2,696.96
5 V	30.00	10.00	30.00	20.00	10.00	2,944.84
6 V	10.00	30.00	30.00	20.00	10.00	3,036.80
7 V	10.00	10.00	10.00	60.00	10.00	5,259.56
8 V	30.00	10.00	10.00	40.00	10.00	4,260.64
9 V	10.00	30.00	10.00	40.00	10.00	4,352.60
10 V	10.00	10.00	30.00	40.00	10.00	5,077.58
11 V	10.00	10.00	10.00	40.00	30.00	4,012.76
12 C(1)	10.00	10.00	10.00	50.00	20.00	4,636.16
13 C(1)	10.00	10.00	30.00	30.00	20.00	3,320.36
14 C(1)	10.00	10.00	20.00	50.00	10.00	4,601.66
15 C(1)	10.00	10.00	20.00	30.00	30.00	3,354.86
16 C(1)	10.00	30.00	10.00	30.00	20.00	3,729.20
17 C(1)	10.00	30.00	20.00	20.00	20.00	3,071.30
18 C(1)	10.00	30.00	20.00	30.00	10.00	3,694.70
19 C(1)	10.00	20.00	10.00	50.00	10.00	4,806.08
20 C(1)	10.00	20.00	10.00	30.00	30.00	3,559.28
21 C(1)	10.00	20.00	30.00	20.00	20.00	2,866.88
22 C(1)	10.00	20.00	30.00	30.00	10.00	3,490.28
23 C(1)	10.00	20.00	20.00	20.00	30.00	2,901.38
24 C(1)	30.00	10.00	10.00	30.00	20.00	3,637.40
25 C(1)	30.00	10.00	20.00	20.00	20.00	3,070.82
26 C(1)	30.00	10.00	20.00	30.00	10.00	3,602.74
27 C(1)	30.00	20.00	10.00	20.00	20.00	3,183.76
28 C(1)	30.00	20.00	10.00	30.00	10.00	3,681.18
29 C(1)	30.00	20.00	20.00	20.00	10.00	3,149.26
30 C(1)	20.00	10.00	10.00	50.00	10.00	4,760.08
31 C(1)	20.00	10.00	10.00	30.00	30.00	3,513.28
32 C(1)	20.00	10.00	30.00	20.00	20.00	2,820.88
33 C(1)	20.00	10.00	30.00	30.00	10.00	3,444.28
34 C(1)	20.00	10.00	20.00	20.00	30.00	2,855.38
35 C(1)	20.00	30.00	10.00	20.00	20.00	3,229.72
36 C(1)	20.00	30.00	10.00	30.00	10.00	3,853.12
37 C(1)	20.00	30.00	20.00	20.00	10.00	3,195.25
38 C(1)	20.00	20.00	10.00	20.00	30.00	3,059.80
39 C(1)	20.00	20.00	30.00	20.00	10.00	2,990.80
40 C(2)	10.00	10.00	20.00	40.00	20.00	3,978.26
41 C(2)	10.00	30.00	16.67	26.67	16.67	3,049.10
42 C(2)	10.00	20.00	10.00	40.00	20.00	4,182.68
43 C(2)	10.00	16.67	30.00	26.67	16.67	3,226.24
44 C(2)	10.00	23.33	23.33	20.00	23.33	2,946.34
45 C(2)	10.00	20.00	20.00	40.00	10.00	4,148.18
46 C(2)	10.00	16.67	16.67	26.67	30.00	3,272.23

Vertex (V) Centroid (C)	5 actual mixtures with limitations. N of user-defined limitations: 0 N of initial limitations for the mixture:					
	Common origanum (Oríganum vulgáre)	Black currant (*Ríbes nígrum*, leaves)	Little duckweed (Lémna mínor, leaves)	Bilberry (Vaccinium myrtillus L., leaves)	Common thyme Thimus serpyllum L).	Flavonoids
47 C(2)	30.00	10.00	16.67	26.67	16.67	3,406.77
48 C(2)	30.00	16.67	10.00	26.67	16.67	3,543.12
49 C(2)	30.00	16.67	16.67	20.00	16.67	3,104.30
50 C(2)	30.00	16.67	16.67	26.67	10.00	3,520.11
51 C(2)	20.00	10.00	10.00	40.00	20.00	4,136.68
52 C(2)	16.67	10.00	30.00	26.67	16.67	3,195.57
53 C(2)	23.33	10.00	23.33	20.00	23.33	2,885.05
54 C(2)	20.00	10.00	20.00	40.00	10.00	4,102.18
55 C(2)	16.67	10.00	16.67	26.67	30.00	3,241.56
56 C(2)	16.67	30.00	10.00	26.67	16.67	3,604.41
57 C(2)	16.67	30.00	16.67	20.00	16.67	3,165.59
58 C(2)	16.67	30.00	16.67	26.67	10.00	3,581.40
59 C(2)	23.33	23.33	10.00	20.00	23.33	3,157.54
60 C(2)	20.00	20.00	10.00	40.00	10.00	4,306.60
61 C(2)	16.67	16.67	10.00	26.67	30.00	3,377.91
62 C(2)	16.67	16.67	30.00	20.00	16.67	2,893.10
63 C(2)	16.67	16.67	30.00	26.67	10.00	3,308.91
64 C(2)	23.33	23.33	23.33	20.00	10.00	3,111.59
65 C(2)	16.67	16.67	16.67	20.00	30.00	2,939.09

Table 3 - Results of variance analysis of the developed antioxidant phytocomposition mixture

Model	Analysis of variance; CRM: Flavonoids (mixtures: 1 experiments: 65) 5 actual plan for mix.; total value mix. =100, 65 experiments Last adjustment of models of incr. complexity						
	SS Effect	cc Effect	MS Effect	SS Error	F	p	R-quad.
Linear	21,339,793	4	5,334,948	1,365,211	234.4671	0.000	0.939872
Quadratic	359,957	10	35,996	1,005,254	1.7904	0.086	0.955725

Using the procedure *Plans for surfaces and mixtures with limitations* of the *Planning experiments* module, with the number of factors (components) equal to 5 and the limitations specified in Table 1, an experiment schedule was built, which consisted of 65 experiments (Table 2).

For each experiment, studies were performed to determine the total content of flavonoids. To determine the optimum mixture composition, for which flavonoids reach their maximum values when the limitations on the component composition of the mixture are met, by means of the procedure *Plans for mixtures*, mathematical models were built in the form of linear and quadratic functions that described the relationship of the dependent variable (response) – *concentration of flavonoids* from fractions of mixture components. The *Analysis of variance* procedure showed the adequacy of the linear model only. Table 3 shows that the model was statistically significant since the significance level of the Fisher's test was (*F*) $p = 0.00$ and took a value less than the accepted critical level of significance for statistical hypotheses, 0.05. The quadratic model was not statistically significant, since the significance level of the Fisher's test (*F*) $p = 0.086$ took the value that was greater than the critical level of significance.

Let us explain the mathematical meaning of other statistics that also describe adequacy of the model, by introducing notations: $\tilde{Y}_i$ – variable response of *flavonoids'* concentration predicted by the model values, $\tilde{Y}$ – their average; Y_i – experimentally determined concentrations of *flavonoids*, and $\overline{Y}$ – their average. Then:

– *SS effect* is calculated by the formula – SS = $\sum_{i=1}^{65}(\tilde{Y}_i - \tilde{Y})^2$ = 21,339,793;

– *MS effect* is the ratio of *SS* effects to the number of degrees of freedom (SS =4) 21,339,793:4 = 5,334,948;

SS error is equal to the sum of squares of residuals, i.e. *SS error* = $\sum_{i=1}^{65}(\tilde{Y}_i - Y_i)^2$ = 1,365,211,

– the coefficient of determination R^2 is the main indicator of the regression model adequacy calculated by the formula:

R^2 = 1– *SS error* / *total SS* = 0.94,

where *total SS* = $\sum_{i=1}^{65}(Yi - \overline{Y})^2$ = 21,339,793;

The R^2 determination coefficient specifies the share from the initial variability of the response relative to the mean value, which can be explained by the regression model. Value R^2=0.94 means that the model explains approximately 94% of the response variability from the average. This means that the dependence between the *flavonoids' concentration response* and the components of the mixture (predictors of the model) is close to the linear model, and the response values corresponding to 65 experiments are located near the five-dimensional response - hyperplane, since a linear model has been built.

Table 4 shows the character designations of A, B, C, D, E component of the mixture, point estimates of the coefficients of linear regression equation, standard errors (stand. error), values of the *t*-test with significance levels *p*, and interval estimates of the coefficients in the form of 95% confidence intervals. Since *p* is less than 0.05, all factors in the model are statistically significant.

Table 4 - Coefficients of the linear regression equation

Factor	Coefficient (initial comp.); CRM.: Flavonoids; R-sq=0.9399; Speed. 0.9359 (mixtures: 1 experiments: 65) 5 actual plan for mix.; total value mix. =100, 65 experiments SN Flavonoids; Residual. SS=22,753.51					
	Coeff.	Standard error	t(60)	p	-95.% Confidence limit	+95.% Confidence limit
(A) Common origanum (Oríganum vulgáre)	23.931	2.0124	11.891	0.000	19.905	27.956
(B) Black Currant (*Ríbes nígrum*, leaves)	26.449	2.0124	13.143	0.000	22.424	30.475
(C) Little duckweed (Lémna mínor, leaves)	12.714	2.0124	6.317	0.000	8.688	16.739
(D) Bilberry (Vaccinium myrtillus L., leaves)	76.535	1.390	55.048	0.000	73.754	79.316
(E) Common thyme (Thimus serpyllum L).	11.234	2.012	5.582	0.000	7.209	15.260

In accordance with the character designations of predictors and, additionally, having denoted *flavonoids' concentration with* character Z, the regression linear equation takes the following form:

$$Z = 23.931 \cdot A + 26.45 \cdot B + 12.714 \cdot C + 76.536 \cdot D + 11.235 \cdot E \quad (1)$$

Limitations of the model predictors may be represented as a system of linear inequalities (2):

$$\begin{cases} 10 \le A \le 30 \\ 10 \le B \le 30 \\ 10 \le C \le 30 \\ 20 \le D \le 60 \\ 10 \le E \le 30 \\ A + B + C + D + E = 100 \end{cases} \quad (2)$$

Equation (1) with conditions (2) represents a mathematical formulation of a linear programming problem and, since R^2=0.94, represents an adequate model of flavonoids' content dependence on the shares of mixture components.

Unfortunately, developers of the program did not provide the possibility of displaying the optimum composition of the mixture that ensured the highest response value with limitations of the factors. In the procedure *Plans for mixtures,* there are various methods of reaching an approximate solution to the problem (1-2). Let us consider a solution with the use of the Pareto diagram.

In the Pareto diagram (Fig. 1), factors of the model are sorted in the descending order of their contribution to the response, the vertical line denoting the level of significance p = 0.05. If the column crosses the vertical line, the factor in the model is statistically significant. Contribution to the response corresponds to the height of the column, which is equal to the value of *t*-test (Table 4) of the mixture component.

Assuming that the shares of components of the mixture are proportional to their contribution to the response, and using the fact that

39.885+41.132+34.35+ 84.162+33.619 = 233.152 = 100%,

let us determine the shares (%) of components A, B, C, D, E: common origanum (lat. Oríganum vulgáre) = 17.109; black currant (lat. Ríbes nígrum, leaves) = 17.642; little duckweed (lat. Lémna mínor, leaves) = 14.733; bilberry (lat. Vaccinium myrtillus L., leaves) = 36.097; and common thyme (thyme) (lat. Thimus serpyllum L) = 14.419.

The computed values of predictors satisfy conditions (2). The module provides the possibility to automatically calculate response at arbitrary predictor values, provided that their sum equals 100. In table 5, line *predicted* shows the approximately optimal value *of flavonoids' concentration* calculated by the program, which is equal to 3,986.218 with a 95% confidence interval (3,937.114; 4,035.233). This clearly shows that value 3,986.218 found by the program exceeds the preset value of 3,500 mg/100 g.

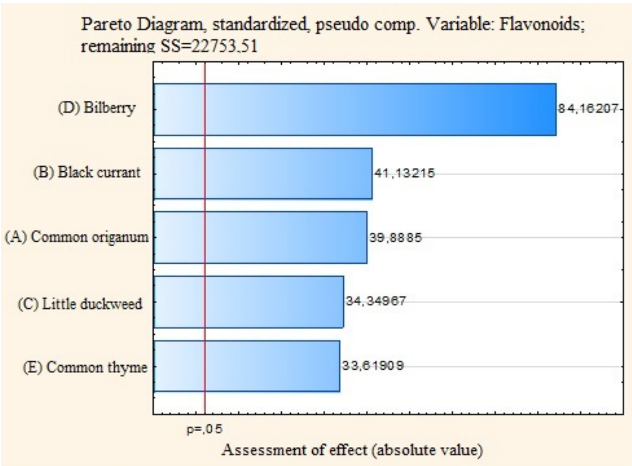

Figure 1 - Pareto Diagram for the developed antioxidant phytocomposition mixture

Table 5 - Predicted flavonoids' content by the Pareto diagram

Factor	Predicted value; CRM.: Flavonoids; R-sq=**0.94**; Speed. **0.936** (mixtures: 1, experiments: 65) SN **flavonoids' concentration**; Residual. SS=22,753.51			
	Coeff.	Pseudo comp.	Coeff. * Val.	Init. comp.
(A) Common origanum (Oríganum vulgáre)	3,231.270	0.175	565.472	17.000
(B) Black Currant (*Ríbes nígrum*, leaves)	3,332.014	0.200	666.403	18.000
(C) Little duckweed (Lémna mínor, leaves)	2,782.583	0.125	347.823	15.000
(D) Bilberry (Vaccinium myrtillus L., leaves)	5,335.451	0.400	2,134.180	36.000
(E) Common thyme (Thymus vulgaris L).	2,723.400	0.100	272.340	14.000
predicted			3,986.218	
-95.% Conf.			3,937.114	
+95.% Conf.			4,035.323	

Table 6 - Predicted values of flavonoids’ concentration according to the profiles’ diagram

Factor	Predicted value; CRM.: Flavonoids; R-sq=0.93987; Speed. 0.93586 (mixtures: 1, experiments: 65) SN Flavonoids; Residual SS=22,753.51			
	Coeff.	Pseudocomp.	Coeff. *Val.	Init.comp.
(A) Common origanum (Oríganum vulgáre)	3,231.270	0.000000	0.000	10.00000
(B) Black Currant (*Ríbes nígrum,* leaves)	3,332.014	0.000000	0.000	10.00000
(C) Little duckweed (Lémna mínor, leaves)	2,782.583	0.000000	0.000	10.00000
(D) Bilberry (Vaccinium myrtillus L., leaves)	5,335.451	1.000000	5,335.451	60.00000
(E) Common thyme (Thymus vulgaris L).	2,723.400	0.000000	0.000	10.00000
predicted			5,335.451	
-95,% Conf.			5,208.642	
+95,% Conf.			5,462.260	

Table 7 - Remainders between the experimental response values and those predicted by the model

Plan	Observed, predicted values and residuals (mixtures: 1, experiments: 65) 5 actual plan for the mix.; total value mix. =100, 65 experiments; SN Flavonoids; R-sq=0.9399; Speed.0.9359		
	Observed	Predicted	Remainders
1	3,013.840	2,977.335	36.505
2	3,105.800	3,027.707	78.093
3	3,353.680	3,281.642	72.038
4	2,696.960	2,752.991	-56.031
5	2,944.840	3,006.926	-62.086
6	3,036.800	3,057.299	-20.499
7	5,259.560	5,335.451	-75.891
8	4,260.640	4,283.360	-22.720
9	4,352.600	4,333.733	18.867
10	5,077.580	4,059.017	1018.563
11	4,012.760	4,029.425	-16.665
12	4,636.160	4,682.438	-46.278
13	3,320.360	3,406.004	-85.644
14	4,601.660	4,697.234	-95.574
15	3,354.860	3,391.208	-36.348
16	3,729.200	3,680.720	48.480
17	3,071.300	3,042.503	28.797
18	3,694.700	3,695.516	-0.816
19	4,806.080	4,834.592	-28.512
20	3,559.280	3,528.566	30.714
21	2,866.880	2,905.145	-38.265
22	3,490.280	3,558.158	-67.878
23	2,901.380	2,890.349	11.031
24	3,637.400	3,630.348	7.052
25	3,070.820	2,992.130	78.690
26	3,602.740	3,645.143	-42.403
27	3,183.760	3,129.488	54.272
28	3,681.180	3,782.501	-101.321
29	3,149.260	3,144.284	4.976
30	4,760.080	4,809.406	-49.326
31	3,513.280	3,503.380	9.900
32	2,820.880	2,879.959	-59.079
33	3,444.280	3,532.971	-88.691
34	2,855.380	2,865.163	-9.783
35	3,229.720	3,154.675	75.045
36	3,853.120	3,807.687	45.433
37	3,195.250	3,169.470	25.780
38	3,059.800	3,002.521	57.279
39	2,990.800	3,032.112	-41.312
40	3,978.260	4,044.221	-65.961
41	3,049.100	3,472.913	-423.813
42	4,182.680	4,181.579	1.101
43	3,226.240	3,289.769	-63.529
44	2,946.340	2,945.999	0.341
45	4,148.180	4,196.375	-48.195
46	3,272.230	3,270.041	2.189
47	3,406.770	3,422.540	-15.770
48	3,543.120	3,514.112	29.008
49	3,104.300	3,088.634	15.666
50	3,520.110	3,523.976	-3.866
51	4,136.680	4,156.393	-19.713
52	3,195.570	3,272.978	-77.408
53	2,885.050	2,912.417	-27.367
54	4,102.180	4,171.189	-69.009
55	3,241.560	3,253.250	-11.690
56	3,604.410	3,547.694	56.716
57	3,165.590	3,122.216	43.374
58	3,581.400	3,557.558	23.842
59	3,157.540	3,095.561	61.979
60	4,306.600	4,308.546	-1.946
61	3,377.910	3,344.822	33.088
62	2,893.100	2,939.072	-45.972
63	3,308.910	3,374.414	-65.504
64	3,111.590	3,115.289	-3.699
65	2,939.090	2,919.344	19.746

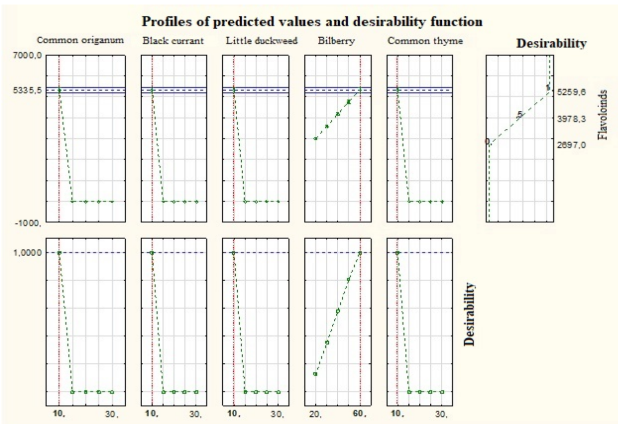

Figure 2 - Profiles of the predicted values and desirability function for the developed antioxidant phytocomposition mixture

Using graphical tools of the module, in particular, the *Profiles for predicted values and desirability function* diagram, it was possible to improve the result. Figure 2 shows diagrams of response behavior – *flavonoids' concentration* (top) and desirability function (bottom), when the mixture components' shares change within acceptable limits with average values of other components. Desirability function in the range from 0 to 1 assesses the degree of response preference to the predicted value with the appropriate value of this factor with average values of other factors. The horizontal line in the diagrams denotes the greatest (optimal) value *of flavonoids' concentration* equal to 5,335.5. The horizontal line in the diagrams of desirability refers to the highest achieved value of desirability, which is equal to 1. Vertical lines correspond to the optimal values of factors – mixture components, with which the maximum values of response and desirability are achieved. The optimal values of the components, with which the desirability function and the response reach the maximum values, are shown in the base of the desirability diagrams: common origanum = 10; Black currant = 10; Little duckweed = 10; Bilberry = 60; and Common thyme = 10. It should be noted that the approximate optimal value is reached at the border of the mixture component share ranges (corresponds to experiment v7).

Table 6 shows the achieved near-optimal response value of 5,335.451 and the values of the predictors corresponding to it.

Note that value 5,335.451 differs from the experimental value 5,259.56, which is easily explained by the fact that the obtained value is a model value as calculated by linear model (2). Table 7 shows the difference between the experimental response values and those predicted by the model.

CONCLUSION

1. The use of data analysis methods implemented in software package STATISTICA in the development of a phytocomposition mixture has allowed to:

– develop plans for experiments with the factors that are components of the mixture;

– build a linear model based on the dependence of total flavonoids' content on the shares of mixture component; and

– to establish the optimum shares of the mixture components, with which the total content of flavonoids is the highest.

2. A mathematically valid formulation of the antioxidant phytocomposition mixture has been developed, with the optimal shares of the components of the mixture being the following: common origanum (lat. Oríganum vulgáre) = 10; black currant (lat. Ríbes nígrum, leaves) = 10; little duckweed (lat. Lémna mínor, leaves) = 10; bilberry (lat. Vaccinium myrtillus L., leaves) = 60; and common thyme (thyme) (lat. Thimus serpyllum L) = 10. Such mixture corresponds to the predictive value of the total content of flavonoids, which is equal to 5,335.451 mg/100 g.

Given the fact that according to the established formulation of the phytocomposition mixture the total content of flavonoids is 3,500 mg/100 g, the use of the module *Planning experiments* allowed to increase the total content of flavonoids in the developed formulation by more than 50%.

The obtained formulation of the phytocomposition mixture may be used in the technology of antioxidant functional beverages.

ACNOWLEDGEMENTS

The research was performed in the framework of the Federal target program "Research and Development on Priority Areas of Developing the Scientific-Technological Complex of Russia in 2014-2020" on the topic "Development of Technologies for Production of High-quality and Safe Functional Drinks with the Use of the Biologically Active Components of Nonconventional Vegetable Raw Materials of the North-Caucasian region", Agreement No. 14.574.21.0174.

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