INVESTIGATION AND ISOLATION OF A PROTEIN–VITAMIN CONCENTRATE FROM PINE NEEDLES

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At the present time, ever-increasing attention is being devoted to the prospects of the use of the green matter of trees. This is due in the first place to the fact that the components of which it is composed – chlorophyll, vitamins, enzymes, lipids, sugars, etc. – belong to those substances which are incomparably more active participants in the metabolism of the organism than the carbohydrate–lignin complex (wood). They can be used successfully in medicine, perfumery, agriculture, and other branches of the national economy.

A number of technological schemes exist which enable a chlorophyll–carotene paste, sodium chlorophyllin, a medicinal pine extract, needle flour, essential oils, and other products enjoying great demand to be obtained from the green matter of trees [1, 2]. In addition to the components mentioned, the green matter of trees includes proteins. On the basis of the results of our investigations and an analysis of literature information it has been established [2] that the amount of protein in pine needles is fairly considerable (8-14%) and its amino-acid composition is diverse. However, in spite of this, not one of the technological schemes existing at the present time provides for the extraction of protein from the technical green matter.

The isolation of protein from needles is a little-studied process, and therefore the development of the optimum conditions for aqueous extraction of the needles requires the performance of a special experiment. The process of extraction of the needles is also affected by controllable factors such as the temperature, the degree of comminution of the needles, the time of extraction, the solvent, and the extraction ratio [3]. Let us consider the influence of the most important of them.

As is well known, extraction is a diffusion process. Because of this, the surface of contact of the substance with the solvent, i.e., the degree of comminution of the material extracted, has a great influence on it. In the present case, the process of extraction takes place simultaneously with comminution, and therefore the degree of comminution of the raw material will depend on the time of extraction.

Fundamental factors in the extraction process are the nature of the solvent and its concentration. The solvent ratio and the temperature of the process must be chosen correctly. The solvent ratio determines the main motive force of the diffusion process – the difference in concentrations. In its turn, the temperature intensifies this process, since raising the temperature leads to a shortening of the time of extraction, but with a rise in the temperature valuable biological substances are destroyed.

The aim of the present investigation was to study the composition of the proteins of pine needles and of the protein–vitamin concentrate obtained, and also to find the conditions for its isolation. Having studied the distribution of the proteins in their extraction with water, alkali, and sodium carbonate, we established the nature of each extractant. Then, by mathematical planning, we found the optimum conditions of the process.

To confirm the calculated value of the functions obtained on a computer, we determined a number of experimental values, and these agreed well with the theoretical figures.

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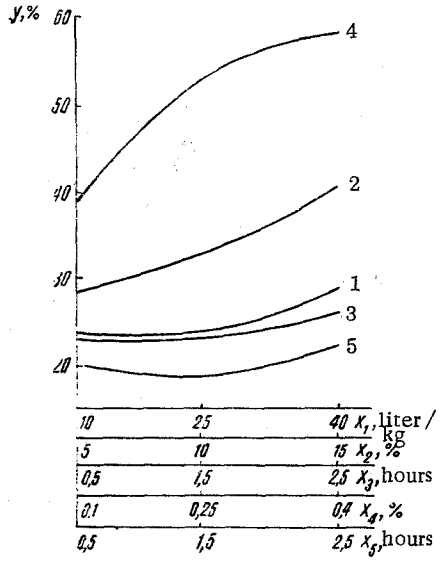

Fig. 1. Influence of the variable factors on the yield of protein: dependence of the extraction of protein on: 1) the liquor ratio; 2) the concentration of salt solution; 3) the time of extraction by the salt solution; 4) the concentration of alkali; 5) the time of extraction with alkali.

TABLE 1. Fractional Composition of the Needle Proteins and of the Protein–Vitamin Concentrate

Index	Amount of the fraction, %					Residue, %	Total, %
	water-soluble	salt-soluble	alcoholic	borate	alkali		
Needle proteins							
total nitrogen	0,30	0,03	0,04	0,06	0,30	0,90	1,69
protein	1,88	0,19	0,25	0,38	1,88	5,63	10,19
Proteins of the vitamin–protein concentrate							
Total nitrogen	4,40	0,50	0,05	0,05	0,60	3,50	9,19
protein	27,50	3,12	0,31	0,31	3,75	21,87	56,86

Figure 1 shows the influence of the variable factors on the yield of protein. The greatest influence is shown by the concentration of alkali (curve 4), which is confirmed by the value of the regression coefficient attached to the corresponding variable in the equation (it is several orders of magnitude higher than the coefficients attached to the other variables). A maximum extraction of protein is observed at a concentration of alkali of 0.3%, and it scarcely changes with a further increase in the concentration.

The chemical composition of the protein–vitamin concentrate obtained under the optimum conditions was as follows: water 10.81%; dry matter 89.19%; fat 11.80%; cellulose 12.18%; protein 55%; Ca 1.30 g; P 0.70 g; carotene 115.10 mg [sic].

In order to determine the food value of the protein–vitamin concentrate it was investigated for its fractional and amino-acid compositions (Tables 1 and 2, respectively).

TABLE 2. Amino Acid Composition of the Proteins

Amino acid	Amount % on the absolutely dry needles	
	protein–vitamin concent.	needle proteins
Lysine	3,91	0,411
Histidine	1,36	0,056
Arginine	3,27	0,301
Aspartic acid	4,41	0,459
Threonine	3,35	0,221
Serine	2,38	0,274
Glutamic acid	4,05	0,636
Proline	0,31	0,405
Glycine	2,82	0,321
Alanine	3,08	0,349
Cystine	0,85	0,130
Valine	3,02	0,247
Methionine	0.92	0,046
Isoleucine	2,01	0,331
Leucine	3,70	0,407
Tyrosine	1,31	0,103
Phenylalanine	1·94	0,171
Tryptophan	0,80	0,120
Total	43,5	4,888

EXPERIMENTAL

Extraction of the Proteins. The proteins were extracted from the needles successively with water (albumins), dilute solutions of neutral salts (globulins), ethanol (prolamines) and solutions of alkali and borate buffer (glutenins). The distribution of the proteins over these fractions is shown in Table 1.

Mathematical Planning of the Extraction Conditions. The results obtained permitted the nature of the extractants for the isolation of proteins to be determined: aqueous, alkaline, and salt solutions.

On the basis of what has been said, the following factors were included in the planning process:

X_1 — water:needle mass ratio, liter/kg;

X_2 — concentration of salt solution, %;

X_3 — time of extraction with salt solution, h;

X_4 — concentration of alkali, %; and

X_5 — time of extraction with alkali, h.

As the optimization criterion Y we took the degree of extraction of protein, which was estimated in percentages of its amount in the initial absolutely dry needles.

To solve the optimization problem we used the Hartley-5 plan [4, 5]. Such a plan has the minimum number of experiments — 27 —, and in the values of its statistical characteristics it is superior to all rotatable plans. In the value of the determinant of the matrix it is close to D — the optimum plan — and in the value of the variance of the predicted value it is superior to it.

The amounts of total nitrogen and of protein in the extracted needles were found by the Kjeldahl method [6].

In accordance with the plan for central points, the effective variables X adopt the code designation ±1. The basic zero levels were set on the basis of literature information [2, 6, 7] and the results of preliminary experiments. The coordinates of the zero point were as follows: 25 liters/kg; 10%; 1.5 h; 0.25%; 1.5 h.

TABLE 3. Planning Conditions and Results of the Experiments

Level of variation	Factor						
	X_1, l/kg	X_2, %	X_3, h	X_4, %	X_5, h	Y	$\hat{Y}$
	Planning conditions						
Basic X_{0l}	25,00	10,00	1,50	0,25	1,50		
Interval λ_l	15,00	5,00	1,00	0,15	1,00		
Upper (+)	10,00	15,00	2,50	0,40	2,50		
Lower (—)	40,00	5,00	0,50	0,10	0,50		
	Results of the experiments						
	+1	+1	+1	+1	+1	61,05	61,57
	—1	+1	+1	+1	—1	62,50	62,33
	+1	—1	+1	+1	—1	58,10	58,32
	—1	—1	+1	+1	+1	63,65	64,61
	+1	+1	—1	+1	—1	61,55	61,41
	—1	+1	—1	+1	+1	60,35	60,95
	+1	—1	—1	+1	+1	57,00	58,00
	—1	—1	—1	+1	—1	58,50	58,80
	+1	+1	+1	—1	—1	52,15	51,13
	—1	+1	+1	—1	+1	58,85	58,57
	+1	—1	+1	—1	+1	47,35	47,46
	—1	—1	+1	—1	—1	51,20	50,61
	+1	+1	—1	—1	+1	53,50	53,10
	—1	+1	—1	—1	—1	54,60	53,66
	+1	—1	—1	—1	—1	43,00	42,45
	—1	—1	—1	—1	+1	47,45	47,64
	0	0	0	0	0	57,35	57,42
	—1	0	0	0	0	60,50	60,39
	0	+1	0	0	0	58,20	59,83
	0	—1	0	0	0	57,15	55,48
	0	0	+1	0	0	58,45	58,47
22	0	0	—1	5	0	56,60	66,34
23	0	0	0	+1	0	60,25	59,92
24	0	0	0	—1	0	43,45	46,47
25	0	0	0	0	+1	63,35	60,45
26	0	0	0	0	—1	55,95	58,80
27	0	0	0	0	0	57,35	57,47

The planning conditions and the results of the experiments are shown in Table 3. All the experiments were duplicated and were randomized in time. Each value of Y in Table 3 is a mean.

The realization of the plan (experiments 1-27) gave regression equations having the form

$$Y = 24.9227 - 0.1686x_1 + 1.0180x_2 + 0.7799x_3 + 188.4185x_4 - 5.5103x_5 + \\ + 0.6664x_{11}^2 + 0.0076x_2^2 + 0.0394x_3^2 - 250.4669x_4^2 + 2.1644x_5^2 - \\ - 0.0060x_1x_2 + 0.0481x_1x_2 - 0.2486x_1x_4 - 0.0019x_1x_5 - 0.1206x_2x_3 - \\ - 1.8125x_2x_4 - 0.0231x_2x_5 - 1.3541x_3x_4 + 0.4031x_3x_5 - 1.9375x_4x_5.$$

The variance of the mean value and the residual variance were, respectively:

$$S^2\{Y\} = 8.2690,$$
$$S_{res}^2 = 31.6775.$$

The errors in the determination of the regression coefficients were

$$S^2\{b_0\} = 1.139, \quad S^2\{b_{ii}\} = 3.375,$$
$$S^2\{b_i\} = 0.458, \quad S^2\{b_{ij}\} = 0.516.$$

The adequacy of the model was estimated by means of the Fisher criterion. The value of the latter at the 5% level of significance is 3.84. The calculated value of the ratio for Y is

$$F_{ad} = 3.8308.$$

Consequently, the mathematical model based on planning adequately reflects the results of the experiments.

The mathematical planning of the experiment enabled us to find the optimum conditions for the extraction process. They correspond to the following values:

ratio of water to needle mass	40 liters/kg;
concentration of the salt solution	15%;
time of extraction by the salt solution	2.3 h;
concentration of alkali	0.3%; and
time of extraction with alkali	2.3 h.

Under these conditions the maximum extraction of protein (65.8%) is achieved.

Amino-Acid Analysis. The total amino-acid compositions of the proteins of the pine needles and of the protein–vitamin concentrate isolated were determined on a Hitachi KLA-3B automatic analyzer. The proteins were first hydrolyzed with hydrochloric acid by a standard method [6]. The identification of the amino acids and the calculation of their amounts were performed by the method of additives.

SUMMARY

1. The fractional and amino-acid compositions of needle proteins and of a protein–vitamin concentrate isolated from an extract have been determined.

2. The optimum conditions of the protein-extracting process have been found. The dependence of the influence of various factors on the degree of extraction of the protein from the needles has been shown.

3. The results obtained enable the protein–vitamin concentrate to be recommended as a valuable additive to fodder for agricultural animals.

LITERATURE CITED

1. F. N. Shcherbak and F. T. Solodskii, "The laws of the development of science and the new wood-biochemical direction. The use of the living elements of wood," Trudy LTA, No. 119, Series No. 1, 13 (1969).
2. R. I. Tomchuk and G. N. Tomchuk, The Green Matter of Wood and Its Utilization [in Russian] (1970), p. 295.
3. A. K. Slavyanskii and F. A. Mednikov, The Technology of Wood-Chemical Products [in Russian], Moscow (1970), p. 300.
4. R. Z. Pen and E. M. Mencher, Statistical Methods in the Pulp and Paper Industry [in Russian], Moscow (1973), p. 29.
5. P. F. Andrukovich, T. I. Golikova, and S. G. Kostina, New Ideas in the Planning of Experimental Work [in Russian], Moscow (1969), p. 140.
6. A. I. Ermakov, V. V. Arasimovich, et al., Methods for the Biochemical Investigation of Plants [in Russian], Leningrad (1972), p. 267.
7. A. P. Dmitrichenko, in: Plant Proteins and Their Use in the Feeding of Agricultural Animals [in Russian], Leningrad (1964), p. 23.