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OPTIMIZATION OF THE ELIMINATION OF LIPIDS FROM THE MICROALGA *Chlorella vulgaris*

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The use as food of the biomass of *Chlorella* without chemical treatment is impossible because of the presence of toxic substances in the lipids. According to the literature [1], the lipid fraction contains a number of specific substances including fatty acids of the hexadecatrienoic type and substances possessing biological activity — chondrillosterol and ergosterol. It has been shown [2, 3] that the use of native *Chlorella* in the fodder and food ration leads to pathological changes in the organs of animals and to signs of allergy in man. These changes are the result of the toxic action of the ethanol-soluble substances of the lipid fraction. The possibility has been shown previously of eliminating the lipids by oxidation with peracids [4, 5]. Hydrogen peroxide is a specific oxidizing agent of lipids, since in the absence of catalysts it has practically no effect on the other components of the cell.

The aim of the present investigation was to find the optimum conditions for eliminating the lipids by peroxide oxidation. Since definite requirements are also set for the color of the food product, the color of the protein–carbohydrate product was chosen as a second optimization parameter.

EXPERIMENTAL

For oxidation we used the pasty biomass of the microalga *Chlorella vulgaris* (moisture content 80%) obtained in the L. V. Kirenskii Institute of Physics of the Siberian Branch of the Academy of Sciences of the SSSR. The experiment was based on a Box–Hunter second-order rotatable plan, which corresponds to the requirements of central composition planning. This satisfies well-known criteria for the optimum nature of the plans. The optimization parameters selected were the lipid content in the protein–carbohydrate product [Y_1] and its color [Y_2]. In view of the fact that in the cell the magnesium is present mainly in the chlorophyll, the color of the complex was expressed in terms of its magnesium content.

In a series of preliminary experiments, we worked out the three main factors affecting the process, their basic levels, and the intervals of variation:

Factor	Symbol	Basic level, X_{cch}	Interval of variation, λ	Upper level	Lower level
Time of oxidation,	X_1	5.5	2.5	8.0	3.0
Oxidation temperature, °C	X_2	45	15	60	30
Concentration of H_2O_2, %	X_3	17.0	8.0	25.0	9.0

Oxidation was performed in a glass reactor fitted with a stirrer, a jacket for heating, and two nozzles for the feed of hydrogen peroxide and the removal of the reaction gases, all

*N. D. Barabash took part in the work.

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TABLE 1. Planning Matrix of the Experiment and Results of Its Realization

X_1	X_2	X_3	Y_1	Y_1	Y_2	Y_2	Y_3	Y_3
+1	+1	+1	5,8	4,476	0,137	0,1160	58,30	56,640
−1	+1	+1	7,5	7,276	0,258	0,2779	69,95	68,593
+1	−1	+1	15,3	12,821	0,412	0,3828	75,80	76,678
+1	+1	−1	9,2	8,103	0,374	0,3038	66,60	64,112
+1	−1	−1	13,4	12,648	0,455	0,4395	76,60	17,626
−1	+1	−1	7,7	9,203	0,302	0,3356	73,80	72,590
−1	−1	+1	14,1	14,220	0,464	0,5387	76,65	78,806
−1	−1	−1	12,0	12,348	0,440	0,4654	74,95	76,279
1,682	0	0	7,60	3,208	0,200	0,2830	66,65	67,825
−1,682	0	0	14,1	12,591	0,530	0,4410	77,45	76,744
0	1,682	0	3,0	3,208	0,091	0,1156	50,30	54,132
0	−1,682	0	11,7	12,871	0,480	0,4492	77,45	74,087
0	0	1,682	8,2	10,052	0,408	0,3838	76,60	76,430
0	0	−1,682	12,0	11,528	0,462	0,4800	79,95	80,589
0	0	0	9,4	9,894	0,400	0,3803	73,30	75,262
0	0	0	10,3	9,894	0,340	0,3803	76,60	75,262
0	0	0	10,3	9,894	0,361	0,3803	76,60	75,262
0	0	0	10,2	9,894	0,362	0,3803	75,20	75,262
0	0	0	9,7	9,894	0,436	0,3803	76,40	75,262
0	0	0	9,7	9,894	0,380	0,3803	75,70	75,262

Note. The values of Y_2 and Y_1 are given in percentages of the protein–carbohydrate complex, and Y_3 in percentages of the absolutely dry weight of the initial *Chlorella*.

the changes in the selected factors being performed in accordance with the planning matrix (Table 1). After oxidation, the reaction mixture was cooled to 15-20°C and was extracted with 80% acetone to eliminate the reaction products and precipitate the protein substances. The protein–carbohydrate residues washed on a Buchner funnel with anhydrous acetone and was dried under a vacuum of 580-600 mm Hg at 55-60°C for 4 h. The yield of protein–carbohydrate complex was determined gravimetrically. The analysis of the initial *Chlorella* and of the protein–carbohydrate complex was performed by known methods [6] with the determination of the amounts of lipids, magnesium, and carbohydrates and of protein by Lowry's method and of total nitrogen by the Kjeldahl method. The amount of nucleic acids was determined by Spirin's spectrophotometric method [7].

The identification and quantitative determination of the amino acids was performed on a Hitachi amino-acid analyzer.

After the calculation of the regression coefficients and a statistical analysis of the experimental results (see Table 1), the following second-order regression equations describing the process of elimination of the lipids and the change in the color of the protein–carbohydrate complex were obtained by a known method [8] on a "Minsk-22" computer:

$$Y_1 = 9.894 - 0.6248 X_1 - 2.8725 X_2 - 0.4386 X_3 + \\ + 0.584 X_1^2 - 0.6553 X_2^2 + 0.3166 X_3^2 - 0.350 X_1 X_2 - 0.425 X_1 X_3 = \\ = 0.95 X_2 X_3, \quad (1)$$

$$Y_2 = 0.3803 - 0.0469 X_1 - 0.0991 X_2 - 0.0286 X_3 - \\ - 0.0065 X_1^2 - 0.0346 X_2^2 + 0.0182 X_3^2 - 0.0325 X_1 X_3 - \\ - 0.0328 X_2 X_3. \quad (2)$$

The results of the analysis show that the mathematical models, represented by second-order polynomials, are not only adequate but are also effective. The adequacy of the model was confirmed by comparing the variance ratio

$$F_{ad} = \frac{S_{res}}{S_{sup}}$$

with the tabular value of the Fisher criterion $F_{tab} = 5.05$ at a probability coefficient of 95% by the condition $F_{ad} \leq F_{tab}$.

For Eq. (1): $F_{ad_1} = 2.8545/0.64 = 4.46$;

For Eq. (2): $F_{ad_2} = 0.0033/0.0025 = 1.3$.

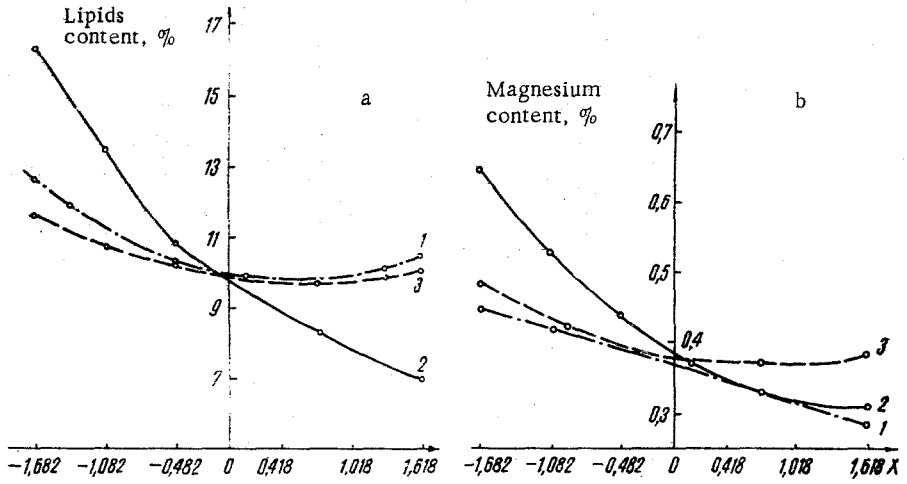

Fig. 1. Influence of the parameters to be optimized on the amounts of lipids (a) and of magnesium (b) in the protein–carbohydrate complex: 1) time of oxidation; 2) temperature of the process; 3) concentration of hydrogen peroxide (values of the parameters along the X axis are given in their coded form; the formula for recalculation being $X = X_{cod}\lambda + X_{rel}$).

The effectiveness of the model was confirmed if $F_{eff} \geq F_{tab}$, where

$$F_{eff} = \frac{S_{yn}}{S_{res}}, \quad F_{tab} = 2.77.$$

For Eq. (1): $F_{eff_1} = 9.0878/2.8545 = 3.184$;

For Eq. (2): $F_{eff_2} = 0.0132/0.0033 = 3.982$.

Correlation analysis showed that a strong linear relationship exists between the values of Y_1 and Y_2 (correlation coefficient r = 0.90). This enabled us to limit ourselves to the solution of one regression equation in order to find the optimum conditions. By solving Eq. (1) on the "Minsk-22" computer, we found the following optimum conditions for Y_1: X_1 = 10 h; X_2 = 72°C; X_3 = 31.4%.

An evaluation of the influence of each factor on the oxidation of *Chlorella* can be made from a graphical analysis of the regression equations (Figs. 1 and 2). As can be seen from the graphs, the temperature of performing the process and its time have the greatest influence. At the beginning of the region, at low values of the variables, a small increase in the temperature and time of oxidation leads to a sharp change in the contents of lipids (Fig. 1a) and magnesium (Fig. 1b). On approaching the top levels of the graph, these functions have small angles of inclination to the X axis. The graphs of the dependence of the yield of product on the factors taken have a different appearance. A sharp fall in the yield of protein–carbohydrate product is observed on approaching the top levels.

Starting from technological considerations and evaluating the contribution of each factor, we adopted a compromise solution for the optimum conditions of oxidation: temperature 65°C, time 7 h, concentration of hydrogen peroxide 30%. A further increase in these factors does not lead to large changes in the color and lipids content but it greatly decreases the yield of protein–carbohydrate complex. Furthermore, a rise in the temperature above 65°C is undesirable because of the denaturation of the protein.

Below we give the results of a determination of the biochemical composition of the initial *Chlorella* and of the protein–carbohydrate complex obtained under the otpimum conditions adopted (in percentages on the absolutely dry weight):

	Total nitrogen	Crude protein	Lowry protein	Total amino acids	Lipids	Nucleic acids	Carbohydrates
Initial Chlorella	8.3	51.9	48.2	42.9	20.0	4.3	19.7
Protein–carbohydrate complex	9.3	58.1	61.2	56.6	0.4	2.0	15.5

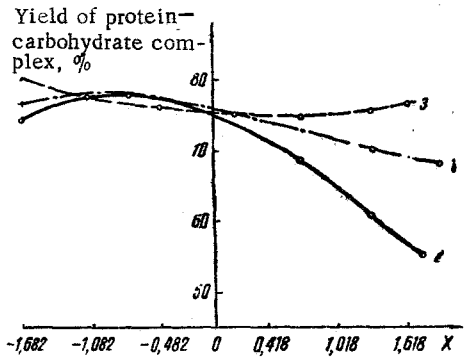

Fig. 2. Influence of the parameters to be optimized on the yield of protein–carbohydrate complex: 1) time of oxidation; 2) temperature of the process; 3) concentration of hydrogen peroxide.

As can be seen from the figures given, the protein–carbohydrate product obtained under the optimum conditions is considerably enriched in protein and its lipids content has been reduced practically to a minimum. Furthermore, in comparison with the initial *Chlorella* it has only half the amount of nucleic acids. The ratio of the majority of amino acids remains unchanged, with the exception of tryptophan, which has been destroyed. The yield of protein–carbohydrate complex under the optimum conditions of working is 55% of the dry matter of the initial biomass. Calculated on the initial protein this amounts to 75%.

The physicochemical properties of the protein product isolated give grounds for recommending it for medical and biological tests as a food raw material.

SUMMARY

Using the microalga *Chlorella vulgaris* as an example, the possibility has been shown of eliminating the lipids and pigments by oxidizing it with hydrogen peroxide without changing the compositions of the protein–carbohydrate complex. The influence of the conditions of performing the oxidation of the *Chlorella* biomass have been studied and it has been described mathematically. The optimum conditions for eliminating the lipids and pigments have been found and the general chemical properties of the protein product isolated have been determined.

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