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Extraction of Chlorophyll From Technologically Prepared Spinach Leaves

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Abstract **—** *The purpose of this research is to improve the processes of obtaining chlorophyll extract from technologically prepared spinach leaves, which will eventually produce fat-soluble chlorophyll paste. According to this objective, the authors solved a number of problems. In particular, they selected rational technological solutions for preparing plant raw materials in order to extract photosynthetic pigments from them. The authors also identified specific features of the use of ultrasound at this stage, which resulted in the choice of rational temperature conditions when it affects plant material. Additionally, the identified kinetic regularities of the extraction process are presented, and a rational approach to extracting the target component from the object of study is proposed on their basis. As a result, due to the analysis of the structures of extraction plants for processes in the solid-liquid system, the article provides practical recommendations for implementing the research results. Namely, a two-section original installation for obtaining alcohol extract with the specified indicators of the final volume concentration of the target component in it is proposed.*
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Keywords — *Chlorophyll, extraction, ultrasound, process kinetics, kinetic coefficients.*

I. INTRODUCTION

One of the promising directions for the development of the food and processing industry in Russia is the search for new technological solutions in obtaining natural healthy food additives, which at the same time are coloring substances. Such premixes-dyes (PC), as a rule, can be independently consumed in granular or encapsulated form and used at food enterprises as a coloring PC in order to obtain materials with specified specific characteristics, in particular, certain taste parameters, texture, color, smell, period of use. An option of such substances is the additive E-140 (chlorophylls and chlorophyllins), which is absolutely harmless and can have a positive effect on the human body. In the food industry, E-140 is used as a natural dye that can give the product an olive hue. Due to the positive effect of chlorophyll on human health and the absence of negative consequences from its use, natural supplement E-140 is allowed in the world [13]. Materials of plant origin or waste from their processing are mainly used as raw materials for PC manufacturing [4, 5, 6, 17]. Chlorophyll is a stable pigment in plant tissue. However, in its extracted form (liquid and dried extracts), it undergoes degradation under light and thermal effects, contact with oxygen, acids, and enzyme preparations [15], chemical interaction of dyes and food components. When decomposed, chlorophyll derivatives such as pheophytin, chlorophyllide, and pheophorbide are formed. To preserve chlorophyll and minimize its conversion to pheophytin, the chlorophyll enzyme is chemically inactivated, and to obtain more color-resistant metallochlorophilic complexes, magnesium, copper, or zinc salts are added to the composition, and the pH and temperature of the extract are controlled [11]. The development of new methods is aimed at increasing environmental safety by reducing the application of synthetic and organic chemicals and improving the quality of the extract, reducing the processing time. To increase the total yield of chlorophyll from plant raw materials, ultrasound, pulsed electric field, enzymatic splitting, microwave heating, and supercritical extraction are used.

The color stability of PCs during storage depends, in addition to their origin, on the method of their isolation from raw materials. It is negatively affected by a small fraction of colorants in the initial product (2 ÷ 5%), as well as the presence of impurities, in particular polyphenolic substances, sugars, and their modifications, pectin substances and proteins, their decomposition products, organic acids, and mineral salts, moreover, as a rule, their amount exceeds the dye content. The low content of PC in raw materials determines the relatively high cost of PC due to the relatively high consumption of raw materials [4, 5, 6, 15].

Extraction is the most important stage in the isolation of various types of biologically active compounds from plants [10]. The main problems of conventional extraction are the long process time, the need for an expensive and highly pure solvent and its evaporation in large quantities, low selectivity of extraction, and thermal decomposition of thermolabile compounds. Along with traditional approaches to the extraction of biologically active materials from raw materials of plant nature, many new methods have been created, but so far, none of the methods is considered the standard. The effectiveness of traditional and unconventional extraction methods mainly depends on critical input parameters, understanding the nature of the plant matrix [12], the chemistry of bioactive compounds, and scientific expertise [3].

Based on the foregoing, the purpose of this study is to improve the processes for obtaining chlorophyll extract from plant materials with a high concentration of the target component in its composition, for example, spinach leaves, which will ultimately produce a fat-soluble chlorophyll paste. In order to achieve this goal, it is necessary to solve the following tasks: to identify rational technological solutions for preparing spinach leaves for extraction, specific features of the use of ultrasound, which results in an increase in temperature, and the need to comply with technological restrictions; to investigate kinetic laws of extracting chlorophyll from prepared spinach leaves and propose a rational technological way to extract it; determine the mass transfer coefficients taking into account the extraction technology in the field of centrifugal forces and conduct a simulation of the extraction process; give practical recommendations for the implementation of the research results.

II. MATERIALS AND METHODS

The choice of spinach leaves as an object of study for obtaining a natural dye-based on chlorophylls, in particular species a and b, is due to the fact that this vegetable is a food raw material rich in this pigment and, in addition, a good source, biologically active in various functional properties. substances [16, 20]. Both chlorophylls in spinach foliage are combined with proteins, and such a common plant complex is called chlorohydrin. Both species are soluble in organic solvents, but their solutions are unstable. Therefore, after distillation of the extractant, chlorophyll is advisable to dissolve in fats, solutions of which can already be stored. The mass fraction of chlorophylls in spinach leaves, according to various sources, ranges from 185mg to 620 mg per 100g of the product [1, 7]. In addition, the preference given to spinach is due to the relatively intensive frequency of harvesting (after every 25-35 days from sowing).

The technological need to prepare the object of study for the subsequent extraction of chlorophyll from it is confirmed by microscopic examination of the spinach leaf before and after preliminary operations with a biological microscope (Altami BI O2 (ALTAMI). The study of temperature conditions for compliance with technological restrictions during ultrasonic exposure to the material after its processing was carried out experimentally and theoretically. Since it is practically impossible to experimentally determine the temperature inside a fine particle suspended in an aqueous emulsion, it is advisable to resort to modeling by solving the differential equation of heat energy transfer using a known numerical method [2] under appropriate boundary conditions. The adequacy of the solution obtained in the first approximation was determined by the temperature of the water-emulsion suspension after the completion of the technological operation on the original experimental setup to determine the degree of temperature increase under ultrasound exposure.

The order of the experiment was as follows. An aqueous emulsion suspension is prepared in which the percentage of solid-phase should be 27%, which corresponds to a mass of 0.07 kg of feedstock with a cylinder volume of 0,00025 m^3. The temperature of the prepared suspension should be within 20 ± 0.5 ° C, after which the ThermoChart program on the computer is switched on, which fixes it and reflects it on the computer monitor using the LT-300 electronic thermometer. When the installation with the sample is ready, the temperature sensor is connected to the computer, and the temperature is displayed on the monitor, the ultrasound probe is placed inside the suspension located in the cylinder, the ultrasound device is turned on for a fixed period of time and the specified power of the supplied ultrasonic radiation. During the experiment, the ThermoChart program captures the temperature change, thereby providing the necessary information to determine the functional dependence of the temperature increase during ultrasonic exposure to the aqueous emulsion suspension.

To implement the mathematical model of heat transfer, information is needed on the thermophysical characteristics (TPC), and density of the chopped spinach leaves, as well as the amount of additional thermal energy (internal heat source), which can be taken as a first approximation equal to the work spent on the formation of a new surface, experimentally measuring the energy consumption for this operation and after a series of calculations. The complex thermal characteristics of the samples were studied using the express method based on the thermal inertia characteristics of the thermal sensor [19]. This technique makes it possible to quickly calculate the heat λ and thermal diffusivity a, as well as the specific mass heat capacity c_M of the sample for a short period of time (up to 1 minute), accompanied by the thermal action of the puree mass of the sample on the thermocouple. In the whole known set of probe methods, there are no methods that make it possible to find the TPC in the dynamics of a technological operation.

The kinetic laws of the process of extracting chlorophyll from technologically prepared spinach leaves were studied experimentally on a KFK-3 photoelectric photometer, where the wavelength was used as the working quantity λ at which the maximum optical density for the extract was observed in a state of dynamic equilibrium.

In the case of extraction in the field of centrifugal forces, it is practically impossible to determine the kinetic laws of concentration change empirically, both in the extract and in the raffinate, and, consequently, the time it takes for the process to reach the maximum extraction of the target component. In this regard, it is advisable to resort to mass transfer modeling, for which it is necessary to identify the mathematical dependence of the transfer coefficients and the driving force in both phases on the time of the process. It is possible to determine the desired dependencies during extraction in the field of centrifugal forces according to the methods described in [8], but with the preselected rate of suspension entering the centrifugal apparatus and its internal working diameter. Also, in the case of applying the methods of [8], a distribution coefficient φ will be needed, equal to the ratio of the contents of the target product in the contacting phases, calculated on the basis of the process statics

The distribution coefficient is found from the system of equations of material balance (1):

$$\begin{cases} m_{СП} + m_{ШП} = m_{Р} + m_{Э} \\ m_{ШП} c_{ХЛОР}^{ШП} = m_{Р} y + m_{Э} x' \end{cases} \qquad /1/$$

where $m_{СП}$ – extractant mass, kg; $m_{ШП}$ – the mass of chopped spinach leaves, kg; $m_{Р}$ – raffinate mass, kg; $m_{Э}$ – extract mass, kg; $c_{ХЛОР}^{ШП}$ – chlorophyll content in spinach, kg / m^3; y – raffinate chlorophyll content (residual spinach raw material after extraction), kg / m^3; x – chlorophyll content in the extract, kg / m^3.

For the mathematical interpretation of empirical data on the kinetics of the extraction process under consideration, it is advisable [2] to use its representation in the form of a functional exponential dependence:

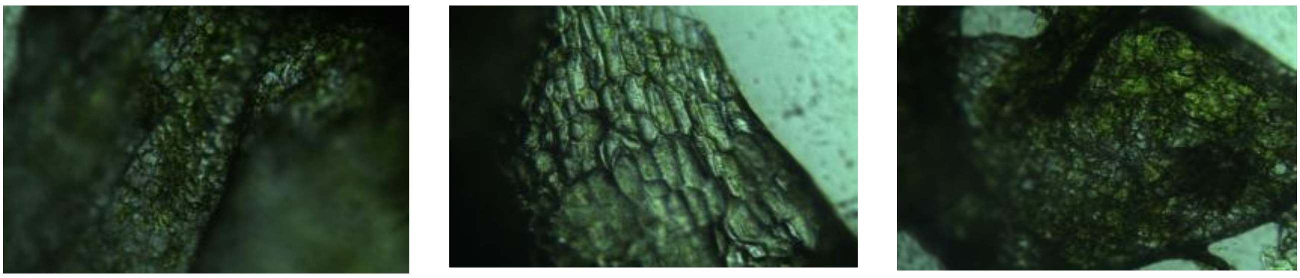
$$C_C(\tau) = C_{CH} e^{-k\tau}, \qquad /2/$$

where C_{CH} – the content of the target component in the extractant at the outlet of the unit, kg/m^3; k – exponential indicator, 1/s; τ – the duration of movement of the processed raw material from the introduction to the device to the current point (section) of the operation, s.

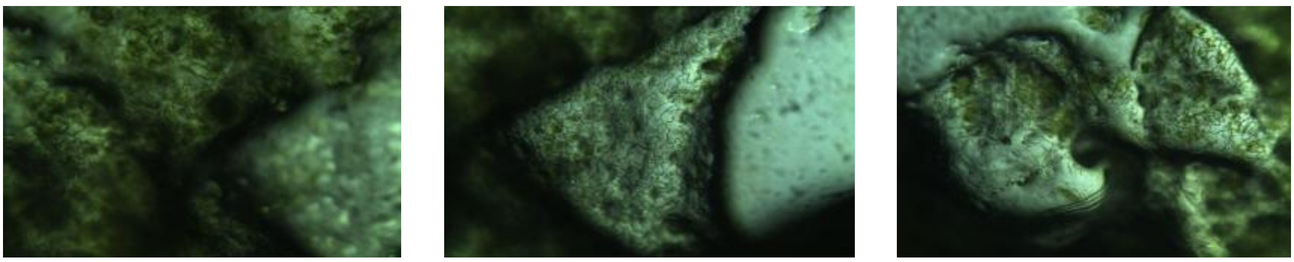
Thus, using methods known and adapted to the object of research, it is possible to theoretically and experimentally prove the need to improve the processes for obtaining chlorophyll extract from technologically prepared spinach leaves, which will allow obtaining the most purified semi-finished product distributed in fats of various origin in the form of an oil extract.

III. RESULTS

To solve the problem of complete extraction of chlorophyll from chopped spinach leaves, it is advisable to subject them to preliminary short-term soaking in water / organic reagent emulsion [9] under ultrasonic treatment. Micrographs of particles of plant material before and after the soaking process in a specially prepared emulsion of water/sodium stearoyl polylactic (E-481) or water / yantol (Fig. 1) clearly show the effectiveness of this physicochemical treatment. If in the micrographs (Fig. 1a), the target component of chlorophyll, which has a green color, is collected in agglomerates, then in the photographs (Fig. 1b), after the process is carried out under ultrasonic treatment, they are broken up into smaller parts. Thus, the feasibility of this Physico-chemical treatment of spinach leaves is confirmed.



A



B

Fig. 1 Micrograph of a spinach particle before the soaking process (a) and after (b) in the field of ultrasonic waves

After the soaking procedure, it is necessary to remove part of the moisture from the spinach with a lower energy intensity compared to thermal processes, for example, centrifugation, after which the material can be considered ready for extraction. Considering that the aqueous emulsion spinach suspension is thermolabile, it is necessary to observe temperature restrictions since when a certain critical temperature is exceeded, the probability of chlorophyll decomposition is high with the loss of its consumer properties. From literature, it follows [14, 18] that such a critical temperature is in the range from 40 to 60 ° C.

Due to the fact that it is practically impossible to experimentally determine the temperature inside a finely dispersed particle suspended in an aqueous emulsion, it is advisable to resort to modeling internal and external heat transfer by solving the differential equation of heat energy transfer under appropriate boundary conditions (Fig. 2).

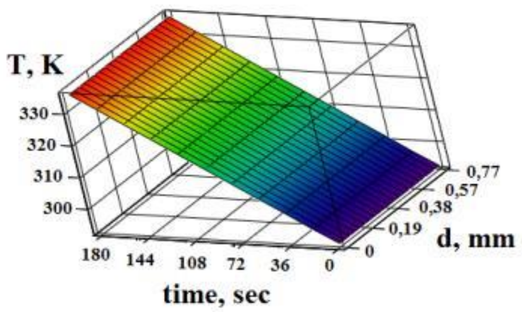


Fig. 2 Transformation of temperature fields in a chopped spinach particle

The adequacy of the obtained solution can be determined to a first approximation by the temperature of the aqueous emulsion suspension after the completion of the technological operation (Fig. 3).

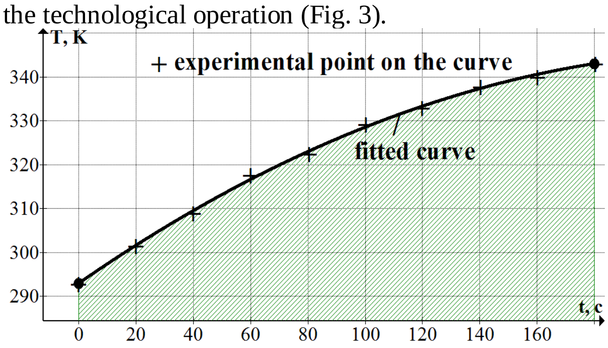


Fig. 3 The empirical curve of the increase in temperature of the suspension under ultrasound exposure

To solve the problem of modeling internal and external heat transfer, the necessary thermophysical characteristics for the object of study were experimentally determined. As a result, for spinach leaves with their moisture content of 92%, the following values of the desired values were obtained, presented in table 1.

Table 1. The results of the experimental determination of the TFC of the object of study

Humidity W ,%	Specific heat c_M , J / (kg · K)	Thermal diffusivity $a \cdot 10^8$, m²/s	Coefficient of thermal conductivity, λ , W / (m · K)
≈92	≈3347	≈27	≈0,96

An analysis of the transformation of temperature fields in a crushed spinach particle shows that during the whole process, small temperature differences are observed, not more than 1K in the depth of the material, which is due to the presence of an internal source of thermal energy under ultrasonic action, as a result of which the temperature of the inner layers rises and tends to the temperature at the boundary phase separation.

An empirical study on the extraction of the target component from technologically prepared raw materials under the above process conditions revealed that the duration of the experiments is no more than 20 minutes (Table 2).

Table 2. The results of experimental studies to determine the optical density depending on the duration of the extraction process at $\lambda = 400$ nm

Extraction time τ , s	5	10	15	30	60	120	300	600	900	1200
The optical density of the extract D_{cp}	1,52	2,27	2,42	2,58	2,76	2,89	2,95	2,98	3,0	3,0

Based on the data obtained, presented in table 2, and a calibration graph for converting the optical density D to the concentration of chlorophyll in the C_x extract, a curve was constructed for the extraction of chlorophyll (Fig. 4) from technologically prepared crushed spinach leaves with ethyl alcohol. Correlation of the obtained data with the results of similar studies shows that they do not radically contradict them in the duration of extraction, and the nature of the curve is slightly different, which confirms the need for further studies.

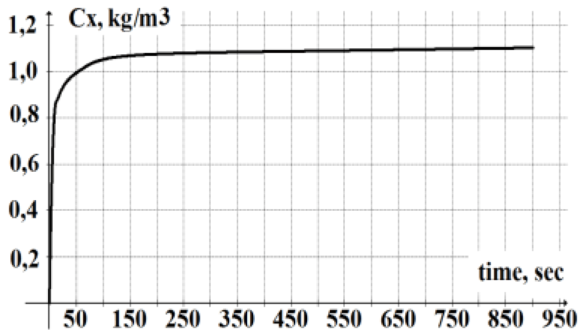


Fig. 4. Extraction curve

The equilibrium state in the process of extraction is determined by the parameter φ , which was calculated according to formulas 1-5 and is 0.11. The numerical value of this coefficient is necessary to determine the driving force of mass transfer on the basis of the generally accepted assumption that equilibrium is observed at the phase boundary, i.e., its resistance can be neglected. With this assumption, the concentration at the boundary in the extract can be calculated using the concentration of the raffinate at a given point in time and vice versa.

The analysis of the mass transfer mechanism during the extraction of chlorophyll from spinach leaves is based on the functional dependence of the extraction rate (Fig. 5) on the concentration of the extract obtained by mathematical description of the empirical curve (Fig. 4).

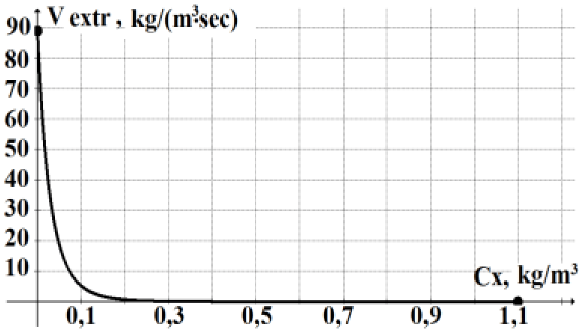


Fig. 5 Extraction rate curve

An analysis of the kinetic laws of internal mass transfer showed that extraction in the first period only in the field of centrifugal forces is not rational due to the length of the process and the need to use multiple cyclones during cascade extraction. Therefore, the process in a hydrocyclone is economically justified to carry out at the last stage of extraction of the first period with a decrease in the driving force and approaching the concentration in the particle to the equilibrium to accelerate this operation.

In the initial stage of extraction in the first period with a large driving force, it is advisable to carry out, due to the need to reduce the residence time of the suspension in the hydrocyclone, in a coil, spiral device, relying on the data of Figure 6, for 10 seconds.

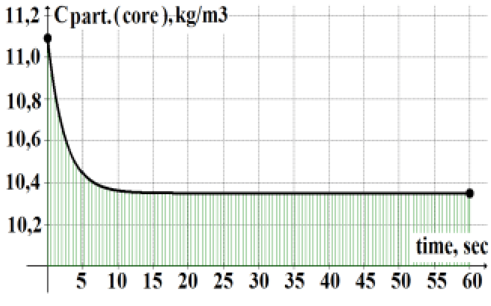


Fig. 6 Graph of changes in the volume concentration of chlorophyll in the nucleus of a particle

As a result, this will limit the time of the operation in the hydrocyclone to 5 seconds (Fig. 7), but only relying on the simulation result, which should confirm the rationality of this approach.

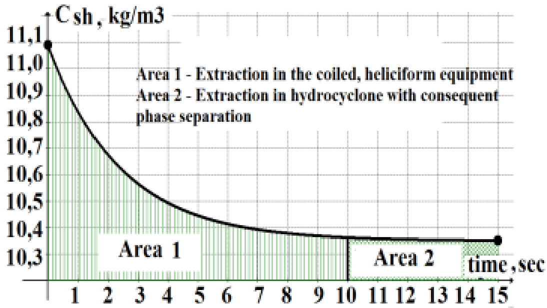


Fig. 7 Kinetics of extraction in the case of a complex spiral-cyclone installation

Thus, the total duration of extraction can be reduced by using an integrated spiral-cyclone plant from 60 to 15 seconds. The use of a hydrocyclone allows not only to reduce the extraction time, which is due to the active hydrodynamic regime but also to separate the dispersed (raffinate) and dispersion (extract) phases. The effectiveness of the proposed variant of the organization of the process flow can be confirmed by modeling the extraction process, by solving the differential equation of internal mass transfer, which requires information on the magnitude of the diffusion coefficient, under given boundary conditions of the second kind, which are determined by the magnitude of the driving force and mass transfer coefficient.

The diffusion coefficient $D(\tau)$ was determined from empirically established kinetic dependences on the duration of the process. The expression for its definition is obtained in the form:

$$D(\tau)=\frac{6.27\cdot10^{-9}}{\left[(10.35+0.74e^{-0.41089\tau})-(9.145-9.145e^{-0.38406\tau})\right]} \quad /3/$$

The mass transfer coefficient $\beta(\tau)$ was calculated from the criteria equation linking the Biot mass transfer criterion $Bi'(\tau)$ with the Prandtl and Reynolds criteria. The parameters included in them, some of which were determined experimentally, and the values of the others were given, were selected from technical considerations when organizing the process, and rational values were obtained by analyzing the solution of the mathematical model of the extraction process.

$$\beta(\tau)=1290.3\cdot Bi'(\tau)\cdot D(\tau)\,, \quad /4/$$

where

$$Bi'(\tau)=12.79\cdot\sqrt[3]{1.39\cdot10^{-6}\,D(\tau)^{-1}}\,.$$

To find non-stationary concentration fields, it is necessary to solve the differential equation of mass transfer, which in the one-dimensional problem has the form [8]. While solving it, it is assumed that during the review process, it is equivalent inside the object or on the boundary of the phase section across the surface of the particles with known geometry, which determines the possibility of using one coordinate value as the depth of the diameter of the particles. For the 2nd coordinate in the finite-difference version, when numerically solving the equation using the implicit scheme, we can take the extraction duration, s in the range (Fig. 7) from 10 to 15 s.

The mass transfer rate at the interface of the phase section to the core of the external phase is determined by the gradient of the content of the transferred component in it. As a rule, in convective diffusion, the regularity of the mass transfer rate is determined by a simplified Newton dependence, which is included in the formulation of the modeling problem in the form of a second-kind boundary condition. The problem of modeling the process of extracting chlorophyll from crushed spinach leaves in the field of centrifugal forces was solved in Mathcad Professional. As a result of this procedure, the evolution of concentration fields along with the particle depth during extraction is obtained (Fig. 8).

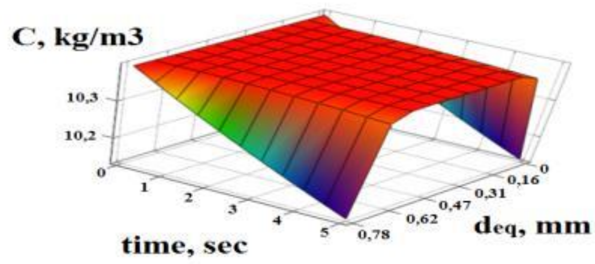


Fig. 8 Transformation of concentration fields from the duration of extraction of chlorophyll from a spinach particle

The graph shows that the average volume content of chlorophyll in a spinach leaf particle falls almost to the equilibrium concentration in 5 seconds. The obtained result allows performing operations of final extraction and separation of inhomogeneous phases in one hydrocyclone, provided that chlorophyll is pre-extracted for 10 seconds in the spiral section of the extractor, the principle of operation of which is proposed by the authors and schematically presented in figure 9.

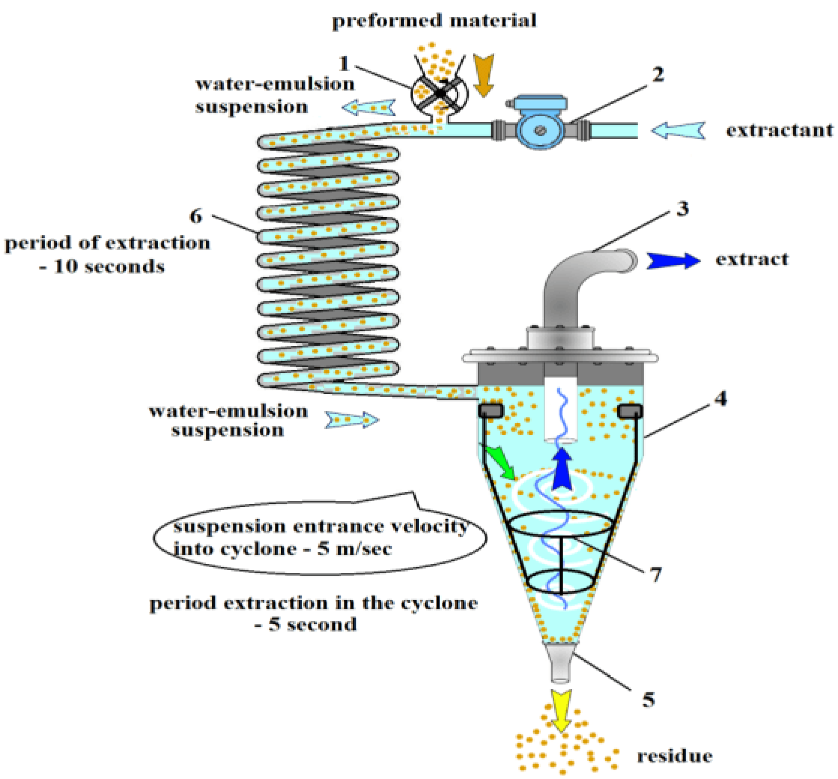


Fig. 9 Scheme of a cascade spiral-cyclone plant for extracting chlorophyll from technologically prepared ground spinach leaves: 1. - feed dispenser; 2. - pump for supplying extractant; 3 - yield of extract; 4. - hydrocyclone; 5. - sediment yield; 6. - spiral section of the cascade extractor; 7. - node cleaning sediment from the inner surface of the hydrocyclone

The use of the proposed design for two-stage processing of plant raw materials will ensure synchronization of technological processes and reduce the duration of the main operation for extracting chlorophyll by four times due to the use of cyclone technology at the second stage. It should be noted that when adjusting the operating parameters, this cascade extractor is applicable to a wide range of food plant raw materials.

IV. CONCLUSIONS

1. Ways to improve the processes for obtaining chlorophyll extract from plant materials with a high concentration of the target component in its composition, for example, spinach leaves, which will allow obtaining a fat-soluble chlorophyll paste, are proposed. When distributing it in fats of various origins, an oil extract can be obtained, where its lipid component is selected depending on the final destination of the finished PC.

2. Rational technological solutions were selected for preparing spinach leaves for extraction of chlorophyll from them, and specific features of the use of ultrasound at this stage were revealed, the result of which is the choice of rational temperature conditions when it affects plant material within the framework of technological limitations;

3. The kinetic laws of the process of extracting chlorophyll from technologically prepared spinach leaves have been identified, and on their basis, a rational approach to extracting the target component from the object of study has been proposed;

4. The mass transfer coefficients are determined taking into account the proposed extraction technology in the field of centrifugal forces and a mathematical model of the process of extraction of chlorophylls from it in the field of centrifugal forces is adapted to the object of study;

5. Based on the analysis of extraction methods and designs for their implementation in a solid-liquid system, practical recommendations are given for implementing the research results, and a two-section original installation (spiral extractor and hydrocyclone) for producing an alcohol extract with specified indicators of the final volume concentration of the target component is proposed in it, with a decrease in the duration of the process to 15 seconds.

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