Modern methods for extraction of biologically active compounds

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Abstract. The article describes the characteristics of modern methods of extracting biologically active compounds from various types of plant materials. The characteristics of the advantages and disadvantages of such methods as: ultrasonic extraction method, extraction with liquefied gases, extraction with liquefied gases, electro-explosive extraction of biologically active compounds, electric discharge extraction, extraction using vibration exposure are given.

1. Introduction

It is relevant in this direction that the new method of obtaining BAS does not violate the chemical structure of BAS, maintains their biological activity with the previously required properties, and significantly increases the rate of processing of raw materials.

Some of the above factors are implemented in one of the main methods of making tinctures – maceration. The crushed raw material with the prescribed amount of extractant is placed in a sealed vessel and infused at a temperature of 15–20 °C, stirring from time to time. If the period is not specifically stipulated, then the infusion is carried out within 7–10 days.

After infusion, the extract is drained, the residue is wrung out, washed with a small amount of extractant, squeezed again, the squeezed extract is added to the drained extract, after which the combined extract is brought to the volume required in each case with the extractant.

At present, maceration in the “classical” form does not meet the tasks of intensifying production and is mainly used to obtain one-time tinctures.

A more perfect way of obtaining tinctures is percolation – continuous passage of the extractant through the layer of raw materials and collection of percolate. Various variations can be introduced into the percolation process in order to maximize the intensification of the extraction process. A combination of processes, infusion and circulation is often used. In this case, the first extract, as sufficiently concentrated, is collected separately, completely letting it out of the percolator. After that, the percolator is filled with fresh extractant, which, after 3–6 hours of infusion, is again passed through the percolator several times, saturating it with active ingredients to the maximum extent.

Known and vortex extraction method (turboextraction), based on intensive mixing and simultaneous grinding of raw materials in an extractant medium using high-speed stirrers equipped with sharp blades.
The disadvantage of this method is the increase in temperature during the operation of the mixers and over-grinding of raw materials with the subsequent complication of cleaning [1, 2, 3].

2. Main part

2.1. Ultrasonic treatment - a method for intensifying the extraction process of BAS
In the chemical-pharmaceutical, food industry, in the production of juices from fruits and berries, among other methods of processing plant raw materials, a certain place is occupied by ultrasound (Muradov, Daudova, Ramazanova, 2000) which at different stages of processing has certain effects on both the extraction process and the products obtained.

The processing of raw materials with ultrasound, as a rule, intensifies the extraction, without which no production associated with the isolation of biologically active compounds from plants can do [3, 4, 5, 6, 7, 8].

For example, in the works of E.V. Pantyukhina. In order to intensify the mass transfer process of extracting phenolic compounds from 40% polyethylene oxide-400 (PEO-400) from sweet clover grass (Melilotus officinalis), a combined method was used - three-stage remaceration with preliminary ultrasonic treatment of the "solid-liquid" system at a temperature of 200 C for 20, 30, 60 and 120 minutes (the total time was distributed over all three stages of remaceration) with a frequency of 20 kHz and a power of 50 W. As a result of research, the system of raw materials-extractant came to equilibrium after 60 minutes of sonication, facilitating the extraction of phenolic BAS from Melilotus officinalis with an efficiency of 85%, the concentration of flavonoids and coumarins increased by 37.5% by 34.3% in comparison with the traditional technology [3].

![Figure 1. Scheme of an ultrasonic device for the production of biologically active compounds (BAS).](image)

2.2. Laser radiation as a way to intensify the BAS extraction process
To intensify the extraction process, such compounds as anthocyanins are widely used physical impact – laser radiation (LR) [1].

Under the action of LR, singlet oxygen is formed, which has the ability to transfer its energy to an aqueous medium, as a result of which the yield of anthocyanins increases by 46.5%. But LR is a rather expensive method.

2.3. Extraction with liquefied gases
In recent years, liquefied gases are increasingly used in production as extractants. The processing of plant raw materials with liquefied gases for the purpose of extracting individual components in unchanged form can be attributed to new highly efficient technological processes capable of providing an integrated use of raw materials and materials.
This method can be used to obtain high quality flavors, fragrances, biologically active substances. With the help of liquefied gases, fatty and essential oils, flavonoids, some alkaloids are well extracted, worse - glycosides, vegetable resins, water-soluble ballasts [4, 5, 6].

2.4. Electro-explosive extraction
Among the many rapidly developing technological processes of extraction, the most significant is supercritical extraction, which can be carried out at temperatures that provide a gentle regime for such components as antioxidants, fatty acids, vitamins, amino acids, proteins. At the same time, it is necessary to increase the pressure in the extraction reactor to significant values, sometimes up to 500 atm. The domestic industry does not produce pumps for creating pressures in the range of 200-500 atm. This leads to the need to use multipliers, precision non-standard plunger pumps, non-traditional receivers, which dramatically increases the cost of extraction devices. Along with the unique CO2-superextraction, steam-gas, alkaline and other types of high-pressure extraction are used. In a number of cases, a recycle of the extractant is used with differential pressure modes, with jet-barrier devices. One of the promising directions for solving the problem of energy-saving high-pressure organization is associated with the use of the Yutkin electrohydraulic effect [5].

If energy is accumulated in an electric or magnetic storage device, and then, with the help of a spark gap, an electric discharge is organized between the electrodes placed in a sealed reactor, then a plasma electric explosion of the medium occurs in a liquid medium. In the region of a high-current discharge, the liquid immediately boils. A shock wave is formed, propagating to the walls of the reactor and reflected from them, leading to shifts of the particles of the medium. An ultrasound wave of large amplitude is formed, a powerful acoustic effect is observed. After the collapse of the vapor-gas region of the plasma discharge, the pressure in the volume is reduced to a value determined by the presence of gas bubbles in the liquid medium. They appear as a result of mechanochemical reactions in the environment, the release of air from the particles of the extracted raw materials and chemical transformations of the extractant. With a repeated electric explosion, the collapse of the bubbles leads to cavitation, which destroys the raw material. The extraction is accelerated due to the leaching of biologically active substances from the destroyed cells [1].

Figure 2. Scheme of ion movement in the inter-electrode gap.

In addition, the resulting cavities constantly pulsate, causing an increase in the rate of movement of the extractant near the particles of the raw material and increasing the rate of extraction due to an increase in the coefficient of convective diffusion. Electrodynamics extraction is an intensification of the classical extraction processes.
Figure 3. Scheme of an electrodynamic extractor.

1) Transformer - rectifier. Provides constant current;
2) High voltage capacitor. Accumulates charge;
3) Remote control panel. Setting parameters;
4) Gas spark gap. Discharge control;
5) Extractor. Efficient extraction by discharge;
6) Single power casing. Isolation;
7) Rotary pump. Provides circulation of the mixture.

Despite the fact that electric explosive extraction technologies have been successfully tested over the past twenty years in the pharmaceutical industry, they have not become widespread in this and other industries. This is due to the fact that rather complex processes of electrohydraulic extraction are successfully implemented if a wide range of different specialists are involved in the implementation of the corresponding projects. In particular, the problem of optimizing electronic equipment for extractors has not yet been solved.

2.5. Electric pulse extraction, the possibility of using it to obtain various types of chemical compounds

The electro-pulse method of processing materials is based on an electric discharge in a liquid [4, 5].

The high value of the instantaneous power, which is released during a pulsed electrical breakdown of a liquid, creates around a rapidly expanding spark channel: pulsed electromagnetic radiation; high pulse pressure arising in the medium as a result of the formation of shock waves and reaching tens of thousands of atmospheres; movement of liquid at a speed of hundreds of meters per second, polydisperse ultrasonic radiation, creating pulsations of gas inclusions; impulse cavitation in a large volume of the medium; exposure to plasma sparks, carrying in its spectrum a wide range of infrared, ultraviolet, hard radiation.

When the spark channel expands, the liquid seems to be thrown away from it, creating a powerful flow of the medium, in which discontinuities of its continuity, cavitation gas cavities are formed. When reflected from the walls of the apparatus, shock waves interfere, creating additional turbulence. In fact, the electrohydraulic shock caused by the discharge should be attributed to the type of microexplosion and the mechanism of its action in the field of hydrodynamics should be considered from these positions [4, 5, 6, 7].
The energy of the diverging liquid and shock wave is precisely the factor that is successfully used in various technological processes: crushing and grinding of ores, drilling wells, stamping, and, with an appropriate approach, can be used in the extraction of plant materials.

Electric pulse extraction is a non-warm alternative method for obtaining BAS.

Exposure to an electric pulse field applied to plant cells (i.e. tissue cells) causes dielectric breakdown and dramatically increases the permeability of the cell membrane so that substances are more easily released from the inside of the cells. And as a result, a higher yield of substances, a reduction in the total processing time of raw materials.

Were analyzed the work on obtaining BAS: pectins, alkaloids, flavonoids using pulsed electric discharges.

In the process of extracting medicinal substances using electrical pulse discharges, the following scheme was used (figure 4). The extraction process with the use of high voltage electrical discharges takes place under "harsh" conditions in comparison with classical methods, water is used as a solvent.

![Figure 4. Scheme of electrical pulse processing of materials.](image)

where: raw material - 3, in a certain ratio with the extracting liquid - 2, is placed in the extraction chamber - 6, after which a series of voltage pulses is supplied to electrodes - 1 and 4. Pulse formation is carried out by a pulse voltage generator (GVP), which serves to initiate the discharge and form a conducting channel, and a pulse current generator (PCG), which provides an energy contribution to the formed breakdown channel – 5 [5, 6, 8, 11].

Analysis of scientific literature has shown the undoubted promise and advantages of the method of intensive extraction of biologically active compounds using pulsed electric discharges.

- when isolating polysaccharides:
  - pectin
    a) the yield of the target product increases up to 2 times;
    b) the yield of protopectins increases - up to 26% (instead of 11%);
    c) the duration of extraction is reduced by 6 times;
  - alkaloids:
    a) the yield of the sum of alkaloids increases by 25-30%);
    b) the extraction time for alkaloids is reduced from 7-10 days to 7.5 hours;
    In both cases, toxic, expensive extractants are replaced by aqueous solutions.
- phospholipids
  a) obtaining native phospholipids by soft dynamic and electromagnetic effects
  b) their quality improves;
  c) the drying temperature of these compounds is reduced by 20 - 25C °;
  d) the number of undesirable hydrolytic and oxidative processes in phospholipids decreases [4, 5, 6].
In addition, the analysis of later sources showed the possibility of the influence of the electromagnetic field on the molecular structure and properties of some ALS, i.e. their modification.

When processing an aqueous solution of beet pectin with pulses of an electric field (pulse frequency $f = 25$ kHz, voltage at the electrodes $Ue$ from 4 to 8 V, current density from 2.9 to 8.5 mA / cm$^2$), an increase in molecular weight occurs with a decrease in the number of carboxyl groups in the voltage range $Ue = 6 - 8$ V, which contributes to an increase in the gelling properties of pectin. The purity of pectin increases and the number of free carboxyl groups in the voltage range $Ue = 4 - 6$ V increases, which contributes to an increase in the sorption properties of pectin [5, 6].

In addition, electric pulse extraction makes it possible to use more fully the waste of the pharmaceutical and food industries (meal, etc.).

Electrical impulse action is effective in microbial inactivation without significant changes in the quality of BAS and their physical properties, such as color, viscosity and electrical conductivity.

Dielectric breakdown of the cell membrane in microorganisms has been widely accepted as a mechanism corresponding to microbial inactivation by an electric pulse field. The electric pulse field significantly inactivates E. coli 8739 cells ($p <0.05$).

The inactivation of food enzymes has been extensively studied since the 1960s. The effect of inactivation of the electric pulse field depends on both the dosage and the structures of the target enzymes [5, 6].

An electric pulse field effective in inactivating natural flora can significantly expand the microbial stability of the resulting soy milk enriched cow milk concentrates.

In addition, the method of electric pulse extraction makes it possible to obtain BAS with greater biological activity (BA). Water-soluble polysaccharide complexes adsorb vitamins and microelements, such as vit. C, vit. groups B, Ca, K, etc. With the temperature method of extraction vitamins, vit. C are lost, which means that the biological activity of the complexes decreases.

The development of the extraction technology based on the forceful action of a pulsed electric field of high intensity has received a new branch, undesirable phenomena must be limited as far as possible the duration of the pulse to a level that negates the thermal effects. The bell-shaped voltage pulse in Fig. 5 corresponds to these conditions. 2, formed on the electrodes of the extraction chamber by a pulsed high-voltage source, based on a pulse transformer [4].

![Figure 5. Oscillogram of a voltage pulse (timestamps 10 ns).](image)

The choice of the design of the high-voltage electrode and its shape was carried out by measuring the low-voltage resistance of the water gap with electrodes of different configurations. The developed design has the form shown in figure 6.
2.6. Intensification of the BAS extraction process by means of raw material pretreatment with low and ultra-low frequency electromagnetic fields

The works of V.N. Savin [4] it is shown that electromagnetic fields of extremely low and ultra-low frequencies cause various effects in plant objects with a radiator power of 50 mW or less, for example, an increase in the yield of valuable components from it, etc.

The papers highlight the environmental issues of using BAS obtained by this method as a dietary supplement.

The advantage of this extraction method makes it possible not only to obtain biologically active compounds with small amounts of substances used to extract biologically active compounds, but also to reduce microbial contamination, inactivate enzymes and hormones, which makes it possible to reduce energy consumption by 1.5-2 times during the extraction process [9].

The rapid release of biologically active compounds is based on the destructive effect of electric current on the protein-lipid membranes of plant tissues while maintaining the integrity of the cell membranes, a maximum increase in cell permeability occurs, which increases the release of substances into solution. But this method, despite its promising potential, requires additional research.

2.7. Extraction of BAS by means of vibration exposure

Vibrating reactors and extractors are currently relevant in continuous and batch modes of extraction in the “solid-liquid” system.

We have developed an experimental sample of a vibration extractor with replaceable blades in the form of a prism and two interconnected circles with perforated holes 10 mm in diameter figure 7.

The optimal technological parameters of the vibration impact of the extraction of anthocyanins have been determined [8, 9, 10].
The parameters that increase the yield of anthocyanins were determined - the value of the intensity of the oscillations

$$\lambda = \frac{a}{g}$$

(1)

where \(a\) – the amplitude value of the vibration acceleration; \(g\) - acceleration of gravity.

**Figure 8.** Diagram of the dependence of the degree of extraction (DE) of anthocyanins on the intensity of fluctuations (\(\lambda\)), for different fractions, mm 0.8 - 2.0 - 1.2.3.4.6; 0.8 - 2.5 - 7; 0.8 - 3.0 - 5; temperature, °C: 45 - 7, 50 - 6, 55 - 5, 60 - 4, 70 - 3.2.1; duration, min: 10 - 6, 15 - 2.3,4,5,7; 30 - 1; pH: 1 - 1.2.3; 2.5 - 4.5.6; 5.1 - 7.

Our experimental results show that the use of vibration impact accelerates the combined process of hydrolysis - extraction, contributing to the degree of extraction of the target product - the anthocyanin extract.

3. Conclusion
A review of modern extraction methods made it possible to conclude that these methods have both advantages and disadvantages.

Electric pulse extraction is the most non-warm alternative method for obtaining biologically active compounds. It is necessary to carry out a comparative analysis of the combined method of electrical impulse and vibration exposure to determine the optimal extraction method.

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