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**MICROWAVE RADIATION, ITS INFLUENCE
FOR SOLUTIONS AND USE FOR EXTRACTIONS
OF COMPONENTS OF PLANT MATERIALS**

2-nd Report.

The interaction of microwave radiation with plant material and factors affecting the process of extraction of natural compounds

Abstract. Plants produce an amazing diversity of secondary metabolites, which have multiple functions throughout the plant's life cycle. Extraction is one of the crucial steps for research and development of plant secondary metabolites. The efficiency of processing and extraction of substances is determined by the speed and completeness of the physical and chemical processes underlying them. The analytical procedure, as a rule, consists of two stages: the first is the preparation of a sample using various extraction procedures (extraction in the Soxhlet apparatus, maceration, percolation, etc.), and the second stage is associated with direct analysis of substances (spectrophotometry, chromatography, spectrometry and others). If the analysis is usually completed after a few minutes, the extraction is the most limiting stage of the process, a lengthy and laborious process. That involves the use of large volumes of solvent, energy-intensive costs, especially at the concentration stage to extract the final extract. It also includes the risk of thermal decomposition of thermolabile active compounds. Various modifications of extraction procedures, which include the replacement of traditionally used solvents, the use of various physical factors, including ultrasonic and microwave processing and other areas, have been investigated and introduced.

Microwave extraction is currently one of the areas of analytical chemistry, which quickly gained the position of one of the most effective and modern methods to increase the efficiency of extraction of biologically active compounds from vegetable raw materials. The review considers the effect of microwave radiation on plant material. Generalized material is on the use of microwave extraction for the selection of natural compounds, as well as the influence of various factors on the extraction process. The advantages and disadvantages of the presented method are considered.

Keywords: microwave radiation, natural compounds, microwave extraction.

Introduction. Currently, areas related to the perfecting of complex processing of vegetable raw materials and the creation of highly efficient methods for isolating biologically active substances (BAS), which include improving the technical equipment of the process, replacing the conditions of the reaction and extraction, as well as using new chemical, physical and chemical, physical and biological approaches that will comply with the principles of "green chemistry" [1-4]. Over the past decades, various modifications of extraction procedures have been investigated and implemented, which include not only the replacement of

solvents [5,6], but also the application of various physical factors, including ultrasonic and microwave processing [7–14].

One of the modern methods of increasing the efficiency of extraction of biologically active compounds from plant materials is microwave processing (microwave-assisted extraction (MAE)) [15-21]. The heating effect of microwaves depends on both the characteristics of microwave energy and the dielectric properties of the material to be heated. The effect of microwaves on many liquids and solid consists of polar molecules and ions that leads to their heating. The reason for this phenomenon is the interaction of the electrical component of the electromagnetic field with the molecules of the substance. This interaction contains several physical effects, of which mainly two mechanisms lead to heat release, namely, dipole rotation and ionic conductivity. At the same time, the effect of microwave exposure during extraction consists in a substantial acceleration of the process, as well as in an increase in the degree of extraction of the components. A specific feature of microwave extraction is not only a significant increase in the temperature of the extraction system by several tens of degrees, but also selective extraction of compounds, which is achieved by selectively heating the phase or individual components, or by selecting a solvent that selectively extracts one or another component [15-21] and also in reducing the risk of thermal decomposition of thermolabile active compounds [22].

In general, MAE systems are classified into a multimodular system and a focused mode system (monomodule) [18–20]. A multimode system produces a random dispersion of microwave radiation in the cavity, while a focused system (monomod) allows microwave radiation to be focused on a limited area in the cavity. Typically, a multi-mode system is associated with high pressure, while a monomodular system is used at atmospheric operating pressure, although it can also operate at high pressure. To avoid confusion in the MAE classification, to refer to the system used, such terminology as “closed system” and “open system” is used, which operates under pressure and at atmospheric pressure, respectively. Over the past decades, MAE methods have been investigated and implemented to allow the process with power, pressure, temperature control, as well as new modified microwave extraction methods that combine extraction with ultrasonic processing, allow MAE without the use of organic solvents, and other technologies [23-28].

The great interest of researchers in microwave analytical chemistry is confirmed by the fact that over the past 10 years, according to [29], more than 3000 papers have been published on the use of microwave extraction by chemical analysis, including more than 900 works on microwave extraction of plant materials for the isolation of natural and biologically active compounds.

1. Application of microwave radiation for extraction of natural compounds. Microwave radiation is used in the extraction of various low molecular weight organic compounds from natural raw materials [15-21, 23-28, 30-127]. Its use is described for the isolation of total groups of substances. For example,

phenolic compounds [30, 32-64, 90, 97, 99, 100, 111, 112, 121, 127], as well as certain groups of substances that belong to such classes as phenolic acids [46, 49, 51, 52, 54-59, 91, 108, 111, 112, 116, 121, 127], flavonoids [36, 39, 43-45, 49, 63, 101, 106, 118], isoflavones [102], anthocyanins [47, 48, 124], coumarins [60-62], anthraquinones [65, 98], tannins [66,67], xanthonoids [68], lignans [69], alkaloids [63,70-77], carotenes [78], stilbens [79, 80], saponins [81, 82, 107], triterpenes [83, 84], lipids [85, 86], carbohydrates [88], essential oils [90-96], tanshinons [105], etc.

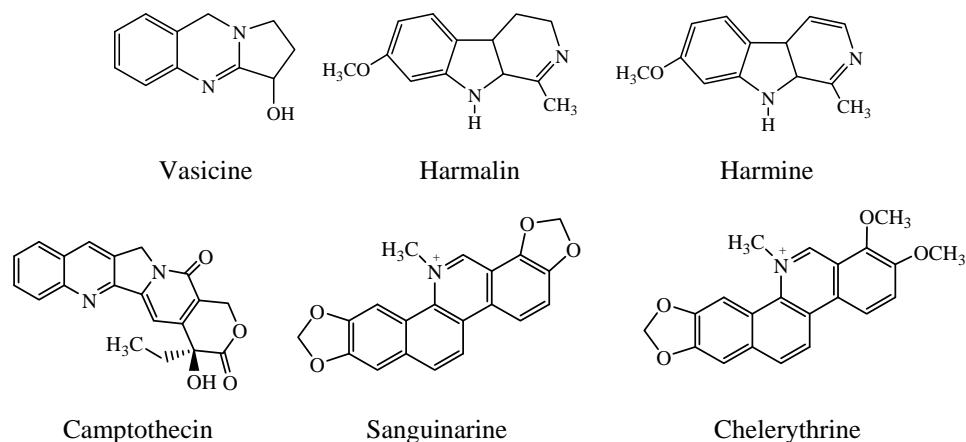
Microwave extraction was used to isolate tyrosol and its glycoside from the genus *Rhodiola* [52], curcumin from *Curcuma longa* [53], rosmarinic acid from *Melissa officinalis* [54, 55], carnolic acid [55], chlorogenic acid [56-59], coffee [56], genepozidovoy [57], coumaric and melototic acids [59], coumarin [60] and its derivatives: decursin from the roots of *Angelica gigas* [61] and isofraxidine from *Sarcandra Glabra* [62], flavonoid vitexin and iovitexin from crotal sis syrin [63], quercetin and its glycosidized derivatives [64], alkaloids: senkirkinine and senestionin in *Tussilago farfara* [70], vazitsina, harmali on and Garmina from *Peganum harmala L.* [71], camptothecin from *Nothapodytes foetida* [72], sanguinarine and chelerythrine from the fruits of *Macleaya cordata* [73], theobromine and caffeine from cocoa [77], β -carotene [78], saponins: diosgenin [81] and astragaloside [82], pentacyclic triterpenoids of a number of lupane – lupeol [83], betulin [84], glycyrrhizic acid [109], cocaine [115], caffeine [116], etc.

At the same time, in the majority of works, quantitative evaluation is carried out both to determine the total amount of substances in the raw material under study [31-49] and individual BAS [50-84], using high performance liquid chromatography (HPLC) [30-32, 38, 39, 42, 51, 58, 60, 109, 123, 124, 127], in various hardware design, for example, with UV detection [33] or detection using a photodiode array HPLC-PDA [40,51], HPLC-UV/DAD [54,60], RP-HPLC-DAD [127], HPLC-MS [124], in combination with mass spectrometric analysis HPLC-DAD-ESI-MS/MS [34], DAD/MS [55], LC-MS-MS [99], using nano-LC-ESI mass spectrometry [59], high-speed counter-current chromatography (HSCCC) [62], gas chromatography-mass spectrometry [115], etc.

A number of studies present the results of a comparative analysis of microwave extraction with traditionally used extraction methods and ultrasonic extraction, as well as their synergistic action. Thus, a comparative analysis of extraction methods, including microwave and ultrasonic, classical and extraction in the Soxhlet apparatus, on the yield, composition of phenolic compounds and antioxidant activity from cherry leaves and fruits (*Cherry*) was presented in a study [42]. The highest yield of extractives was obtained by extraction in the Soxhlet apparatus. However, the extracts obtained by microwave extraction contained the greatest amount of phenolic and flavonoid compounds and showed the best antioxidant activity. The main phenolic compound in all extracts was chlorogenic acid, and o-coumaric acid, quercetin 3-glucoside, luteolin 7-glucoside, apigenin 7-glucoside, kempferol 3-glucoside and naringenin were first detected in the

leaves, while the presence of vanillic acid, caffeic acid and rutin was confirmed in all extracts.

Microwave extraction was also used to extract three alkaloids from *Peganum harmala* L. - the vasicine, as well as the harmalin and harmine alkaloids [71], camptothecin from *Nothapodytes foetida* [72] for sanguinarine and chelerythrine from *Macleaya cordata* [73].



Analysis of various extraction methods (mixing, extraction in the Soxhlet apparatus, ultrasonic and microwave extraction) for extraction showed that microwave processing showed the best results in terms of quantitative characteristics of the final yield and time spent on extraction.

2. Impact of microwave on plant materials. Despite the fact that, in most cases, dried plant material is used for extraction, plant cells contain the smallest traces of moisture, which serve as a target for microwave processing and extraction. The moisture contained inside the plant cell when heated due to the microwave effect evaporates and generates tremendous pressure on the cell wall [97]. The pressure pushes the cell wall from the inside, which provokes its rupture and facilitates the release of active components from the destroyed cell wall. This phenomenon can be even more intense if the plant matrix is impregnated with solvents. The higher temperature achieved by the action of microwave radiation can hydrolyze the essential bonds of cellulose, which is the main component of the plant material. Increasing the temperature during MAE enhances the dehydration of cellulose and reduces its mechanical strength, and this in turn helps the solvent to easily penetrate into the compounds inside the cell, improving extraction.

According to Pavlin's kinetic model [13] (established for supercritical fluid extraction), the compounds are extracted in several stages: desorption from the matrix surface, diffusion in the porous organic layer to the solvent, and solubilization in the solvent. In this case, the extraction process can be limited to one or several steps. In the case of a well-chosen solvent, the solubility of the component

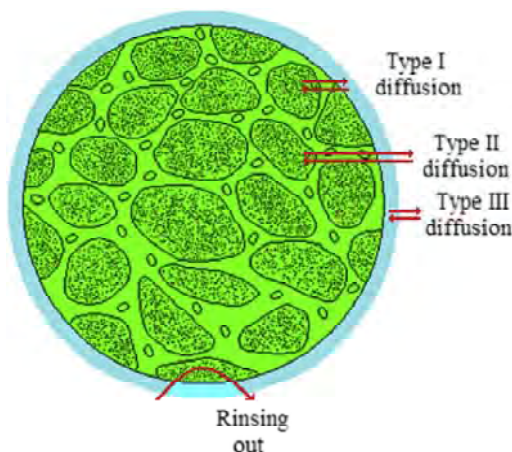


Figure 1 – Image of a swollen plant particle surrounded by a layer of solvent and types of diffusion [24]

is not a limiting factor, and desorption is more important to consider, since the extraction must overcome the interactions between the analytes and the extracted matrix.

As soon as the plant particle is surrounded by a layer of solvent, the following processes begin to occur:

- 1) Type I diffusion - diffusion of plant components towards the outer layer;
- 2) Type II diffusion - diffusion of plant components into a solvent;
- 3) Type III diffusion - diffusion from the outer layer in the direction of the main solvent;
- 4) Leaching of contents from the destroyed (lost integrity) cells.

Microwave heating differs from the traditional method of high volumetric and temporal gradient, as well as the unequal ability of the solutions and components of the heterogeneous system is differing in composition to absorb radiation energy [11], whereas conventional heating occurs outside and requires heat outside when in contact with a hot surface. In case of microwave radiation, heating occurs from the inside throughout the entire volume of the sample. The impact on the substance of the electromagnetic wave of the microwave range leads to a continuous reorientation of polar molecules, due to the continuously changing direction of the vector of the electrical component of the radiation, which, due to intermolecular interactions, leads to the release of heat. The water contained in the plant cells is heated under the action of microwave irradiation and this internal change leads to an increase in pressure inside the plant cells, which further violates their integrity and contributes to the release of molecules. Schematically, this process is illustrated in figure 2 for solvent-free microwave extraction (SFME) [27].

The effect of MAE strongly depends on the dielectric susceptibility of both the solvent and the solid plant matrix. In some cases, selective heating of the

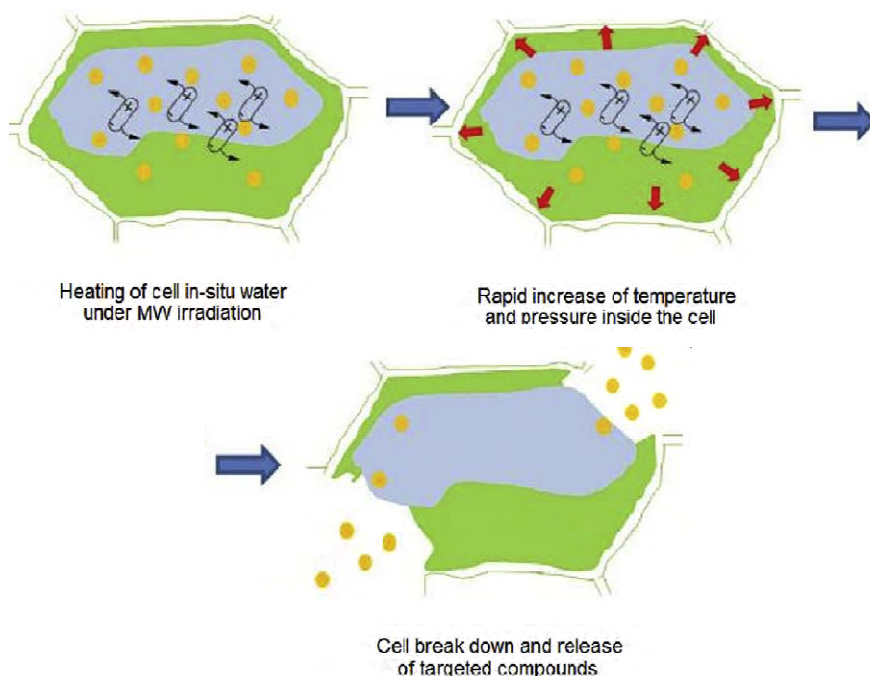
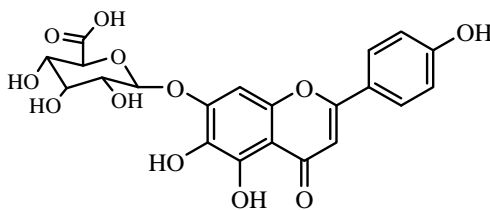


Figure 2 – Schematic image of the mechanism microwave extraction without solvent (solvent-free microwave extraction (SFME)) [27]

sample matrix is created by immersion in a “clear” solvent, which can be non-polar solvents, chloroform, used as a specific reaction medium. This approach is especially useful for thermolabile components to prevent their degradation [13, 97].

For a better understanding of the effect of microwave extraction on plant material, the study of structural changes in samples is necessary. In order to provide evidence to elucidate the mechanism of microwave assisted extraction of components from plant materials, structural changes of vegetable samples after extraction were observed by light microscopy, scanning electron microscopy, transmission electron microscopy and atomic force microscopy [19].

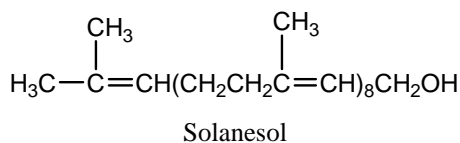
Thus, in [30], a comparative analysis of *Erigeron breviscapus* samples processed and untreated by microwaves was carried out with the aim of isolating scutellarin. Scanning electron microscopy (SEM) or electron micrographs are used for this purpose.



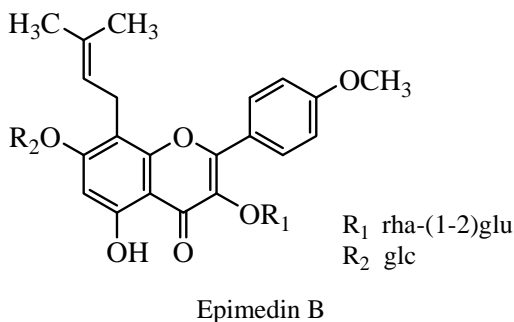
Scutellarin

It was shown that microwave treatment impaired the integrity of the *E. Breviscapus* tissue, which contributed to the release of chemicals from cells into the extractant. On the contrary, the structure of the samples when applying heat treatment did not significantly differ from the parameters of the untreated samples, and several small gaps were observed on their surface. In addition, the particle size after microwave treatment was smaller than that of untreated samples and samples after thermal extraction, which increased their specific surface area, and as a result, contributed to a better flow of extraction processes.

Similar conclusions were obtained in the study using scanning electron microscopy of the surface of tobacco samples. The MAE method has been applied to isolate solanesol, which is the starting material for many high value biochemicals, such as vitamin K analogues and coenzyme Q₁₀. The surfaces of tobacco samples after microwave extraction were destroyed, while on the surface of the samples during heat treatment, only small gaps were observed [31].



The effect of the MW treatment is most clearly demonstrated in figure 3, which shows the results of electron microscopy, which clearly show a difference in the samples of fresh leaves of *Epimedium koreanum*, without microwave processing and using microwave extraction to extract epimedin B [32].



After microwave-assisted extraction *Epimedium* leaf samples, the number of the chloroplasts decreased slightly and extractant turned from colorless to green. These observations suggest that microwave assisted extraction can result in the damage of the plant tissues and/or cells (even organelles) [19].

Scanning electron microscopy (SEM) also provided visual evidence of the effect of microwave radiation on the destruction of natural material when saponins (diosgenin) were extracted from *Dioscorea zingiberensis* C.H. Wright [81] (figure 4). The sample after microwave extraction had a highly porous structure, which contributed to the rapid and effective release of steroids into the extraction solvent.

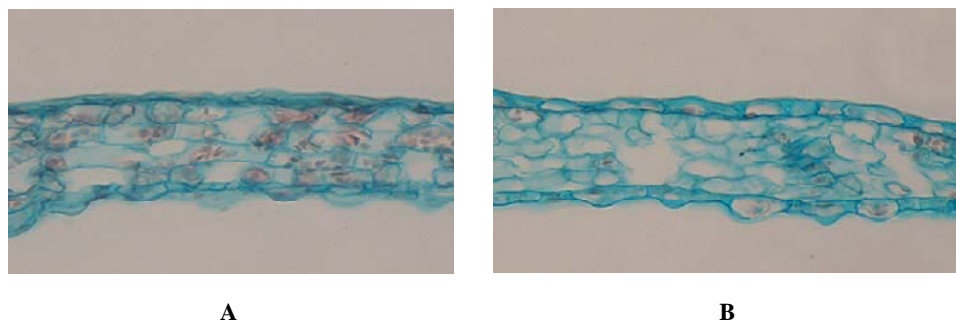


Figure 3 – Epimedium leaf samples [32]:
(A) without the use of microwave processing; (B) after applying the microwave treatment

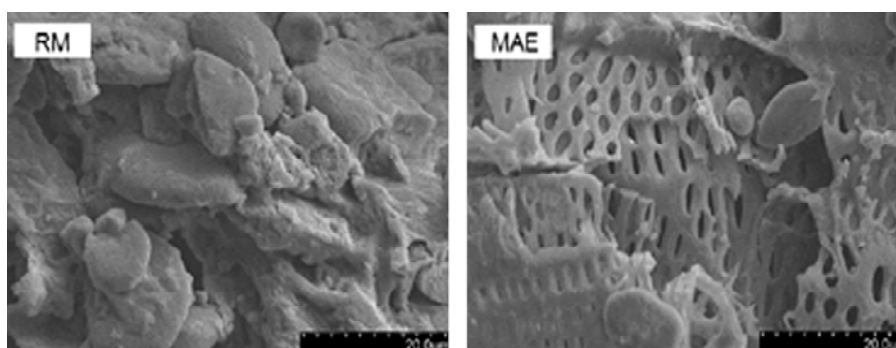


Figure 4 – Sample source material *Dioscorea zingiberensis* C.H. Wright before (RM) and after microwave extraction (MAE) [81]

3. Experimental variables affecting the microwave-assisted extraction.

The efficiency of microwave extraction of plant secondary metabolites can be affected by various factors, such as: type and concentration of solvent, solid-liquid ratio, microwave power and frequency, extraction temperature, microwave heating time, moisture content of raw materials, particle size of plant samples, extraction pressure and the number of extraction cycles [16-20, 23, 24, 97].

3.1. Effect of solvent. Choosing the right solvent is considered to be one of the most important parameters for microwave extraction and is fundamental for getting an optimal result. The choice of solvent for MAE is dictated not only by the solubility of the extractable components, but also by the interaction between the solvent and the plant matrix, and by the microwave absorption properties of the solvent. Preferably, the solvent should have a high selectivity with respect to the extracted substances, so that undesirable components are excluded from the extraction process. The dielectric properties of the solvent play a key role in microwave extraction, since both the efficiency and selectivity of MAE strongly depend on the dielectric constant of the extracting solvent or its mixture. For example, in the case of extraction of furanocoumarins from plant tissues *Pastinaca sativa* [98], petroleum ether is usually used, while more polar coumarins

containing hydroxyl groups are extracted with methanol. Since the use of petroleum ether in MAE is ineffective, 80% methanol was used. However, the yields of furanocoumarins when using it were significantly lower than during extraction in the Soxhlet apparatus and in ultrasonic extraction. As a rule, when MAE in most cases, solvents or solvent mixtures are used with good heating efficiency under the action of microwaves (with a high $\tan \delta$ value).

In some cases it is effective to use a water-organic solvent, since the presence of water can improve the penetration of the solvent into the sample matrix and, thus, increase the heating efficiency. Another important aspect is the compatibility of the extracting solvent with the chromatographic analytical stage.

The use of methanol and its water-alcohol mixtures with various percentages [33, 50, 51, 56, 58, 60, 67, 70, 76, 99], ethanol and its water-alcohol mixtures [34, 37, 38, 40, 41, 53, 57, 64, 71, 74, 75, 79, 81, 84, 99, 100, 106-108], acetone [33, 55, 98], ethyl acetate [33, 50], acetonitrile [98] et al.

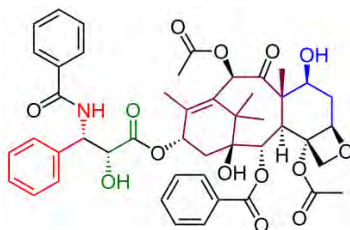
Thus, in article [33], the usual (reflux) and microwave extraction was studied to extract phenolic substances from *Rosmarinus officinalis*. It was shown that the amount of extractable phenolic substances using the traditional method decreased with decreasing solvent polarity: 60% methanol, 60% acetone and ethyl acetate-water (60:30, v/v), and during microwave extraction: 60% acetone, 60% methanol, water, ethyl acetate-water. It has also been proven that microwave extraction is a more effective method compared to the conventional method, by reducing the extraction time and the amount of solvent, as well as increasing the amount of the extracted phenolic compounds.

The type of solvent used (acetone, ethanol, methanol, and acetonitrile) during microwave extraction of *Morinda citrifolia* influenced the yield of anthraquinones [98]. It was shown that for the maceration method, the percentage of extraction of substances decreases in the order acetone-methano-ethanol-acetonitrile, and in the case of microwave extraction: methanol-acetone-ethanol-acetonitrile. This difference is due to the fact that during maceration, the extractability depends mainly on the solubility of the compound in the solvent used, the kinetics of the mass transfer of the product, and on the strength of the interaction of the solute/matrix.

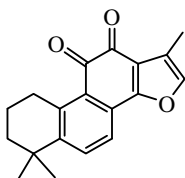
The separation efficiency and the selectivity of MAE for the isolation of color pigments from *Capsicum annum* were investigated using 30 extraction solvents and it was found that both the efficiency and selectivity of MAE depend significantly on the dielectric constant of the extracting solvent mixture. As a rule, in most cases solvent mixtures with good heating efficiency at MAE, such as aqueous methanol and ethanol, showed the best extraction results [103].

The percentage of alcohol-water of the extracted solvent mixture also influences the extraction efficiency. The methanol – water mixture (90:10) turned out to be better with MAE of paclitaxel from *Taxus baccata* [104].

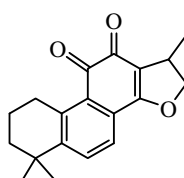
Among various ethanol concentrations, the use of 95% (v/v) ethanol showed the best optimal results with MAE tanshinons (tanshinone IIA, cryptotanshinone, tanshinone I) from *Salvia miltiorrhiza* roots [105]. If MAE takes only 2 minutes,



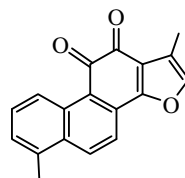
Paclitaxel



Tanshinone IIA



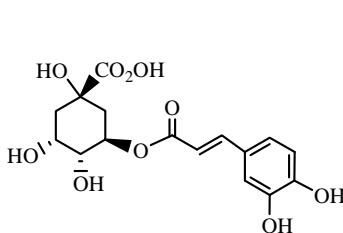
Cryptotanshinone



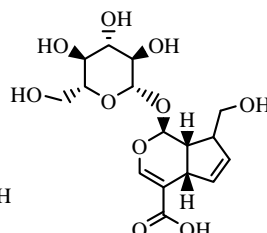
Tanshinone I

then extraction at room temperature, ultrasonic extraction, and Soxhlet extraction require 24 hours, 75 minutes, and 90 minutes, respectively.

A small amount of water in the extract solvent can easily penetrate into the cells of the plant matrix and promote better heating of the plant matrix. This in turn increases the mass transfer of the active components to the extracting solvent. The use of 80% aqueous methanol (v/v) was the optimal solvent extraction composition for the MAE extraction of chlorogenic acid and genoposidic acid from *Eucommia ulmoides Oliv.* [58].



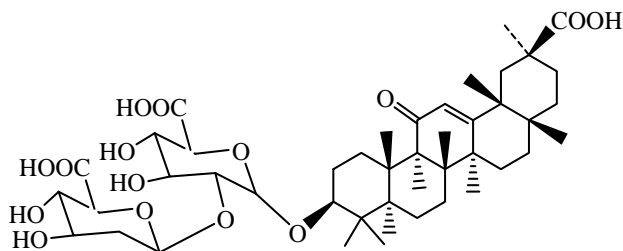
Chlorogenic acid



Genoposidic acid

When studying the concentration of ethanol to isolate flavonoids from *Cyclocarya paliurus (Batal.)* [106], it was shown that the yield of flavonoids increased when the concentration of aqueous ethanol ranged from 30% to 70%, and when the concentration of ethanol was above 70%, the yield decreased. Similar patterns were found with MAE saponins from *Panax ginseng* [107], when there was an increase in the yield of derivatives with varying ethanol concentration from 30% to 60%, and a decrease in yield when using 75% and 90% ethanol, as with a microwave power of 88 W, and at 162 watts. In the case of extraction of phenolic compounds from *Morus alba L.* [108], the highest yield of derivatives was

achieved using 40% ethanol, while using 30%, 40%, 50% and 60% ethanol, the quantitative yield was lower. MAE phenolic compounds from *Stevia rebaudiana* [41], an increase in the yield of derivatives was observed when the ethanol concentration was varied from 0% to 75%, and the use of 100% ethanol decreased the indices.



Glycyrrhizic acid

The study of the concentration of ethanol for the separation of glycyrrhizic acid [109] showed that an increase in the concentration of ethanol from 0 to 50-60% leads to an increase in the degree of extraction, whereas at concentrations from 60% to 100% the output sharply decreases. To improve the extraction, the use of 1-2% ammonia with 50-60% ethanol was tested and it was shown that the yield of glycyrrhizic acid was significantly increased due to the formation of salt having better solubility in the solvent system used.

Some more examples of the use of a high concentration of a solvent that absorbs microwave radiation are presented for the MAE coumarin [60], safflower yellow [110], and phenolic compounds [32, 33, 101, 111].

Solvent toxicity is also assessed when selecting a suitable solvent for MAE. For example, methanol was used to extract phenolic compounds from grape skins and seeds. When using it, a higher polyphenol yield was obtained compared to using ethanol, but the latter extract had stronger antioxidant properties [112]. In general, ethanol is by far the most used solvent for MAE, which is suitable for extracting many active compounds from plants [34, 37, 38, 40, 41, 53, 57, 64, 71, 74, 75, 79, 81, 84, 99, 100, 106-109].

With MAE, the same solvent can be used as with conventional extraction methods, however, optimal microwave extraction can not always be carried out with the same solvent.

For example, diethyl ether, which is widely used to extract steroids of the *Saxifragaceae* family, is not suitable as a solvent for MAE [113]. The way out may be the use of a modifier that can be added to the solvent to increase its overall performance. Thus, water was added as a modifier to diethyl ether to increase the efficiency of microwave heating during the extraction of steroids from *Rodgers aesculifolia Batal.* [113].

Sometimes with MAE, solvent mixtures characterized by low and high microwave absorption are used to ensure optimal extraction results.

A mixture of solvents consisting of ethanol and hexane, characterized by a high and low value of the dielectric constant (ϵ') was used to isolate solanesol (solanesol). Ethanol is a relatively good solvent that absorbs microwave energy ($\epsilon' = 25.7$), but is not considered as a good extraction solvent for solanesol, and hexane for it is a good extraction solvent, but has ($\epsilon' = 2.0$). Therefore, ethanol and hexane were mixed in different ratios for the MAE process. The ratio of hexane to ethanol (1: 3) gives the best percentage of solanesol extraction from tobacco leaves among the tested combinations [31].

Another example of the effect of a solvent in MAE extraction is the results of a study presented in [114]. Thus, the use of hexane in the MAE ginger as a solvent, gives smaller quantities than when extracted in the apparatus of Soxhlet. On the other hand, the use of ethanol as an extraction solvent at MAE shows a significantly higher yield than with ethanol in Soxhlet extraction. This difference can be explained due to the difference in dielectric properties of the solvent. Hexane is “transparent” to microwaves and therefore does not heat up in a microwave oven, whereas ethanol has a good microwave absorption capacity and, therefore, heats up faster and can improve the extraction process. Thus, the dielectric properties of the solvent plays an important role in microwave extraction.

3.2. Effect of solid to liquid ratio. Another factor affecting the efficiency of microwave extraction is the solid to liquid ratio. In traditional extraction methods, large volumes of solvent are commonly used, which generally affects the ability to extract biologically active ingredients. With regard to microwave extraction, a number of authors investigated and described the influence of the ratio of solids to liquids on the final quantitative characteristics of the process.

Thus, in a study [30], it was shown that the yield of scutellarine during the extraction of *Erigeron breviscapus* decreased with an increase in the ratio of solid to liquid from 2.5:100 (g/ml) to 15.0:100 (g/ml). Similarly, in article [100] it was noted that the yield of phenols in the extraction of peanut shells decreases with increasing solid-liquid ratio from 1.5:37.5 (g/ml) to 3.5:37.5 (g/ml) with extraction with 30% aqueous ethanol. At the same time, the influence of the microwave power (10%, 50%, 90% of the nominal), the exposure time (30, 90, 150 s) and the sample mass (1.5, 2.5, 3.5 g) were also studied. It was shown that the yield of flavonoids in *Radix Astragali* increased from a solid to liquid ratio from 1.25:50 to 1.67:50 (g/ml), and the maximum yield of flavonoids with MAE was obtained by double extraction using 90% ethanol 25 ml/g of material at 110 °C for 25 min [100]. The percentage of glycyrrhizic acid extraction increased with increasing liquid-solid ratio from 5:1 to 20:1 (ml/g) and ranged from 1.88 to 2.58% [109].

Possible reasons for a lower extract yield with a higher solid-to-liquid ratio are the fact that, firstly, microwave energy is absorbed and dispersed by larger particles of plant material [30], and secondly, an increase in the mass of extractable material reduces surface area for penetration solvent and, as a consequence, reduces the solubility [100]. The fact of a decrease in the yield of extraction substances with an increase in the volume of the solvent can also be explained by the excessive swelling of the plant material [101]. Another reason for influencing

the extraction process may be the use of mechanical stress factors (shaking, stirring). You should also consider the efficiency of heating the solvent at MAE, since evaporation of the solvent or how quickly it heats up under the action of microwaves will depend on this. Thus, the optimization of this parameter is of paramount importance in the MAE.

As a rule, liquid/solid ratios are reported in MAE. Thus, the study [106] showed that the yield of flavonoids tended to increase (from 14.56 ± 0.22 to 15.42 ± 0.45 mg/g) with an increase in the ratio of solvent to material from 10:1 to 20:1, however, the recovery percentage rapidly declined at a ratio above 20:1. This is probably due to inadequate mixing of the solvent under microwave exposure. On average, the ratio of liquid/solid ratio is in the range of 10 to 20-25 ml/g [13].

3.3. The influence of the particle size of the plant matrix. The size of the extractable particles of plant material and the state in which it is presented for MAE can have a strong influence on the efficiency of the extraction process.

The volume of solvent must be sufficient to ensure that the plant matrix is completely immersed in the solvent during the entire extraction time. In most cases, a higher ratio of solvent volume to solid matrix can be effective with conventional extraction methods, however, with MAE, a higher ratio can lead to lower sample extraction, which may be due to inadequate mixing of the solvent when exposed to microwaves. Thus, the effect of particle size in MAE tannins showed that the material used was crushed and sieved to select a particle less than 1 mm in the range: 1-0.8; 0.8-0.4; 0.1-0.05 mm. Extraction yields and colorimetric results showed that the amount of extracted substances increases with decreasing particle size of crushed bark to 400 μm . Similar patterns are presented for MAE ginseng saponins [107] and MAE for cocaine [115].

Fine powders can increase the extraction process, providing a large surface area that provides better contact between the plant matrix and the solvent. As well as smaller particles can improve or significantly increase the deeper penetration of microwaves. Here the ability of the plant material to swell should also be taken into account, which will make it difficult to mix the extracted raw materials during extraction. The disadvantage associated with the use of smaller particles is the difficulty of separating the plant matrix from the solvent after microwave irradiation, as well as the subsequent processing process, especially at the filtration stage, creating some technical problems. Particle size is usually in the range of 100 μm to 2 mm [97].

3.4. Effect of the types of plant raw material. Many types of plants can be used to extract different valuable compounds. The most important properties of the plant submitted to extraction are:

- The cell structure which contain the valuable components. To further understand the mechanism of microwave-assisted extraction, it is essential to study the sites of the biosynthesis, transport and accumulation of secondary metabolites in the plant tissues, cells or organelles. Some compounds (for example essential oils) are found in special cell structures either on the surface of the plant or in the

plant tissues. The simplest structure is a single secretion containing cell where it is only the actual content that distinguishes it from adjacent non-secretory cells. However, it may be larger than the other cells or have a thick cuticularized lining. These types of compounds are extracted more easily, since the structures are located at the surface of vegetal material and their walls can be disrupting easy. Another compounds, such as phenolics, are more difficult to extract because they are stockpiled in the central vacuoles of guard cells and epidermal cells as well as subepidermal cells of leaves and shoots [27]. Immuno-localization experiments revealed that most of the flavonoid biosynthesis-related enzymes were loosely bound to the cytoplasmic face of the endoplasmic reticulum, possibly in a multi-enzyme complex [19].

- Another feature is the type of binding between the main derivatives derived from MAE and the accompanying compounds contained in the plant material. For example, polyphenolic compounds are rarely found in free form and most of them found covalently linked to the plant cell wall and are found in the form of glycosylated form.

- In addition to the particle size of the vegetable matrix used for extraction, the important point is the type of raw material used, its condition, in what form, dried or freshly harvested, it is used. Extraction typically uses dried plant materials, but plant cells contain small traces of moisture that serve as targets for microwave heating. Moisture present in the sample matrix can have a strong influence on the extraction efficiency. The presence of water, naturally contained in the plant material or added during its pre-soaking, leads to an increase in the polarity of the extractant, which will affect its ability to absorb microwaves, thus facilitating heating. The presence of water can cause swelling of the plant matrix, making the analytes more available accessible to the extractant.

Due to the microwave effect, when the moisture is heated inside the plant cell, it evaporates and creates tremendous pressure on the cell wall from the inside, which leads to its destruction and helps the components from the destroyed cells to enter the surrounding solvent.

In many cases, the natural moisture content in the matrix improves the extraction of extracts. This is used, for example, in MAE extraction without using a solvent, for example in solvent-free microwave extraction (SFME) [27.90-96]. This extraction method uses microwave heating of plant material containing natural water. However, in the case of dried raw materials, in order to use microwave heating, it must be rehydrated by adding a sufficient amount of water, which is usually achieved by pre-soaking.

Pretreatment of the sample to MAE can provide efficient and selective heating of the matrix. In some cases, soaking the dried plant material in an extracting solvent to MAE leads to an improved yield. Dried samples that have been pretreated with higher microwave absorption solvents combined with extraction solvents such as ethanol or methanol provide heating, at least using two competing mechanisms, namely direct heating from the interaction of microwaves with

solvent and heating with diffusion excess heat resulting from the interaction of microwaves with a pretreated matrix [97].

Increase the pre-leach time from 4 minutes to 90 minutes. The extraction of polyphenols from green tea leaves increased by 1.53%, and the extraction of caffeine increased by 0.49% [116]. These results indicate that raw material pretreatment can be a strategy for improving extraction efficiency in MAE.

3.5. Effect of microwave power and microwave efficiency. When extracting plants, a high microwave power can lead to a decrease in yield due to the degradation of heat sensitive compounds. In general, the extraction yield increases in proportion to the increase in microwave power to the limit before the increase becomes insignificant or decreases [43, 55-57]. The microwave power provides localized heating in the sample and acts as the MAE driving force required to destroy the plant matrix, so that the analyte can diffuse and dissolve in the solvent. The increase in power usually improves the extraction yield and leads to a shorter extraction time. The variables microwave power and time of exposure to radiation have opposite effects; thus, using a high microwave power affords a decreased exposure time, whereas using a low power requires irradiating the sample for a longer time if the same amount of energy is used.

A higher power level does not contribute to the study of the interaction of microwaves and extraction solvent with the sample. Thus, the extraction of saponins using the MAE method [107] took 30 seconds at a power of 250 W and 180 seconds at a power of 88 W, whereas with conventional thermal extraction at 80 °C it took 3 hours.

A combination of low or moderate power with a longer exposure may be a more reasonable extraction approach. Thus, the number of ginsenosides extracted by the MAE method in different microwave conditions [117] was studied in this work and it was shown that, in general, the extraction efficiency was increased by increasing the microwave power from 30 to 150 W. The difference in ginsenosides extracted at a power of 30 to 150 W was more significant with a shorter extraction time than with a longer extraction time. Gao M. et al. [118] showed that varying the power from 400 W to 1200 W had no significant effect on the release of flavonoids from *Saussurea medusa* [118].

High power with long exposure is always associated with the risk of thermal degradation. Reasonable choice of power used avoids excessive temperature, which can lead to destruction of the sample and excessive pressure inside the vessel, especially in the case of a closed MAE system. In MAE, it should be taken into account that exposure to microwave radiation, even at low operating power or low temperature, can reduce the extraction yield due to the degradation of the chemical structure of the active compounds [119, 120].

Since the power level alone does not provide sufficient information about the absorbed microwave energy in the extraction system, Alfaro and his staff [114] introduced the term known as energy density, power per mass for a certain unit of time, to study the effect of microwave power at MAE. This variable represents the microwave irradiation power for a given unit of extraction volume and is compa-

ratively more significant than the microwave power level in optimizing MAE conditions. However, the irradiation power for microwave heating (power density) does not reflect the actual power absorbed in the extraction system. They reported that as soon as the plant matrix is destroyed by microwave radiation, the active compounds will be released.

The amount of energy absorbed for localized heating in the extraction system can be considered by using two intensity-related MAE variables, namely: the absorbed power density and the absorbed energy density, which reflect the amount of microwave power absorbed and the heating energy of the extraction system, respectively. These variables are mutually independent and were recently successfully used to replace microwave irradiation power and extraction time in modeling MAE of active compounds from cocoa leaves at various extraction scales [128, 129]. Also, some extraction models were adapted on the basis of the amount of microwave energy absorbed during extraction in order to predict MAE extraction profiles at a variable irradiation power (100-600 W) and extractant load (100-300 mL) [130].

Microwave efficiency is also dependent on the input microwave frequency, which, together with the dissipation factor of the irradiated material, affects the penetration depth of microwave energy. At a given input frequency, the greater the dissipation factor of a sample is, the less it will be penetrated by microwave energy. In large samples with high dissipation factors, heating beyond the penetration depth of the microwave energy is due to thermal conduction; as a result, temperatures are higher at or near the surface of the irradiated material.

3.6. Effect of temperature. Another parameter affecting the efficiency of microwave extraction is temperature. The temperature and power of the microwave are interrelated, since high microwave power can raise the temperature of extraction of the system. As a rule, a higher extraction temperature is advantageous for extraction due to increased solubility. The choice of extraction temperature depends on the stability and extraction of the desired active compound. The use of low and medium microwave power with a longer exposure to the material being extracted may be a more reasonable approach. Rapid destruction of the cell wall occurs at a higher temperature and at higher power, as a result, along with the desired components, the associated substances also pass into the solvent. At low power levels, cell wall rupture can occur gradually, which makes it possible to conduct selective MAE. On average, at MAE, the optimum temperature range varies from 30 to 60-140°C [13].

With MAE anthraquinones from *Morinda citrifolia*, it was found that increasing the temperature of the solvent from 60 to 120 °C significantly increases the efficiency of extraction, due to the fact that a higher temperature causes an increase in the solubility of the derivatives. An increase in temperature leads to the destruction of the cell frame, and, consequently, to the availability of anthraquinones for extraction. In addition, at high temperatures, the viscosity of the solvent decreases and the diffusion coefficient increases, thus increasing the extraction efficiency. So, with an exposure time of 5 to 30 minutes and a extraction

temperature of 60 °C, the percentage of anthraquinone extraction was approximately 42-60%, whereas at T = 120 °C - 81-90% [98].

Thus, the effect of microwave power (450–600–900 W) and duration of irradiation (30–210 s) on the total concentration of phenols was studied in [121] and showed that the effect of the ratio of solids to liquids during microwave extraction of phenols from tea is related with the temperature of plant samples. Tsubaki S. and colleagues [122] during the extraction of phenolic compounds from tea showed that the phenolic content of the extract increases with the extraction temperature and the optimum temperature was equal to 170 °C. A further increase in the extraction temperature gave a lower yield. The authors of article [101] demonstrated that with a high microwave power of 1000 W when extracting flavonoids from the roots of *Radix astragali*, the yield of extractive substances decreases if the extraction temperature is higher than 110 °C due to the instability of flavonoids at these temperatures. They also showed that excessive exposure to microwaves will lead to the loss of flavonoids due to their thermal degradation.

The article [106] provides data on the effect of temperature T = 40, 50, 60, 70, 80 and 90 °C, respectively, on the yield of flavonoids from *Cyclocarya paliurus* (*Batal.*), with a fixed extraction time of 5 minutes, the ratio of solvent to material 20:1 and using 90% ethanol as a solvent. At a solvent temperature of 40 to 80 °C, the extraction efficiency significantly increased (from 6.17±0.32 to 12.99±0.25 mg/g), which may be due to the fact that a higher temperature leads to an increase in molecular interactions and increase solubility. However, higher extraction temperatures T = 80-100 °C did not show a significant improvement in extraction, and further heating may have negative effects caused by the degradation or conversion of analytes.

3.7. Effect of exposure time. The power of the microwaves and the time of exposure are two such factors that greatly influence each other. As in the case of traditional extraction methods, microwave extraction time is another parameter whose influence must be taken into account. In general, by increasing the extraction time, the amount of extractables increases, although there is a risk of destruction of the derivatives. On average, according to [13], the duration of the MAE varies from 15-30 seconds to 15-20 minutes. So, in the work [58] 40 second microwave extraction of *Eucommia ulmodies Oliv.* gave an excellent result.

In [116], it was found that the amount of polyphenols and caffeine from green tea leaves at MAE increased with extraction time up to 4 minutes, and then decrease with increasing time. Similar observations on the effect of MAE time from *Salvia militiorrhiza* [105]. Thus, a study [123] estimated the duration (5, 10, 15, and 20 min) of the extraction of capsaicinoids (capsaicinoids) from pepper, which showed a clear increase in the extraction rates of capsaicinoids with an optimal value of 5 minutes.

Thus, 15-20 minutes is sufficient for the extraction of phenolic compounds using aqueous ethanol from *Malus domestica* [40], leaves of *Stevia rebaudiana Bertoni* [41] and *Cyclocarya paliurus* (*Batal.*) [106], when using aq. HCl with

MAE *Dactylis glomerata*, *Festucarubra L.*, *Festuca ovina L.*, *Bromus inermis* and *Bromus marginatus*, *Hypericum perforatum*, *Thymus vulgaris L.*, *Tilia cordata*, *Uncaria tomentosa* [111], using 15 M HCl:95 % ethanol in the ratio (15:85) in the extraction of anthocyanins from *Zea mays L.* [124], 80% ethanol to extract anthraquinones from the roots of *Morinda citrifolia* [98] and water to extract alkaloids (corilagin, geraniin) from *Geranium sibiricum Linne* [66]. However, there are studies that have demonstrated that it took 60 minutes to extract the active ingredients from *Pistacia lentiscus var* [125], silymarin from *Silybum marianum L.* [126] or phenolic compounds from *Quercus robur L.* [67].

If a longer extraction time is required, the risk of thermal decomposition can be reduced by extraction cycles. This process can be modified by either adding fresh solvent to the residue or repeating the extraction step to ensure that the extraction is completed. To isolate 3-Hydroxy-4-phenylmethoxy-stilbene-2-carboxylic acid and pinostobin from *Pigeonpea leaves (Cajanus cajan (L.) Mill sp.)*, 80% ethanol and 2 extraction cycles of 1 min each were used [127]. When optimizing the extraction of flavonoids from the roots of *Radix astragali*, it took 2 extraction cycles with a duration of 25 min [101], and Yan et al. [82] found that 3 extraction cycles of 15 minutes are best suited for the extraction of astragalosides from *Radix astragali*. Extraction of coumarin, o-coumaric acid and melilotic acid from *Melilotus officinalis L.* was carried out 50% ethanol in 2 extraction cycles of 5 min each [60].

Optimization of extraction time is an important factor in process efficiency, since the extraction time can vary depending on the part of the plant used and the type of microwave extraction. The dielectric properties of the solvent also affect the exposure time. According to Chan et al. [20] and Veggi et al. [131], thermal degradation and oxidation of sensitive compounds are associated with microwave exposure time. Solvents such as ethanol, methanol and water can be very hot with longer exposure, thereby increasing the risk of destruction of the thermolabile components. Thus, in the study of MAE compounds from *Morus alba L.* [108], it was shown that when the irradiation time was extended from 1 to 7 minutes, maximum contents of polyphenols and flavanoids were recorded, however, longer heating reduced the yield of derivatives due to thermal decomposition of polyphenolic components.

Conclusions. Since microwave irradiation was used in the recovery of the important components from plant materials, there have been numerous reports on microwave assisted extraction of plant secondary metabolites. This technique uses microwave radiation to cause polar molecules and ions to move and dipoles to rotate in order to heat solvents and promote transfer of target compounds from the sample matrix to the extractant. A lot of examples suggested that microwave-assisted extraction has some considerable merits such as shorter extraction time, higher extraction yield and less solvent consumption compared to conventional extraction methods. Microwave-assisted extraction was currently regarded as a robust alternative to traditional extraction techniques, especially in the case of the sample preparation for analytical purpose. The main variables affecting micro-

wave heating include nature and amount of the matrix, microwave power and temperature, solvent properties and volume, stirring, extraction time, extraction pressure and the number of extraction cycles. Traditional MAE usually involves the use of toxic and hazardous organic solvents. The trend is to reduce the amount of solvents or replace them by others of greener nature.

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Резюме

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МИКРОТОЛҚЫНДЫ СӘУЛЕЛЕНДІРУ, ОНЫҢ ЕРІТІНДІЛЕРГЕ ӘСЕР ЕТУІ ЖӘНЕ ӨСІМДІК МАТЕРИАЛДАРЫ КОМПОНЕНТТЕРІНІҢ ЭКСТРАКЦИЯСЫ ҮШІН ҚОЛДАНЫЛУЫ

2–ші хабарлама

Микротолқынды сәулелендірудің өсімдік материалымен өзара әрекеттесуі және табиғи қосылыстардың алу процесіне әсер ететін факторлар

Қазіргі уақытта микротолқынды экстракция аналитикалық химия салаларының бірі болып табылады, бұл дегеніміз өсімдік шикізатынан биологиялық белсенді қосылыстардың өнімділігін арттырудың ең тиімді және заманауи әдістерінің бірі болуы үшін жылдам күрескен әдіс. Зерттеуде микротолқынды сәулеленудің өсімдік материалына әсерін қарастырады. Табиғи қосылыстарды бөліп алу үшін микротолқынды экстракцияны қолдану, сондай-ақ түрлі факторлардың өндіру үрдісіне әсері туралы жалпыланған заттар қарастырылған.

Түйін сөздер: микротолқынды сәулелендіру, табиғи қосылыстар, микротолқынды экстракция.

Резюме

Т. В. Харламова

**МИКРОВОЛНОВОЕ ИЗЛУЧЕНИЕ,
ЕГО ВЛИЯНИЕ НА РАСТВОРЫ И ИСПОЛЬЗОВАНИЕ
ДЛЯ ЭКСТРАКЦИИ КОМПОНЕНТОВ РАСТИТЕЛЬНОГО СЫРЬЯ**

Сообщение 2.

Взаимодействие микроволнового излучения с растительным материалом
и факторы, влияющие на процесс экстракции природных соединений

Микроволновая экстракция в настоящее время является одним из направлений аналитической химии, которая быстро завоевала позицию одного из наиболее эффективных и современных методов повышения эффективности экстракции биологически активных соединений из растительного сырья. В обзоре рассматривается влияние микроволнового излучения на растительный материал. Обобщен материал по использованию микроволновой экстракции для выделения природных соединений, а также влияние различных факторов на процесс извлечения.

Ключевые слова: микроволновое излучение, природные соединения, микроволновая экстракция.