Modern Extraction Methods of Biologically Active Components in Food Biotechnology
# TABLE OF CONTENTS

Preface ........................................................................................................................................ x

Chapter I ........................................................................................................................................ 1
Modern methods for extraction and encapsulation of biologically active components

1. Introduction ................................................................................................................................. 1
2. Importance of extraction processes in the food industry and modern methods of extraction .......................................................... 3
   2.1. Ultrasound-assisted extraction (UAE) ................................................................................ 7
       2.1.1. Parameters affecting UAE ....................................................................................... 11
           2.1.1.1. Power and frequency ......................................................................................... 11
           2.1.1.2. Intensity ............................................................................................................ 12
           2.1.1.3. Design (shape and size) of ultrasonic reactors ............................................ 13
           2.1.1.4. Solvent ............................................................................................................. 14
           2.1.1.5. Temperature ..................................................................................................... 15
   2.2. Microwave-assisted extraction (MAE) .............................................................................. 15
       2.2.1. Parameters affecting MAE ....................................................................................... 17
           2.2.1.1. Solvent .............................................................................................................. 17
           2.2.1.2. Extraction time ................................................................................................. 19
           2.2.1.3. Design of microwave reactors ........................................................................ 19
           2.2.1.4. Temperature ..................................................................................................... 20
           2.2.1.5. Power .............................................................................................................. 21
   2.3. Supercritical Fluid Extraction (SFE) ............................................................................... 22
       2.3.1. Mathematical modeling of supercritical extraction ............................................. 25
   2.4. Supercritical carbon dioxide extraction (SC-CO2) ......................................................... 29
       2.4.1. Parameters affecting SC-CO2 ................................................................................. 31
           2.4.1.1. Pressure and temperature ............................................................................... 31
           2.4.1.2. Flow ................................................................................................................ 34
           2.4.1.3. Modifiers .......................................................................................................... 34
   2.5. Solvent extraction under pressure ..................................................................................... 35
   2.6. Subcritical water extraction (SWE) ............................................................................... 37
       2.6.1. Parameters affecting SWE ...................................................................................... 40
           2.6.1.1. Temperature .................................................................................................... 41
           2.6.1.2. Pressure ......................................................................................................... 42
           2.6.1.3. Flow rate and extraction time ........................................................................ 42
Table of Contents

2.6.1.4. Modifiers................................................................. 43
2.6.1.5. Degradation.......................................................... 43
2.6.1.6. Other parameters................................................. 44
2.7. Methods for extraction optimization............................... 45
3. Encapsulation of the extracted biologically active components ...................................................................... 46
  3.1. Carriers for encapsulation.............................................. 50
    3.1.1. Maltodextrins....................................................... 51
    3.1.2. Inulin.................................................................... 52
    3.1.3. Protein whey.......................................................... 52
    3.1.4. Alginates............................................................... 53
  3.2. Encapsulation techniques.............................................. 54
    3.2.1. Spray drying.......................................................... 56
      3.2.1.1. The principle of spray drying process.............. 57
      3.2.1.2. Challenges in drying.................................. 64
      3.2.1.3. Carriers in drying....................................... 66
    3.2.2. Spray congealing............................................... 70
    3.2.3. Lyophilization.................................................... 71
      3.2.3.1. Lyophilization steps................................. 73
    3.2.4. Electrostatic extrusion....................................... 77
Bibliography.................................................................................. 80

Chapter II.................................................................................... 97
The most important biologically active components in food
  1. Introduction................................................................... 97
  2. The importance of bioactive components in daily nutrition... 98
  3. Polysaccharides in food.................................................. 107
    3.1. The most important sources of polysaccharides...... 112
      3.1.1. Mushrooms..................................................... 112
      3.1.2. Blackbary (Rubus fructicosus).................... 117
      3.1.3. Peach kernels (Prunus persica).................. 118
      3.1.4. Potato (Solanum tuberosum).................... 120
      3.1.5. Milk.............................................................. 122
  4. Proteins in food.......................................................... 124
    4.1. Bioactive peptides.................................................. 136
    4.2. Alternative sources of proteins............................. 138
      4.2.1. Mushrooms................................................... 140
      4.2.2. Wheat (Triticum aestivum).......................... 142
      4.2.3. Soybean (Glycine max)............................. 146
      4.2.4. Potato (Solanum tuberosum).................... 148
      4.2.5. Pumpkin seeds (Cucurbita pepo)................. 150
Modern Extraction Methods of Biologically Active Components in Food Biotechnology

4.2.6. Proteins from by-products ........................................... 152
4.3. Milk as a source of proteins ........................................... 154

5. Polyphenolic components in food ........................................ 159
5.1. The most important sources of polyphenolic components .... 168
  5.1.1. Mushrooms .......................................................... 168
  5.1.2. Raspberry (Rubus idaeus) ...................................... 170
  5.1.3. Blackberry (Rubus fruticosus) ................................. 172
  5.1.4. Apple (Malus domestica) ....................................... 175
  5.1.5. Plum (Prunus domestica) ....................................... 178
  5.1.6. Peach and peach kernels (Prunus persica) ............... 179
  5.1.7. Pomegranate (Punica granatum) ............................. 180
  5.1.8. Quince (Cydonia oblonga) ..................................... 183
  5.1.9. Tomato (Solanum lycopersicum) ............................. 184
  5.1.10. Pepper (Capsicum annuum) ................................... 185
  5.1.11. Basil (Ocimum basilicum) ..................................... 187
  5.1.12. Soybean (Glycine max) ....................................... 188
  5.1.13. Cocoa (Theobroma cacao) ................................... 191
  5.1.14. Chocolate .......................................................... 194
  5.1.15. Grape (Vitis vinifera) and wine ............................. 196

Bibliography ................................................................................. 200

Chapter III ......................................................................................... 233

Functional properties of bioactive components and their effect on consumer health

1. Introduction ........................................................................... 233
2. Antioxidant activity .............................................................. 235
  2.1. Antioxidants ...................................................................... 236
    2.1.1. Classification of the antioxidants .............................. 237
    2.1.2. Mechanism of action of antioxidants ...................... 239
  2.2. Antioxidant protection enzyme activity ......................... 247
3. Antimicrobial activity ............................................................ 253
  3.1. Antibacterial activity ...................................................... 254
  3.2. Antiviral activity ............................................................. 263
  3.3. Antifungal activity ............................................................ 265
4. Immunomodulatory activity .................................................... 267
5. Neuroprotective activity ........................................................ 272
6. Anti-inflammatory activity ....................................................... 278
7. Antidiabetogenic activity ........................................................ 281

Bibliography ....................................................................................... 286
# Appendix A

Bio soups – the future of industrial production of functional and safe food

Bibliography

Biography of the authors
To eat is a necessity, but to eat intelligently is an art.

~ François de la Rochefoucauld
Food quality and safety have become a global concern due to the established relationship between food and health. To improve health, it is crucial to ensure the availability of safe food with defined quality parameters. This issue is being addressed by both developed and developing countries, as it is a common problem that affects everyone.

Traditional methods of food protection try to ensure its safety, by applying more effective synthetic preservatives or, on the other hand, by applying more drastic physical treatments during the production process, such as high temperatures. However, it was found that these procedures have a lot of drawbacks: the toxicity of many of the most commonly applied synthetic preservatives was proven, a change in the nutritional and sensory properties of the food was observed due to the application of elevated temperatures, while consumer demands were also directed towards new trends in consumption food – high quality, absolutely safe, but minimally processed, without traditional chemical additives. The fact is that synthetic additives (sorbates, benzoates, nitrates, etc.) represent reliable food preservation factors because they have a pronounced antimicrobial effect. However, these compounds do not meet the concept of natural and healthy food consumers insist on. Therefore, replacing synthetic preservatives with natural antimicrobial compounds is a relevant alternative in cases where it is necessary and possible.

Despite all that, the food industry still dominates the opinion that the antimicrobial properties of various natural additives are questionable because they need to be added in large quantities to get a positive result. However, with the application of modern procedures for the extraction of various bioactive substances from natural products and their concentration, substances with a proven GRAS status (Generally Recognized as Safe) have been obtained, which can be applied in the food industry.

In line with that, the application of not only certain microorganisms, their metabolites and herbal extracts, but also extracts of some higher fungi (edible as well as medicinal) represents the basis of the development of new technologies of biological preservation and food protection, the application of which can contribute to the standardisation of the production process of foodstuffs with uniform and improved quality parameters, on the one hand, and the other – the creation of safe products with an extended shelf life and
the possibility of preventing the occurrence of certain diseases or protecting the health of the population. At the same time, the application of extracts in food products offers the possibility of creating a new, so-called functional food, which at the same time possesses biological potential.

Accordingly, the Monograph *Modern Extraction Methods of Biologically Active Components in Food Biotechnology* represents a unique and creative symbiosis of three very important segments in food biotechnology: extraction, biologically active components in various food products and biological potential of the extracted compounds. Namely, the first chapter elaborates on the most important modern methods of extraction of biologically active components from food. The choice of the appropriate method is the first and key segment in obtaining a quality extract, which could further be used in the various segments of the food industry to obtain functional food, in the pharmaceutical, cosmetic industry, etc. Emphasis is placed on modern, rather than traditional methods due to numerous advantages. In the second chapter, the most significant biologically active components and the food products that contain them have been moved. In this chapter, readers will learn about important sources of functional components in food and how to choose the right foods for their meals. The third chapter deals with the biological effect of biologically active compounds on the health of consumers. This section talks about the importance of the daily consumption of functional food and the ways to influence the long-term improvement of the health status of consumers. Finally, as Appendix A, readers will have the opportunity to get acquainted with an innovative product, Bio-Soup, i.e. the first functional industrially produced dehydrated vegetable soup enriched with lyophilized extracts of edible and medicinal mushrooms.

This Monograph not only brings immense scientific benefits but also presents a new and innovative perspective on the industrial production of food that is completely safe, high-quality, and functional. This food is the future of building a healthier and better nation, contributing to the well-being of people worldwide.

This Monograph is perfect for a diverse range of readers, ranging from researchers, scientists, and professors to students and employees in the food industry. Additionally, it is suitable for anyone who is looking to learn how to eat healthier and improve their life habits.

... *Because science is the poetry of reality.*

~ Authors
CHAPTER I

MODERN METHODS FOR THE EXTRACTION
OF BIOLOGICALLY ACTIVE COMPONENTS

1. Introduction

Functional food has become a global trend in recent years. Apart from providing nutrition, it also has medicinal properties. Due to the modern lifestyle which often results in inadequate nutrition and the increasing prevalence of chronic diseases, there is a growing need to explore and utilize natural substances and antioxidants that can provide targeted protection to the body.

Plant extracts have been found to have significant biological activity, as determined by biological, chemical, and pharmacological tests. As a result, active components can be isolated and characterized, many of which are now used in modern pharmacotherapy. The incorporation of medicinal plants and plant extracts can enhance the quality and nutritional value of food, making it particularly relevant for the development of innovative food and functional products.

Phytonutrients are becoming increasingly popular as a substitute for chemical additives due to their antioxidant and antimicrobial properties. This is particularly relevant for the food and pharmaceutical industries, which are actively seeking alternative preservatives to enhance product safety and quality. Natural components like phenolic or sulfur components derived from various types of onions have great potential to be used as preservatives due to their antimicrobial properties and broad spectrum of activity against foodborne pathogens.

Obtaining natural plant products, particularly plant extracts, has traditionally involved conventional extraction methods. However, these methods have several drawbacks, such as low efficiency, high energy and time consumption, use of toxic solvents, thermal degradation of compounds, negative impact on the environment, and production of low-quality extracts. Therefore, the modern production of plant extracts focuses on developing extraction procedures that use “green” non-toxic solvents, which reduce
solvent consumption and produce a higher yield of uniformly high-quality extracts. This shift towards using “green” solvents aligns with market demands for biodegradable, uncontaminated extracts that remain undenatured.

Significant advancements in technology and scientific knowledge have made it possible to extract pharmacologically active compounds from natural sources. With modern instrumentation, it is now possible to conduct a detailed qualitative and quantitative analysis of the extracted components. The choice of appropriate extraction techniques, solvent, and extraction parameters plays a crucial role in obtaining high-quality extracts with the desired bioactive compounds. The extraction technique chosen can significantly affect the quality and content of the extract. Modern trends in pharmaceutical and food technology involve the use of safe solvents, such as ethanol and water, or subcritical and supercritical fluids, and incorporating ultrasound technology to increase the content of isolated bioactive components in the extract. Extracts obtained using these techniques can be directly used as semi-finished or finished products in food and pharmaceutical technology.

The food industry has rapidly developed and as a result, there has been an increase in the amount of by-products and waste. Unfortunately, the disposal or destruction of these by-products is not done correctly, which has a negative impact on the environment. However, recent research has shown that by-products in the food industry can be used to create functional food that not only has nutritional value but also medicinal properties. By-products obtained during the production of plant-based food are especially promising as they contain a large number of bioactive compounds, depending on the raw material used. Having that in mind, the by-product obtained in this way represents a significant source of bioactive compounds that could potentially be used for application in functional food and dietary supplements.

It is vital to carefully select the methods for extracting bioactive compounds and optimize the necessary parameters to obtain stable and high-quality extracts. With modern extraction techniques, a broad range of applications is possible, irrespective of the raw material type and the type of bioactive components extracted. These methods ensure a good yield and stable biological potential.
2. Importance of extraction processes in the food industry and modern methods of extraction

In the process of obtaining bioactive compounds from raw materials, extraction is the first and crucial step. Many of the secondary plant metabolites are expensive or impossible to synthesize in labs, so extraction from natural sources is the best option for their separation and concentration. There are various techniques for extraction, ranging from traditional to modern, each with its own advantages and disadvantages. The extraction technique chosen affects the composition and isolation of the obtained extract as well as the effectiveness of the final product. Therefore, extraction is a vital step in obtaining different preparations.

The extraction process is a technological operation of separating pharmacologically and biologically active compounds of plant and animal tissues from inactive or inert components, using selective solvents and standard methods. Extracts are preparations of liquid (liquid extracts and tinctures), semi-solid (soft extracts and oleoresins) or solid consistency (dry extracts), which are usually obtained from dried plant or animal material (Vuleta et al., 2012).

The basic requirement that the extraction should fulfil is the maximum yield of the target group of compounds, with as little disruption of their activity as possible. Considering the chemical nature of the components, the preservation of their activity is considered one of the critical points when choosing an extraction method. In addition, the choice of extraction method is influenced by the expected quality of the product, as well as the purpose of its further application. Numerous factors affect the efficiency of extraction, among which the most important are the solvent, extraction method, temperature, duration of the extraction process, degree of fragmentation of the material and pH of the extractant. Solvent selection is based on the chemical nature of one or a group of components to be extracted.

Bioactive components from plant material can be extracted using classical extraction methods, such as Soxhlet extraction, maceration, hydrodistillation water or alcoholic extraction. During the extraction of plant material, in the first phase, there is wetting, dissolution and rapid transfer of components from the destroyed cells, by the mechanism of turbulent diffusion. A period of rapid extraction is followed by slow diffusion of ingredients from intact plant cells and mass transfer by molecular diffusion (Čujić et al., 2016). The transfer of active components from the plant material to the extract is directly proportional to the diffusion coefficient, the contact surface of the drug and the solvent, the concentration
gradient and the extraction time, and inversely proportional to the thickness of the diffusion layer (Vuleta et al., 2012). The goal of extraction can be the extraction of total extractable substances or the extraction of a specific component. Factors that significantly affect the yield of extracted components are the characteristics of the plant material (growing conditions, collection period, part of the plant, degree of pulverization of the herbal drug), drug: solvent ratio, type of solvent, extraction time, temperature, pressure, pH, as well as chosen extraction method.

To produce high-quality herbal extracts, it is important to carefully select the right herbal drug, choose an appropriate extraction method, use the necessary equipment, and select a suitable solvent. The best way to obtain large quantities of plant material for extract production is through organized plantation farming. This method reduces the impact of external factors, ensures optimal growth and development of the plant species, and guarantees a sufficient supply of plant raw material that is of consistent quality while also protecting natural resources (Vuleta et al., 2012).

Traditional methods for making herbal drug extracts include maceration, percolation and digestion. Maceration is a one-time extraction of a properly crushed drug with a prescribed solvent at room temperature. The advantage of this extraction technique is reflected in its simplicity and the use of a cold solvent, which makes it suitable for the extraction of thermolabile compounds. However, the extraction time is long and large amounts of solvent are needed for a better extraction yield. The duration of the maceration can be different, most often from 4 to 10 days, with mixing 3 times a day, because rest leads to a decrease in the rate of diffusion of active principles. Diffusion continues until the concentrations of active substances are equalized intracellularly and extracellularly. The resulting balance is disturbed by intensive mixing during the entire extraction process, which shortens the time to 10–30 minutes. Intensive mixing leads to an increase in the concentration gradient in the solvent and an acceleration of diffusion, with a consequent increase in the extraction yield. It is important to note that with maceration it is not possible to exhaust the material and complete extraction. That's why double maceration is applied to herbal drugs with a rough consistency, achieving a higher degree of material utilization, because the already extracted drug is poured with fresh solvent and the concentration gradient is re-established (Vuleta et al., 2012). When applying elevated temperature, the efficiency of extraction increases as follows - damage to the plant cell leads to an increase in membrane permeability and the breaking of intermolecular bonds (polyphenols-lipoproteins), which increases the solubility of active substances, accelerates the thermal movement of molecules in the liquid phase and the
Modern methods for extraction and encapsulation of biologically active components

extraction kinetics. An important limitation of this method is the physico-chemical characteristics of the active principles because it is not possible to apply them to plant material with thermolabile components.

The main disadvantages of conventional extraction techniques are long extraction time, low yield, insufficient selectivity, need for subsequent processing and purification, degradation of thermolabile components, use of solvents that are harmful to the environment, as well as the possibility of their remaining in the final product. The mentioned shortcomings created the need for the development and implementation of new technologies, which are developed within the concept of green extraction.

The process of extracting bioactive compounds, known as valorization, requires the use of significant amounts of solvents. However, most of these solvents are hazardous to human health and the environment. In 1991, Paul Anastas, an American scientist, recognized the need to reduce the use of harmful solvents. To address this issue, he developed a program called Green Chemistry. This program established principles for reducing the use of hazardous solvents. The concept of Green extraction of natural products aims to protect the environment and human health while promoting competition among industries for the development of innovative methods that are both economically feasible and environmentally acceptable.

Today, a wide range of solid-liquid extraction procedures are available for the extraction and isolation of functional compounds, so it is almost impossible to find a production line in the food, pharmaceutical or cosmetic industry that does not use one of the classic extraction procedures. Traditional extraction methods include solid-liquid extraction, maceration, maceration with continuous mixing, percolation, digestion or Soxhlet extraction. These methods are characterized by a long extraction time, low yield, insufficient selectivity, the need for subsequent processing and purification, degradation of thermolabile components, the use of solvents that are harmful to the environment, as well as the possibility of their remaining in the final product. The mentioned shortcomings, as well as the very concept of “green chemistry”, imposed the need for the development and implementation of new technologies.

The main goals of modern extraction methods are to maximize the yield of desired compounds without or with minimal impact on their characteristics and at the same time minimize the extraction of undesirable compounds. The use of toxic chlorinated (chloroform, carbon tetrachloride, tetrachlorethylene) and non-chlorinated (acetone, methanol, acetonitrile) solvents is common for conventional extraction procedures, leaving them behind in the extract (Tiwari, 2015). The implementation of a solvent-free process is emerging as an ideal solution. However, solvents are unavoidable
due to their crucial role in solid phase dissolution, mass and heat transfer, as well as in separation and purification operations. That is why we strive to use green solvents, primarily water, which are not harmful to the environment. Water is already used in industrial plants, mainly in emulsion polymerization and hydrodistillation. Despite this, the low selectivity of water and the high energy requirements for its removal limit its wider use and lead to the consideration of alternative “green” solvents. To overcome these shortcomings and improve existing extraction techniques, modern extraction methods have been developed, which include ultrasonic extraction, microwave extraction, supercritical fluid extraction and subcritical water extraction. These new techniques offer tremendous potential to reduce or eliminate the use of toxic organic solvents while increasing extraction yield and extract quality. They are also known as “cold” extraction techniques because the temperature during the extraction process is relatively low and does not affect the stability of the extracted compounds.

When extraction of certain components is required, it is important to define the ratio of drug to solvent and extraction time to maximize the yield. The extraction time depends on the molecular weight of the active substances. In the case of high molecular mass of active components, the extraction process is prolonged due to difficult diffusion. Also, the speed of diffusion of active principles depends on the viscosity of the medium, that is, on the viscosity of the solvent and the amount of ballast substances present (mucus and resin). Increased viscosity of the medium leads to a decrease in the diffusion rate due to the hindered movement of particles (Vuleta et al., 2012).

Considering that most plant extracts are used for food and pharmaceutical products, the choice of extraction solvent is an important step. The choice of solvent depends on the characteristics of the plant material, the physico-chemical characteristics of the desired active principles and the applied extraction method. An ideal solvent has the following properties: selectivity, physiological indifference, non-toxicity, non-flammability, chemical inertness, suitable viscosity and low cost. Also, under ideal conditions, to achieve high extract purity and selectivity, the active principles should have good solubility in the selected vehicle, while other components are insoluble or minimally soluble in it. The most commonly used extraction agents are water, ethanol, methanol, propylene glycol, acetone, ether, chloroform and fatty oils. Due to the diversity of polyphenols and differences in polarity, a wide range of solvents are used for their extraction: water, ethanol, methanol, acetone, ethyl acetate and
hexane (Aziz and Rehman, 2008; Stojanova et al., 2022; Stojanova et al., 2023a).

From a toxicological point of view, in the food and pharmaceutical industry, water and ethanol are safer and more convenient than other solvents, so their mixtures are most often used in the extraction process (Tauchen et al., 2015; Stojanova et al., 2023a). Depending on the solubility of the desired components, it is necessary to determine the optimal ratio of water and ethanol. Water extracts a large number of pharmacologically active principles of herbal drugs, but also a large amount of ballast substances. In addition, it is a suitable environment for the development of microorganisms and the occurrence of oxidative and hydrolytic reactions, which cause the degradation of active substances. Also, water causes intense swelling of the plant material, and the active principles remain behind in the drug and are poorly extracted (Stojanova et al., 2023b). Due to the high boiling point, removing water from the obtained extracts is a long and uneconomical process, which threatens the stability of the active ingredients. On the other hand, ethanol is a highly selective solvent, which does not cause swelling of cell membranes. It causes protein deposition and inhibits enzymatic degradation of active principles. It has a low boiling point, and therefore its removal from the extract is facilitated. However, ethanol also has several disadvantages: easy flammability, high cost and possible presence of impurities such as methanol.

The goal is to improve the efficiency of the extraction process by increasing the yield and selectivity of desired components, minimizing or eliminating the use of toxic solvents, and minimizing energy consumption. As a result, recent extraction techniques have been directed towards developing modern methods that meet these requirements.

2.1. Ultrasound-assisted extraction (UAE)

Extraction with ultrasonic waves is an economical, simple and effective alternative to conventional extraction methods, with the use of an ultrasonic bath or an ultrasonic probe. In the last two decades, there has been an expansion of the use of ultrasonic waves with a frequency of 20 kHz to 10 MHz, for the extraction of bioactive components in the chemical and food industry. Ultrasound-assisted extraction – UAE is a key technology to achieve the principle of sustainable green extraction. The advantages of ultrasound extraction are greater reproducibility, extraction at lower temperatures, shorter extraction duration and, most importantly, higher yield and lower environmental toxicity. Also, this relatively simple
technique requires significantly less investment than other extraction techniques.

Ultrasound is defined as sound with a frequency above 15 kHz, which is the threshold for human auditory detection. The output source of ultrasound is usually a vibrating body, which causes the surrounding medium to vibrate, and then the ultrasound wave transmits energy to other neighbouring particles. Ultrasound is known to have a significant effect on the speed of various processes in the chemical and food industries. Using ultrasound, extractions are reproducible, last only a few minutes and result in reduced solvent consumption, ensuring higher yields of extracts with a higher degree of purity, eliminating the post-treatment of effluents and at the same time consuming only part of the energy required for some of the conventional extraction procedures with simple manipulation (Chemat et al., 2017).

Ultrasound waves allow the formation of bubbles in the extraction medium and cause an increase in temperature and negative pressure. The bubbles grow and burst, causing changes in the solid surface of the plant material. The mentioned mechanical and thermal effects cause damage to cell walls, release of cell contents, greater penetration of solvents inside the plant matrix and intensification of mass transfer.

Ultrasound can be used as a pretreatment or during the extraction of various molecules and biomaterials, including polysaccharides, essential oils, proteins, peptides and pigments, and bioactive molecules of commercial interest (Tiwari, 2015).

The mechanism of ultrasonic extraction is based on various physical and chemical phenomena that lead to the acceleration of mass transfer to the liquid phase, such as cavitation, vibration, crushing, mixing, friction, pressure, decompression and free radical formation (Wen et al., 2018). Cavitation, thermal and mechanical effects have a significant influence on the ultrasonic extraction process. Acoustic cavitation is the main driving force in ultrasonic extraction. Ultrasound results from a series of high-pressure (compression) and low-pressure (decompression) wave cycles induced in the molecules of the medium through which it passes. At a sufficiently high power, the decompression cycle can overcome the attractive forces of the liquid molecules and then the formation, growth and implosion of air bubbles occur. Bubbles formed in a homogeneous liquid are symmetrical and undergo symmetrical implosion leading to a sudden local increase in temperature and pressure (Mason and Peters, 2002).

A sudden jump in temperature and pressure on the surface of the plant material leads to cell wall damage. By destroying the cell wall due to the mechanical effects of ultrasonic cavitation, micro-cracks and pores are
formed on the surface of the wall, thus increasing the permeability of the wall. The intracellular contents become accessible to the solvent facilitating the release and diffusion of the target compounds from the solid to the liquid phase. When cavitation bubbles undergo implosion near a solid phase (vessel wall or suspended plant material in a liquid), they are deformed due to asymmetric implosion and a high-velocity (> 400 km/h) jet is formed that impinges on the surface of the solid phase and removes particles from it or causes its destruction (Lauterborn and Ohl, 1997). This is also the main effect of cavitation that must be taken into account when defining UAE mechanisms because it makes this process extremely efficient (Fig. 1-1).

Fig. 1-1. Ultrasonic cavitation

A cavitation bubble does not contain a vacuum because it undergoes “rectified” diffusion (this process involves the slow growth of a pulsating gas bubble due to the time-averaged mass flow into the bubble) and can grow unhindered (Crum and Fowlkes, 1986). The solvent vapours and gases (dissolved in the solvent) contained in the bubble are exposed to the extreme conditions created by the implosion. If water vapour is present in the bubble, its implosion will lead to homolytic cleavage of water molecules, producing reactive radicals HO• and H• (Schmitt et al., 1929). Newly formed radicals undergo reactions that lead to the formation of H₂O₂ and other active agents (Arzeni et al., 2012):

\[
\begin{align*}
\text{H}_2\text{O} & \rightarrow \text{HO}• + \text{H}• \\
\text{H}• + \text{O}_2 & \rightarrow \text{HO}_2• \\
2\text{HO}• & \rightarrow \text{H}_2\text{O}_2 \\
2\text{HO}_2• & \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\end{align*}
\]
Although the amount of oxidizing agents thus formed is small, they may cause the degradation of the extract if the sonication lasts for a long period (Fig. 1-2). When mixtures of water and ethanol are used as extractants in the extraction, the generation of reactive oxidation agents resulting from the breakdown of water molecules is reduced (Vinador et al., 2017). The reason for this is that during the growth of cavitation bubbles in the water-ethanol mixture, due to “corrected diffusion”, not only water enters the bubble, but also ethanol, which is more stable in terms of homolytic decomposition.

Fig. 1-2. Ultrasonic extraction from plant cells: The microscopic transverse section (TS) shows the mechanism of actions during ultrasonic extraction from cells (magnification 2000x) (Sitthiya, et al., 2018).

Fig. 1-3. Schematic representation of the major processes during ultrasonic extraction (Wen et al., 2018).
Modern methods for extraction and encapsulation of biologically active components

The application of higher ultrasound power leads to greater intensity of mixing, and more intensive destruction of the cell wall (Fig. 1-3), however, it can also lead to the degradation of sensitive compounds, and the method is usually optimized in such a way that the best results are obtained with as little power as possible (Bermúdez-Aguirre et al., 2011). Also, using a lower frequency usually results in a higher extraction yield, and frequencies from 20 to 100 kHz are mostly used (Chemat et al., 2017).

2.1.1. Parameters affecting UAE

The ability of ultrasound to cause cavitation depends on the properties of the radiation itself (frequency and power), the properties of the solvent (viscosity and surface tension) and ambient conditions (temperature and pressure). Considering that ultrasound is a mechanical wave, its characteristics can affect the acoustic cavitation and therefore the extraction process. Physical parameters such as the design (shape and size) of the ultrasonic reactors and the shape of the ultrasonic probe also affect the process (Pingret et al., 2013). The choice of solvent, drug/solvent ratio, temperature and extraction time are parameters whose influence on the extraction yield, as well as on the yield of certain bioactive compounds, has been thoroughly investigated through numerous studies (Ramić et al., 2015; Hosseini et al., 2016; Tomšík et al., 2016).

2.1.1.1. Power and frequency

The influence of power and frequency of ultrasonic waves on extraction yield and transfer phenomena is not easy to measure. Most methods are based on the approximation of energy transfer by measuring physical or chemical changes in the medium through which the ultrasound passes. Most often, physical methods are acoustic pressure measurements using hydrophones or optical microscopes, Al foil methods, and calorimetric methods. Among chemical methods, indirect measurement of OH• radicals formed by sonoluminescence and measurement with chemical dosimeters are most often used (Suslick et al., 2011).

Several studies describe that high ultrasound power causes drastic changes in the tested materials (Bermúdez-Aguirre et al., 2011), while others insist that power variation results in increased selectivity towards target molecules (Chemat et al., 2004). The most commonly used frequencies in UAE processes range from 20 to 100 kHz. The use of higher frequencies in the UAE has been investigated in only a few studies. Toma et al. (2001) noted a reduced physical impact on the structure of the plant.
material when they applied high frequencies (500 kHz) compared to low frequencies (20 kHz), while Chukwumah et al., (2009) detected different chemical composition of extracts obtained at different frequencies (25 and 80 kHz). At lower frequencies (20–40 kHz) mechanical effects are dominant including enhanced mixing, particle size reduction and solvent penetration. These mechanical effects are thought to affect the texture of the plant material and represent the processes behind the enhanced extraction capabilities of ultrasound through the formation of microjets due to non-uniform cavitation collapse near the solid surface. At higher frequencies (> 200 kHz), the shorter life of the cavitation bubble results in the release of radicals, which are inside the bubble, into the surrounding environment, where chemical effects dominate, especially at frequencies close to 850 kHz. Here, radicals affect plant material by enhancing oxidative processes, which can be beneficial or detrimental to antioxidant properties (Paniwnyk, 2017).

2.1.1.2. Intensity

Ultrasonic intensity (UI) is expressed as transmitted energy per second per square meter of emitting surface. According to UI, ultrasound can be classified into two types of sonification (Tiwari, 2015):

- low intensity sonification (< 1 W/cm²);
- high intensity (10–1,000 W/cm²).

This parameter is directly correlated with the amplitude of the ultrasound emitter and consequently with the amplitude of the sound wave pressure (Santos and Capelo, 2007). With increasing pressure amplitude, bubble implosion is more intense. To reach the cavitation threshold, a minimum ultrasonic intensity is necessary. From the extraction aspect, UI represents a relevant input value that significantly affects its efficiency (Tiwari, 2015). An increase in UI results in an increase in sonochemical effects (Mason and Lorimer, 2002). As the increase in amplitude is directly proportional to UI, it should be emphasized that high amplitudes lead to rapid deterioration of the ultrasound emitter, which results in agitation instead of liquid cavitation and poor transmission of ultrasound through the liquid medium. However, the amplitude should be increased when highly viscous fluids such as oils are used (Santos and Capelo, 2007).
2.1.1.3. Design (shape and size) of ultrasonic reactors

There is relevant scientific evidence supporting the suitability of the ultrasonic system for intensification of extraction in a shorter time, compared to the duration of conventional solid-liquid extraction, using pure "green" solvents. To fully utilize the potential of ultrasonic technology, it is necessary to optimize the process parameters. The primary parameters that should be paid attention to when using an ultrasound system are ultrasound power, ultrasound intensity and acoustic energy density because they relate to the input value of energy into the system. Today, two types of devices are in use, an ultrasonic bath and an extractor with an ultrasonic probe, commercially available for extraction processes at different frequencies. Both devices are based on the use of transducers to generate ultrasound.

![Fig. 1-4. Schematic view of the ultrasound-assisted extraction system (Moradi et al., 2018).](image)

There are two types of transducers most commonly used in ultrasound technology: piezoelectric and magnetostriective. Transducer positioning plays a vital role in determining extraction efficiency, process intensification and energy losses. Transducers can be placed on both sides of the extraction vessel so that the ultrasound waves are transmitted through the outer wall of the vessel. The main advantage of this positioning of the transducers is that they are not in direct contact with the sample, although losses of acoustic energy occur on the vessel walls and the environment. Multiple transducers are used in larger-scale flow extraction systems and continuous extraction systems. When the transducers are in direct contact with the
sample in the appropriate solvent, extraction efficiency is increased, while acoustic energy losses are minimized. Recently, the use of ultrasonic baths has been supplanted by the more frequent use of extractors with an ultrasonic probe that provides more efficient extraction in the context of higher yields in a shorter time. The type and volume of the examined material are two key factors that determine the choice of the shape of the ultrasonic probe. There are several different probe shapes, including the uniform and exponential cylinder, and the cone, which can be linear or stepped. When choosing a high-intensity probe, which can heat the medium up to 85 °C, it is necessary to take into account the temperature sensitivity of the target compounds, as well as the flammability of the solvent (Chemat et al., 2017).

Depending on the extractor type (Fig. 1-4), there are discontinuous and continuous systems. A continuous sonoreactor with several serially connected probes can work in continuous and pulse mode. Numerous authors examined the influence of these two modes of operation on the extraction yield and antioxidant activity of dried pomegranate peel. It was found that the UAE process in pulse mode is superior due to lower energy consumption, significant reduction of extraction time and increase of extraction yield and antioxidant activity (Pan et al., 2012).

2.1.1.4. Solvent

Solvent selection in the UAE process is determined by the solubility of the target metabolites, but also by physical parameters such as viscosity, surface tension and vapor pressure of the solvent. The mentioned physical parameters affect the phenomenon of acoustic cavitation, especially the cavitation threshold (Mason and Lorimer, 2002). The initiation of cavitation in a liquid requires that the negative pressure during the decompression cycle overcomes the attractive forces between the liquid molecules. An increase in viscosity or surface tension induces an increase in these molecular interactions, thus significantly increasing the cavitation threshold. In this way, when more viscous samples are being tested, the amplitude should be increased to ensure the necessary mechanical vibrations that cause cavitation. For UAE processes, a solvent with lower vapour pressure values is recommended, as bubble implosion is more intense compared to solvents with higher vapour pressure values (Flannigan and Suslick, 2010).
Modern methods for extraction and encapsulation of biologically active components

2.1.1.5. Temperature

Temperature directly affects the properties of the solvent. An increase in temperature causes a decrease in viscosity and surface tension and at the same time an increase in vapour pressure, whereby solvent vapours enter the interior of the bubbles to a greater extent, which will implode less violently and thus reduce the effects of sonification (Santos and Capelo, 2007). Therefore, sonochemical effects are favoured at lower temperatures, whose control is carried out to limit its increase (Salisová et al., 1997). From the aspect of extraction, temperature mainly contributes to its efficiency by affecting the increase in yield. In the case of UAE, some authors point out the positive effect of the temperature increase from 20 to 70 °C compared to conventional extractions (Shirsath et al., 2012). This effect is explained by an increase in the number of cavitation bubbles, a larger solid-liquid contact surface, and improved solvent diffusion with an increase in desorption and solubility of compounds. However, this effect is reduced when the temperature is close to the boiling point of the solvent, and therefore some authors suggest a positive influence of low temperature (below 30 °C) on the UAE process (Palma and Barroso, 2002; Zhang et al., 2008; Esclapez et al., 2011).

2.2. Microwave-assisted extraction (MAE)

The use of household microwave ovens in the laboratory was first mentioned in 1975 for the treatment of plant material for trace metal analysis (Abu-Samra et al., 1975), while the first extractions of organic compounds using microwaves were performed in 1986 (Ganzler et al., 1986). Since then, many laboratories have explored the possibilities of using microwaves for analytical purposes.

Microwave heating is used to extract organic compounds such as pesticides, polychlorinated biphenyls, phenols, polycyclic aromatic hydrocarbons, etc.

Microwave extraction enables rapid delivery of energy to the entire volume of solvent and plant material, with consequent heating of both the liquid and solid phases. The water inside the plant cell absorbs the microwave energy, which leads to internal heating and damage to the cell. The mentioned changes facilitate the desorption of components from the matrix and increase the release of active principles into the extraction medium. Also, the migration of dissolved ions increases the penetration of the solvent into the plant material and thereby further increases the efficiency of microwave extraction (Wang and Weller, 2006).
The main advantages of microwave extraction compared to conventional techniques are shortening the extraction time and reducing the amount of solvent. Then, reduction of solvent waste, reduced environmental impact and human exposure to the harmful effects of solvents are also advantages of this extraction technique.

Microwaves are non-ionizing electromagnetic waves with a frequency of 300 MHz to 300 GHz, positioned between X-ray and infrared waves in the electromagnetic spectrum. Microwave-assisted extraction (MAE) is based on localized, dielectric heating of water present in all natural materials, especially in plant materials. In contrast to heat transfer in conventional extraction processes, which depend on conduction/convection phenomena, in the MAE process the mass and heat gradients are equally directed (inside to outside), providing rapid heating within the solid phase where the dissolution of the constituents takes place (Fig. 1-5) (Criminna et al., 2017). The principle of microwave heating is based on two phenomena:

- ion conduction;
- dipole rotation.

![Fig. 1-5. Mass and heat transfer gradients in conventional and microwave-assisted extraction (Gomez et al., 2020).](image)

Ion conduction represents the electrophoretic migration of ions under the influence of changes in the electric field. Ionic conduction occurs because the migration of ions in the solution due to the action of the electromagnetic field causes resistance and friction, which further leads to heating (Chemat et al., 2015). As the dipole rotates, heat is generated due to friction between
the rotating molecules. These two mechanisms indicate that only dielectric solvents with permanent dipoles can be heated by microwaves. The effectiveness of microwave heating of the solvent depends on the dissipation factor, which is a measure of the ability of the solvent to absorb microwave energy and transfer it as heat to the surrounding molecules.

2.2.1. Parameters affecting MAE

2.2.1.1. Solvent

Proper solvent selection is essential to optimize the microwave extraction process. The choice of solvent is dictated by the solubility of the target analyte, the interaction between the solvent and the plant matrix, and ultimately the absorbing properties of the solvent. The solvent should have a high selectivity towards the analyte, excluding the extraction of unwanted components of the plant matrix. Solvent properties can be modified by combining different solvents, which also leads to different solvent selectivities for different compounds. For the extraction of thermolabile compounds, a combination of solvents with relatively lower dielectric properties can be used to ensure that the temperature of the solvent remains lower to cool the analytes released into the solvent. In this case, microwave energy acts favourably on the plant matrix (Kaufmann and Christen, 2002) leading to an efficient release of the compound into the solvent. Both polar (Li et al., 2004; Casazza et al., 2010) and non-polar solvents (Barnabas et al., 1995) can be used in microwave extraction. Benzene, chloroform, ether, and ethyl acetate are common solvents for extraction of less polar components, while acetone, ethanol, methanol, and water are common solvents for microwave extraction of polar compounds (Terigar et al., 2010; Tsukayama et al., 2010). Variations in solvent concentration also affect the extraction of flavonoids as observed in the case of MAE quercetin and rutin using different concentrations of ethanol. The extraction yield was found to increase when the ethanol concentration increased from 30% to 50% and decreased with further ethanol dilution (Zhang et al., 2008).

Many other examples support the fact that extraction efficiency is affected by the type of solvent used for extraction (Kiss et al., 2000; Escribano-Bailon and Santos-Buelga, 2003; Song et al., 2007) along with the different dilution levels obtained by water use (Chan et al., 2007). It has been observed that not only the composition of the solvent but also the pH value of the solvent can significantly affect the different extraction efficiency. MAE can be performed with the same solvent used for conventional extraction, although the choice of solvent for MAE should not be based on the solvent used in the conventional procedure. Microwave
extraction of ginger with hexane as solvent gave a lower yield compared to Soxhlet extraction (Alfaro et al., 2003).

On the other hand, the use of ethanol as an extractant in the MAE process provided a significantly higher yield compared to Soxhlet extraction with ethanol. This can be attributed to the difference in the dielectric properties of the solvent. Ethanol has a good absorption capacity for microwaves and therefore heats up faster and can improve the extraction process, while hexane is transparent to microwaves and therefore does not heat up with microwaves. Thus, it was established that the efficiency and selectivity of the MAE process depend to a large extent on the dielectric constant ($\varepsilon_r$) of the solvent. In most cases, solvent mixtures with good microwave heating efficiency are used, and different concentrations of ethanol and methanol are used for this purpose (Talebi et al., 2004). Among the various tested concentrations of ethanol, the use of 95% ethanol showed the best results in the microwave extraction of *Salvia miltiorrhiza* (Pan et al., 2001). A small amount of water in the extractant can easily penetrate into the cells of the plant matrix and thereby enable better heating of the matrix. This increases the mass transfer of active principles to the extractant. 80% methanol proved to be the optimal extractant for microwave extraction of chlorogenic and geniposidic acid (Li et al., 2004). Also, authors of other studies report these solvents as suitable for microwave extraction of coumarins, anthraquinones and phenolic compounds (Sterbova et al., 2004; Martino et al., 2006; Hemwimon et al., 2007). Sometimes binary mixtures of high and low absorbing solvents give optimal results. Ethanol is a relatively good absorber ($\varepsilon_r = 25.7$) of microwave energy, but it is not an adequate solvent for the extraction of solanesol. However, hexane is a good extractant for solanesol, but not a good absorber of microwave energy ($\varepsilon_r = 2.0$). Therefore, a mixture of hexane and ethanol in a ratio of 1:3 (v/v) gives the highest yield of solanesol by microwave extraction (Zhou and Liu, 2006).

The drug/solvent ratio is also a critical factor. It is known that the volume of solvent must be sufficient to ensure complete immersion of the plant material throughout the MAE process. There are many different studies regarding the drug/solvent ratio used. In general, while a higher drug/solvent ratio is effective in conventional extraction methods, in MAE processes it may be the cause of lower yields due to inadequate microwave mixing of the solvent (Wang and Weller, 2006). When examining the effect of different drug/solvent ratios on pectin yield in the MAE process (Wang et al., 2007), it was found that a lower ratio gives a higher pectin yield. Some studies advocate the opposite view, as in the case of microwave extraction of *Aretimisia annua* where a higher yield is achieved with a larger volume of solvent (Hao et al., 2002).