# GREEN EXTRACTION TECHNIQUES IN FOOD ANALYSIS

Editors:
**Merichel Plaza**
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# Current and Future Developments in Food Science

# (*Volume 3*)

# Maria Luisa Marina (Ed.)

# *Green Extraction Techniques in Food Analysis*

Edited by

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# PREFACE

In the last year, society has increased its interest in green technology due to higher awareness about the environment. Analytical chemistry plays a valuable role in the sustainable development of the planet. It is not just for pollutant monitoring in the environment, but also for the development of more sustainable processes. In this sense, the replacement of existing extraction techniques with more environmentally sustainable extraction techniques is an important step to the final greenness of the entire process. Also in food analysis, it is one of the main strategies to make it greener. One of the main aspects to be considered when processing to change from a current extraction method to a greener one is the choice of solvent. The degree of environmental impact changes according to the type of solvent used because it depends on the way natural resources are harvested, energy usage, and emissions to air and water from the production and the use of solvents, transportation, and disposal or recycling.

This book is composed of 14 chapters providing the reader with the last trends in the development and applications of green extraction techniques in food analysis. Chapter 1 presents the current state of the art of Green Analytical Chemistry and its main strategies for improving the sustainability of analytical methods, reducing their environmental impact, and offering solutions to the needs that arise from food analysis. Chapter 2 introduces the principles of green extraction techniques and makes an overview of the main green extraction techniques (such as microwave-assisted extraction, ultrasound-assisted extraction, subcritical water extraction, and supercritical fluid extraction). Chapter 3 provides an overview of the environmentally friendly solvents most commonly used in food analysis (including water, carbon dioxide, ethanol, ionic liquids, natural deep eutectic solvents, surfactants, and switchable solvents), in which the advantages and limitations of the use of these solvents in food analysis from the perspective of green analytical chemistry are described. In addition, some applications of the combination of environmentally friendly solvents with assisted extraction techniques and miniaturized techniques are discussed. In Chapters 4-6, recent applications of deep eutectic solvents, ionic liquids, and supramolecular solvents, respectively, in food analysis are described in depth. While Chapters 7-14 are focused on the applications of supercritical fluid extraction, gas-expanded liquids extraction, pressurized liquid extraction, microwave-assisted extraction, enzyme-assisted extraction, pulsed electric field, high-voltage electrical discharges, and high hydrostatic pressure in food analysis, respectively. Also, the main fundamentals/principles, instrumental setups, as well as, the advantages and drawbacks of the use of these green extraction techniques are broadly explained and detailed in these chapters.

This book is intended for a large audience comprising Ph.D. students and experts working on different topics related to food analysis with new technological and methodological approaches to make extraction techniques greener and more sustainable.

We would like to sincerely thank all authors who have collaborated in writing this book for their excellent contributions. We wish to express our scientific gratitude to them for sharing all their knowledge and experience on the development of more efficient green extraction processes.

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# CHAPTER 1

# Green Analytical Chemistry

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**Abstract:** Food analysis demands are mandatory from quality, safety, and authentication point of view, and there is an increase in analytical activity in both the control laboratory and research and development. This chapter presents the current state-of-the-art of Green Analytical Chemistry and its main strategies for improving the sustainability of analytical methods, reducing their environmental impact, and offering solutions to the needs that arise from food analysis. Direct analysis is presented as the ideal method that avoids the use of solvents or reagents and the generation of waste. Miniaturization, automation, and the use of sustainable solvents, in addition to reducing energy consumption, are the basic strategies that allow us to achieve the objectives of Green Analytical Chemistry. The reduction of single-use plastic laboratory material and their waste has also been considered an objective for analytical method greenness.

**Keywords:** Agro-solvents, assisted extraction, Automation, Bio-solvents, Chemical imaging, Direct analysis, Eco-scale, Energy consumption, Food authentication, Food quality, Food safety, Green features, Greenness, Microextraction, Miniaturization, Plastic waste, Solvent consumption.

## INTRODUCTION

Green Analytical Chemistry is a challenging strategy focused on the modification of conventional analytical methods in order to avoid or reduce the deleterious effects on both, users and the environment [1]. This green trend is of particular importance in the food analysis area, where an extremely high number of samples must be daily analysed all around the world in order to assess the food quality and food safety of raw and manufactured products. Moreover, food analysis is a diverse task and it may involve the determination of physico-chemical parameters and macronutrients, to the determination of micronutrients, bioactive components, and residues of pesticides. It also involves the detection of food adultera-

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tion, fraud, and the geographical origin of food products. Most of these studies are carried out by using reference methods and guidelines from the *Codex Alimentarius* Commission of the Joint FAO/WHO Food Standards Programme [2], the Association of Official Agricultural Chemists (AOAC) [3], and European Food Safety Authority (EFSA) [4], which typically use conventional analytical techniques that are characterized by long analysis time, laborious sample preparation, and high consumption of reagents and solvents that very often involve the generation of toxic wastes [5].

The concept of Green Analytical Chemistry was firstly proposed by de la Guardia and Ruzicka in 1995 with the novel idea of environmentally conscientious Analytical Chemistry through miniaturization, containment, and reagent replacement [6]. Later in 1999, de la Guardia proposed the "integrated environmentally friendly approach", considering the side effects of chemical measurements that can be reduced by using new strategies for sampling, sample treatment, and chemometrics [7]. In this frame, methodologies like in-field sampling, on-line analysis, microwave-assisted treatment, automation through flow analysis, decontamination or passivation processes, and surface analysis were proposed in order to achieve excellent analytical figures of merit, but also considering external factors such as environmental safety, health, and social problems. Since that time, the Green Analytical Chemistry concept has been expanded and widely discussed in several books authored by researchers, such as Anastas in 1999 [8], Koel and Kaljurand in 2010 [9], de la Guardia and Armenta in 2011 [10], de la Guardia and Garrigues in 2012 [1], and, more recently, Płotka-Wasylka and Namieśnik in 2019 [11].

Since its inception, Green Analytical Chemistry concept has been running in parallel to Green Chemistry, focusing on chemical analysis and processes, respectively. Thus, the 12 principles of Green Chemistry defined by Anastas in 1998 [12] were adapted to an analytical focus by Galuzska *et al*. in 2013 [13]. The 12 principles of Green Analytical Chemistry are shown in Fig. (**1**), and they summarize the diverse trends proposed to reduce the environmental impact of analytical procedures. From a Food Analysis perspective, the main principles to have into account are those related to the implementation of *in situ* measurements and direct analytical techniques in order to avoid or reduce sample treatment, the reduction of the number of samples to be analysed, the use of automated and miniaturized methodologies, and the preferential use of multianalyte methods, improving in all cases the level of information obtained from sample measurements.

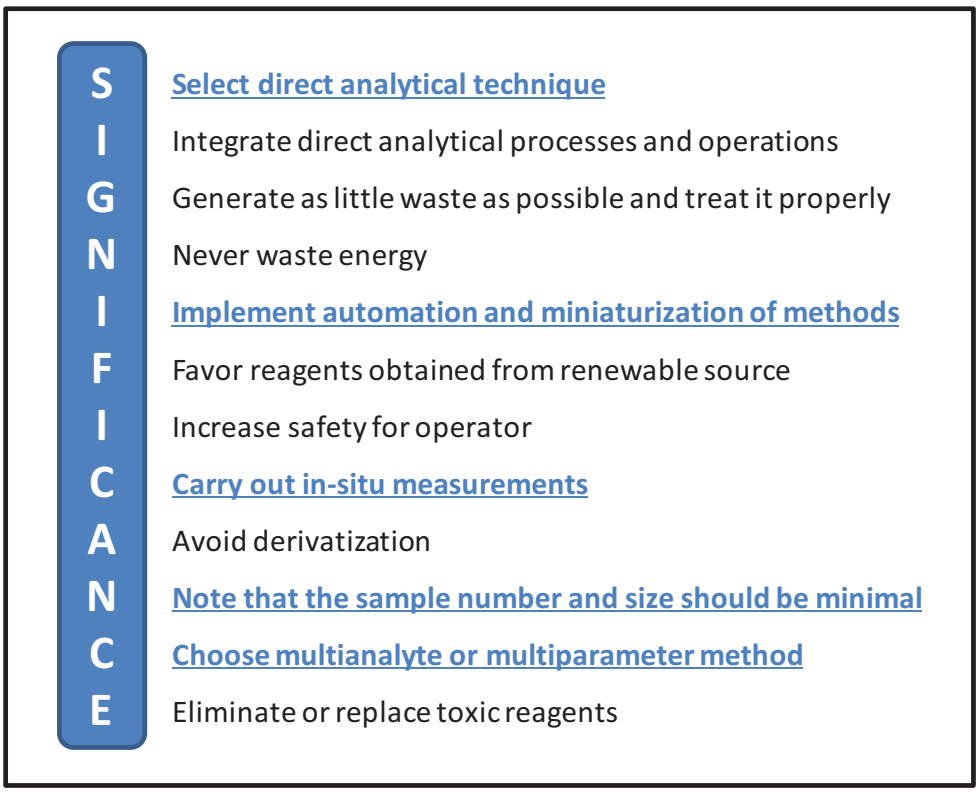


**Fig. (1).** The 12 principles of Green Analytical Chemistry (adapted from reference [13]).

During these years, several criteria have been proposed to evaluate the green character of an analytical method, such as: the National Environmental Methods Index (NEMI) [14], Green Assessment Profile [15], color scale adapted-NEMI [16], penalty points and Eco-scale [17], Green Motion tool [18], E-factor [19], Green Certificate [20], and Green Analytical Procedure Index (GAPI) [21]. However, today there is not still a common agreement among analytical chemists about what is the definitive criterion to evaluate the green character of an analytical methodology. Thus, a homogeneous guideline is still required to quantify the green features of analytical methods and evaluate their environmental impact. Nevertheless, after two decades since the inception of the Green Analytical Chemistry concept, it can be confirmed that the environmental concern has increased with a significant positive balance.

Vibrational spectroscopy is widely applied in both, food laboratories and production lines using techniques based on infrared, Raman, and Hyperspectral Image System, usually associated with chemometrics [22]. These techniques

# CHAPTER 2

# Green Extraction Techniques

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**Abstract:** Green extraction of natural products was and will always remain an important research subject in various fields. It is based on developing techniques that meet the six principles of eco-extraction. This concept responds to the challenges of the 21st century, aiming to protect the environment, the operator, and the consumer by reducing hazardous solvent consumption and by favoring the use of more environmentally friendly methods. In this chapter, we review the principles of eco-extraction in detail, followed by an overview of four methods widely used in extraction, namely ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), subcritical water extraction (SWE), and supercritical fluid extraction (SFE).

**Keywords:** Green extraction techniques, Microwave-assisted extraction (MAE), Principles, Subcritical water extraction (SWE), Supercritical fluid extraction (SFE), Ultrasound-assisted extraction (UAE).

## INTRODUCTION

Sustainable development is one of the most commonly used terms in today's debates. It is considered a development that satisfies the needs of the present without compromising the ability of future generations to respond to their own needs. This demand affects all areas of society, including chemistry. It should reflect on how chemistry can contribute to greater sustainability in our society, now and in the future.

One of the contributions of chemistry to meet the challenge of greater sustainability in the development of our society is promoting sustainable chemist-

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ry in research and industrial production. Under the name of green chemistry (or in Europe also sustainable chemistry), many efforts have been made to make the chemistry of tomorrow less toxic and less dangerous. Green chemistry aims to make chemistry more energy efficient, reduce waste disposal, and/or produce innovative products using fewer natural resources. Alternative processes and reaction routes are designed, and new materials and products are developed, helping to ensure our current requirements, but taking greater account of the interests of future generations.

Extraction is considered a key step in food processing which consists of separating the desired compounds from the raw material and transferring these compounds into a solvent. It includes several methods, such as solvent extraction, ultrasound-assisted extraction, microwave-assisted extraction, Soxhlet extraction, supercritical fluid extraction, and subcritical water extraction. In the main, natural product extraction goes through the following phases: (1) the solvent penetrates the solid matrix; (2) the solute dissolves in the solvents; (3) the solute is diffused out of the solid matrix; (4) the extracted solutes are collected. The efficiency of the extraction is conditioned by various parameters, including particle size, the extraction solvent, the solvent-to-solid ratio, the extraction temperature, and duration.

These extraction methods allow for faster and more sustainable component separation, as fewer toxic solvents [1] and energy [2] are used. In addition, they simplify manipulation and sample preparation, give high purity and yield of the final extract, and eliminate post-treatment of wastewater [3]. Numerous classes of compounds such as vitamins, sugars, proteins, lipids, fibers, aromas, pigments, antioxidants, and other organic and mineral compounds have been extracted from various matrices, mainly insects [4], plant materials [5 - 8], and animal tissues [9].

This chapter provides an overview of existing knowledge on innovating methods of sample preparation of natural products. It gives the fundamental theoretical framework and a few details about the extraction using some of the most innovative, green, fast techniques such as ultrasound-assisted extraction, microwave-assisted extraction, subcritical water extraction, and supercritical fluid extraction by detailing their principles, instrumentations, and applications in food analysis.

## GREEN EXTRACTION: DEFINITION AND PRINCIPLES

Despite the prejudicial opinions of some world leaders today, global environmental awareness continues to be on the increase. Terms such as green, biorefinery, and sustainability, are increasingly important in all facets of global development. This idea is closely associated with the principles of green

extraction, which can be defined as a process of obtaining an extract using minimal hazardous / petroleum solvents by reducing energy consumption and waste as well as ensuring safe and high-quality extracts. It is a concept that seeks to meet the challenges of the 21st century by protecting the environment and the consumers, and at the same time, increasing competition between universities and industries to be more environmentally, economically, and innovative [2, 10, 11].

According to Chemat *et al.* (2012), The list of the "Six Principles of Green Extraction of Natural Products" can be consulted by industry and scientists as a direction to establish an innovative and green label, charter, and standard, and as a reflection to innovate not only in the process but in all aspects of solid-liquid extraction. The principles have been identified and described not as rules but more as innovative examples to follow, discovered by scientists, and successfully applied by industry [2].

**Principle 1: Innovation by A Selection of Varieties and Use of Renewable Plant Resources**

The rising claim of natural products and extracts to respond to the need of food, cosmetic and pharmaceutical industries is resulting in the over-exploitation of plant resources. Using agricultural by-products became trendy, providing additional income to primary producers and industries and improving the overall agricultural value chain. Nowadays, trends in botanical by-products are to increase their added value, from organic fertilizers and raw material pellets to functional ingredients [10, 12].

Plant/crop-based resources are defined as raw materials derived from the natural flora and the transformation processes in numerous industries (food, feed, fiber, *etc*.). An underlying hypothesis is that these resources are renewable over a short period, using annual crops, perennials, and short-rotation woody species. The reuse of these plant resources as raw materials for industrial production or as a source of energy is quite limited. This is due to the poor adaptation of the hydrocarbon processing system, which, unlike these materials, was developed in a more advanced way to use fossil fuels.

Renewable plant-based resources represent a strategic option to fulfill the growing need for industrial components, enabling economic, environmental, and societal benefits. The opportunity is advantageous. Nevertheless, it requires a foresight perspective, stakeholders' integration, investment in new approaches, and coordination of research to generate a safe future.

An example can be given with the plant breeding technique. Medicinal plants for instance play an important role in prevention and treatment. As stated by the

# CHAPTER 3

# Environmentally Friendly Solvents

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**Abstract:** The present chapter aims to provide a brief overview of the environmentally friendly solvents most commonly used in food analysis, including water, carbon dioxide, ethanol, ionic liquids, (natural) deep eutectic solvents (NA)DES, surfactants, and switchable solvents. A general outlook of their properties, production sources, and classification is provided. The advantages and limitations of the use of these solvents in food analysis are evaluated from the point of view of Green Analytical Chemistry. Some recent applications have been selected to illustrate the potential of environmentally friendly solvents in combination with assisted extraction techniques and miniaturized techniques for the development of green extraction methods in food analysis.

**Keywords:** Analytical Chemistry, Environmentally friendly solvents, Food analysis, Green Chemistry, Green extraction, Green solvent, Sample preparation.

## INTRODUCTION

Environmentally friendly solvents, commonly designated as “green” solvents, respond to the need of minimizing the impact of chemical processes in the environment. These solvents show better EHS (environmental, health, and safety) properties than the traditional hazardous ones, but other aspects are also taken into consideration, such as energy flows and costs related to their production, performance, and disposal. In this context, an environmentally friendly solvent may be defined as one solvent that fulfills some of the following twelve criteria [1]: (1) availability at a large scale, (2) competitive price, (3) recyclability, (4) purity grade, (5) synthesis, (6) negligible toxicity, (7) biodegradability, (8) perfor-

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mance, (9) thermal and chemical stability, (10) flammability, (11) transport and storage, (12) renewability. The use of these solvents is encompassed under the principles of Green Analytical Chemistry (GAC) [2], which aim, among other goals, to reduce the amounts of solvents and reagents used in analytical methods along with the waste generated, and the replacement of toxic solvents. In this regard, sample preparation, which consumes more than 80% of the analysis time, is a key step in the development of any analytical application [3]. It is obvious that the greenest approach is the direct analysis of samples, but even though fields such as ambient mass spectrometry have developed in recent years, direct analysis of food is still a complex issue [4]. Therefore, the current approaches to make methods for food analysis and foodomics greener are more focused on the miniaturization of analytical techniques and the use of green solvents [5, 6].

In terms of solvent consumption, the employment of environmentally friendly solvents instead of more hazardous ones is critical in studies aiming at the recovery of bioactive compounds from natural sources to produce food-related products. The most common conventional techniques used for the extraction of valuable compounds from food and other natural sources are solid-liquid extraction (SLE) and liquid-liquid extraction (LLE). These techniques are characterized by simplicity and the use of not expensive instrumentation, but one of their main drawbacks is the consumption of considerable amounts of organic solvents [7]. Among the strategies followed to seek greener alternatives to these methods, the use of the so-called assisted extraction techniques is a widely used approach. The application of ultrasounds, microwaves, or high pressures allows the reduction of extraction time and solvent consumption. Another approach to reducing the consumption of organic solvents is the use of miniaturized extraction techniques, such as liquid-phase micro extraction techniques (LPME), or miniaturized solid-phase extraction (μSPE) [8].

According to GAC principles, the reduction of the amount of solvents used is not sufficient, but additionally, toxic solvents should be replaced by greener ones, preferably obtained from renewable sources. Therefore, it is important to have some tools that allow the classification of environmentally friendly solvents into groups, helping the analyst in the selection of the most suitable solvent or solvent mixture for a particular food application. In the next section, different selection guides are presented, and the considerations to be taken into account are discussed. Following, a detailed description of different solvents and their applications in food analysis is presented.

## CLASSIFICATION AND SELECTION OF ENVIRONMENTALLY FRIENDLY SOLVENTS

The selection of an environmentally friendly solvent for a particular application is a challenging task. Considering that the concept of green solvent is based on the fulfillment of a sort of criteria related to green chemistry [1], it is easy to figure out that there is not a straightforward definition and classification of green solvents. In addition, a one-to-one replacement of conventional-to-green solvents is difficult and has to be based on a two-side comparison: on one hand, the green solvent should own better EHS properties than the solvent to be replaced and, on the other hand, the physicochemical properties and analytical performance of the new solvent should be at least similar to the one to be replaced. To this aim, efforts have been taken to develop different computer-aided selection tools that put environmentally friendly solvents into groups.

Among the classification tools based on EHS characteristics of solvents, it is worth mentioning the solvent selection guide CHEM21, although it was designed for the pharmaceutical industry [9]. It establishes a scoring system based on environmental issues such as volatility, recyclability or bioaccumulation, health issues related to occupational hazards, and safety issues such as flammability. One of the strengths of this guide is that it is based on easy-to-access information contained in the solvents Safety Data Sheets. The outcome of the CHEM21 guide is a ranking that groups the solvents into four categories, namely highly hazardous, hazardous, problematic, and recommended solvents. Another approach is the combination of EHS information with life cycle assessment (LCA) methodology, which considers energy use during solvent production and waste treatment [10]. LCA methodology may be also applied to the entire chemical process (and not only to the solvents), which is the so-called “cradle-to-grave” approach [11]. Likewise, the “analytical Eco-Scale” is a tool designed for GAC that evaluates the analytical method as a whole, considering not only the hazards associated with the solvents but also the energy consumed by the instrumentation and the waste generated [12]. This scale starts from 100 points, from which penalty points are subtracted, thus being the greenest approach the highest scored one. It should be noted that in the last years the trend is moving towards the development of green metrics that assess the entire method of analysis, instead of focusing on a simple replacement of solvents and reagents for greener ones [13 - 15].

Regarding the tools available for the comparison of solvent properties, Hansen solubility parameters (HSP) [16, 17] or Kamlet-Taft solvatochromic parameters [18, 19] can be used to compare solvent properties. Both models consider molecular interactions such as hydrogen-bond donating ability (acidity),

# CHAPTER 4

# Recent Applications of Deep Eutectic Solvents

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**Abstract:** Among the different strategies applied in recent years for the development of green extraction techniques in food analysis, the design and use of deep eutectic solvents (DESs) have aroused the utmost attention due to the advantages provided by these materials in terms of sustainability and versatility. Different types of DESs have been applied in this field including hydrophilic and hydrophobic mixtures, natural DESs, or polymeric-DESs. In this sense, the great availability of components and the wide range of possible combinations constitute potential tools to increase the selectivity and enhance the extraction capacity of the procedures, which is an important concern when complex food samples are tackled. This broad spectrum of possibilities has allowed the extraction of diverse compounds including not only contaminants such as pesticides, plastic migrants, heavy metals, or pharmaceuticals, among others, but also the extraction of biomolecules from food and food by-products. However, despite the advantages of these materials, there are important drawbacks like their high viscosity and low volatility that limit their application. In this context, an important effort has been carried out by the study of different combinations and the development of numerous approaches. In this chapter, the most relevant applications of DESs in the last five years in food analysis have been compiled and discussed in order to provide a global view of the advantages and limitations of the application of these green extraction solvents in the field. Additionally, the current trends and future perspectives in the use of DESs in food analysis are also pointed out.

**Keywords:** Biomolecule, Bioactive compound, Extraction technique, Food safety, Green solvent, Sustainability.

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## INTRODUCTION

Solvents represent the largest proportion of substances used in chemical protocols. Thus, it is not surprising that, within the framework of green chemistry, several efforts have done to develop green solvents [1] due to the contaminant power and typical intrinsic toxicity of the traditional ones obtained from fossil feedstocks. In this regard, apart from water and $CO_2$, several bio-based molecular solvents [1] (substances available from low value and extensively accessible biomass resources), supramolecular solvents [2] (nanostructured liquids composed of three-dimensional amphiphilic aggregates), and low transition temperature mixtures [3] (LTTMs, solvents made from mixtures of compounds with a low transition temperature between liquid and solid-state) have been proposed.

Different families of substances can be classified as LTTMs [3], including ionic liquids (ILs) and DESs (from the Greek word "ευτύπος" *eutēktos* -easily melted). Generally speaking, LTTMs share similar physicochemical properties (*e.g.*, low vapor pressure, high density, low volatility, *etc*.) and components. This is the reason why some of these solvents are sometimes classified as DESs or ILs [3], depending on the particular application. Although some of them are formed by a similar process (and for this reason, there is some confusion in the nomenclature), ILs are normally recognized as molten salt liquid at room temperature. Meanwhile, DESs can be defined as mixtures of at least two constituents (a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD)) that remain liquid over a wide range of temperatures (also room temperature) [4]. The main force driving the formation of DESs is strong hydrogen bond interaction, but other forces, such as Van der Waals, dipole, alkyl-alkyl, halogen bonds, and other electrostatic interactions, are also involved [4]. As a result of those interactions (and the corresponding charge delocalization), the eutectic temperature is significantly lower than the melting point of the specific constituents and also much lower than expected from the known enthalpies of fusion of the pure components. Strictly speaking, the eutectic point is only achieved at a particular molar ratio of the individual constituents (Fig. **1**) and, thus, the term DESs should be only employed for this mixture. However, different mixtures present melting temperatures lower than the predictable, which are frequently included in this category (although some authors prefer the term "eutectic mixtures"). It should be noted that certain particular DESs (for example those containing $SnCl_2$, $AlCl_3$, and $FeCl_3$ mixed with imidazolium chlorides) have two different eutectic points [5].

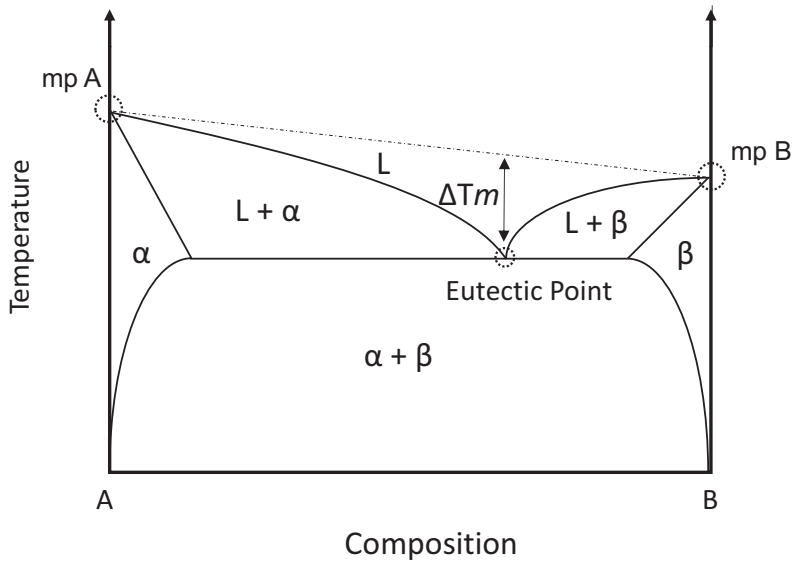


**Fig. (1).** Schematic representation of the phase diagram for a binary eutectic mixture. L: liquid phase, α: solid rich in component A, β: solid rich in component B.

Typical HBDs are alcohols, amides, amines, azole, carboxylic acids, imidazole, phenol, thiourea, urea, and water, whereas conventional HBAs comprise dication-based, imidazolium-based, quaternary phosphonium, and quaternary ammonium salts, inner salts, and molecular imidazole and its analogs.

Archetypally, DESs can be described by the formula $C^+X^-zY$, where $C^+$ is a cation of a salt, $X^-$ refers to a Lewis base (such as the halide anion of the salt), and z is the number of units of a Y Lewis or Brønsted acid or HBD molecule [5]. Initially, the vast majority of DESs were classified into four different types [5], attending to the nature of the components. This way, type I DESs are those formed by a salt and metal halide; type II are composed of salts and metal halide hydrates; type III by salts and HBDs; and type IV contain metal chloride hydrates and HBDs. Moreover, the search for non-ionic DESs, that overcome the issues associated with salt-based DESs, led to the emergence of type V, which are characterized by a mixture of molecular components. It should be mentioned that in those DESs formed by non-ionic components, it is not easily distinguishable which component acts as HBD and which one as HBA.

Apart from the nature of the components, DESs can also be classified according to their hydrophobicity and to the origin of their components. As the name

# CHAPTER 5

# Ionic Liquids

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**Abstract:** The significant potential of ionic liquids (ILs) in the extraction and separation of valuable products from food samples is deeply discussed in this chapter, where the main studies on the application of ionic liquids to food analysis are presented. The novel extraction strategies reviewed in this chapter have the potential to significantly enhance the extraction yield, in particular when the combination of ionic liquids with accelerated and green extraction techniques, such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) or subcritical water extraction (SBWE) are used. ILs are considered environmentally-friendly solvents and they offer some advantageous properties which are particularly relevant in extraction systems in food matrices, such as their low toxicity and volatility and different polarity, hydrophobicity and selectivity. A particular section is devoted to microextraction techniques with ionic liquids, which have shown great performance in the extraction of valuable compounds for a variety of food samples. This chapter summarizes and gives an overview of the latest developments and applications of ILs in the extraction of bioactive compounds from food.

**Keywords:** Bioactive compounds, Food analysis, Green extraction techniques, Ionic liquids, Microextraction, Sustainable solvents.

## INTRODUCTION

One of the main reasons for the gradual implementation of green extraction techniques in analytical procedures is the increasing awareness of the use of high amounts of potentially toxic and non-sustainable organic solvents in traditional extraction methods. The replacement of solvents with emerging green alternatives, such as deep eutectic solvents, ionic liquids, and non-ionic surfactants, is in line with sustainable development strategies [1]. Ionic liquids (ILs) have been proposed as environmentally-friendly alternatives in their use as extraction solvents in food analysis. Their use in combination with advanced and sustainable extraction techniques, such as microwave-assisted extraction (MAE),

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ultrasound-assisted extraction (UAE) and supercritical/subcritical extraction techniques is considered an adequate alternative to traditional solid-liquid methods. In particular, their use in food matrices can be considered a very promising possibility with multiple applications. ILs have been the subject of a number of interesting and recent reviews and they have raised great attention since their introduction during the 90s [2]. Most of these reviews deal with general issues in the use of ILs under different extraction conditions [3 - 5] and specific applications, including food analysis [6 - 9].

It is noteworthy that ILs can also be considered as one of the most fascinating "green" solvents for food analysis owing to their high thermal stability (higher than 300 ºC in most of the cases) along with very low vapour pressure and the huge number of possibilities in their application, including their use in miniaturized systems [10]. ILs are organic salts with low melting temperature (below 100 ºC), consisting of large organic cations and organic or inorganic anions. They exhibit unique characteristics, such as high ionic conductivity, low flammability and non-volatility among others, making them environmentally-friendly alternatives to traditional organic solvents commonly used in the extraction of food components. Additionally, their high viscosity and surface tension lead to the formation of stable phase boundaries, enhancing the easy extraction of analytes.

These outstanding properties make ionic liquids the ideal candidates as green solvents and hardeners in many extraction processes. In particular, halogen-free ILs are more advantageous for environmental reasons due to their low toxicity, being excellent solvents for food analysis. As an example, Husson *et al.* [11] indicated that acidic ionic liquids are good solvents for fast esterification of many food components, particularly lipids, without the need of any catalyst. Hence, the use of ILs as green solvents for the selective extraction of polyacids from food samples can modify the food structure without any additional catalyst in the esterification process [12]. The main aim of this chapter is to perform a general overview of the use of ionic liquids in food analysis, particularly in the extraction of valuable components coupled with advanced and sustainable extraction methods.

## CHEMICAL STRUCTURE AND PROPERTIES OF IONIC LIQUIDS

Ionic liquids are liquid organic salts with low melting point, which are usually made up of a large organic cation and a smaller inorganic or organic anion [4, 6]. Those ILs with melting points at or below room temperature are defined as room temperature ionic liquids (RTILs) [13] and they are highly valuable since their use leads to significant energy savings during extraction. The origin of ILs dates back

to 1914, when Walden [14] succeeded in the synthesis of ethylammonium nitrate ($[C_2NH_3][NO_3]$); with a melting point of 12 °C [6]. However, the interest in the use of ILs as alternative to conventional solvents has raised only in the last three decades and their research and development have become a milestone in different scientific applications, including their use as catalysts in organic synthesis and separation processes, as part of lithium-ion batteries and sensors, thermal fluids, biomass solvents, lubricant additives or as a $CO_2$ absorption medium [15].

Some of the most common organic cations that make up the structure of ILs are those based on imidazolium, pyridinium, pyrrolidinium, triazolium, thiazolium, piperidinium, sulfonium, phosphonium or ammonium. In the same way, anions can be both inorganic and organic, including magnetic anions. Inorganic anions include halides (such as $Cl^-$, $Br^-$ and $I^-$), tetrafluoroborate ($BF_4^-$) and hexafluorophosphate ($PF_6^-$). Organic anions include acetate, bis(trifluoromethylsulfonyl)imide ($[NTf_2]^-$), trifluoro-methanesulfonate ($[OTf]^-$), trifluoroacetate, bis(pentafluoroethylsulfonyl) imide ($[BETI]^-$) and many others. In addition, the incorporation of paramagnetic components, including metal ions (Mn, Fe, Co and Ni) and rare earths (Gd and Dy), into the IL structure has raised some interest in recent years [16, 17]. Some examples include tetrachloroferrate, tetrachloromanganate$^{2-}$ and tetrachlorocobaltate$^{2-}$. The structure and nomenclature of the most common cations and anions in ILs are shown in Fig. (**1**).

The large number of potential combinations of cations and anions in the formation of ILs results in their unique and characteristic properties, including their broad range of working temperatures, high polarities, variable viscosities, low charge densities, reasonable ionic conductivities and low electrical conductivities [18]. Their miscibility with water and organic solvents can be controlled by the different cation/anion combination, their ratio or by incorporating certain functional groups to the IL structure, resulting in tailor-made compounds for specific applications. In addition, their very low vapour pressure (associated with low combustibility and high thermal stability), as well as their non-explosive and minimally corrosive character, give ILs a clear environmentally-friendly nature [4]. However, not all ILs can be classified as fully sustainable, since some of them have been reported as toxic when incorporated into the environment [19 - 22].

A summary of those unique properties of ILs, which make them especially valuable as extraction media is shown in Fig. (**2**). As mentioned above, one of the most important characteristics of ILs is their negligible vapour pressure, making unnecessary the inclusion of distillation processes in the overall extraction methods. However, it has been observed that vapour pressure should not be considered negligible at very low pressures and temperatures above 200 or 300

# CHAPTER 6

# Supramolecular Solvents

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**Abstract:** Supramolecular solvents (SUPRASs) are becoming more and more demanded for sample preparation in food analysis. Their inherent properties (*e.g.* different polarity microenvironments, multiple binding sites, discontinuous nature, easy tailoring of their properties, *etc*.) make them highly efficient for the extraction of single- and multi-class contaminants in food matrices. Likewise, they offer numerous opportunities for the development of innovative sample treatment platforms not attainable by conventional solvents. In this chapter, the fundamentals underlying the production of SUPRASs and their more relevant properties regarding their application to the extraction of food contaminants are discussed. An overview of representative developments in this field is given based on the different types of SUPRASs applied so far in food analysis. Major achievements attained, mainly related to the extraction of single- and multi-components prior to their quantification by liquid chromatography coupled to different detection systems, are critically presented. The main challenges to be faced in order to get SUPRAS-based methodologies that meet European requirements for screening/quantification of contaminants in food and promote their use in food control labs are discussed.

**Keywords:** Coacervates, Cloud point, Extraction, Food analysis, Liquid chromatography-mass spectrometry, Supramolecular solvents.

## INTRODUCTION

Supramolecular solvents (SUPRASs), tailored and green nanostructured liquids consisting of self-assembled amphiphilic aggregates, have gained momentum in recent years for sample treatment in analytical chemistry [1]. Major driving forces for their increasing demand in this field are the potential of SUPRASs for facing the challenges that the detection/quantification of contaminants have in areas such as food quality assurance [2], environmental analysis [3], anti-doping control [4], and so on.

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Developing effective sample treatments in food analysis has to cope with highly demanding requirements. Among them, the high chemical structural variability of the analytes, the required low detection limits, the need for high specificity to avoid false-positive and negative findings; and the high number of samples to be analysed while delivering results in a short time, are worth noting. Thus, regulated and unregulated chemicals in food are very diverse and originate from different sources along the food chain (crop, transport, storage, processing, packaging, *etc*.). Regulated chemicals in food include a variety of contaminants such as mycotoxins, dioxins, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), residues of veterinary medicines, hormones, pesticides and so on. Unregulated food contaminants are not routinely controlled, despite they could exert a risk to public health. One of the most important sources of unknown potentially toxic food contaminants is food contact materials (FCMs) [5], which constitute a hot topic and a priority for the European Food Safety Authority (EFSA) [6]. Global lists for FCMs do not usually take into account the so-called non-intentionally added substances (NIAS), impurities, by-products or degradation products that originated during food processing or come from recycling and that could constitute the majority of chemicals present in a product [7]. So, effective food quality control requires *multi-methods* for dealing with the huge number of chemicals under regulation, as well as proactive approaches to cope with the potential harm of unknown chemicals and fraudulent practices.

Nowadays, analytical method requirements for food control of regulated substances are well established [8, 9]. Mass spectrometry (MS), combined with liquid chromatography (LC) and gas chromatography (GC), has become the primary technique for confirmatory methods in food control since they provide suitable identification points for legislated chemicals. For this purpose, LC-MS/MS using electrospray (ESI) or atmospheric pressure chemical ionisation (APCI) and GC-MS using electron impact (EI) are routinely used, whose selection mainly depends on the compound polarity and technique availability [10]. GC-head space (HS) injectors have gained popularity in routine analysis, given their potential to perform clean-up in an easy automated way through the selective desorption of analytes from the matrix.

Suspect and non-target screening analysis is nowadays of great interest for identifying emerging food safety issues at their early stages. To address this interest, LC-MS with time-of-flight (TOF, QTOF) and Orbitrap analysers have become the most suitable techniques due to their high capacity for compound identification on the basis of mass accuracy and isotopic pattern [11, 12]. On the other hand, ambient mass spectrometry (AMS) is an emerging approach based on new ion sources capable of desorbing the analytes directly from the sample with minimal or no sample treatment and of ionising chemicals under ambient

conditions. AMS is gaining interest in food fraud detection given the possibility of generating results quicker than conventional MS techniques [13, 14].

On the whole, sophisticated processes are mostly required for food quality control. So, there is a need for quick but accurate screening and quantification methods in order to make them affordable and not hamper trade [15]. In this respect, comprehensive MS-based screening and confirmation analytical platforms able to identify and quantify as many as possible *single- or multi-class analytes* in a run should be developed. To fulfil this demand, suitable sample treatments for efficient isolation of a wide polarity range of chemicals while providing also effective sample clean-up should be developed [16]. In short, there is a need for greener, simple and quick matrix- and analyte-independent sample treatments, which are compatible with LC- and GC- combined with MS, and emerging techniques such as AMS. In this respect, green SUPRASs, tailored to meet specific functionalities, represent a great promise for developing innovative sample treatments.

This book chapter deals with some fundamentals and characteristics of SUPRASs relevant for their application to the extraction of contaminants in food, the achievements attained so far in this area, and the ongoing challenges that need to be confronted to make SUPRAS competitive extractants in food analysis.

## SYNTHESIS OF SUPRAMOLECULAR SOLVENTS

The synthesis of SUPRASs occurs through a bottom-up approach in which amphiphilic molecules in colloidal solutions undergo self-assembly and coacervation under environmental conditions where amphiphile-amphiphile interactions predominate over amphiphile-solvent interactions [17]. The general synthesis procedure comprises two steps (Fig. **1**). First, amphiphilic molecules are in an aqueous or organic solution above the critical aggregation concentration (*cac*), forming a colloidal suspension. The aggregates in suspension are usually aqueous or reversed micelles or vesicles. Second, the size of the supramolecular aggregates can be controlled by changing the environmental conditions of the colloidal suspension through the action of a coacervation-inducing agent (*e.g*. pH or temperature modification, the addition of inorganic and organic salts or poor solvents for the amphiphile). As a result, the aggregates grow into oily droplets, which spontaneously associate in clusters of individual droplets. The density of these clusters differs from that of the synthesis solution, so they are separated into a new liquid phase (SUPRAS) by flocculation or precipitation. The SUPRAS is not a continuous phase but is composed of coacervate droplets clearly visible by optical microscopy. This phase is in equilibrium with the solution from which it formed (bulk solution), which contains the amphiphile at the *cac* [18].

**CHAPTER 7**

# Supercritical Fluid Extraction

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**Abstract:** In this chapter, we highlight the basic concepts behind the use of SFE to select molecules present in food matrices, *e.g.*, carotenoids, essential oils, waxes, and phenolic compounds. Also, we highlight the SFE equipment setup, the methods for process intensification, and mass transfer mechanisms involved in the process, besides the advantages and drawbacks. Supercritical fluids have been suggested as a powerful tool to improve the performance of analytical methods in terms of reduced steps for sample preparation and waste generation, besides enhanced precision and recovery of analytes detected. The offline association of SFE with analytical detection has been elucidated for decades. Currently, many efforts have been made to reach the miniaturization of equipment as well as the online hyphenation between extraction and analytical detection with supercritical fluids as a novel method for sample preparation to detect food analytes in real time with accuracy and robustness.

**Keywords:** Analytical chemistry, Chromatography, Food bioactive, Overall extraction curves, Sample preparation, Supercritical $CO_2$.

## INTRODUCTION

Supercritical fluid extraction (SFE) is a green technology widely used to extract active principles present in varied matrices for multiple applications, including foods, pharmaceuticals, and cosmetics. A significant number of works have sho-

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wn that supercritical fluids are more effective in extracting target compounds than conventional methods that use organic solvents (methanol, acetone, and hexane) [1, 2].

Supercritical carbon dioxide (SC-$CO_2$) is the most used supercritical fluid due to its mild critical point (31.1 °C and 7.38 MPa), low cost, and non-flammability. Compared with conventional solvent extraction and steam distillation, $CO_2$ does not interact with the analytes in the sample, providing extracts of high quality in terms of target compounds, including carotenoids, phenolic compounds, and tocotrienols, which are extensively required by food industries.

In food analysis, SFE has been used as a sample preparation protocol before detecting target compounds in an analytical instrument. In chromatographic methods, the use of SC-$CO_2$ as mobile phase reduced retention times of compounds, and enhanced sensitivity over other methods, including HPLC and GC. Such advantages supported works on the optimization of supercritical fluid chromatography (SFC) and the implementation of direct hyphenation of SFE of matrix with the SFC or other analytical instruments for the real-time acquisition of data [3].

Efforts have been made to develop robust and accurate analytical methods to identify and quantify target compounds in food samples, including the miniaturization of SFE hyphenated with chromatographic techniques [4 - 6]. The direct hyphenation of SFE and analytical detection has been proposed to reduce the analysis time and generation of waste solvents. However, direct hyphenation represents a difficult task because of damage to the detector or lack of stability in the baseline induced by high pressure [7, 8].

In this chapter, we introduce recent findings on the use of SFE to obtain target compounds present in food samples, as well as the theoretical aspects behind SFE and the equipment used. Also, we discuss the factors that justify the selection of SFE to analyze foods, and the current developments to include supercritical technology as an analytical method to improve the reliability of methods used in food analysis.

## FUNDAMENTALS ABOUT SFE

### Properties and Procedure

A substance becomes a supercritical fluid when subjected to temperature and pressure above its critical values [1]. At a supercritical state, there are no distinctions between the liquid and vapor phases [9]. Some properties can be

significantly tuned by increasing the temperature and/or pressure above their critical values.

Supercritical fluids have a density close to a liquid, whereas their viscosity is close to that of gas. In contrast, its diffusivity between liquid and gas values enhances transport properties, favoring solute extraction from the solid matrix [3, 4]. Likewise, the solvent density is easily tuned by temperature and pressure changes, which effectively modifies the solubility of the solute in the solvent, inducing high selectivity [10-12]. Thus, the flexibility of SFE that allows modification of the solvating power and its selectivity are the attractions of the process [13-15].

Specifically, the thermodynamic properties of carbon dioxide ($CO_2$) attract attention and make it the most commonly used solvent in food applications. Carbon dioxide presents a mild critical point (Tc = 31.1 °C, Pc = 7.38 MPa); it is nontoxic, nonexplosive, has low cost, commercially available, and has an environmentally friendly nature [3, 4, 7 - 9]. In addition, $CO_2$ was designated as a GRAS (generally recognized as safe) solvent for the food industry. Additionally, $CO_2$ is a gas at room temperature, and 1 atm (0.1 MPa), making it possible to obtain a solvent-free extract by depressurization, which is applicable while making bioactive compounds [16]. For these reasons, $CO_2$ has been used as a supercritical solvent in over 90% of SFE applications [13].

On the other hand, the physical properties of $CO_2$ limit its use in the extraction of compounds with low polarity or nonpolar compounds. A polar cosolvent (also known as a modifier) can be added to $CO_2$ in small amounts (usually 1 to 15%) [17] to improve the solubility of polar compounds in $CO_2$. Due to its low toxicity, anhydrous ethanol or aqueous ethanol is the most common cosolvent reported in food applications [18 - 21]. There are also reports in the literature regarding the use of water, methanol, ethyl ether, formic acid, acetic acid, acetone, and isopropanol as cosolvents [12, 14]. Likewise, cosolvents such as ethyl lactate, vegetable oils, and ethyl acetate combined with $CO_2$ (less than 10% of the total) have been applied [22].

Concerning the process, the SFE of solid matrices comprises two essential steps: extraction and separation. Firstly, the solvent must be tuned to a supercritical state, *i.e.*, the fluid is heated and pressurized, reaching the desired operating temperature and pressure. Once the fluid has reached the supercritical state, it flows through the extractor filled with the pretreated sample, extracting the solid matrix's solute by convection and diffusion. Afterward, the dissolved compounds pass through the separator, where they are separated from the solvent. The solute

# CHAPTER 8

# Gas-Expanded Liquids Extraction

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**Abstract:** Gas Expanded Liquids (GXL) are mixtures of liquid solvents (organic, water) and gases or supercritical fluids with diverse physicochemical properties halfway between pure liquids and supercritical fluids. The possibility of changing their properties by introducing small changes in pressure, temperature, and/or solvent/gas ratio, makes these solvents a very interesting and appropriate option for developing green extraction protocols for food analysis. In general, GXLs have similar densities as the solvent used in their composition, while having improved mass transfer through reduced viscosity, increased solute diffusivity, and decreased interfacial tension. Some other advantages are related to the wide range of polarities that can be obtained, depending on the liquid selected. Moreover, the substitution of a liquid fraction for a gas reduces the final use of organic solvent, thus improving the green character of GXLs. In the present chapter, the physicochemical properties of GXL are addressed along together with the description of applications in the food science and technology area.

**Keywords:** Biorefinery, By-products, Food analysis, Gas-expanded liquids, Green solvent, Natural compounds.

## INTRODUCTION

Sustainable Development Goals (SDGs) are at present guiding the action of countries, governments, industries, and people since we have great challenges in front that are necessary to address for the future of humankind. The high levels of pollution, food waste, increasing poverty, and the accelerated degradation of natural resources are only some of the challenges we are facing; the shift towards

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SDGs in all areas is necessary, and food analysis can contribute by greening the different steps involved in any analytical protocol and linking them to Green Chemistry and Green Analytical Chemistry (GAC) principles, as suggested in a very recent opinion paper [1].

According to United Nations (https://www.un.org/sustainabledevelopment/), energy is the main contributor to climate change, accounting for about 60% of all global greenhouse gas emissions. In fact, in 2015, only 17.5% of final energy consumption worldwide came from renewable sources. In this sense, research has the challenge of developing increasingly cleaner and safer processes that incorporate high energy efficiency, while companies need to move forward in decreasing energy consumption or using renewable energy sources as a competitive strategy towards sustainable industrialization.

Therefore, green technologies (considered this way not only the use of green solvents but also energy-efficient practices) are at the forefront when applied to the extraction of bioactive metabolites and to food analysis.

This book chapter is structured in three parts: 1) some general features about GXL solvents such as fundamental principles, parameters, and basic equipment, 2) some applications of GXL extraction in natural sources as highly efficient alternative for obtaining bioactive metabolites from natural sources and by-products, food wastes and agricultural residues, and 3) examples of multi-fluid platforms using GXL involved in biorefinery approach that can be run at analytical or processing scale.

## GREEN EXTRACTION TECHNIQUE: MEAN FEATURES

In the previous chapter, the reader has entered deeply into the Supercritical Fluid Extraction world, which is highly connected with the Gas Expanded Liquid Extraction (GXLE). Before knowing more about extraction using Gas Expanded Liquids (GXL), this type of solvents will be introduced to let the reader know more about the composition, types, physical properties, and the effects of physical parameters such as pressure, temperature, and gas molar ratio on solvents' properties.

### Fundamentals of GXL

An organic solvent that dissolves, partially or totally, a compressible gas or a supercritical fluid, composes GXLs. The gas phase is commonly carbon dioxide, ethane, or ethylene, but due to the environmental and economic advantages, and safety of $CO_2$, it is the main fluid used in GXLs. When carbon dioxide is employed under GXLs conditions, it is named as carbon dioxide expanded-liquids

(CXL). The transformation from a pure solvent to a GXL by increasing pressure and addition of gas is shown in Fig. (**1**). GXL can be defined as *switchable solvents* since they can be easily turned back to a pure solvent just by decreasing pressure or removing the gas phase. In fact, this switchability of GXL and the common use of $CO_2$ have been used to define GXL as "*a type of switchable solvents that is halfway from pressurized liquids to supercritical fluids by increasing the amount of compressed* $CO_2$ [2-4]". They are often referred to as "enhanced fluidity solvents" when liquid phase is under subcritical conditions [5]. The advantages of using GXLs are similar to those described for supercritical fluids, such as higher gas diffusivity and enhanced transport properties in solvents, but the extraction conditions using GXL can be milder than those required for SFE. The possibility offered by GXL of introducing a gas or a fluid such as supercritical $CO_2$ for producing multiple immiscible phases with enhanced fluidity is also interesting.

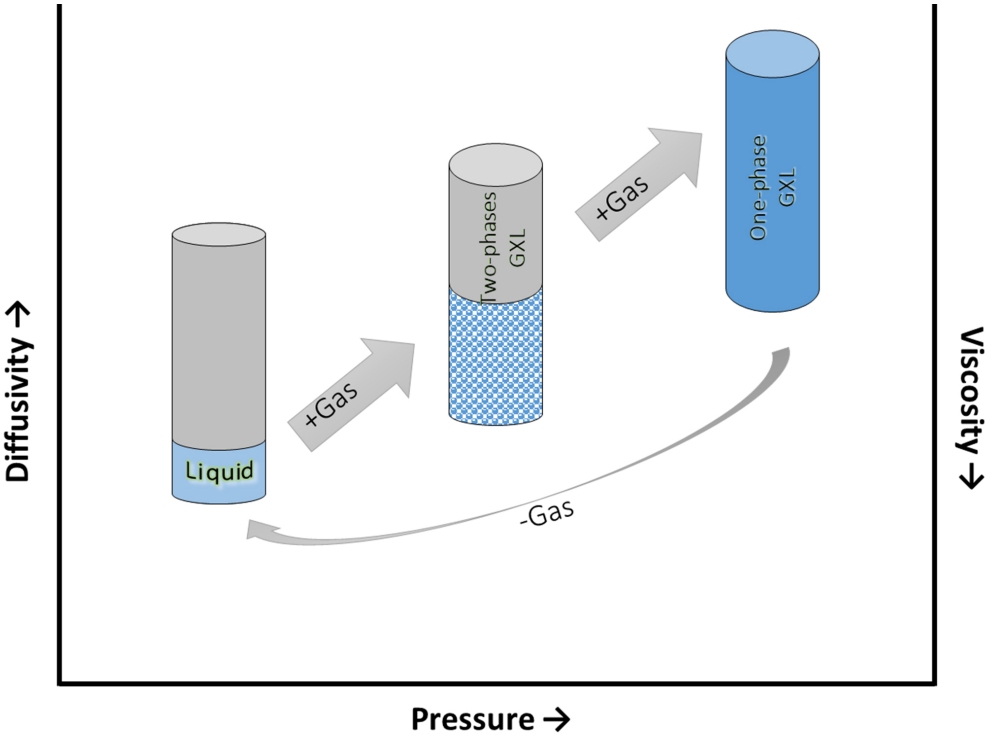


**Fig. (1).** Scheme of GXL formation from neat liquid and main physicochemical parameters affected.

It is important to note that in this chapter we will talk about gas phase or supercritical phase indistinctly in contrast with the liquid phase. As can be seen in

# CHAPTER 9

# Pressurized Liquid Extraction (PLE)

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**Abstract:** Pressurized liquid extraction (PLE) is regarded as an emergent extraction technique; it is an appropriate tool to obtain green extracts from foods or related samples. Studies on the content of contaminants in foods or food raw materials can be carried out by PLE. In the same way, studies on the obtention of bioactive extracts from classic and emerging foods and their by-products can be carried out by PLE too. Besides sequential process combinations of PLE with other innovative extraction techniques could generate benefits for the food industry. The objective of this chapter is to clearly define the role that this technique plays in food analysis, as well as the updated spectrum of some of its applications during the last lustrum.

**Keywords:** Bioactive extracts, Food analysis, Green extraction, Instrumental analysis techniques, Pressurized liquid extraction.

## INTRODUCTION

The pressurized liquid extraction (PLE) technique was developed in 1995 by the Dionex Corporation under the name Accelerated solvent extraction (ASE®). It is also known as pressurized hot solvent extraction (PHSE) and if it uses water as the solvent, it can also be called subcritical water extraction (SWE) or pressurized hot water extraction (PHWE) [1]. PLE is used in chemical analysis to undertake exhaustive extractions from solid samples in short periods of time with small amounts of solvent [2]. With the growth of green chemistry, the use of environmentally friendly solvents under PLE allows the recovery of harmless

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extracts and extraction by-products [3]. Fig. (**1**) shows some basic applications of PLE in food analysis, classic and emerging foods, or their by-products. These matrices can be subjected to a PLE to obtain an enriched extract with the contaminants, or the bioactive compounds from the initial sample.

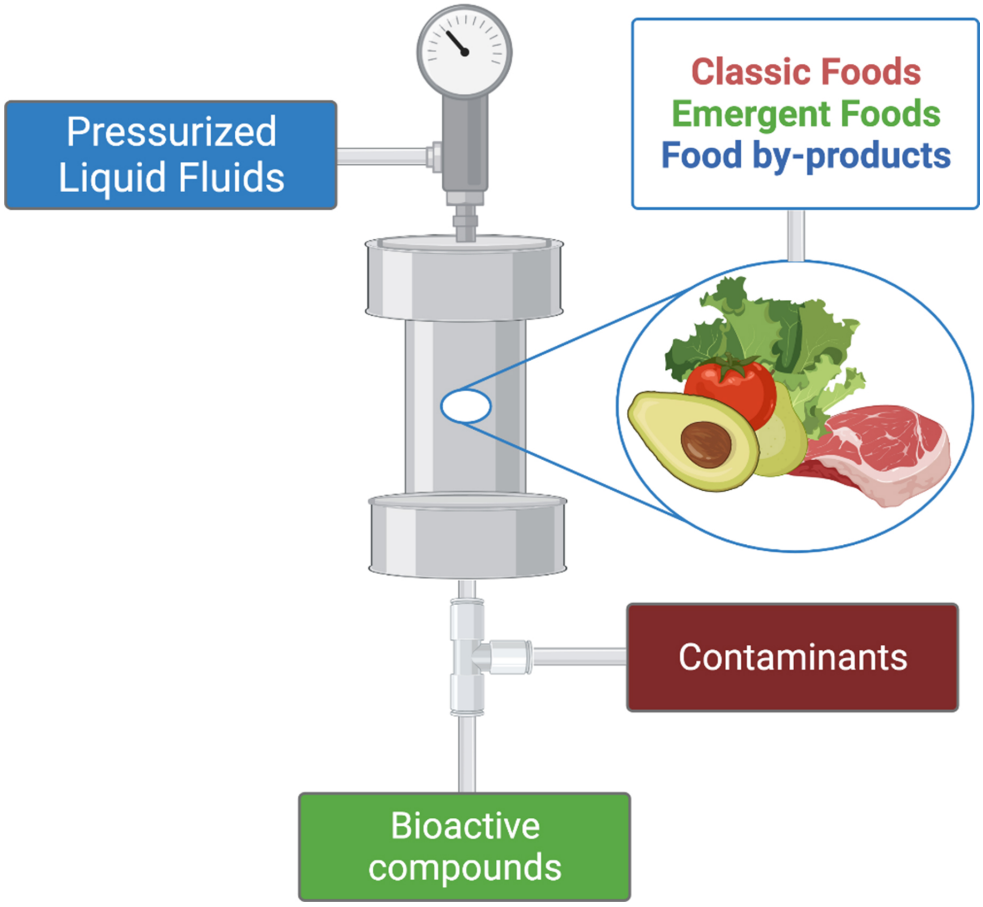


**Fig. (1).** PLE basic applications in food analysis. *Created with BioRender.com*.

PLE consists of exposing the sample for extraction to pressurized solvents (pressures lower than the critical pressure) and temperatures higher than those of their boiling point, thus ensuring that the solvent remains in its liquid phase [4]. This technique was initially employed in the analysis of soils contaminated with organic compounds (PCBs, PHAs) [5]. The Environmental Protection Agency (EPA) of the U.S. government has now established the use of PLE in the analysis of some contaminants in soil samples [6]. More recently the use of PLE has been

growing relative to other emerging extraction techniques such as supercritical fluid extraction (SFE). As shown in Fig. (**2**), two decades ago the relationship of works that "mentioned SFE" topic to those that "mentioned PLE" topic was 2:1 approximately. Nowadays that relationship is close to 1:1. At this time, the works that mention the "PLE" topic has increased from 293 to 2414.

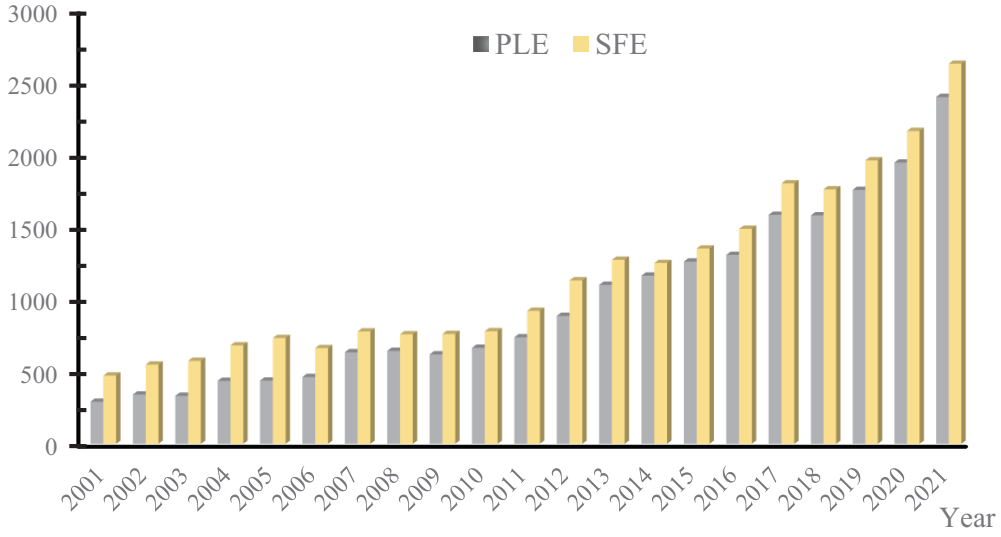


**Fig. (2).** Ratio of works that mention the PLE topic vs the SFE topic.

The main uses of PLE in food analysis consist of obtaining extracts from biomasses commonly used for food preparation, emerging biomasses, or their corresponding by-products. Different applications can be given to PLE using green solvents [3, 7]: Food valorization using green techniques [8], recovery of bioactive compounds or extracts from biomasses [9], obtention of antioxidants from agro-industrial by-products [10], isolation of organic contaminants from food samples or food used materials [11, 12], as is the case of ethylene polymers content [13, 14].

In this chapter, we will deal with the basic principles of PLE and its experimental conditions as well as the equipment and the extraction methods used in PLE. We will consider the combination of PLE with other extraction and analysis techniques, and we will finish by reviewing the use of PLE to obtain extracts from traditional or promising foods and from their by-products as well as from inputs used in the food industry, for example, the raw packaging materials.

**CHAPTER 10**

# Microwave-Assisted Extraction (MAE)

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**Abstract:** Microwave assistance is an optimum strategy to shorten time, solvent, and energy consumption during the extraction of target solutes from different sources. This intensification strategy has been successfully applied to laboratory methods to enhance the extraction performance of a number of bioactive compounds of interest for food, cosmetic and pharmaceutical applications. This chapter presents an overview of the fundamentals, equipment configurations, combinations with other techniques, and some representative applications for the extraction of compounds from food products and byproducts.

**Keywords:** Bioactive compounds, By-products, Biomass, Green extraction, Microwave treatment.

## INTRODUCTION

Plant bioactives are an excellent resource that is gaining commercial interest and in order to provide standardized products, reliable extraction methods both for analysis and for industrial production are needed. Understanding the cellular and subcellular localization of plant metabolites is essential to study their extraction. Usually, the target solutes are not readily accessible to the extracting solvent and a previous pretreatment aimed at breaking the solid matrix structure is needed. Efficient solvent extraction techniques aim to reduce mass transfer limitations to enhance rates and to increase the yield. The use of intensification strategies can speed up the process without undesirable effects on the quality of the products. Their combination could also provide interesting and synergistic effects [1].

The benefits of microwaves in extraction processes are in relation to selective hea-

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ting and thermal effects on the physicochemical and transport properties of solvent and solutes, as well as thermal-induced structural damage of the matrix. In analytical applications, microwave extraction offers a number of advantages over conventional methods, particularly in relation to the shortened extraction time, lower solvent consumption, improved efficiency and selectivity, as well as reproducibility and precision for analytes recovery [2, 3]. Furthermore, the costs and pollution are lower compared to conventional techniques and as a result, this technique is commonly found in laboratories and has been included in some standardized extraction protocols.

This chapter presents a short survey on the fundamentals, major types of extractor configuration, combination with other intensification strategies as well as representative examples of microwave-assisted extraction of bioactives from vegetal biomass and also from other sources.

## GREEN EXTRACTION TECHNOLOGIES

Bioactive compounds from foods can be obtained using different extraction strategies which can be classified as conventional or non-conventional methodologies. The main advantage of the conventional extraction process is simplicity, but among the several disadvantages can be mentioned low selectivity, low yield, high temperature, long time, high energy consumption, and sometimes the use of organic solvents increases the cost. Besides, a possible thermal degradation of the compounds could happen. The common traditional techniques are: soxhlet extraction, maceration, and hydrodistillation [4]. Nowadays, innovative extraction technologies, focused on the reduction of energy consumption with the purpose of reducing the environmental impact by fostering eco-friendly technologies have been studied and developed. The environmental impact can be lower because these eco-friendly methodologies use a limited amount of toxic chemicals, which could be replaced using green solvents (*e.g.* ethanol solutions, ionic liquids, deep eutectic, and supramolecular solvents or water), therefore their resultant hazardous wastes are significantly reduced [5, 6].

In this context, the recent tendency of society towards consuming products obtained through environmentally sustainable processes is growing [7]. This trend fits into the "green chemistry" concept, which can be defined as a model aimed at designing products and processes that are less harmful to the environment and people [8]. On the other hand, a definition related to green extraction techniques has been proposed by Chemat *et al.* [8] as "*processes in which energy consumption and the use of harmful solvents will be reduced, in order to obtain a high quality and sustainable extract/product*".

A general overview of the conventional and innovative extraction technologies and their possible combinations is summarized in Fig. (**1**), the novelty of these green extraction techniques is presented in this section. Some examples of green extraction processes, defined by the extraction technique and the greener character of the solvents, have been summarized in Table **1**. The operation time can be significantly shortened compared to those of the conventional process and these innovative techniques can also be valid for the extraction of compounds from other biomass, not only from plant sources.

**Table 1. Examples of green extraction of edible materials.**

| Extraction Technique | Solvent | Raw material | Compound extracted | Operation conditions T(°C), P(MPa), T(min), P(W), F(kHz) | References |
|---|---|---|---|---|---|
| EAE | Water | Fish scale | Collagen | 135 ˚C; 360 rpm | [31] |
| EAE* | Water | Kinnow peel | Naringin | 90 ˚C; 10 min | [32] |
| | Water | Lentils and white beans | Protein | 50 ˚C; 180 min | [33] |
| | Water/EtOH | Vanilla green poods | Glucovanillin | 8-70 ºC | [34] |
| HHP | EtOH | Seafood (boone) | Astaxanthin | 0.1-600 MPa; 0-20 min | [35] |
| | Water | Black garlic | Melanoidins | 25 ˚C; 200 - 500 MPa; 5, 15, 25 min | [36] |
| PEF | Water | Tomato | Carotenoids | 60 ˚C; 0.5 kV/cm; 1 kJ/kg | [37] |
| | EtOH | Purple-fleshed potato | Anthocyanins | 40 ˚C; 3.4 kV/cm | [38] |
| | EtOH | Grape seeds | Polyphenols | 5 kV/cm | [39] |
| SFE | EtOH/$CO_2$ | Strawberry | Phenolic compounds | 30 ˚C; 5 MPa; 60 min | [40] |
| | $CO_2$ | Wine grapes (seeds) | Flavonoids and phenolic acids | 39.85 ˚C; 35 MPa; 240 min | [41] |
| | EtOH/$CO_2$ | Mushrooms | Antioxidants | 35-55 ˚C; 10-30 MPa; 30 min | [42] |
| | EtOH/$CO_2$ | Grape bagasse (stems, skins and seeds) | Anthocyanins, catechins, glucosides of flavonols | 40 ˚C; 20 and 35 MPa; 300 min | [43] |
| | EtOH | Coffee (grounds and husk) | Phenolic compounds | 20-20 MPa; 50-60 ˚C | [44] |

# CHAPTER 11

# Application of Enzyme-Assisted Extraction for Food Analysis and Release of Natural Products

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**Abstract:** The transition to a circular bioeconomic model that incorporates sustainable extraction processes such as enzyme-assisted extraction (EAE) is motivated by climate change, population growth, and changing diets to address food security and safety, and preserve natural resources (land, and water) and biodiversity. EAE can be applied to extract nutrients and bioactive molecules for food analysis and profiling, and for industrial exploitation of bioactive compounds from novel feedstocks. Commercial extraction processes require high recovery of the targeted compounds and must guarantee the preservation of the biological activity of the products, which is difficult to achieve using conventional methods. EAE is a possible alternative to preserve the quality of final products while reducing the industrial footprint in the food sector at a larger scale. This chapter describes the parameters that impact the extraction yield obtained in the EAE process and provides recent examples of its successful application for the extraction of polymers and bioactive compounds of very diverse matrices (plant, animal, mushrooms, yeast, food waste, and insects), with emphasis on process conditions. This chapter also identifies the challenges and opportunities of EAE and the emerging areas of research to facilitate the economic feasibility of the enzymatic extraction of bioactive molecules. Costs related to enzyme production and its use are one of the main impediments to the industrial application of the EAE process. Recent research progress suggests that reduction of EAE costs can be achieved by a holistic approach considering all steps: enzyme production (by using cheap enzyme production media, in-house enzyme production), selection of feedstock (*i.e.*, food byproducts), enzyme recycling (enzyme immobilization, nano-biocatalysts), the search of novel enzymes (marine degrading polysaccharides), more robust enzymes (*i.e.*, extremozymes) and/or enzyme improvement (bioengineering), and EAE process optimization (minimum optimal enzyme dosage). EAE technology for food analysis and production of bioactive molecules keeps building momentum as it is sustainable, environmentally friendly, and innovative.

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**Keywords:** Bioactive compounds, Biorefinery, Biocatalysis, Environmentally friendly enzyme technology, Enzyme-assisted extraction, Sustainable natural sources.

## INTRODUCTION

Enzymes have been applied as tools for research and development in almost every industrial sector [1]. Enzyme technology is one of the keystones of biotechnology, particularly industrial or white biotechnology, with an expanding market projected to reach $10,519 million by 2024 [2]. It involves the application of enzymes as catalysts in diverse industrial processes for the manufacture of products and thus fulfilling human needs. The primary goal of enzyme technology is the development of more sustainable processes to generate novel products and/or conventional products from traditional or new raw materials. Enzyme technology has important applications in the food and beverage industry as bioprocessing agents and as tools for food analysis. Factors that are driving the global food enzymes market are also related to changes in consumer preferences. There is a growing demand for: natural preservatives, antioxidants, and colorants in place of synthetic ones from health concerns [3, 4]; novel and exotic flavors; nutritious foods; and functional foods (*i.e.*, food enrichments, fortifiers). These increased requirements could be met by the extraction of bioactive compounds from different sources, including food waste [5]. Enzyme-assisted extraction (EAE) is gaining popularity owing to more stringent regulations over the industrial extraction of bioactive compounds and its impact on the environment and health [6]. The increasing weight of biocatalysis in the food and beverage sector is reflected by the estimated expansion of its enzyme market from about USD 2.8 billion in 2020 to around USD 3.7 billion by 2026 [7].

Enzymes were discovered in the 1830s when scientists demonstrated the hydrolysis of starch using an enzyme present in germinated cereal grains, the enzyme that we know these days as amylase. The production of rennet constitutes another example of biocatalysis where enzymes (aspartic protease) from calves' stomachs were used to coagulate the proteins in the milk for cheese making. The food industry has capitalized on enzyme applications not only for cheese products but also in other applications such as the clarification of fruit juices (pectinases). The microbial enzymes started to be applied in the food industry by 1960, with major applications in the starch industry. The starch industry is a good example of the adoption of a greener technology based on enzymes, α-amylases, and β-glucoamylases, in substitution of the acid hydrolysis process for the conversion of starch into glucose. The innovation of enzyme technology increased abruptly with the development of DNA technology that relies on the progress of other enzyme applications. Simultaneously, the discovery of more and more enzymes through

metagenomics and the continuous progress in enzyme engineering is favoring the transition from conventional chemical processes to greener enzyme-based conversion technologies [1].

Enzymes are specialized proteins able to increase the speed of a chemical reaction (reduction of activation energy) up to more than 1010 times, promoting the transformation of different compounds into specific products of interest. There is an enzyme-catalyzed reaction corresponding to almost every type of known organic reaction. The nomenclature committee of the International Union of Biochemistry and Molecular Biology (IUBMB) classified the enzymes into six classes according to the reaction they catalyze: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. The IUBMB assigned a code of four digits to the enzymes, designated as Enzyme Commission number (EC number). This EC number also considers substrate specificity and the functional groups involved. The first digit refers to the enzyme class, the second digit refers to the sub-class and its substrate specificity, the third number corresponds to the functional group donor and the fourth one refers to the acceptor molecule. EC numbers do not identify specific enzymes, but enzyme-catalyzed reactions, meaning that different enzymes that catalyze the same reaction will share the same EC number. Databases dealing with enzyme nomenclature are ExplorEnz [8] and IntEnz [9]. Although the EC classification has been broadly used, it presents some limitations since it does not consider enzyme structure, its amino acid sequence, or mechanisms of action, all of which are significant parameters for elucidating the enzymatic reactions. Thus, enzyme databases have been created to cover a wide range of aspects including enzyme function and properties, distribution, kinetics of catalyzed reactions, structure (three-dimensional folds) and amino acid sequence, or metabolic function. BRENDA [10] supplies vast information of all classified enzymes whereas KEGG [11], EzCatDB [12], MEROPS [13], MetaCyc [14], REBASE [15], CAZy [16], ESTHER [17], RedoxiBase [18], and KinBase [19] in either certain aspects of enzyme function or specific enzyme classes, organisms, or metabolic pathways. There are other interesting databases on enzyme application that can be used to predict the cleavage sites of proteins of known sequence and structure when subjected to proteases, as well as to predict the potential biological activity of the products of the reaction [20].

The different enzyme classes and some examples used in the food industry are given below:

1. Oxidoreductases (EC 1) catalyze oxidation-reduction reactions where there is a transfer of electrons from an electron donor (reductant) to an electron acceptor molecule (oxidant). This group of enzymes usually utilizes nicotinamide adenine

# CHAPTER 12

# Pulsed Electric Field

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**Abstract:** This chapter reviews the fundamentals of the Pulsed Electric Field (PEF) and its applications to the extraction of high-added value substances from food matrices. The electroporation process on the cell membrane is explained and the most recent works dealing with the use of PEF for extracting essential molecules for the human body such as lipids, phenolic compounds, carotenoids, proteins, carbohydrates, and vitamins, from food and plant matrices, and food waste, are described in detail. The combination of PEF with other extraction techniques is a common practice and improves the extractability of specific compounds to increase the recovery yields.

**Keywords:** Electroporation, Extraction, Food matrices, Food waste, High-added value substances, Pulsed electric field.

## INTRODUCTION

Pulsed electric field (PEF) technology consists of an electric treatment of repetitive short pulses with high voltage to a sample placed between two electrodes. The application of an electric field leads to the formation of pores on the cell membrane due to the electroporation phenomenon. This process improves cell permeability, increasing mass transfer by the diffusion of solutes located inside the cells toward the solvent. In this way, PEF enhances the extraction yield of intracellular compounds. Moreover, the application of electric pulses at short time periods allows for decreasing the processing time avoiding the elevation of

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temperature, so this non-thermal technique reduces the degradation of heat-sensitive compounds. In addition, this technology facilitates obtaining purified extracts due to the release of selective molecules and easy separation between the solid particles and the extracted compounds in the solvent. In addition, it reduces the economic and energetic impact and the extraction times, so it is considered an environmentally friendly technique [1, 2].

PEF technology presents numerous applications in food processing. The main applications are the extraction of intracellular compounds and the food preservation, especially juices. PEF enhances the diffusion extraction, osmotic treatment, pressing extraction, drying, and freezing [2, 3]. PEF has also been applied to pasteurization and sterilization processes, inactivation of pathogenic and spoilage microorganisms, and enzyme deactivation, among others [2, 4 - 6].

The extraction of compounds by PEF takes place in a treatment chamber in batch or continuous flow systems [7]. Special attention must be paid to the chamber to avoid fouling and corrosion of the electrodes [8]. The PEF technology is used for treating liquid, solid, and semisolid foods. Food waste has also been treated by PEF. The valorisation of food residues supposes a big challenge from the environmental, economic, and social point of views. Food residues are cheap sources of valuable compounds whose recovery contributes to reach the target of a circular economy (zero residues). In order to increase the recovery yields of specific compounds, many researchers have combined a PEF pre-treatment with other extraction techniques such as solid-liquid extraction, ultrasounds, and high hydrostatic pressure, among others [9 - 11].

This chapter reviews the action mechanism of a pulsed electric field based on the electroporation phenomenon and focuses on the application of PEF to extract essential compounds for the human body from different food matrices.

## MECHANISM OF PULSED ELECTRIC FIELD

PEF is based on the electroporation phenomenon. This phenomenon is related to the formation of pores inside the cell membrane under the influence of an external electrical field. The electroporation process increases the electric conductivity and improves the cell permeability and the extraction of intracellular compounds [12]. PEF is an environmentally friendly technique that enhances mass transfer in food products.

### Generator of Electrical Pulse and Pulse Waveforms

A PEF equipment is composed of a pulsed power generator and a treatment chamber. PEF treatments are based on the application of electric pulses for very

short periods of time (μs) [13]. Different types of pulses can be produced by a PEF system. The exponential decay and square wave pulses are the most used, but each one is produced by a different electrical circuit (Fig. **1**) [14]. Fig. (**1a**) shows an electrical circuit formed by a high-voltage power supply, a capacitor connected in series with a charging resistor, a high-voltage switch, and a treatment chamber. When the switch is opened, the high-voltage power supply charges the capacitor of energy. When the trigger signal is applied, the switch is closed and the electrical energy stored in the capacitor flows toward the treatment chamber. In that moment, the exponential decay pulses are produced due to the voltage is unidirectional and it rises quickly to a maximum value. Then, it decays slowly to zero [14, 15]. These pulses offer short peaks of high electric field intensity and long tails of low electric field strength [16]. On the other hand, square wave pulses are more energetic than the exponential decay pulses. Square waveforms offer width peaks with fast rising and fast falling. This electrical circuit is formed by an array of capacitors, inductors, and switching devices (Fig. **1b**) [14, 15]. In both cases, exponential decay and square wave pulses, the duration of the pulse is determined by the control signal of the switch, because the pulse starts by closing the switch and it ends by opening it [13].

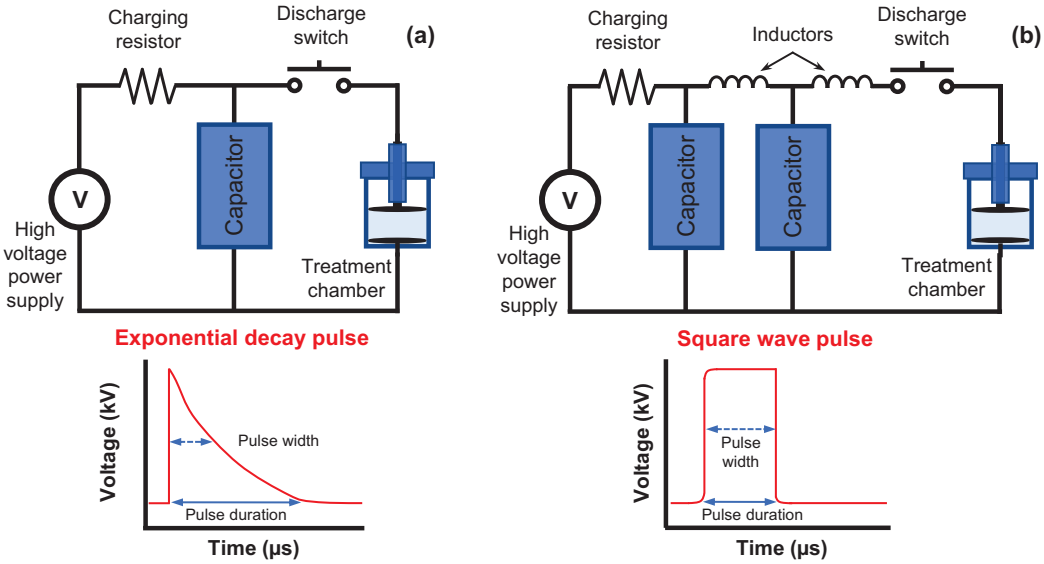


**Fig. (1).** Schemes of electrical circuits employed to generate exponential decay pulse (a) and square wave pulse (b).

The application of a pulsed electric field can take place in batch or static systems and in dynamic or continuous flow systems [17]. Batch systems are the most employed at the lab and pilot scale. The treatment chamber has two parallel plate

# CHAPTER 13

# High-Voltage Electrical Discharges

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**Abstract:** High-Voltage Electrical Discharges (HVED) are considered an emergent extraction technique based on the application of high-pulsed voltages. The aim of this chapter was to review its fundamentals for applications at laboratory and industrial scales. The configuration of devices and employed electrodes is described. Moreover, main steps required for using HVED and most important factors affecting this technique are also highlighted. Extraction of high added-value compounds from food waste and plant matrices using batch HVED has been the most usual application in last five years. In many cases, the low selectivity of the technique has made the use of a solid-liquid extraction step after HVED application necessary.

**Keywords:** Application, Electrodes, Extraction, Food, High-voltage electrical discharges, Treatment chamber.

## INTRODUCTION

High-voltage electrical discharges (HVED) is a non-thermal technology based on the application of high-pulsed voltages within a liquid phase. Main applications of this technique are the preservation of foods and the extraction of compounds. The application of HVED to a liquid phase can lead to the fragmentation of cell membranes and the permeation of cell walls. This fact has been applied for increasing food shelf life by the deactivation of microorganisms, which constitutes an interesting alternative to other thermal methods that can result in sample degradation. Moreover, HVED technique is also an interesting tool for the extraction of compounds by promoting the availability of intracellular compounds [1]. Indeed, HVED enhances mass transfer and increases extraction yields.

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Additionally, it is considered an environmentally friendly technique since it requires low solvent and energy consumptions and short treatment times [2]. Furthermore, HVED is suitable for extracting thermolabile compounds since it can use mild conditions and temperatures.

Nevertheless, this technique also shows some limitations. It can disintegrate food particles, making more difficult the separation of target compounds. Moreover, it is not a selective technique and can generate reactive oxidizing species [3, 4]. In order to overcome these drawbacks, some researchers have suggested the application of electrical discharges at low energy followed by an additional step to promote mass transfer and increase the extraction yield of compounds [5]. Thus, HVED has often been used as a pre-treatment followed by a conventional solid-liquid extraction [6 - 8], enzymatic hydrolysis [4] or other non-conventional techniques such as high-pressure homogenization [9].

This chapter reviews the main designs and theoretical aspects related to this technique and the application of HVED to the sustainable extraction of compounds.

## DEVICE CONFIGURATION

HVED can be applied in three different regimes: batch, continuous, and circulating. The design of the treatment chamber, the geometry of electrodes, and the process scale limit the kind of regime. The batch HVED system is the most used when the aim is the extraction of high added-value compounds from food matrices, at the lab or pilot scale. In this case, electrical discharges are generated in a treatment chamber usually constituted by two needle-plate geometry electrodes (point-plane) working in an intermittent way [1]. The continuous HVED system is preferred for industrial applications. In this case, the material and solvent are continuously pumped through a cooling system into a treatment chamber where electrical discharges are applied. After the application of HVED, the treated material enters again into the cooling system. There are two continuous systems with different treatment chambers: the converged electric field system and the annular gap system. The converged electric field system is constituted by a pair of parallel plates electrodes, separated by a small gap where electrical discharges are produced. The annular gap system presents a treatment chamber that consists of two concentric electrodes of different diameters with an annular gap between them for hosting the material to treat [4]. Samples with high amounts of solids could provoke the clogging of the converged electric field system, being the annular gap system the best choice in this case [10]. Finally, the circulating HVED system contains a needle electrode located in the centre of a ring electrode. The material and solvent are introduced into the bore region between electrodes for their treatment. Afterward, the treated material is diffused into an extraction

tank, located at the end of the treatment chamber. After extraction, a valve enables the separation of the supernatant from the residues, which can be driven into the treatment chamber again [4]. This system can enhance the extraction yield since the material can be exposed to electrical discharges several times [10].

## THEORETICAL FUNDAMENTALS

HVED technology is based on the phenomenon of electrical breakdown in water. The application of high-voltage electrical discharges generates physical processes and chemical reactions that lead to the fragmentation of the cell membrane promoting the extraction of intracellular compounds. It is considered a green extraction technique since it is a non-thermal and low energy technique [11]. The batch extraction system has been the most applied at lab and pilot scale [4]. The mechanism, in this case, involves the generation of the electrical pulse, the streamer propagation and the electrical arc formation, the fragmentation of the raw material, and the extraction of compounds. A brief description of these steps is given below.

### Generation of the Electrical Pulse

HVED are generated through a system connected to a pulsed power generator. This generator consists of a high voltage power supply, a high energy storage capacitor, and a high voltage switch (Fig. **1**) [12]. The power source supplies a high voltage and generates electrical energy that is stored in the capacitor. The maximum stored energy in the capacitor depends on its capacitance and can be calculated using the following equation [12]:

$$E_e = \frac{1}{2} C V^2 \quad \textbf{(1)}$$

where $E_e$ is the stored electrical energy (Joules), $C$ is the capacitance (Farads), and $V$ is the charging voltage (V) in the capacitor.

When the capacitor reaches its maximum voltage, the stored energy is transferred towards the switch. The high voltage switch is constituted by two spherical electrodes separated a certain distance. When the voltage reaches a threshold value, an electric arc is formed in the air, and the switch is closed. Then, the electrical energy from the capacitor is transmitted to the chamber electrodes. When the electrical current arrives at the needle electrode, the voltage rises in few seconds (nanoseconds) and the streamer reaches the ground electrode, which leads to the formation of the electrical discharge. After the energy stored in the capacitors is transferred to the chamber, the switch is opened again [12]. This

# CHAPTER 14

# High Hydrostatic Pressure: A Green Extraction Technique for Food and Pharmaceutical Industries

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**Abstract:** High Hydrostatic Pressure (HHP) is a green extraction method, which finds several uses in different branches of science. HHP is a novel non-thermal technique mostly used in food processing. The "high pressure" in HHP states an ultra-high cold isostatic hydraulic pressure, which processes basically at low or mild process temperatures (<45 °C) ranging between 100 and 800 MPa. In some applications, this pressure can extend up to 1000 MPa. In food processing, there are several purposes for using HHP, such as sterilizing, coagulating, and gelatinizing food samples. Alternatively, HHP has many remarkable uses in some branches of science besides food processing. This chapter aims to present the capabilities of HHP as a green extraction technique in the food and pharmaceutical industries.

**Keywords:** Extraction, HHP, High hydrostatic pressure, UHP, Ultra-high pressure.

## INTRODUCTION

High hydrostatic pressure (HHP) or alternatively known as ultra-high pressure (UHP), is one of the novel food processing techniques. It is a non-thermal food processing method that has become popular, especially in the last three decades, due to its wide application potential. As a non-thermal process, it has minor effects on both the quality and nutritional characteristics of processed food [1]. The "high pressure" in HHP states an ultra-high cold isostatic hydraulic pressure [2], which processes basically at low or mild process temperatures (<45 °C) ranging between 100 and 800 MPa. In some applications, this pressure can extend up to 1000 MPa [3, 4].

In HHP processing, water is generally the medium to transfer pressure. As the pressurizing process starts, pressure acts immediately on the whole sample being processed. Thus, HHP is a quicker process than thermal food processing techniq-

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ues, which require a longer time to act on the whole food [5, 6]. In addition, using HHP in food processing does not require to use heat which can damage proteins, enzymes, etc [7]. On the other hand, HHP can cause cellular deformation, membrane damage, and protein denaturation [8 - 11].

In food processing, there are several purposes for using HHP, such as sterilizing, coagulating, and gelatinizing food samples [12]. In addition, some new potential uses of HHP in processing food are under research by several researchers. Alternatively, HHP has many remarkable uses in some branches of science besides food processing [7, 12 - 20].

This chapter aims to present the capabilities of HHP as a green extraction technique in the food and pharmaceutical industries.

## The Definition of Pressure

The particles in a closed container hit the inner walls of this thermodynamic system and create a force on the walls of the container. The total net force on the container's wall is known as pressure [21].

In thermodynamics, pressure, which is quite important, is a characteristic element of a system. It is affected by volume, temperature, entropy, and the number of particles.

Pressure (*p* or *P*) is the amount of force that acts on a unit area (Fig. **1**).

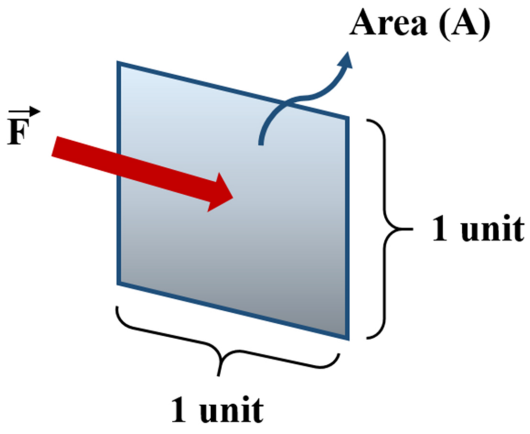


**Fig. (1).** Definition of pressure.

The following formula (1) shows how to express pressure mathematically.

$$p = \frac{\vec{F}}{A} \quad \textbf{(1)}$$

In the above equation, "*p*" stands for pressure, "*F*" for force, and "*A*" for surface area.

The official SI unit of pressure is the Pascal (Pa). It is possible to convert Pascal to other pressure units as follows [22].

1 Pa = $10^{-5}$ bar = 1 $N/m^2$

Since 1 atmosphere (atm) equals 1.01325 bar, the pressure unit Pascal represents a low pressure. For this reason, the megapascal (MPa) is the pressure unit in HHP studies.

1 MPa = $10^6$ Pa

Table **1** shows the conversion of different pressure units.

**Table 1. Conversion of different pressure units (adapted from Rivalain *et al.* [22]).**

| - | Atm | Bar | MPa | PSI* |
|---|---|---|---|---|
| **Atm** | 1 | 0.987 | 9.901 | 0.068 |
| **Bar** | 1.013 | 1 | 10.000 | 0.069 |
| **MPa** | 0.101 | 0.100 | 1 | 0.00689 |
| **PSI** | 14.696 | 14.504 | 145.038 | 1 |

* PSI: $pounds/inch^2$

## What is Hydrostatic Pressure?

Fluid systems are either static or dynamic. Thus, a fluid system can be either defined as hydrostatic or hydrodynamic. In a hydrostatic system, the fluid does not move. Therefore, it is called a static system.

In a static system, the pressure can be isostatic or non-isostatic. Isostatic pressure has the same value at any point in a system and acts in all directions. But a pressure gradient is present in the non-isostatic type. The structure of the pressure-generating equipment and the non-homogeneous compressibility of the medium are the main factors in generating pressure gradients [22].

# SUBJECT INDEX

## A



## B

## C



## D



## E



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## G



## H



## I



## J



## L

## M



## N

## O



## P



## R

## S

## T



## U



## V



## W

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