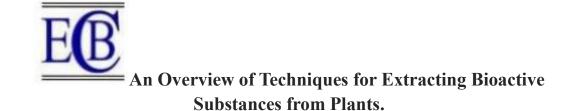
An Overview of Techniques for Extracting Bioactive Substances from Plants

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Abstract:

Since bioactive chemicals are used in a lot of commercial sectors, including the pharmaceuticals, diet, and chemicals manufacturing industries, it is necessary to devise an extraction technique that is both suitable and standardized in order to get these active substances from plant parts. Addition to the traditional approaches, various novel methods have been developed; nonetheless, there is not one method that is universally acknowledged as the standard method for extracting bioactive chemicals produced by plants. The critical input criteria that have the greatest influence on the overall effectiveness of extraction methods, whether conventional or unconventional, include consideration of the chemical nature of the plant, the chemistry of bioactive components, and the required level of scientific experience. This investigation's objective is to investigate a lot of different ways of the of pharmacologically active chemicals extraction from medicinal plants and to investigate the fundamental mechanisms that underlie each approach.

1. Introduction:

Since bioactive compounds are employed in multiple industries, including the pharmaceutical, food, and chemical sectors, it is important to develop an appropriate and standardised process of extraction for obtaining these active components from plant materials. The standard technique for extracting bioactive compounds from plants is not yet agreed upon, despite the fact that several unique ways have been discovered in addition to the old ones. The effectiveness of both traditional and nontraditional extraction processes depends upon a number of input factors, the most important of which are knowledge of the plant material, the biochemistry of bioactive substances, and the requisite degree of scientific expertise. The focus of investigation is on the principles behind various approaches of obtaining Pharmacologically active components from medically effective plants.

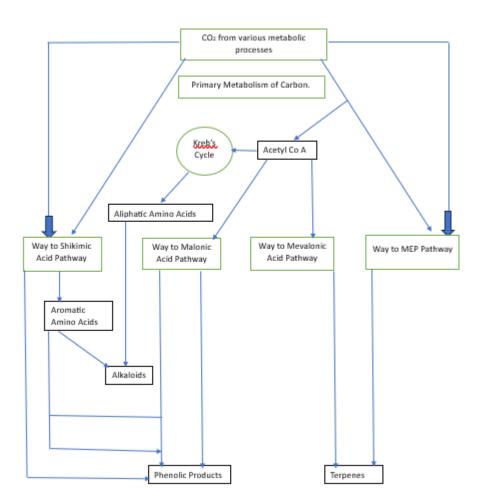
2. Bioactive compounds history and definition:

The usage of plants to benefit humans' dates back to the dawn of civilization. The discovery of plants' therapeutic capabilities transformed them from a just nutritional resource into a valuable tool for improving people's health in a wide range of societies. Thousands of recipes found in ancient Egyptian papyri demonstrate the therapeutic, cosmetic, and preservation value of coriander and castor oil. Hippocrates, Theophrastus, Celsus, Dioscorides, and many others from the ancient Greek and Roman worlds detailed the myriad medicinal use of various plants. The Romanians have a long history of using herbal remedies. For instance, Herodotus (who lived in the fifth century B.C.) recorded the usage of Leonurus cardiaca (Mother wort) by those who resided north of the Danube River. Herbal remedies were originally included in the Romanian pharmacopoeia in the 19th century, while in 1904, first institution of medical plants founded inside Cluj city ⁹⁵. The traditional usage of medicinal plants is a good example of how bioactive molecules have evolved through time. Bioactive chemicals were previously unknown to scientists, yet their applications span a wide range of fields. Secondary metabolites are often the means by which plants manufacture their bioactive substances⁸. From singlecelled bacteria to multi-cellular plants, all living things rely on the biochemical processing of a wide variety of chemicals. The chemicals found in living systems may be broken down to two basic categories. One category is primary metabolites, which include nutrients including carbs, proteins, and lipids that are used for growth and the development. Secondary metabolites are a class of molecules distinct from primary metabolites that are thought to improve plants' overall survival and overcome local hurdles via interaction with their environment ³². In other words, secondary metabolites are formed as mixtures of closely related members of a chemical family, having different chemical structures, are produced by specific taxonomic groups and have no role in growth (while they might have function for survival)⁶³. Secondary metabolite production is mostly determined by the species' unique evaluation and survival requirements. Toxic chemical synthesis has developed towards diseases and herbivores to inhibit the development of neighbouring plants ¹⁷, whereas aromatic chemical synthesis by floral species attracts insects for their pollination and fertilisation. Some of the secondary metabolites have effects on biological systems and are therefore classified as bioactive. For this reason, secondary metabolites that can produce medicinal or poisonous effects in human and animal subjects is a straightforward definition of bioactive molecules in plants⁸.

3. Biosynthesis and Classification of Bioactive compounds:

How biologically active compounds are classed may be profoundly affected by the motivation behind a certain classification method. For instance, biosynthetic categories, which aid in the simplification of describing biological synthetic pathways, may not correspond with breadth of classification based on the pharmacology. Croteau and his colleagues identify more than 25,000 different terpenes and terpenoids, around 12,000 different alkaloids, and almost 8,000 different compounds having phenolic ring as bioactive substances found in plants¹⁴. Figure 1 shows the overarching structures shared by numerous groups of bioactive compounds. Because of the biosynthetic process by which they are generated, most bioactive compounds may be grouped into one of several families, each of which has its own distinctive structural features. The four most essential pathways for the production of secondary metabolites (bioactive compounds) are the Mevalonic acid pathway (MAP), the Shikimic acid route (SAR), the malonic acid road (MAR), and the non-mevalonate (MEP) pathway ⁹⁰. The production of alkaloids necessitates the initial synthesis of aromatic amino acids, which may be obtained via the shikimic acid route. Subsequently, the synthesis of aliphatic amino acids is required, which can be achieved through the tricarboxylic acid cycle. The malonic acid route and the shikimic acid route both contribute to

the production of phenolic chemicals. The MEP and mevalonic acid pathways are responsible towards the synthesis of terpenes. In Figure 2, we see a simplified version of pathways that lead to three primary categories of bioactive chemicals. The aliphatic amino acids are synthesised via the tricarboxylic acid cycle, whereas the aromatic amino acids are synthesised through the shikimic acid route. Shikimic acid route and the malonic acid route are involved in synthesis of phenolic chemicals. Terpenes are synthesised via mevalonic acid pathway and the methyl ester transfer protein pathway. Three important classes of bioactive plant chemicals and the many mechanisms involved in their formation are summarised here as shown in the Fig. 1



4. The process of extracting bioactive substances:

Since there are significant variations between pharmacologically active chemicals of the plant species and a huge diversity of plant species are even being identified, It is critical to develop a standardised and rigorous method for filtering out these chemicals with human health advantages. The study of medicinal plants, as stated by Farnsworth et al. (1985), has progressed from the aggregation of common names for these species to their eventual commercialization. Fig. 3 is a flowchart showing the steps that must be taken into the consideration to learn about medicinal plants and the significance of different extraction techniques. Without using a suitable extraction method, it is not feasible to separate, identify, or characterise bioactive chemicals. Experimenting with different extraction techniques is crucial for learning about extraction selectivity from various natural sources. Extracting bioactive compounds may be done in a variety of ways, some of which haven't changed much in hundreds of years. The objectives of these methods are to (A) purify the

pharmacologically compounds of interest from a complex plant sample, (B) Enhance the specificity of analytical methodologies., (C) Enhance the precision of bioassays by the augmentation of the concentration pertaining to the variable of interest., (D) transform pharmacologically active components to make them even more amenable to identification and separation, (E) supply the technique that is robust, reproducible, and resistant towards the changes in the sample material.

5. Traditional extraction methods:

There present a number of standard techniques for extracting biologically active components from plant parts. The majority of these procedures include the use of solvents, which aid in the dissolution of the target substance, in conjunction with additional solubilizing elements such as heat and/or agitation. Traditional methods for extracting biologically active components from plants include Soxhlation, maceration, and hydro distillation.

The proposition of the Soxhlet extractor was put up by German scientist Franz Ritter von Soxhlet in the year 1879. Its original purpose was lipid extraction; however, its use has since broadened. The Soxhlation method was widely used for the extraction of valuable bioactive chemicals from the sources. This model can be used to test the efficacy of novel extraction techniques. The usual sample container for dry materials is a cover. The cover is then kept over a distillation flask containing the chosen solvent. A syphon pulls up the solution when it gets too high in the thimble holder. The syphon re-collects the solution in the original container. The extracted solutes are carried by this solution back to the primary liquid. The solvent is returned to the plant solids via recycling, while the residual solute is accumulated inside the distillation flask. The extraction process is done as many as necessary until no more material can be taken out.

Maceration was the gold standard for producing homemade tonics for a long time. As a very inexpensive approach to generating bioactive compounds and essential oils, it swiftly rose to prominence. Extraction on a smaller scale often involves many maceration processes. To start, plants are crushed into a finer powder to enhance their surface area and improve their ability to combine with the solvent. The second step of the maceration procedure is to add menstruum as the appropriate solvent in a sealed container. In the third step, the marc is subjected to pressing in order to remove the occluded solutions subsequent to the removal of the liquid. The liquid that was extracted via pressing and the liquid that was recovered through straining are mixed and filtered to get rid of any impurities.

Periodically agitating the maceration process might potentially enhance the extraction yield via two mechanisms: (a) facilitating the diffusion rate and (b) displacing concentrated solution from the sample's surface, then replenishing it with new solvent. For millennia, the technique of hydro distillation has been used to extract essential oils and other therapeutic substances from plants. This procedure may be conducted prior to the dehydration of the plant material, since it does not need the use of any organic solvents. There are three distinct forms of hydro distillation that have been officially recognised: water distillation, water and steam distillation, and direct steam distillation ⁹³. The first step involves the introduction of plant materials into a chamber designed for distillation, followed by the addition of an appropriate quantity of water. Subsequently, the whole mixture is subjected to the process of boiling. The use of direct steam injection on the plant sample is a potential approach. The primary factor responsible for the liberation of bioactive chemicals upon application to plant tissue is the thermal energy derived from water or steam. Condensation transpires as a result of the indirect cooling of a water and oil vapour combination by water. The separation of condensed mixes of oil and bioactive chemicals from water may be

achieved by using a separator⁸³. Hydro distillation is predicated upon three fundamental physicochemical mechanisms, namely hydro diffusion, hydrolysis, and thermal degradation. There is a possibility that the loss of some volatile components may occur at elevated extraction temperatures. Due to this limitation, its use is restricted to thermolabile chemical extraction on a limited scale. The efficacy of conventional extraction techniques is strongly contingent upon the meticulous choice of solvents ¹³. Choosing a solvent mostly depends on the polarity of the target compound. Environmental concerns, human and animal toxicity, cost effectiveness, and practicality should all be factored into the decision of which solvent to use for extracting bioactive molecules. Table 1 displays the many extraction techniques used to get bioactive chemicals. The aforementioned methods include aided extraction, supercritical fluid extraction, and pressurised liquid extraction. The classification of some methods as "green techniques" is conducted by the United States Environmental Protection Agency (EPA) (http://www.epa.gov/greenchemistry/pubs/about gc.html). The mitigation of pollution and accidents can be achieved by implementing strategies such as employing less hazardous chemical synthesis, adopting safer chemical design principles, utilising safe solvents and auxiliaries, incorporating energy-efficient design practises, utilising renewable feedstock, reducing the production of derivatives, employing catalysis techniques, implementing degradation prevention design measures, optimising atom economy, conducting time analysis, and embracing inherently safer chemistry principles.

6. Non-conventional extraction techniques:

Poor extraction selectivity, solvent evaporation, and heat deterioration of thermolabile chemicals are only some of the problems that rise as a result of the prolonged extraction times and the necessity for expensive, highly pure solvents ⁵⁶. Problems with traditional extraction methodologies are addressed through promising newer approaches. These tasks stand associated with innovative extraction methods. The most promising extraction methods include the use of supercritical fluids, pressurised liquids, enzymes, microwaves, pulsed electric fields, or ultrasounds. The Environmental Protection Agency (EPA) has designated some of these methods as "Green" due to their low environmental impact (more information is available on the webpage https://www.epa.gov/greenchemistry/pubs/about gc.html). Safer chemical synthesis and design encompasses various strategies such as the utilisation of solvents and auxiliaries with minimal harm, enhanced energy efficiency, incorporation of renewable feedstocks, mitigation of byproducts and derivatives, implementation of catalysis, adoption of degradation prevention design, promotion of atom economy, application of time analysis, and prioritisation of accident prevention.

.6.1. Ultrasound-assisted extraction (UAE):

Human ears are incapable of hearing ultrasound waves. The range of frequency of Twenty kHz to Hundred MHz is used in chemistry. Like other waves, it causes compression and expansion in a material. The process involves cavitation, or the development, growth, and eventual collapse of bubbles. Energy is produced in large quantities by the transformation of motion into bubble heating. Suslick and Doktycz (1990) report that bubbles may reach temperatures of 5000 K, pressures of 1000 ATM, cooling and heating speeds of faster than 1010 K/s. This is foundation upon which the UAE is built. Only when solids are present in a liquid can cavitation occur. The organic and inorganic components in plant matrices are extracted by the ultrasonic energy used in UAE ³⁷. Ultrasound helps in the movement of plant mass and hence the penetration of solvents into plant cells. Ultrasound extraction entails fracturing the cell walls, followed by diffusion over the membrane and washing the present material ⁶⁴. The quality of a process of extraction is affected through various factors such as

sample humidity, milling intensity, size of particles, the solvent material used, time used for performing sonication. Temperature, pressure, frequency, and sonication time all play a role in ultrasound. In order to maximise system performance, it is fairly uncommon to combine UAE with more conventional approaches. To maximise extraction yields, ultrasonic technology is integrated into solvent extraction equipment ¹⁰³. UAE is a resource- and cost-saving mechanism. Extraction with ultrasound radiation allows for improved mixing, transfer of energy, temperature gradients, temperature used for extraction, selective type of extraction process, size of equipment, extraction control response time, fast startup, higher output, and fewer steps ⁹. The UAE is a great herbal extractor. Rostagno and his colleagues in 2003 used mix-stirring in addition to several extraction times and solvents to remove major pharmacological ingredients from soyabean plant. The efficiency of solvent extraction was increased using ultrasound. Ultrasound was utilised by Herrera et al ³⁷ to recover Phenolic chemicals, exemplified as rutin, and kaempferol from the straw berries. As Li and his colleagues in 2005, extracted chlorogenic acid from fresh plant parts of Eucommia unmodish Oliv. using this technique under optimum conditions. Using optimal sonication settings ⁵³, Yang and and his colleagues extracted the beneficial compounds rutin and quercetin from Euonymus alatus ¹¹⁰. The extraction using this method was more productive than the conventional methods. Vindoline, catharanthine, and vinblastine can be extracted from Vinca rosea using ionic liquid-based technique ¹¹⁰. To obtain the most anthocyanin compounds and phenolic analogues out of peels of grape fruits, the United Arab Emirates optimised the extraction temperature of the solvent under use, and time required for the process ²⁵. Rosmarinus officinalis' active components were more efficiently and quickly extracted using UAE based on ionic liquids than using conventional methods¹¹².

6.2 Extraction with the help of enzymes (EAE):

Some active plant chemicals in plant materials are protected from solvents by making them hydrophobic by forming hydrogen bonds, where they are distributed throughout the cellular cytosol or trapped inside the polysaccharide-lignin complexes. Pre-treatment with enzymes is an innovative and efficient technique for releasing such bound molecules which are difficult to extract and increasing production capacity of the process ⁷⁹. Enzymes, such as cellulase, alpha-amylase, and pectinase, may assist in the degradation of the cell wall, structural polysaccharides, and lipid bodies, therefore facilitating their extraction ^{79, 90}. Extraction techniques include extraction with water as a solvent and enzyme assistance commonly called (EAAE) and cold pressing with enzyme assistance commonly called (EACP). Common methods for oil extraction from seeds using EAAE include those developed by Rosenthal and Sharma ^{79, 90}, and Hanmoungja and his colleagues ³⁰. Enzymes cause hydrolysis of seed cell-wall with help of enzymes like cellulase due to the lack of polysaccharide and protein colloids ¹¹. Time required for hydrolysis, water -to- solid ratio, enzyme concentration, size of particles, and nature of the plant material all have a role in the extraction process Dominguez and his colleagues found that the water content of plant materials influenced the rate of hydrolysis by using enzymes. Bhattacharjee and his colleagues recommended EACP for extracting oilseed pharmacologically active components due to its safety and lack of in flammability⁸. More free fatty acids and phosphorus can be extracted from oil processed with enzymes than in oil processed with hexane ^{71, 19,} EAE is green since it uses water rather than organic chemicals to extract bioactive compounds and oil ⁷⁶. Winemaking involves the breakdown of plant cell walls by enzymes, and Meyer and his colleagues found a co-relation between entire phenol output and this process ⁶⁶. Landbo and Meyer along with their colleagues found that enzymes increased the release of phenol components from plant R. nigrum pomace ⁴⁶. Five citrus fruit peels (Lisbon lemon, Valley lemon, pomelo, tangerine, and sweet orange) had their total phenolic contents extracted by EAAE using different enzymes by Li and his colleagues ⁵³. The greatest improvement was shown with Celluzyme

MX. Next significant conclusion from those studies was a higher concentration of enzymes led to a greater extraction of phenolic antioxidants. Using a 2:1 pectinolytic-cellulolytic enzyme combination, Maier along with his colleagues was able to extract pharmacologically active compounds like phenolic acids, non-anthocyanin flavonoids and anthocyanin flavonoids from grapes ⁵⁹. In comparison to extraction assisted with sulphites, productions were higher. When compared to a non-enzymatic control, phenolic antioxidant levels were found to be significantly higher in raspberry solid wastes after enzymatic hydro-alcoholic extraction ⁴⁸. Celluclast, pectinex, and novoferm were utilised by Gómez-Garca and his colleagues in EAE to extract phenol compounds from grapes ²⁷. Novoferm had the most impact. Through the use of enzyme technology, potentially useful chemicals may be extracted from byproducts obtained from agricultural industries.

6.3 Microwave assisted extraction (MAE):

Different materials may have their soluble components extracted into a liquid using the novel technique of microwave-assisted extraction ⁷³. Electromagnetic waves within 300 MHz to 300 GHz range are termed microwaves. To construct them, two electrical and magnetic fields that move in opposing directions are essential. Polar substances may be heated directly in a microwave oven ⁵¹. Ionic conduction and dipole rotation turn electromagnetic energy into thermal energy ⁴². Due to medium's resistance, ionic conduction results in heat production. However, ions are guided by shifting field signals. Molecular collisions and heat are the results of this regular change of orientation. Separation of solutes from active sites of sample matrix under increasing temperature and pressure, diffusion of solvent across sample matrix, and release of solutes from matrix to solvent are the three sequential phases described by Alupului for microwave aided extraction². Accelerating the extraction of bioactive ingredients from plant materials, decreasing temperature gradients, decreasing equipment size, and increasing extract production are all benefits of MAE¹². MAE is superior to conventional approaches for extracting bioactive compounds. Purify organic and organometallic compounds selectively. Because MAE reduces the use of organic solvents, it is environmentally friendly². When the sample is kept for 20 hrs at room temperature, MAE extracted more quantities of polyphenols and caffeine from green tea leaves in just 4 minutes than any other technology ⁷². Extraction of ginseng root to obtain ginsenoside using focused MAE took just 15 minutes, compared to 10 hours using solvent extraction in order to increase the effectiveness of MAE extraction in comparison to Soxhlet and maceration⁸⁷, Dhobi and his other collegues isolated a flavolignin, silvbinin, from Silvbum marianum ^{15B}. Bioactive compounds (E- and Z-guggolsterone, cinnamaldehyde, and tannin) were extracted from a variety of plants using MAE and shown to be more quickly and easily extracted under ideal conditions by ⁶. Different fractions of sorghum and maize bran and flour, which varied in terms of their hardness, were exposed to microwave-assisted extraction (MAE) with the aim of liberating phenolic acids that were bound within the samples ¹⁰. Solvent concentration, extraction duration, and microwave power were modified to optimise flavonoids, phenolics, and electrondonating capacity in Chinese quince (Chaenomeles sinensis) MAE. ^{38B}.

6.4 Extraction of liquids under pressure (PLE):

Richter et al. introduced PLE in 1996. Many various terms are used to refer to this technique depending on the setting ⁷⁰. High pressure does not cause solvent liquids to boil. Pressure helps in the extraction process. Automation has helped speed up the development of PLE-based technologies and cause reduction of solvent use and also the required extraction time. PLE requires less solvent at higher pressure and temperature. Solubility increases with the rise in and rate of mass transfer and temperature also lowers surface tension and viscosity of the solvent in use, leading a much higher rate of extraction ⁴⁰. The process is more efficient, uses less resources than the process of Soxhlet

extraction ⁷⁸. In future, PLE may take the place of supercritical fluid extraction when it comes to polar chemical extraction ^{43-B}. It has been suggested that PLE may be used to remove of organic contaminants in the conditions where the temperature is higher ¹⁰⁶. Extracting bioactive compounds through sea sponges using PLE was discovered by ⁴⁰. The process has a great use in the isolation of natural components ^{43-B}. Ibaezet and colleagues argue that PLE is environmentally friendly since it requires so little organic solvent ⁴⁰. PLE has been used to extract plant-based bioactive chemicals. Optimised extraction conditions prevented PLE degradation during isoflavone extraction from freezedried soyabeans ⁸². According to research conducted by Shen and Shao (2005)⁸⁶, the compounds like sterols, terpenoids etc can be isolated using different techniques like ASE, Soxhlation, and ultrasonication. Time taken for the process of extraction is almost reduced to half, lesser solvent is required, and yield is increased when PLE is used instead of traditional techniques. Extraction of spinach flavonoids was shown to be more successful using combination of the mixture of water and ethyl alcohol in the ratio (70:30) compared to the plain water as solvent at 50-130 C.³⁸. According to Luthria (2008) ⁵⁸, while extracting phenolic compounds from parsley (Petroselinum crispum) flakes using PLE, temperature, the pressure, size of particles, flushing volume, static time frame, and solvent -to- solid ratio. When it came to extracting Amaryllidaceae alkaloids from jonguil (Daffodil), the optimised process found superior to process of extraction using the hot water (HSE), extraction assisted with the microwaves (MAE), and ultrasonic-assisted extraction (UAE)⁶⁸. Ideal conditions for PLE extraction of chemicals like catechin, myricetin, gallocatechol (GCT), epicatechin gallate, caffeic acid and chlorogenic acid, and from Anatolian propolis (40 degrees Celsius, 1500 pressure for 15 minutes; were achieved. 18

6.5 SFE (supercritical fluid extraction):

Although Hogarth and Hannay with their colleagues (1879) pioneered the application of supercritical fluid extraction (SFE), Zosel with his colleagues ¹¹¹ was the first to patent SFE as a method for decaffeinating coffee. Since its introduction, supercritical fluid technology has been used in many different industries, including environmental, pharmaceuticals, polymers, and food analysis ¹¹¹. This method of decaffeinating coffee has been widely used by enterprises for decades ⁶⁹.

Matter may exist in three distinct phases: the solid, the liquid, and the gas. A material can only enter its supercritical state once it reaches its critical pressure and temperature values. At Tc and Pc⁴¹, there is no coexistence of a gaseous and liquid phase. Supercritical fluid can't be turned into a liquid since it has qualities in between those of a gas and a liquid. Supercritical fluids exhibit similar behaviour to gases in terms of diffusion, viscosity, surface tension, density, and solvation power. These features make it amenable to fast and efficient chemical extraction ⁷. Oven with extraction vessel, pressure controller, and trapping vessel. Tank for mobile phase (usually CO2). Pump to pressurise gas. It's possible to employ a variety of metres, including flow, dry/wet gas, and others. A typical symmetric diagram for SFE tools is shown in Figure 4.

Carbon dioxide has the highest solubility for SFE. Carbon dioxide has a lower critical pressure of 74 bars; and a very low critical temperature (31 C; allowing for operations at moderate pressures, usually within 100 and 450 bar ⁹⁵. Due to its low polarity, carbon dioxide is not suitable for most medicines and medication samples, but it works well with lipid, fat, and non-polar substances. As discussed by Lang and wai (2001) and Ghafoor et al. (2010), chemical modifiers may be used to increase the polarity of carbon dioxide. A little increase in carbon dioxide levels may drastically change the polarity of the gas. Improved extraction may be achieved using hydrodistillation using 0.5 ml of dichloromethane (CH2Cl2) for 4 hours (Hawthorne et al., 1994). Sample and target chemical parameters, as well as historical experimental data, are used to determine the best modifier to use.

Adjustable SFE settings for the extraction of bioactive compounds from plants (Raverchon and Marco, 2006). You may get the most out of this strategy by adjusting these parameters. Temperature, pressure, particle size, moisture content, extraction duration, CO2 flow rate, and the solvent-to-feed ratio are the primary factors influencing extraction efficiency ⁹⁵; ⁴⁰.

When it comes to the removal of bioactive compounds, supercritical fluids provide a number of benefits ⁴⁷. When it comes to entering sample matrices and enabling mass transfer, (1) supercritical fluids excel over liquid solvents due to their larger diffusion coefficient, reduced viscosity, and lower surface tension. SFE shortens the duration of the extraction process. Supercritical fluid reflux is used several times to remove the substance (2). Supercritical fluid is more selective than solvent in liquid phase liquid because its solvation strength is more sensitive to changes in temperature and pressure. Because it doesn't involve separating the solute from the solvent, supercritical fluid depressurization has the potential to be more effective than traditional extraction methods. (3) SFE may be used to effectively extract thermolabile compounds at room temperature. Because (4) SFE requires less sample than solvent extraction, time may be saved throughout the experiment by using SFE. Because it requires less organic solvent, SFE is better for the environment. SFE combined with online chromatography may be utilised to analyse volatile chemicals.

Reusing supercritical fluids is an efficient way to reduce garbage. 5) In a lab, an SFE scale can be calibrated to milligrams, and in an industrial context, it can be calibrated to tonnes. (6) Understanding the mechanism and process of extraction is made possible by the SFE method.

Saldaa et al. and his colleagues in (1999) used SFE at 313-343 K & 14-24 K to extract xanthine alkaloids from I. Paraguayans (maté tea leaves). At 9.5 MPa and 58.6 C, using a supercritical CO2 solution modified with 15% ethanol, the naringin flavonoid was extracted from Citrus paradise ²⁶. The liberation of catechin and the epicatechin exceeded 79% when polyphenols and procyanidins were extracted from seeds from grapes using supercritical fluid extraction (SFE) and a mixture of methyl alcohol and the gas carbon dioxide (40%). ⁴⁴. Catharanthus roseus leaves are rich in indole alkaloids, and Verma and his colleagues in 2008 optimised SFE to extract those compounds. Using methanol (6.6% by volume) as a modifier, the optimal conditions for catharanthine recovery lasted 40 minutes at 25 MPa and 80 MPa ¹⁰⁰.

Conclusion:

The need to remove plant bioactive components is on the rise, and with that comes a desire in developing more effective extraction techniques. Several new extraction techniques have emerged as a result of the emergence of chromatography and the growing awareness of the need to safeguard the environment. Most non-conventional extraction techniques rely on diverse mechanisms, and improved process of extraction is the result of a number of processes, thus it is crucial to have a thorough understanding of the whole process. Scientists and engineers should be guided in their pursuit of hybridization by the materials and compounds found in plants. Several of the current methods require adequate experimental confirmation. Efficiency measurements of extraction also benefit from standardisation. The increasing value of biologically active molecules and substances rich in these bioactive chemicals, however, motivate scientists to develop even efficient cutting-edge extraction technologies.

References:

- 1. Ade-Omowaye, B.I.O., Angersbach, A., Taiwo, K.A., Knorr, D., 2001. Use of pulsed electric field pre-treatment to improve dehydration characteristics of plant-based foods. Trends in Food Science and Technology 12 (8), 285–295.
- 2. Alupului, A., 2012. Microwave extraction of active principles from medicinal plants. U.P.B. Science Bulletin, Series B 74(2).
- Ambrosino, P., Fresa, R., Fogliano, V., Monti, S.M., Ritieni, A., 1999. Extraction of azadirachtin A from neem seed kernels by supercritical fluid and its evaluation by HPLC and LC/MS. Journal of Agricultural and Food Chemistry 47 (12), 5252–5256.
- Angersbach, A., Heinz, V., Knorr, D., 2000. Effects of pulsed electric fields on cell membranes in real food systems. Innovative Food Science and Emerging Technologies 1 (2), 135–149.
- Anklam, E., Berg, H., Mathiasson, L., Sharman, M., Ulberth, F., 1998. Supercritical fluid extraction (SFE) in food analysis: a review. Food Additives and Contaminants 15 (6), 729– 750.
- 6. Asghari, J., Ondruschka, B., Mazaheritehrani, M., 2011. Extraction of bioactive chemical compounds from the medicinal Asian plants by microwave irradiation. Journal of Medicinal Plants Research 5 (4), 495–506. Barsotti, L.,
- 7. Cheftel, J.C., 1998. Traitement des aliments par champs electriques pulses. Science des Aliments 18, 584–601.
- Bernhoft, A., 2010. A brief review on bioactive compounds in plants. In: Proceedings from a symposium held at The Norwegian Academy of Science and Letters, Oslo, Norway. Bhattacharjee, P., Singhal, R.S., Tiwari, S.R., 2006. Supercritical carbon dioxide extraction of cottonseed oil. Journal of Food Engineering 79 (3), 892–989. Bryant, G., Wolfe, J., 1987. Electromechanical stress produced in the plasma membranes of suspended cells by applied electrical fields. Journal of Membrane Biology 96 (2), 129–139.
- 9. Chemat, F., Tomao, V., & Virot, M., 2008. In: Otles, S. (Ed.), Handbook of Food Analysis Instruments. Ultrasound-Assisted Extraction in Food Analysis. CRC Press, pp. 85–94.
- 10. Chiremba, C., Rooney, L.W., Trust, B.J., 2012. Microwave-assisted extraction of bound phenolic acids in bran and flour fractions from sorghum and maize cultivars varying in hardness. Journal of Chromatography A 1012 (2), 119–128.
- Concha, J., Soto, C., Chamy, R., Zuniga, M.E., 2004. Enzymatic pretreatment on RoseHip oil extraction: hydrolysis and pressing conditions. Journal of American Oil Chemist's Society 81 (6), 549–552.
- Corralesa, M., Toepflb, S., Butza, P., Knorrc, D., Tauschera, B., 2008. Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: a comparison. Innovative Food Science and Emerging Technologies 9 (1), 85–91.
- Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12 (4), 564–582. Cravottoa, G., Boffaa, L., Mantegnaa, S., Peregob, P., Avogadrob, M., Cintasc, P., 2008. Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. Ultrasonics Sonochemistry 15 (5), 898–902.
- Croteau, R., Kutchan, T.M., Lewis, N.G., 2000. Natural products (secondary metabolites). In: Buchanan, B., Gruissem, W., Jones, R. (Eds.), Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, pp. 1250–1318. Delsart, C.,

Ghidossi, R., Poupot, C., Cholet, C., Grimi, N., Vorobiev, E., Milisic, V., Peuchot, M.M., 2012.

- 15. Enhanced extraction of phenolic compounds from merlot grapes by pulsed electric field treatment. American Journal of Enology and Viticulture 63 (2), 205–211. 15B Dhobi, M., Mandal, V., Hemalatha, S., 2009. Optimization of microwave assisted extraction of bioactive flavolignan–silybinin. Journal of Chemical Metrology 3 (1), 13–23.
- 16. Dominguez, H., Ntiiiez, M.J., Lema, J.M., 1995. Enzyme-assisted hexane extraction of soybean oil. Food Chemistry 54 (2), 223–231.
- 17. Dudareva, N., Pichersky, E., 2000. Biochemical and molecular genetic aspects of floral scent. Plant Physiology 122 (3), 627–633.
- Environmental Protection Agency, USA. Erdogan, S., Ates, B., Durmaz, G., Yilmaz, I., Seckin, T., 2011. Pressurized liquid extraction of phenolic compounds from Anatolia propolis and their radical scavenging capacities. Food and Chemical Toxicology 49 (7), 1592–1597.
- 19. Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D., Guo, Z., 1985. Medicinal plants in therapy. Bulletin of WHO 63, 965–981.
- 20. Fincan, M., Dejmek, P., 2002. In situ visualization of the effect of a pulsed electric field on plant tissue. Journal of Food Engineering 55 (3), 223–230.
- 21. Fincan, M., De Vito, F., Dejmek, P., 2004. Pulsed electric field treatment for solid– liquid extraction of red beetroot pigment. Journal of Food Engineering 64 (3), 381–388.
- Gaur, R., Sharma, A., Khare, S.K., Gupta, M.N., 2007. A novel process for extraction of edible oils: enzyme assisted three phase partitioning (EATPP). Bioresource Technology 98 (3), 696–699.
- 23. Ghafoor, K., Choi, Y.H., Jeon, J.Y., Jo, I.H., 2009. Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants and anthocyanins from grape (Vitis vinifera) seeds. Journal of Agricultural and Food Chemistry 57 (11), 4988–4994.
- 24. Ghafoor, K., Park, J., Choi, Y.H., 2010. Optimization of supercritical carbon dioxide extraction of bioactive compounds from grape peel (Vitis labrusca B.) by using response surface methodology. Innovative Food Science and Emerging Technologies 11 (3), 485–490.
- 25. Ghafoor, K., Hui, T., Choi, Y.H., 2011. Optimization of ultrasound-assisted extraction of total anthocyanins from grape peel. Journal of Food Biochemistry 35, 735–746. Ghafoor, K., AL-Juhaimi, F.Y., & Choi, Y.H., 2012. Supercritical fluid extraction of phenolic compounds and antioxidants from grape (Vitis labrusca B.) seeds. Plant Foods for Human Nutrition. http://dx.doi.org/10.1007/s11130-012-0313-1.
- 26. Giannuzzo, A.N., Boggetti, H.J., Nazareno, M.A., Mishima, H.T., 2003. Supercritical fluid extraction of naringin from the peel of citrus paradise. Phytochemical Analysis 14 (4), 221–223.
- 27. Gómez-García, R., Martínez-Ávila, G.C.G., Aguilar, C.N., 2012. Enzyme-assisted extraction of antioxidative phenolics from grape (Vitis vinifera L.) residues.
- 28. 3 Biotech. http://dx.doi.org/10.1007/s13205-012-0055-7.
- 29. Guderjan, M., Töpfl, S., Angersbach, A., Knorr, D., 2005. Impact of pulsed electric field treatment on the recovery and quality of plant oils. Journal of Food Engineering 67 (3), 281–287.
- 30. Hanmoungjai, P., Pyle, D.L., Niranjan, K., 2001. Enzymatic process for extracting oil and protein from rice bran. Journal of the American Oil Chemists Society 78 (8), 817–821.
- Hannay, J.B., Hogarth, J., 1879. On the solubility of solid in gases. Proceeding of the Royal Society of London 29, 324–326.

- 32. Harborne, J.R., 1993. Introduction to Ecological Biochemistry, forth ed. Academic Press, Elsevier, London, pp. 1–32.
- Hawthorne, S.B., Yang, Yu., Miller, D.J., 1994. Extraction of organic pollutants from environmental solids with sub- and supercritical water. Analytical Chemistry 66 (18), 2912– 2920.
- 34. Heinz, V., Toepfl, S., Knorr, D., 2003. Impact of temperature on lethality and energy efficiency of apple juice pasteurization by pulsed electric fields treatment. Innovative Food Science and Emerging Technologies 4 (2), 167–175.
- 35. Hennion, M.C., Cau-Dit-Coumes, C., Pichon, V., 1998. Trace analysis of polar organic pollutants in aqueous samples: tools for the rapid prediction and optimization of the solid-phase extraction parameters. Journal of Chromatography A 823 (1–2), 147–161.
- Hernández, Y., Lobo, M.G., González, M., 2009. Factors affecting sample extraction in the liquid chromatographic determination of organic acids in papaya and pineapple. Food Chemistry 114 (2), 734–741.
- 37. Herrera, M.C., Luque de Castro, M.D., 2004. Ultrasound-assisted extraction for the analysis of phenolic compounds in strawberries. Analytical and Bioanalytical Chemistry 379 (7–8), 1106–1112. 37 B Herrera, M.C., Luque de Castro, M.D., 2005. Ultrasound-assisted extraction of phenolic compounds from strawberries prior to liquid chromatographic separation and photodiode array ultraviolet detection. Journal of Chromatography 1100 (1), 1–7.
- Howard, L., Pandjaitan, N., 2008. Pressurized liquid extraction of flavonoids from spinach. Journal of Food Science 73 (3), C151–157. 38 B Hui, T., Ghafoor, K., Choi, Y.H., 2009.
- 39. Optimization of microwave-assisted extraction of active components from Chinese quince using response surface methodology. Journal of Korean Society of Applied and Biological Chemistry 52 (6), 694–701.
- 40. Ibañez, E., Herrero, M., Mendiola, J.A., Castro-Puyana, M., 2012. Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. In: Hayes, M. (Ed.), Marine Bioactive Compounds: Sources, Characterization and Applications. Springer, pp. 55–98.
- Inczedy, J., Lengyel, T., Ure, A.M., 1998. Supercritical Fluid Chromatography and Extraction. Compendium of Analytical Nomenclature (Definitive Rules 1997), third ed. Blackwell Science.
- 42. Jain, T., 2009. Microwave assisted extraction for phytoconstituents an overview. Asian Journal of Research in Chemistry 2 (1), 19–25.
- Jennings, W.G., Rapp, A., 1983. Sample Preparation for Gas Chromatographic Analysis, first ed. Verlagsgruppe Huthig Jehle Rehm GmbH, Heidelberg. 43-B Kaufmann, B., Christen, P., 2002. Recent extraction techniques for natural products: microwave-assisted extraction and pressurized solvent extraction. Phytochemical Analysis 13 (2), 105–113.
- 44. Khorassani, M.A., Taylor, L.T., 2004. Sequential fractionation of grape seeds into oils, polyphenols, and procyanidins via a single system employing CO2-based fluids. Journal of Agricultural and Food Chemistry 52 (9), 2440–2444.
- 45. Lakkakula, N.R., Lima, M., Walker, T., 2004. Rice bran stabilization and rice bran oil extraction using ohmic heating. Bioresource Technology 92 (2), 157–161.
- Landbo, A.K., Meyer, A.S., 2001. Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (Ribes nigrum). Journal of Agricultural and Food Chemistry 49 (7), 3169–3177.
- 47. Lang, Q., Wai, C.M., 2001. Supercritical fluid extraction in herbal and natural product studies—a practical review. Talanta 53 (4), 771–782.

- Laroze, L., Soto, C., Zúñiga, M.E., 2010. Phenolic antioxidants extraction from raspberry wastes assisted by-enzymes. Electronic Journal of Biotechnology 13 (6). http://dx.doi.org/10.2225/vol13-issue6-fulltext-12.
- 49. Latif, S., Anwar, F., 2009. Physicochemical studies of hemp (Cannabis sativa) seed oil using enzyme-assisted cold-pressing. European Journal of Lipid Science and Technology 111 (10), 1042–1048.
- 50. Lebovka, N.I., Bazhal, M.I., Vorobiev, E., 2002. Estimation of characteristic damage time of food materials in pulsed-electric fields. Journal of Food Engineering 54 (4), 337–346
- Letellier, M., Budzinski, H., 1999. Microwave assisted extraction of organic compounds. Analusis 27 (3), 259–270. Li, H., Chen, B., Yao, S., 2005.
- 52. Application of ultrasonic technique for extracting chlorogenic acid from Eucommia ulmodies Oliv. (E. ulmodies). Ultrasonics Sonochemistry 12 (4), 295–300.
- Li, B.B., Smith, B., Hossain, M.M., 2006. Separation and purification in the food industry extraction of phenolics from citrus peels: II. Enzyme-assisted extraction method. Separation and Purification Technology 48 (2), 189–196.
- 54. López, N., Puértolas, E., Condón, S., Álvarez, I., Raso, J., 2008. Effects of pulsed electric fields on the extraction of phenolic compounds during the fermentation of must of Tempranillo grapes. Innovative Food Science and Emerging Technologies 9 (4), 477–482.
- 55. López, N., Puértolas, E., Condón, S., Raso, J., Álvarez, I., 2009. Enhancement of the extraction of betanine from red beetroot by pulsed electric fields. Journal of Food Engineering 90 (1), 60–66.
- Luque de Castro, M.D., Garcia-Ayuso, L.E., 1998. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Analytica Chimica Acta 369 (1–2), 1–10.
- 57. Lusas, E.W., Watkins, L.R., 1988. Oilseeds: extrusion for solvent extraction. Journal of the American Oil Chemists' Society 65 (7), 1109–1114.
- Luthria, D.L., 2008. Influence of experimental conditions on the extraction of phenolic compounds from parsley (Petroselinum crispum) flakes using a pressurized liquid extractor. Food Chemistry 107 (2), 745–752.
- 59. Maier, T., Göppert, A., Kammerer, D.R., Schieber, A., Carle, R., 2008. Optimization of a process for enzyme-assisted pigment extraction from grape (Vitis vinifera L.) pomace. European Food Research and Technology 227 (1), 267–275.
- Majors, R.E., 1999. An overview of sample preparation methods for solids. LC-GC Europe 17 (6), 8–13.
- 61. Majors, R.E., 2003. HPLC column packing design. LC-GC Europe 16 (6), 8-13.
- 62. Marr, R., Gamse, T., 2000. Use of supercritical fluids for different processes including new developments—a review. Chemical Engineering and Processing 39 (1), 19–28.
- 63. Martin, J.F., Demain, A.L., 1978. The filamentous fungi. In: Smith, J.E., Berry, D.R. (Eds.), Developmental Mycology, vol. 3.
- 64. Edward Arnold, London. Mason, T.J., Paniwnyk, L., Lorimer, J.P., 1996. The uses of ultrasound in food technology. Ultrasonics Sonochemistry 3 (3), 253–260.
- 65. Meireles, A., Angela, M., 2003. Supercritical extraction from solid: process design data (2001–2003). Current Opinion in Solid State and Materials Science 7 (4–5), 321–330.
- 66. Meyer, A.S., Jepsen, S.M., Sørensen, N.S., 1998. Enzymatic release of antioxidants for human low-density lipoprotein from grape pomace. Journal of Agricultural and Food Chemistry 46 (7), 2439–2446.

- 67. Moldoveanu, S.C., David, V., 2002. Sample Preparation in Chromatography, first ed. Elsevier, Amsterdam.
- 68. Mroczek, T., Mazurek, J., 2009. Pressurized liquid extraction and anticholinesterase activitybased thin-layer chromatography with bioautography of Amaryllidaceae alkaloids. Analytica Chirica Acta 633 (2), 188–196.
- 69. Ndiomu, D.P., Simpson, C.F., 1988. Some applications of supercritical fluid extraction. Analytica Chimica Acta 213, 237–243.
- Nieto, A., Borrull, F., Pocurull, E., Marcé, R.M., 2010. Pressurized liquid extraction: a useful technique to extract pharmaceuticals and personal-care products from sewage sludge. TrAC Trends in Analytical Chemistry 29 (7), 752–764.
- 71. Niranjan, K., Hanmoungjai, P., 2004. Enzyme-aided aquous extraction. In: Dunford, N.T., Dunford, H.B. (Eds.), Nutritionally Enhanced Edible Oil Processing. AOCS Publishing.
- 72. Pan, X., Niu, G., Liu, H., 2003. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. Chemical Engineering and Processing 42 (2), 129–133.
- 73. Paré, J.J.R., Bélanger, J.M.R., Stafford, S.S., 1994. Microwave-assisted process (MAP[™]): a new tool for the analytical laboratory. TrAC Trends in Analytical Chemistry 13 (4), 176–184.
- 74. Paulsen, B.S., 2010. Highlights through the history of plant medicine. In: Proceedings from a Symposium Held at The Norwegian Academy of Science and Letters, Oslo, Norway. Poole, S.K., Dean, T.A., Oudsema, J.W., Poole, C.F., 1990. Sample preparation for chromatographic separations: an overview. Analytica Chimica Acta 236 (1), 3–42.
- 75. Puértolas, E., López, N., Saldaña, G., Álvarez, I., Raso, J., 2010. Evaluation of phenolic extraction during fermentation of red grapes treated by a continuous pulsed electric fields process at pilot-plant scale. Journal of Food Engineering 119 (3), 1063–1070.
- 76. Puri, M., Sharma, D., Barrow, C.J., 2012. Enzyme-assisted extraction of bioactives from plants. Trends in Biotechnology 30 (1), 37–44.
- 77. Raverchon, E., Marco, I.D., 2006. Review: supercritical fluid extraction and fractionation of natural matter. Journal of Supercritical Fluids 38 (2), 146–166.
- Richter, B.E., Jones, B.A., Ezzell, J.L., Porter, N.L., Avdalovic, N., Pohl, C., 1996. Accelerated solvent extraction: a technology for sample preparation. Analytical Chemistry 68 (6), 1033–1039.
- 79. Rosenthal, A., Pyle, D.L., Niranjan, K., 1996. Aqueous and enzymatic processes for edible oil extraction. Enzyme Microbial Technology 19 (6), 402–420.
- Rosenthal, A., Pyle, D.L., Niranjan, K., Gilmour, S., Trinca, L., 2001. Combined effect of operational variables and enzyme activity on aqueous enzymatic extraction of oil and protein from soybean. Enzyme and Microbial Technology 28 (6), 499– 509.
- Rostagno, M.A., Palma, M., Barroso, C.G., 2003. Ultrasound-assisted extraction of soy isoflavones. Journal of Chromatography A 1012 (2), 119–128
- Rostagno, M.A., Palma, M., Barroso, C.G., 2004. Pressurized liquid extraction of isoflavones from soybeans. Analytica Chimica Acta 522 (2), 169–177.
- Saldaña, M.D.A., Mohamed, R.S., Baer, M.G., Mazzafera, P., 1999. Extraction of purine alkaloids from maté (Ilex paraguariensis) using supercritical CO2. Journal of Agricultural and Food Chemistry 47 (9), 3804–3808.
- Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M., Latha, Y.L., 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional Complementary and Alternative Medicines 8 (1), 1–10.
- 85. Sharma, A., Khare, S.K., Gupta, M.N., 2002. Enzyme-assisted aqueous extraction of peanut oil. Journal of American Oil Chemist's Society 79 (3), 215–218.

- Shen, J., Shao, X., 2005. A comparison of accelerated solvent extraction, Soxhlet extraction, and ultrasonic-assisted extraction for analysis of terpenoids and sterols in tobacco. Analytical and Bioanalytical Chemistry 383 (6), 1003–1008.
- 87. Shu, Y.Y., Ko, M.Y., Chang, Y.S., 2003. Microwave-assisted extraction of ginsenosides from ginseng root. Microchemical Journal 74 (2), 131–139.
- 88. Sihvonen, M., Järvenpää, E., Hietaniemi, V., Huopalahti, R., 1999. Advances in supercritical carbon dioxide technologies. Trends in Food Science and Technology 10 (6–7), 217–222.
- Silva, L.V., Nelson, D.L., Drummond, M.F.B., Dufossé, L., Glória, M.B.A., 2005. Comparison of hydro distillation methods for the deodorization of turmeric. Food Research International 38 (8–9), 1087–1096.
- Singh, R.K., Sarker, B.C., Kumbhar, B.K., Agrawal, Y.C., Kulshreshtha, M.K., 1999. Response surface analysis of enzyme-assisted oil extraction factors for sesame, groundnut, and sunflower seeds. Journal of Food Science and Technology 36 (6), 511–514.
- 91. Smith, R.M., 2002. Extractions with superheated water. Journal of Chromatography A 975 (1), 31–46.
- 92. Smith, R.M., 2003. Before the injection—modern methods of sample preparation for separation techniques. Journal of Chromatography A 1000 (1–2), 3–27. Soxhlet, F., 1879.
- Die gewichtsanalytische Bestimmung des Milchfettes. Dingler's Polytechnisches Journal 232, 461–465. Suslick, K.S., Doktycz, S.J., 1990. The effects of ultrasound on solids. In: Mason, T.J. (Ed.), Advances in Sonochemistry, vol. 1. JAI Press, New York, pp. 197–230.
- 94. Szumski, M., Buszewski, B., 2002. State of the art in miniaturized separation techniques. Critical Review of Analytical Chemistry 32 (1), 1–46.
- 95. Temelli, F., Güçlü-Üstündag, Ö., 2005. Supercritical Technologies for Further Processing of Edible Oils. Bailey's Industrial Oil and Fat Products. John Wiley & Sons, Inc. http://dx.doi.org/10.1002/047167849X.
- 96. Tiaz, L., Zeiger, E., 2006. Secondary metabolites and plant defense. In: Plant Physiology, 4th ed. Sinauer Associates, Inc., Sunderland, Massachusetts, pp. 283–308 (Chapter 13).
- 97. Toepfl, S., Mathys, A., Heinz, V., Knorr, D., 2006. Review: potential of high hydrostatic pressure and pulsed electric fields for energy efficiency and environmentally friendly food processing. Food Review International 22 (4), 405–423.
- 98. Torssell, K.G., 1997. Natural Product Chemistry. A Mechanistic, Biosynthetic and Ecological Approach, second ed. Apotekarsocieteten–Swedish Pharmaceutical Society, pp. 12–40.
- 99. Vankar, P.S., 2004. Essential oils and fragrances from natural sources. Resonance 9 (4), 30–41.
- 100. Verma, A., Hartonen, K., Riekkola, M.L., 2008. Optimisation of supercritical fluid extraction of indole alkaloids from Catharanthus roseus using experimental design methodology—comparison with other extraction techniques. Phytochemical Analysis 19 (1), 52–63.
- 101. Vinatoru, M., 2001. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrasonics Sonochemistry 8 (3), 303–313.
- 102. Vinatoru, M., Toma, M., Radu, O., Filip, P.I., Lazurca, D., Mason, T.J., 1997. The use of ultrasound for the extraction of bioactive principles from plant materials. Ultrasonics Sonochemistry 4 (2), 135–140.
- 103. Vinatoru, M., Toma, M., Filip, P., Achim, T., Stan, N., Mason, T.J., Mocanu, P., Livezeanu, G., Lazurca, D., 1998. Ultrasonic Reactor Dedicated to the Extraction of Active Principles from Plants. Romanian Patent, Nr. 98-01014.

- 104. Vorobiev, E., Lebovka, N.I., 2006. Extraction of intercellular components by pulsed electric fields. In: Raso, J., Heinz, V. (Eds.), Pulsed Electric Field Technology for the Food Industry: Fundamentals and Applications. Springer, New York, pp. 153–194.
- 105. Vorobiev, E., Jemai, A.B., Bouzrara, H., Lebovka, N.I., Bazhal, M.I., 2005. Pulsed electric field assisted extraction of juice from food plants. In: Barbosa-Canovas, G., Tapia, M.S., Cano, M.P. (Eds.), Novel Food Processing Technologies. CRC Press, New York, pp. 105–130.
- 106. Wang, L., Weller, C.L., 2006. Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology 17 (6), 300–312.
- 107. Wang, L.J., Weller, C.L., Schlegel, V.L., Carr, T.P., Cuppett, S.L., 2008. Supercritical CO2 extraction of lipids from grain sorghum dried distillers' grains with solubles. Bioresource Technology 99 (5), 1373–1382.
- 108. Wnk, M., 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64 (1), 3–19.
- 109. Yang, Y., Zhang, F., 2008. Ultrasound-assisted extraction of rutin and quercetin from Euonymus alatus (Thunb.) Sieb. Ultrasonics Sonochemistry 15 (4), 308–313.
- 110. Yang, L., Wang, H., Yuan-gang, Zu., Zhao, C., Zhang, L., Chen, X., Zhang, Z., 2011. Ultrasound-assisted extraction of the three terpenoid indole alkaloids vindoline, catharanthine and vinblastine from Catharanthus roseus using ionic liquid aqueous solutions. Chemical Engineering Journal 172 (2–3), 705–712.
- Zosel, K., 1964. German Patent: 1493,190. Zougagh, M., Valcárcel, M., Ríos, A., 2004. Supercritical fluid extraction: a critical review of its analytical usefulness. Trends in Analytical Chemistry 23 (5), 399–405.
- 112. Zu, G., Zhang, R., Yang, L., Ma, C., Zu, Y., Wang, W., Zhao, C., 2012. Ultrasoundassisted extraction of carnosic acid and rosmarinic acid using ionic liquid solution from Rosmarinus officinalis. International Journal of Molecular Science 13 (9), 11027–11043