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Novel extraction techniques towards the recovery of plant derived secondary metabolites – a review

Zastosowanie nowoczesnych technik ekstrakcyjnych w izolacji roślinnych metabolitów wtórnych – praca przeglądowa

INTRODUCTION

Liquid-liquid extraction (also known as solvent extraction) involves the separation of the constituents (solutes) of a liquid solution by contact with another insoluble liquid. Solutes are separated based on their different polarities and solubilities in different liquids. Separation is achieved when the substances constituting the original matrice is transferred from the original solution to the other liquid solution [19,34]. Temperature, extrahent's volume, pressure, flow, as well as the surface of extracted matrice and duration of extraction process remain crucial for the effectiveness of this process. Fine powdering process can enhance the recovery, as the limiting step of extraction may be connected with a weak diffusion of chemicals out of the plant matrice. Larger surface area of fine powder provides better contact between the source and a solvent [44].

The effectiveness of extraction is strongly bound with two processes – dissolution and diffusion. Both of them proceed easier in the increased temperature, although such conditions can be used for thermostable compounds only.

In sum, complete recovery of both main and minor secondary metabolites with concurrent loss in ballast substances from a plant remains the aim of extraction.

Traditional extraction methods (maceration, percolation, diacolation, or centrifugal extraction) are performed in the room temperature. Although they belong to the first choice methods in the recovery of thermolabile natural products, they require high amounts of solvents and remain time-consuming.

Modern analytical techniques provide higher recoveries in a shorter time and with use of significantly smaller amounts of extrahents [34].

In the current review, three extraction techniques: Accelerated Solvent Extraction, Supercritical Fluid Extraction and Microwave Assisted Extraction will be characterized. Several examples of plant-derived metabolites' recovery will be presented in the attached figures.

CHARACTERISTICS OF MODERN EXTRACTION METHODS

SUPERCRITICAL FLUID EXTRACTION

Since the first applications of SFE were published by Zosel in 1978, this extraction technique has developed into a key method for the separation of contaminants from both sediment and biological matrices. Extraction of natural products by means of supercritical fluids has found numerous large-scale applications in food, perfume and medicine industries. Decaffeination of coffee beans, extraction of bitter principles from hops, recovery of natural food colorants or volatiles from plants are possible thanks to Supercritical Fluid Extraction. The method enables total or selective extraction of unpolar and polar (after the introduction of a gradient) compounds from the source [5]. Supercritical Fluid Extraction belongs to highly automated, rapid, selective and non-toxic methods. It uses clean, safe, environment-friendly and nonpolluting solvents, which can be easily recycled. SFE is based on the ability of certain substances to be transformed into a supercritical state, which are characterized of both gaseous and liquid properties [13, 14, 32,46].

When a gas is compressed to a sufficiently high pressure, it becomes liquid. Then, the gas is heated over a specific temperature as no compression of this gas will liquefy it. The

values of critical temperature and critical vapor pressure define a critical point, which is unique to a given chemical substance [42].

The state of the substance is called supercritical fluid (SCF) when both the pressure and temperature exceed the critical point values.

Supercritical fluid holds the properties of both gas and a liquid. High diffusivity (one to two orders of magnitude higher than those of other extrahents), viscosity and lower surface tension are responsible for better permeability and penetration of plant material, as well as for its favorable soluting properties [50]. Carbon dioxide is the most common solvent for extraction of plant metabolites. As an inert, inexpensive, odorless, tasteless and GRAS (generally regarded as safe) it is the gas of first choice (see Tab. 1) [52].

Table 1. Physical properties of some common solvents used in SFC state [32]

Fluid	Normal boiling boiling (°C)	Critical Constants		
		Pressure (bar)	**Temperature** (°C)	**Density (g/cm^3)**
Carbon dioxide	-78.5	73.8	31.1	0.468
Ethane	-88.0	48.8	32.2	0.203
Ethylene	-103.7	50.4	9.3	0.200
Propane	-44.5	42.5	96.7	0.220
Propylene	-47.7	46.2	91.9	0.230
Benzene	80.1	48.9	289.0	0.302
Toluene	110.0	41.1	318.6	0.290
Chlorotrifluoromethane	-81.4	39.2	28.9	0.580
Trichlorofluoromethane	23.7	44.1	196.6	0.554
Nitrous oxide	-89.0	71.0	36.5	0.457
Ammonia	-33.4	112.8	132.5	0.240
Water	100.0	220.5	374.2	0.272

Described technology has been recently gaining importance over the conventional methods in the process of natural products' extraction.

For many supercritical fluids, the solubilities of the compounds of interest, even in the high-density region, may be too low for the practical application. This limitation can be overcome through the use of a co-solvent. Early on, researchers discovered that the addition of small amounts of a co-solvent could dramatically enhance the solubility of various analytes.

Addition of polar co-solvents (modifiers) to the supercritical solvent is known to increase the solubility of polar compounds significantly [5]. Chosen modifiers are selected to interact strongly with the compounds of interest. Methanol and ethanol belong to the most frequently used modifiers. They undergo dipole–dipole interactions and hydrogen-bonding with polar functional groups of chosen secondary metabolites. A number of SFE applications employing polar modifiers for the extraction of moderately polar to strongly polar natural products have been published, including alkaloids [14], cardiac glycosides, and various phenolics and phenolic glycosides [33,34,45,46].

The values of dielectric constant, density, and the solvating power of supercritical carbon dioxide depends on its pressure and temperature [34]. Pressure increase enhances the solubility of solutes. At very high pressure values it is possible to increase the solvating power of the extraction fluid. Most SFE applications have been carried out in the pressure range between the critical pressure of carbon dioxide and *ca.* 300 bar [34].

The intrinsic features of SFE are ideal for the extraction of natural products from plant materials. Use of SFE is particularly indicated for thermolabile compounds, as the extractions are carried out at low temperature values. It provides more clean plant extracts without the occurrence of artifacts, which are normally observed by lenghy exposure to hight temperatures. Furthermore, no oxygenation of secondary metabolites have been stated while working with carbon dioxide. Additionally disturbing chlorophylls are not soluble in carbon dioxide and remain in the plant material.

In this context, SFE has a great potential for replacing older extraction methods, e.g. Soxhlet extraction. Despite important limitations connected with the unpolar character of carbon dioxide, with help of special strategies for the extraction of moderately polar and highly polar analytes, the SFE range of applications will dramatically increase in the near future [45,47].

There are several examples of natural products' extraction by means of SFE listed in the Table 2.

ACCELERATED SOLVENT EXTRACTION

ASE (Accelerated Solvent Extraction) is a relatively new extraction method that packs solid and semisolid samples into an extraction cell with liquid solvents. It uses elevated temperatures (above boiling point) and pressures (10 – 14 MPa) to increase the efficiency of the extraction process. Increased temperature accelerates the extraction kinetics, and elevated pressure keeps the solvent below its boiling point, enabling rapid extractions. Under these conditions, the solvent has properties favoring the extraction process, such as low viscosity, high diffusion coefficients and dissolving ratios, as well as high solvent strength. Desorption of analytes from the cellular wall and organelles is of higher extent [6,39]. Disruption of the strong solute-matrice interaction caused by van der Waals forces, hydrogen bonding and dipole interactions between solute molecules and active sites of the matrix is also observed [41].

Table 2. Recovery of different secondary metabolites by means of SFE

Plant species	Group of metabolites	SFE conditions	Comments	
Tabernaemontana catharinensis Apocynaceae	Alkaloids	CO_2+4.6%ethanol T=45ºC p=250bar	The SFE kinetics, the yield, and the content of coronaridine and voacangine were determined for different conditions.	[35]
Sophora flavescens Ait. Fabaceae	Alkaloids	CO_2+75% ethanol T=50ºC p=250bar	The extraction yield of three quinolizidine alkaloids: matrine, oxysophocarpine, oxymatrine differed significantly according to the set conditions.	[25]
Torresea cearensis Fabaceae	Coumarins	CO_2+50%ethanol T=45.2 ºC p=240bar	Solubility of coumarin was highest in the given conditions	[38]
Zea mays L. Poaceae	Flavonoids	CO_2+20% ethanol T=58ºC p=418bar	The amount of flavonoids in *Maydis stigmata* increases with the polarity of extraction mixture. Their extraction yields reached their maxima in 20% aqueous solution of ethanol.	[27]
Scutellaria baicalensis Georgi Lamiaceae	Flavonoids	CO_2-Methanol-Water (20:2.1:0.9) T=50ºC p=200bar	SFE led to the isolation of significant amounts of the followind flavonoids: baicalein, baicalin and wogonin	[24]
Pistachia vera L. Anacardiaceae	Phenols	CO_2+15%MeOH T=45ºC p=355bar	The best results were obtained for solvent extraction (water) and ultrasonic extraction (water). Total phenolic content (Folin-Ciocalteu test) calculated for SFE fractions was 5-fold smaller than the one of the methods listed above.	[12]
*Eucalyptus camaldulensis*Dehnh. Myrtaceae	Phenols	CO_2, T=50ºC p=200bar,	Antioxidant activity was greater in supercritical fluid extracts than in hydrodistillation extracts. Higher concentration of sesquiterpenes, p-cymen-7-ol, thymol and oxygenated compounds in SF fractions.	[10]
Tamarindus indica L. Leguminosae	Phenols	CO_2+ 10% ethanol	Raise of antioxidant activity was observed with the increase of temperature and pressure. 10% addition of ethanol increased the antioxidant activity. Ethanolic extracts were found to be more rich than SF fractions.	[47]
Zingiber officinale Roscoe Zingiberaceae	Phenols	CO_2/ CO_2+ ethanol T=25-35ºC p=200-250bar	Highest antioxidant activity (related to the preferential extraction of gingerols and shogaols) obtained with a modifier At low tempeatures, low pressures and long extraction simes.	[51]
Olive oil *Olea europaea* Oleaceae	Phenols	CO_2 T=40ºC p=350bar	SC-CO2 was confirmed to be an efficient solvent for recovering phenolic compounds with relatively high antioxidant activity from olive oil mill waste.	[21]
Phyllanthus niruri Linn. Phyllanthaceae	Tannins	CO_2+50%ethanol T=100ºC p=200bar	SFE gave good results in tannins' extraction, although Soxhlet extraction and pressurized solvent extraction were found preferable.	[28]
Terminalia catappa L. Combretaceae	Volatiles	CO_2+10%methanol T=40ºC p=200bar		[22]
Perilla frutescens (L.) Britton Lamiaceae	Volatiles	CO_2 T=45ºC p=300bar Static=10min Dynamic=60min	At a given pressure, the higher extraction yield was generated at a higher temperature Use of higher pressures and temperatures, led to the co-extraction of heavy compounds with large retention indices, and a higher extraction temperature also led to heat degradation of the sensitive compounds	[11]

Table 3 Recovery of plant derived secondary metabolitem by Accelerated Solvent Extraction

Plant species	Group of compounds	ASE conditions	Comments	
Narcissus jonquilla 'Pipit' Amaryllidaceae	Alkaloids	Solvent= 1% tartaric acid methanolic solution T=120ºC Static time=10min p=60bar static cycle=1	Optimized ASE was of higher effectiveness than MAE, UAE and hot-solvent extraction	[31]
Coptis chinensis Franch. Ranunculaceae	Alkaloids	Solvent=80% aqueous ethanol + 0.5%HCl T=100ºC Static time=10min Extraction cycles=2	berberine, palmatine and jatrorrhizine were recovered from the plant material	[7]
Houttuynia cordata Thunb. Saururaceae	Flavonoids	Solvent=50% ethanol T=70ºC Static time=15min p=80bar static cycle=1	The conditions given led to high yields of flavonoids (higher than in ultrasonic extraction)	[53]
Glycine max (L.) Maxx Fabaceae	Flavonoids	Solvent=90% aqueous methanol T=80ºC p=150bar Static time=10min Extraction cycles=3	ASE coupled with 1min UAE gave the best results concerning the recovery of chosen flavonoids from plant material	[2]
Pastinaca sativa L. Apiaceae	Furanocoumarins	Sample mixed with neutral glass Solvent= methanol T=100ºC p=60bar	the yield of furanocoumarins was highest by use of ASE method as well as by ultrasonification at 60 ◦C	[49]
Rosmarinus officinalis L. *Origanum majorana* L. Lamiaceae	Phenols	Solvent=56% methanol T=129ºC Static time=5min p=103bar static cycle=1 flush volume=60%	The antioxidant activity yields of the optimal ASE extracts were significantly ($p<0.05$) higher 37 than solid/liquid extracts.	[15]
Ginseng radix *Panax ginseng* L. Araliaceae	Saponins	Sample+diatomaceous earth (2:1) Solvent=methanol T=150ºC Static time=15min p=103bar static cycle=1	Extraction of ginsenosides from *Panax ginseng radix et folium*. Complete extraction after first cycle	[36]
Oak wood *Quercus spp.* Fagaceae	Volatiles	Solvent=DCM T=150ºC Static time=7min p=200bar	Extraction of volatile and semi-volatile constituents	[45]
Angelica spp. Apiaceae	Volatiles	Solvent=n-hexane T=80ºC p=103bar Static time=10min Extraction cycles=2	Samples were mixed with sea sand standard (5:2)	[8]
Tobacco *Nicotiana spp.* Solanaceae	Volatiles and semi--volatiles	Solvent=dichloromethane T=100ºC Static time=5min Extraction cycles=2 p=103bar	ASE has been used as a pretreatment method for chemical fingerprinting of volatile and semi-volatile components in cut tobacco	[23]

ASE apparatus is highly automated and easily approachable. It gives the opportunity of damp samples' extraction. Another advantage of ASE is the possibility of extraction solvents' choice. They can be adjusted according to the character of extracted material.

Accelerated solvent extraction is considered as a potential alternative technique to SFE for the extraction of polar compounds [3]. Compared with traditional Soxhlet extraction, there is a dramatic decrease in the amount of solvent and the extraction time for ASE [37]. Particular attention should be paid to the accelerated solvent extraction performed with high extraction temperature, which may lead to degradation of thermolabile compounds.

Applications of Accelerated Solvent Extraction remain broad. The extraction of plant derived natural products (see: Table 3), contaminants in environmental matrices, as well as additives in polymers is possible with the use of ASE apparatus [1,48].

The use of ASE decreases the total extraction time and improves the extraction efficiency through the manipulation of parametres such as temperature, time, cycles and solvent.

MICROWAVE ASSISTED EXTRACTION

Microwaves belong to electromagnetic radiations characterized by a frequency of 0.3-300 GHz. They can penetrate biomaterials and interact with polar molecules such as water in the biomaterials to create heat. As a result, microwaves get inside the matrice and heat it homogeneously. It facilitates the desorption of chemicals from the source, improving the recovery of nutraceuticals. The changes caused by microwaves in plant tissues gives a considerable increase in the yield of extractable secondary metabolites (see: Table 4). Furthermore, the migration of dissolved ions enhances the penetration of a solvent into the matrix and thus facilitates the release of the chemicals. The effect of microwave energy is thus strongly dependent on the dielectric susceptibility of both the solvent and the solid plant material [18].

There are two types of MAE systems commercially available: closed extraction vessels (used for extraction under drastic conditions of both temperature and pressure) and focused ovens (characterized of short extraction time, but lower operation temperatures – only around boiling points of used solvents) [9,18].

MAE depends on dielectric susceptibility of solvent and matrix. On the basis of the above information, better recoveries can be obtained by moistening of the samples with a substance possessing a relatively high dielectric constant (e.g. water). In this case, the matrix itself can interact with microwaves and subsequently facilitate the heating process.

The microwave heating leads to the rupture of cell walls and is followed by the liberation of chemicals into the solvent [44]. In this case, the surrounding solvent can have a low dielectric constant and thus remains cold during extraction. This method can be used to extract thermo-sensitive compounds such as essential oils [4].

It was confirmed, that MAE extraction of completely dried samples was not possible [30].

Solvent choice suitable for this technique influenced by solubility of extracts, interaction between solvent and plant matrix, and, finally, by microwave absorbing properties of a solvent (determined by its dielectric constant). Solvents such as ethanol, methanol and water are sufficiently polar to be heated by microwave energy [4]. Nonpolar solvents with low dielectric constants such as hexane and

toluene are not potential solvents for MAE. The extracting selectivity and the ability of the solvent to interact with microwaves can be modulated by using mixtures of solvents (e.g. hexane-acetone) [4,44]. A small amount of water (e.g. 10%) can also be incorporated in non-polar solvents such as hexane, xylene, or toluene to improve the heating rate [43,44].

Temperature – another important factor – is proportional to the recovery of constituents from the matrix. Working with thermolabile compounds special precautions must be undertaken not to cause their decomposition [11].

MAE has been considered as a potential alternative to traditional solid–liquid extraction. It has been used to extract nutraceuticals for several reasons: reduced extraction time and solvent usage as well as improved extraction yields. MAE is also comparable to other modern extraction techniques such as supercritical fluid extraction due to the process simplicity and low costs. By considering economical and practical aspects, MAE is a strong novel extraction technique for the extraction of nutraceuticals. However, compared to SFE, an additional filtration or centrifugation is necessary to remove the solid residue during MAE. Furthermore, the efficiency of microwaves can be very poor when either the target compounds or the solvents are non-polar, or when they are volatile.

Possible applications of MAE in the recovery of natural products are listed in the Table 4.

Table 4 Microwave Assisted Extraction. Conditions suitable for the recovery of chosen secondary metabolites. R = ratio of solid to solvent (g/ml)

Plant species	Group of compounds	MAE conditions	Comments	LIT.
Ginseng radix *Panax ginseng* L. Araliaceae	Saponins	80%methanol R= 1/10 T=75ºC T=0.5min		[36]
Allium cepa L. Aliaceae	Flavonoids	No solvent T=100ºC T=23min	41.9% of flavonoids in obtained extract	[54]
Saussurea medusa DC. Asteraceae	Flavonoids	80%ethanol R= 1/100 T=80ºC t=60min	The lower the R value, the higher flavonoid content in the obtained extracts	[29]
Pastinaca sativa L. Apiaceae	Furanocoumarins	80%ethanol R= 1/100 T=80ºC t=31min	The highest amount of xanthotoxine was obtained by MAE, whereas other coumarins were in higher concentration in ASE and UAE extracts	[49]
Glycine max (L.) Maxx Fabaceae	Isoflavones	50% ethanol R-1/50 T=50ºC t=20min	No decomposition of isoflavones was observed	[40]
Tobacco *Nicotiana spp.* Solanaceae	Terpenes	hexane:ethanol=1:3 R= 1/10 T=60ºC t=40min		[17]
Solanum lycopersicum L. Solanaceae	Pigments (lycopene)	Ethyl acetate R= 1/10.6 T=60ºC t=6min	The percentage of lycopene yield was 97.4% in the obtained extract (higher amount than during the UAE)	[52]

CONCLUSIONS

The need to extract nutraceuticals from plant material stimulates continuous search for economical and environmently friendly extraction technologies. Classical solid-liquid extraction techniques require significant amounts of solvents and are time consuming. It increases the operating costs but also causes environmental problems. The techniques presented above have been developed as an alternative to the conventional extraction, offering advantages such as: higher extraction yields, shorter extraction time, lower solvent consumption. Much research is needed to improve the understanding of secondary metabolites' recovery to remove technical barriers and improve the design of novel systems for analytical and industrial applications.

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SUMMARY

Constant pursuit for new medicines sets higher demands towards the analytical techniques these days. Numerous plant derived secondary metabolites require successful recovery methods to be obtained from the matrix. Proper conditions of extraction guarantee full recovery of secondary metabolites from different parts of a plant in the satisfactory yields, short time and use of non-toxic solvents. In the current paper three novel extraction techniques are thoroughly characterized. Supercritical Fluid Extraction, Accelerated Solvent Extraction and Microwave Assisted Extraction exceed conventional extraction methods. Current knowledge on the extraction conditions of different groups of secondary metabolites is presented here as well.

Keywords: Extraction, Supercritical Fluid Extraction, Accelerated Solvent Extraction, Microwave Assisted Extraction

STRESZCZENIE

Nieustające poszukiwania nowych leków skutecznych w walce z nieuleczalnymi chorobami stawia coraz wyższe wymagania technikom ekstrakcji, separacji i analizy materiału roślinnego. Ekstrakcja ciał czynnych z materiału biologicznego wymaga zaawansowanych technik ekstrakcji. Dobór właściwych technik ekstrakcji zapewnia pełny odzysk metabolitów wtórnych zawartych w różnych częściach substancji roślinnej w zadowalających ilościach, z zachowaniem krótkiego czasu ekstrakcji oraz z zastosowaniem nietoksycznych rozpuszczalników. W niniejszym opracowaniu

wnikliwie scharakteryzowano trzy techniki ekstrakcji. Ekstrakcja nadkrytyczna, przyspieszona ekstrakcja ciśnieniowa oraz ekstrakcja wspomagana promieniowaniem mikrofalowym zdecydowanie przewyższają klasyczne techniki wytrawiania substancji roślinnych. Ponadto przedstawiono wybrane aspekty doboru warunków zapewniających jak najwyższy stopień odzysku metabolitów wtórnych z roślin należących do różnych rodzin.

Słowa kluczowe: Ekstrakcja, ekstrakcja nadkrytyczna, ekstrakcja ciśnieniowa, ekstrakcja wspomagana mikrofalami