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Methods for Obtaining and Analysing Extracts of *Sambucus nigra*

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Abstract: The elderberry (*Sambucus nigra* L.) is known for its high anthocyanin content and strong antioxidant activity, which is why multiple extraction methods are being analysed to choose the most favourable method considering both economic and environmental aspects for extracting the analysed plant matrix. The extract obtained must exhibit a certain stability to be included in various food products as a colorant without losing its beneficial properties on the product. This work aims to select the most suitable extraction methods for the analysed plant matrix to achieve the most beneficial results.

Keywords: antioxidants, extraction, methods, *Sambucus nigra* L.

Introduction

Anthocyanins are of particular interest to the food colouring industry because of their ability to impart vibrant colours. Anthocyanins are the most important pigments among flavonoids; anthocyanidins are linked to one or more sugar molecules (the glycoside form), which consist of an aromatic ring linked to an oxygen-containing heterocyclic ring, which is also linked by a carbon-carbon bond to a third aromatic ring (Albuquerque et al., 2020).

Elderberries (*Sambucus nigra*) contain an abundant amount of anthocyanins, the pigment that gives them a blue-violet colour. The abundance of these anthocyanins and other polyphenols is highly appreciated in today's market for their potential health benefits (Lee and Finn, 2007). These fruits hold high commercial value due to the quantity of anthocyanins, some of which are only present in elderberries. They are used in the industry as a food colouring agent, in jams, jellies, bakery products, ice cream, yogurts, juices, syrups, and alcoholic beverages (Ferreira et al., 2020).

Sambucus nigra has been examined for its potential as a natural colorant and botanical supplement for human nutrition by numerous researchers. Products from *S. nigra* and *S. canadensis* fruits have been studied for their stability in response to heat and light. *Sambucus nigra* has a higher number of acylated anthocyanins, while *S. canadensis* contains more stable acylated anthocyanins.

Due to the health benefits offered by elderberries, there is an interest for the extraction of colour compounds from the fruits using various methods and introduce them into food products, replacing the artificial colorants currently used. This initiative aims to bring a nutritional benefit to the products in which it is introduced.

Sambucus (Caprifoliaceae) is a genus of approximately 20 temperate and subtropical species of small trees, shrubs, and herbs (Atkinson and Atkinson, 2002). *Sambucus nigra* L. (Figure 1) has three subspecies: *S. nigra L. ssp. nigra, S. nigra L. ssp. canadensis, S. nigra L. ssp. cerulea* (Młynarczyk et al., 2017). *Sambucus canadensis*, the American elder is native to the eastern part of North America, it is found from Florida to the northern part of the Gaspe coast of Quebec in Canada, where the wild type and some varieties grow well, but the fruit hardly reaches full maturity because a short growing season. *Sambucus nigra*, the European elder has a distribution that extends further north than its American counterpart, reaching Norway. It reaches its latitudinal and altitudinal limits, where the average temperature in October is around 7°C, which is probably a period for ripening. *Sambucus nigra* has been introduced to various parts of the world, including Asia, North America, New Zealand, and southern Australia (Atkinson and Atkinson 2002). The parts of the plant material that are relevant for production are the fruit and flower extract, in many countries the larger amounts of raw material are collected from the wild. Total fruit production compared to other berry crops is quite small and therefore relevant statistical data are scarce or incomplete (Christensen et al., 2008).

General aspects regarding the elderberry

Elderberry can be grown on a wide variety of soils. Excellent growth and yield can be expected in organic soil (mud). Mineral soil will also provide good conditions for elderberry production. Sandy soils, although capable of supporting limited growth and production, provide few nutrients and insufficient water retention. The most favourable environment for development includes soils rich in bases, nitrogen, and phosphate (Atkinson and Atkinson 2002). Nitrogen can be applied in any form. When the cuttings are placed, all that is required is to manually apply the equivalent of 0.30 kg N near, but not touching, the base of each cutting (Charlebois et al., 2010).

Figure 1. *Sambucus nigra*
Source: https://gradinahobby.blogspot.com/

Ripe elderberries contain carbohydrates, including dietary fibres, mainly hemicelluloses and polygalacturonic acid, pectins and simple sugars (Przybylska-Balcerek et al., 2021). Other carbohydrates found in *Sambucus nigra* are pectic acid, protopectin, Ca-pectate and cellulose (Młynarczyk et al., 2017). In terms of carbohydrates, elderberries contain 7.86–11.50% of total sugar and 2.8–8.55% from the reducing sugar (Przybylska-Balcerek et al., 2021). The main sugars identified by Veberic et al. (2009), were glucose and fructose, while only small amounts of sucrose were present in fruits.

The chemical composition of elderberries depends on various factors, including variety, conditions of environment, processing method and storage conditions. The bioactive compounds found in elderberries are mainly polyphenols and anthocyanins. Due to due to its rich phenolic composition, *S. nigra* extracts show significant antioxidant activity. The content of polyphenols and anthocyanins also depends on the growing season. The main polyphenols in elderberries are chlorogenic acid, neochlorogenic acid, acid cryptochlorogenic, quercetin, quercetin-3-rutinoside, quercetin-3-glucoside (isoquercitrin), kaempferol-3-rutinoside, kaempferol-3-glucoside, isorhamnetin-3-rutinoside and isorhamnetin-3-glucoside. The content of polyphenols changes at different stages of fruit ripening and each compound shows its own pattern of individual change during this process (Młynarczyk et al., 2017). A total of 16 phenolic compounds were identified like those previously described by Uzlasir et al. (2020).

Sambucus nigra fruit contains anthocyanins such as cyanidin-3-glucoside and cyanidin-3-sambubioside. Two other (minor) anthocyanins are cyanidin-3,5-diglucosides and cyanidin-3-sambubioside-5-glucoside. In addition, traces of cyanidin-3-rutinosides, pelargonidin-3-glucosides and delphinidin-3-rutinosides have been identified in the fruits of certain elderberry varieties (Młynarczyk et al., 2017). Lee and Finn (2007) found the same 11 anthocyanins present in each of the *S. canadensis* that tested them: cyanidin 3-(E)-p-coumaroylsambubioside-5-glucoside (major pigment present) cyanidin 3-sambubioside-5-glucoside (second major pigment present), cyanidin 3,5-diglucoside, cyanidin 3-sambubioside, cyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside (traces present),cyanidin 3-(Z)-p-coumaroyl-sambubioside5-glucoside, cyanidin 3-p-coumaroyl-glucoside, petunidin 3-rutinoside (traces present), and cyanidin 3-p-coumaroyl-sambubioside.

Although the fruit elderberry and elderflowers are rich sources of phenolic compounds, exposure to changing oxygen, light, temperature and pH, the presence of metal ions, water, and enzyme activity, can enhance degradation and the loss of their activity (Ferreira et al., 2020).

The number of extractable oils is significant (approx. 30% of dry weight) for this species, and agro-industrial wastes from various producers are used as feed supplements for animals or organic fertilizer, cosmetic agents and in pharmaceutical industries. Waste from processing elderberries could probably be used in a similar way. In fact, the oil content of the pressing residues can reach up to 12%, and these residues are particularly rich in tocopherol. Amounts important amounts of anthocyanins can be extracted from elderberry (Seabra et al., 2010).

Elderberries are characterized by a high antioxidant activity, which ranges from 82.08 at 89.25% inhibition with respect to the DPPH radical. The antioxidant properties of elderberries are attributed primarily to the presence of phenolic compounds and largely depend on the chemical structure of molecules and individual fruit composition. Anthocyanins significantly influence the activity antioxidant in elderberries. As the concentration of anthocyanins increases, the activity also increases antioxidant, but only up to a certain level, after which this parameter begins to decrease (Pliszka et al., 2005).

Anthocyanins can present different chemical forms depending on the pH, being more stable under the acid condition, in conjugated forms (glycosylation and esterification), as well as complexed with other flavonoids (copigmentation). Anthocyanins could be significantly absorbed in the stomach due to the acidic pH (Fig.2.), but anthocyanins present as glycosides are not absorbed in the small intestine, they must transform into aglycone which is more lipophilic and is absorbed by passive diffusion (Ferreira et al., 2020).

Due to the presence of flavonoids, elderflowers primarily demonstrate diaphoretic properties and diuretics. They seal the capillary walls, improve their flexibility, and prevent the infiltration of red cells and plasma outside the vessels, due to the content of compounds (rutin) having the properties of vitamin P. Furthermore, elderflowers show anti-inflammatory and antibacterial properties and are therefore used for gargle to treat sore throats or as compresses to treat conjunctivitis. They are the most often used as dried flower infusions for internal or external application (Krawitz et al., 2011).

Cyanogenic glycosides are secondary metabolites of plants, which consist of an aglycone and a fragment of sugar. Amygdalin can be found in several plant families, including the family *Caprifoliaceae*, which also includes *Sambucus nigra*. An important characteristic of cyanogenic plants is the ability to generate toxic hydrogen cyanide. Although cyanogenic glycosides are not toxic when they are intact, they become toxic when plant enzymes (b-glucosidases and a-hydroxynitrile lyases) come in contact with the cyanogenic glycosides in the plant as a result of tissue damage after bruising or chewing (Bolarinwa et al., 2014).

Sticks, roots, and leaves are not dangerous if properly prepared. Glycosides cyanogenic are not toxic in themselves, but the interaction between enzymes and cyanogenic glycosides in the microflora intestinal in the gastrointestinal tract or plant enzymes promote the cleavage of the glycoside portion of cyanohydrins and then later decompose to hydrogen cyanide (HCN) and aldehyde (Ferreira et al., 2020)

Extraction methods of flavouring and colourings

Considering the diversity of plants and essential tissues, selecting the optimal extraction process can ensure the successful extraction of bioactive substances from plant materials. Traditional methods of extraction, which basically include Soxhlet extraction, steam or hydro distillation, maceration, or solvent extraction, usually contain large amounts of hazardous organic solvents, are non-selective, time extraction time is long and, in some cases, extirpates thermosensitive substances (Garavand et al., 2019).

Conventional extraction techniques

The extraction is a unit operation of mass transfer in which a particular compound or class of compounds is separated from a matrix, solid or liquid, through chemical, physical, or mechanical processes (Prado et al., 2014). These methods can be divided into conventional or non-conventional methods. Conventional methods include extraction with organic solvents using Soxhlet techniques, maceration, agitation, percolation, or centrifugation, and extraction using water through hydrodistillation or steam distillation. Such methods may require the use of potentially harmful organic solvents and high temperatures; they also require high energy input and long processing times, leading to low selectivity and/or yield (Prado et al., 2018).

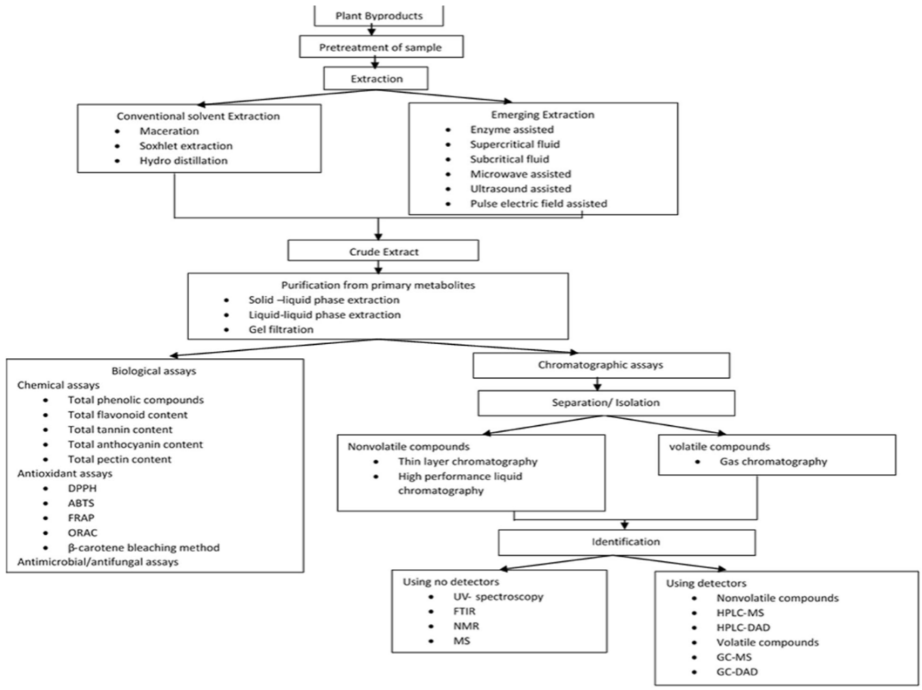

Figure 2. Flow chart showing the extraction and characterization of bioactive compounds from fruit by-products
Source: Patra et al. (2022)

Bioactive substances need to be extracted from various plants in order to be used for foods. Compared to traditional techniques, it is argued that an appropriate extraction method can capture the bioactive target up to 5 times. Extraction strategies, from classical to sophisticated, should be adapted based on the type of bioactive substances present in the examined plant and the inherent characteristics of the plant, such as tissue complexity and heat sensitivity (El Asbahani et al., 2015). Considering the diversity of plants and their essential tissues, selecting the optimal extraction process can ensure the successful extraction of bioactive substances from plant materials. Traditional extraction methods, which principally include Soxhlet extraction, steam or hydrodistillation, maceration, or solvent extraction, usually contain large amounts of hazardous organic solvents, are non-selective, have long extraction times, and in some cases, extract thermosensitive substances (Garavand et al., 2019).

Soxhlet Extraction

Soxhlet extraction has been a widely used and accepted extraction technique for extracting various bioactive compounds from solid materials derived from diverse natural sources. Franz Ritter von Soxhlet, a German scientist, was the first to propose the Soxhlet extractor in 1879. It was primarily created for lipid extraction, although it is no longer limited solely to this purpose. The Soxhlet extraction method has been extensively used for extracting important bioactive substances from a wide range of natural sources. It is used as a benchmark for new extraction alternatives (Anusha Siddiqui et al., 2022). The advantages of this technique include ease of operation (it is a very simple method that requires little training), low investment costs, no need for filtration after extraction, and requiring only a small amount of raw material. Among the main disadvantages of Soxhlet extraction are high temperature and prolonged extraction time, which increase the possibility of thermal degradation, the large amount of solvent required, as well as the toxicity of the solvents used, which can affect the final quality of the obtained extract. Furthermore, it is only feasible for analytical purposes and is not used on an industrial scale (Prado et al., 2014).

Solvent Extraction (Maceration)

Maceration is another extraction technique where plant material is crushed and placed in a closed vessel with a solvent, allowing it to sit for a specific period, occasionally stirring. The process aims to soften and break the plant's cell walls to release soluble compounds. After extraction, the mixture is pressed or filtered. The advantages include reduced costs and ease of handling, while the main disadvantages are the lengthy time required for efficient extraction and the large amount of solvent needed (Prado et al., 2014).

Solvent extraction can be used to extract oils from plant material. It is a well-known and cost-effective method for obtaining basic bioactive components and essential oils. Maceration involves several steps for extraction in the laboratory. Various solvents, including water, organic solvents, and their combinations such as water and ethanol (35-50%), or ethanol and acetone, have been used to extract bioactive components from elderberries. Another study examines obtaining anthocyanins from aronia and elderberries using glycerol-water systems. It was found that glycerol can be used as an alternative solvent to ethanol in anthocyanin extraction, which may not always be suitable for use in food products (Kowalska et al., 2021).

Hydrodistillation

Bioactive compounds and essential oils can be obtained from plant tissues through water distillation. The plant components are placed in a distillation chamber; necessary amounts of water are added into a vessel, which is then brought to a boil. On the other hand, hot water and steam are often mentioned as the most efficient techniques for releasing bioactive compounds from plant tissues. It should be noted that high extraction temperatures lead to the unexpected loss of some bioactive components. This drawback hinders its widespread use for extracting thermosensitive bioactive substances (Garavand et al., 2019).

This method has several disadvantages, including a long processing time, destruction of some polar molecules due to overheating, and cyclization and hydrolysis caused by prolonged interaction with boiling water. A plate column is used for recovering bioactive chemicals present in the vapor. Hydrodistillation has long been considered a viable extraction process for industrial and semi-industrial applications due to reduced costs, ease of handling, and excellent selectivity (El Asbahani et al., 2015).

Green Extraction Technologies

Green analytical chemistry aims to reduce the amount of organic solvents during sample preparation, extraction, purification, and detection. Minimizing the use of organic solvents, regardless of economic and environmental aspects can significantly reduce by-products in extraction and analysis processes, affecting the quality of the final compounds (Ali Redha et al., 2021).

Eco-friendly solvents enhance energy performance, ensuring a high level of safety during laboratory work, minimizing sample usage, and utilizing environmentally friendly analytical methods. Consequently, based on automated, safe, online sensors, remote sensing procedures, and image processing practices, are suggested to meet the requirements of green analytical chemistry to achieve an efficient analysis process (Gałuszka et al., 2013). Milena et al. (2019), reported an efficient extraction technique using green solvents to isolate polyphenols from elderberries. Kowalska et al. (2021), assessed a green chemistry approach through potential use of glycerol to obtain anthocyanin extracts from elderberries. They suggested glycerol as a safe alternative to other solvents like ethanol and reported optimal efficiency of water-glycerol solvents for extracting anthocyanins from elderberries. Green analytical chemistry aspects could be performed at every stage (sample preparation, extraction, purification, and identification) in this procedure. Extraction techniques, from traditional to advanced, should be optimized based on the type of bioactive compounds available in the investigated plant and the intrinsic characteristics of the plant itself, such as tissue complexity, heat sensitivity, etc. (El Asbahani et al., 2015).

Enzyme-assisted extraction (EAE), supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE) or sonication, and microwave-assisted extraction (MAE) are examples of eco-friendly extraction techniques that present suitable potential for extracting bioactive compounds from saffron. Generally, the effectiveness of extraction methods significantly relies on the selection of suitable solvents, considering solvent-solute affinity, and the use of complementary or co-extraction methods.

New extraction techniques such as SFE (Supercritical Fluid Extraction), PLE (Pressurized Liquid Extraction), MAE (Microwave-Assisted Extraction), UAE (Ultrasound-Assisted Extraction), EAE (Enzyme-Assisted Extraction), and UMAE (Ultrasound and Microwave-Assisted Extraction) not only are more environmentally friendly technologies but also with shorter extraction times, can significantly improve the extraction efficiency. PLE and SFE are green methods under pressure suitable for extracting heat-sensitive compounds as they can be performed at low temperatures. Additionally, they exhibit higher selectivity by allowing the obtainment of different extracts using distinct pressure and temperature conditions. However, one of the main drawbacks associated with these techniques is the expensive equipment, and for extracting polar compounds, the use of toxic modifiers is required, while extractions at high temperatures might lead to the degradation of heat-sensitive compounds. Comparing these two techniques, SFE is the preferred procedure for selectively extracting bioactive compounds as it is more flexible in solvent selection. However, this technology might be more time-consuming than other alternative techniques and might present lower extraction yields. A common aspect among techniques like SLE (Solid-Liquid Extraction), PLE, and SFE is the use of high temperatures that can cause targeted bioactive compounds to react during extraction through chemical reactions like Maillard reactions and caramelization. The Maillard reaction efficiently occurs at temperatures above 50°C and is favoured at pH 4-7, while caramelization occurs when certain compounds are heated above their melting points (>120°C) under acidic conditions (pH 3) or alkaline conditions (pH 9) in the absence of nitrogen-containing components. Modern extraction techniques such as UAE, SFE, and MAE have equipment cost-related disadvantages and the potential for deteriorating extraction quality but can offer better yields and be more environmentally friendly and time-efficient. Compared to EAE, which employs enzymes like proteases and carbohydrases to enhance the yield of bioactive compounds from algae, these techniques might also be faster and more efficient but require careful monitoring of enzyme conditions and availability. EAE and MAE provide significant benefits in terms of extraction efficiency, including reduced extraction time, minimized solvent usage, lower energy consumption, and increased yield. Furthermore, UAE has been used on an industrial scale for the efficient extraction of bioactive compounds from natural sources (Quitério et al., 2022).

Microwave-Assisted Extraction (MAE) is considered a novel technology for extracting soluble compounds from various materials into a fluid using microwave radiation. Microwaves are electromagnetic fields with a frequency between 300 MHz and 300 GHz. They consist of two perpendicular oscillating fields, such as electric and magnetic fields. The concept of microwave heating is based on direct effects on polar materials. The rotation of dipoles and ionic conduction processes transform electromagnetic energy into heat (Azmir et al., 2013).

Microwave-Assisted Extraction (MAE) affects molecular movement using microwave energy and induces the formation of permanent dipoles in liquids, resulting in rapid heating of the solvent and the sample. Its high level of automation reduces extraction time, solvent consumption, and improves efficiency (Buldini et al., 2002).

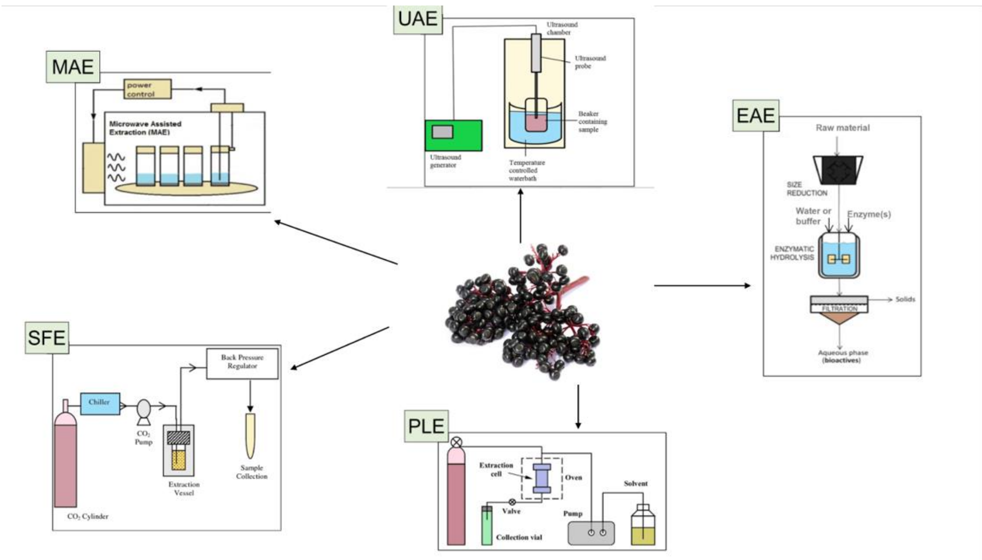

Figure 3. The extraction methods studied for elderberry fruit
Microwave-Assisted Extraction (MAE); Ultrasound-Assisted Extraction (UAE); Supercritical Fluid Extraction (SFE); Enzyme-assisted extraction (EAE)
Pressurized Liquid Extraction (PLE)

Regarding MAE extraction used for elderberries, Milena et al. (2019) investigated the antioxidant activity of phenolic compounds using 50% ethanol or H2O as solvents. According to the results, the extract prepared with 50% EtOH exhibited the highest antioxidant activity, which was consistent with the high phenolic content. The highest inhibitory activity against tyrosinase was also found in this extract.

Ultrasound-Assisted Extraction (UAE) is highly regarded as a sustainable method for recovering polyphenolic substances from plant material. It requires a moderate investment of solvent and energy, is easy to handle, safe, cost-effective, and reproducible. Other significant advantages of UAE include operation under atmospheric pressure and temperature conditions. UAE involves acoustic cavitation, which can lead to the breaking of cell walls, facilitating the release of bioactive compounds and can be highly efficient for obtaining polyphenolic phytochemicals (Vinatoru et al., 2019).

The application of ultrasound in the food industry can be divided into two categories: (i) high frequency and low intensity ($f > 100$ kHz), which do not alter the physical or chemical properties of the material; and (ii) low frequency and high intensity (20 kHz $\leq f \leq$ 100 kHz), inducing ultrasonic effects in the material and can lead to physical, chemical, and mechanical effects. Ultrasound-assisted extraction (UAE) is selective, relatively easy to use, versatile, and flexible. It can also achieve a high extraction yield in a short time using small amounts of solvent and can be less costly than traditional extraction methods. Furthermore, extraction can be performed at low temperatures, preserving thermosensitive compounds. UAE can also be combined with other techniques such as supercritical fluid, pressurized fluid, or microwave-assisted processes to further improve process efficiency. The extraction efficiency with UAE can be influenced by power, intensity, frequency, sonication time, temperature, sample density and moisture, particle size, and the solvent used (Prado et al., 2018).

The ultrasound-assisted method enhances solvent absorption and mass transfer. In this method, a small amount of solvent is required but can efficiently extract the target compounds. Ultrasounds have been reported to be suitable for extracting pigments from various plant sources by exerting a mechanical effect that leads to breaking the cell walls due to cavitation within the tissue matrix (Virot et al., 2010). The extraction of three cyanogenic compounds (amygdalin, prunasin, and sambunigrin) from the leaves, flowers, branches, and fruits of *S. nigra* was optimized by Rodríguez Madrera and Suárez Valles in 2021. High-power ultrasound was used as the extraction method, and acidified water was employed as the extraction solvent. The effect of extraction time, pulse, and amplitude on the extraction yield was investigated. Based on their findings, the optimal extraction condition was determined as follows: 80% sonication amplitude, 55 seconds extraction time, 70% duty cycle, 0.1 g sample mass, and 10 mL of acidified water containing 0.1% perchloric acid. It was emphasized that the sample/solvent extraction ratio is a significant factor influencing the yield of cyanogenic glycosides with β-glucosidase activity. In comparison to previous approaches, the extraction time in this study was significantly reduced, which is particularly important for minimizing amygdalin degradation during the extraction phase (Rodríguez Madrera et al., 2021).

Supercritical Fluid Extraction (SFE) is an extraction method that uses supercritical fluids as extraction solvents. This technique resembles Soxhlet extraction, but instead of conventional solvents, it employs a supercritical fluid, which is a substance above its critical temperature and pressure, offering a unique combination of properties. The entire process takes less than 20 minutes, unlike several hours needed in traditional liquid-solid extractions like Soxhlet. This method can also be applied to thermally unstable analytes by selecting supercritical fluids with lower critical temperatures. Supercritical fluids diffuse through solids like gases but dissolve analytes like liquids, thus improving the extraction rate and causing less thermal degradation. Additionally, many sample pretreatments can be carried out with non-polluting and non-carcinogenic supercritical fluids such as carbon dioxide, which represents an excellent alternative to potentially hazardous and costly solvents used in Soxhlet extraction (Buldini et al., 2002).

Kitrytė et al. (2020) used supercritical carbon dioxide extraction (SFE-CO2) and adjusted its properties (temperature, duration, and pressure) to recover important non-polar components from elderberry juice. From 100 grams of residues, 14.05 grams of the lipophilic fraction were recovered under optimal SFE-CO2 conditions (53°C, 35 MPa, and 45 minutes), including beneficial polyunsaturated fatty acids for health such as linoleic acid (42.0%) and linolenic acid (34.1%). Regarding extraction yields and time, SFE-CO2 was generally more efficient than traditional Soxhlet and SLE but less efficient than PLE and UAE. Compared to hexane-based PLE and UAE, SFE-CO2 offered the advantage of using GRAS CO2 solvent and avoiding the solvent removal operation. After separating the lipid-soluble fraction with supercritical carbon dioxide or hexane, a minor amount of antioxidants was obtained from elderberry residues, while the defatted elderberry husks retained a significant amount (>60%) of these bioactive components. Therefore, high-pressure fractionation and/or ultrasound-assisted fractionation of elderberry husks could be used to separate molecules with higher polarity, having various applications in the food, pharmaceutical, and nutraceutical industries.

Enzyme-assisted extraction (EAE) of biomolecules from plant products is becoming increasingly popular as a viable alternative to traditional solvent extraction techniques due to its performance, safety, sustainability, and environmental friendliness. The ability of enzymes to perform reactions with precise specificity, regional selectivity, and the capability to carry out reactions under moderate conditions while still maintaining their biological potential on bioactive substances, are all critical characteristics of enzyme-assisted extraction. The fundamental premise of enzyme-assisted extraction is to hydrolyse the plant cell wall using an enzyme as a catalyst under ideal experimental conditions to release internal components. The plant cell wall binds the enzyme's active site and allows the enzyme to change its shape so that the substrate fits into its active site, resulting in the best possible interaction between the two. Changes in the enzyme's structure lead to the breaking of cell wall bonds, allowing the active components to be released (Ali Redha et al., 2021).

Enzymes can be more costly in large-scale or industrial production if they are processed with a high enzyme-to-substrate ratio. Ultimately, because enzymes respond differently under various environmental conditions (such as temperature and pH), and since pH and temperature can fluctuate inside an industrial-scale extractor, EAE may be challenging to scale in the industry. If the aforementioned disadvantages can be addressed, EAE could offer a way to improve extraction yields, reduce extraction times, and enhance the quality of extracts by utilizing milder extraction conditions (such as lower extraction temperatures) (Wen et al., 2020).

Pressurized Liquid Extraction (PLE), also known as Accelerated Solvent Extraction (ASE), is based on maintaining solvents with low boiling points in a fluid state under high pressure, thus enhancing the analyte's diffusivity from a solid matrix (Tamkutė et al., 2020). In many cases, PLE optimizes the solvent quantity, temperature, static extraction time, and the number of cycles through which the solvent passes through the matrix. PLE has been utilized effectively to extract thermosensitive phytochemicals from various plant sources (Anusha Siddiqui et al., 2022).

Jonušaite et al. (2021) assessed the antioxidant activity of essential oils from cloves, oregano, and black elderberry (*S. nigra*) extracted in butylated hydroxytoluene (BHT) in salmon burgers stored at 4°C for 14 days after cooking. The flowers of *S. nigra* treated with SFE-CO2 and then extracted through pressurized liquid extraction (PLE) with ethanol, exhibited superior antioxidant properties. The presence of various bioactive chemicals in the phenolic components of essential oils and flower extracts investigated in this study demonstrated a high antioxidant capacity. Concerning phenolic components, *S. nigra* was identified as a plant with high levels of flavonoids (rutin and quercetin) and phenolic acids (gallic acid and gentisic acid). Therefore, it was chosen for preserving salmon burgers.

Microencapsulation Techniques

Phenolic compounds, including flavonoids and phenolic acids, are the most abundant bioactive compounds found in elderberries. These compounds have demonstrated nutritional and pharmaceutical effects, such as antioxidant, anti-inflammatory, and antimicrobial actions, which have various applications in different industries. However, phenolic compounds could undergo degradation and loss of effectiveness when exposed to light, oxygen, variable temperatures, pH, and enzymatic activity (Albuquerque et al., 2020).

The technique of microencapsulation to protect bioactive compounds could be considered an efficient practice and solution to address this issue (Aliakbarian et al., 2015). In recent years, this technique has rapidly advanced across various industries, including food production, pharmaceuticals, textiles, and agriculture. The target compounds are entrapped within a solid or liquid matrix and surrounded by a coating material during the encapsulation process.

The use of natural pigments requires an understanding of their chemical structure and stability to adapt them to usage conditions during processing, packaging, and distribution. It is essential to comprehend how natural pigments react in various environments and under different conditions to ensure the preservation of their colour and properties throughout the product's lifecycle. This may involve selecting encapsulation techniques or other protective methods to maintain pigment stability and prevent their degradation during manufacturing, packaging, and distribution processes. Detailed knowledge of the chemistry and behaviour of natural pigments is crucial to achieving high-quality food products with natural and stable colours (Mateus and de Freitas, 2009).

Generally, there are three distinct capsules depending on size, including macrocapsules, microcapsules, and nanocapsules. Therefore, in implementing the encapsulation technique, the selection of coating materials and their compatibility with the active agent are essential. The choice of an appropriate microencapsulation method for bioactive compounds depends on various factors such as the physical and chemical properties of the core and the encapsulating agent, the release properties of the encapsulated bioactive compounds, and manufacturing considerations (Anusha Siddiqui et al., 2022).

Conclusion

As presented above, extraction methods come in various types, each having multiple advantages and disadvantages depending on their application. Conventional technologies are becoming increasingly rare due to their use of reactive agents, lengthy determination times, and costly budget. Green technologies offer many more advantages over conventional ones, using more environmentally friendly reagents and achieving shorter extraction times. In addition to considering all these influencing parameters, choosing the most suitable and efficient extraction technique should also take into account the nature of the plant matrices.

The type of extraction technique used as well, as the variation in extraction parameters significantly impacts the antioxidant properties of the extracts. However, the greatest benefits of these new eco-friendly technologies can only be obtained by overcoming limitations such as high costs and process selection. Therefore, in order to implement these new technologies on a large scale, removing technical barriers in processes should be a priority to exploit enhanced extraction of bioactive compounds from plant matrices at an industrial level.

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