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REVIEW ARTICLE

Conventional and emerging techniques for extraction of bioactive compounds from fruit waste

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Abstract

Fruit residues (peel, seed, and pulp residues) have in their composition several compounds such as polyphenols, carotenoids, fibres, and lipids which, due to their functional properties, these compounds make them potential sources of natural additives. The great technological challenge is to use the most suitable techniques for extracting these compounds. In this paper, definitions, advantages, and disadvantages of conventional (maceration, soxhlet extraction, hydrodistillation) and emerging (Ultrasonic-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE), Pressurized Liquid Extraction (PLE), and Pulsed Electric Field (PEF)) techniques for the extraction of bioactive compounds from fruit residues will be shown. Some emerging techniques are based on non-thermal process, reduced use of energy and the implementation of no toxic solvents, being considered "green" or "clean" technologies. These ones are particularly interesting to extract heat-labile compounds. In addition, enzyme-assisted extraction and extractions through fermentation processes (submerged or solid-state fermentation) will be highlighted as alternative to promote the release of compounds from fruit residues not extracted by other techniques. These extractions techniques can enhance the content of bioactive compounds by increased their concentrations, as well as new compounds can be formed after these processes. It has been proven that the emerging techniques and fermentative processes, as alternative to conventional methods, are promising to extract bioactive compounds from fruit residues, since that these techniques improved extraction yields, reduced processing times, and reduced environmental damage are achieved.

Keywords: Fruit residue; Solvents; Extraction methods; Fermentation; Enzymes; Phenolic compounds.

Highlights

- Bioactive compounds from fruit residues are commonly extracted by conventional methods
- "Green" extraction techniques have been highlighted as alternative to extract compounds from fruit residues
- Fermentation processes have increased the polyphenolic content extracted from residues
- Enzyme assisted extraction promotes the release of bound compounds from matrix solid

1 Introduction

The agroindustry is an industry sector that generates a considerable amount of organic waste, contributing more than 0.5 billion tons worldwide (Banerjee et al., 2017). Fruit residues such as peels, seeds, and pulp remain are often discarded, despite their excellent nutritional value and the presence of bioactive compounds (gallic acid, quercetin, resveratrol, epicatechin, among others) with functional characteristics (antioxidant, antimicrobial, anticancer, anti-inflammatory, anti-diabetic, and others) (Renard, 2018).

The management of these wastes occurs through traditional methods such as landfill, composting, or incineration, but these methods have negative impacts on the environment and human health due to the emission of greenhouse gases and odorous compounds. On the other hand, over the years, several researchers have studied alternative methods for the use of these residues because they may contain bioactive compounds and nutrients of industrial interest (Banerjee et al., 2017; Renard, 2018; Saini et al., 2019).

Bioactive compounds from fruit residues are commonly extracted by organic solvents (liquid-liquid/liquid-solid) using various conventional methods, such as maceration, Soxhlet extraction and hydrodistillation, which generally use heat and/or agitation. However, these techniques have disadvantages such as the use of solvents with high purity, high cost and potential toxicity, low selectivity in extraction, long periods of extraction, and thermal decomposition of heat-labile compounds (Banerjee et al., 2017). As an alternative, several researchers have studied emerging extraction techniques such as Supercritical Fluid Extraction (SFE) (Reis et al., 2016), Pressurized Liquid Extraction (PLE) (Santos et al., 2019), Pulsed Electric Fields (PEF) (Parniakov et al., 2016) and based on enzyme (Azmir et al., 2013), consisting of non-thermal processes, ultrasound, and microwaves (Renard, 2018).

Emerging techniques are also known as “assisted” extraction techniques where an additional physical phenomenon (ultrahigh pressure, ultrasound, electric fields, use of enzymes) is used to intensify the process. It still considering the requirements related to organic solvents used in some extractions such as, registration, evaluation, authorization and restriction of chemicals regulation in addition to their recovery costs, also alternative solvents as subcritical liquids, ionic liquids and deep eutectic solvents has been studied over the years (Renard, 2018). Some of these techniques possess as advantages the use of less hazardous chemical synthesis, safer solvents, use of renewable feedstock, reduce derivatives, prevent degradation, reduced time analysis and pollution prevention, being considered as “green” techniques (Azmir et al., 2013). The main objectives of emerging extraction processes are related to achieve a faster extraction rate, more effective energy use, increased mass and heat transfer, reduced equipment size, and a reduction in the number of processing steps (Jacotet-Navarro et al., 2016).

Despite most bioactive compounds from fruit residues are extracted by conventional or emerging techniques, several studies have reported that most of polyphenolic compounds cannot be extracted by these methods alone, because they exist as an insoluble bound form conjugate with cell wall components through ester, ether or glycosidic bonds (Dey et al., 2016). Enzyme assisted extraction of phenolics by different cell wall degrading enzymes could be a useful and environment friendly technique, however, it is a high-cost process since the production and purification of the enzymes to be applied is necessary. The phenolic compounds are primarily obtained from plants, although these compounds can also be produced as secondary metabolites by mushroom basidiomycetes, yeasts, and endophytes (Dey et al., 2016). For this reason, fermentation processes have attracted the attention of the researchers to the extraction, as well as the formation of new phenolic compounds from fruit residues (Dey et al., 2016). In this review paper, the definitions and applications of conventional and emerging methods for the extraction of bioactive compounds from fruit residues will be showed, as well as the application of fermentation processes as a tool to increase the content of these compounds in the residues.

2 Bioactive compounds from fruit residues

Bioactive compounds are defined as primary or secondary metabolites that are present throughout the plant kingdom and vital for the maintenance of human health (Patil et al., 2009). In plants, these compounds can be found in cell wall/membrane-bound or in free form, and they can vary with genetic or environmental factors and cultivation techniques. Secondary metabolites, despite not directly participating in photosynthetic or respiratory metabolism, are essential for plant survival (Chikezie et al., 2015). These compounds can act as protective agents of plants against UV radiation (flavonols), as deterrents against herbivores (glucosinolates, some polyphenols), or as attractants for pollination (anthocyanins), among other properties and functions (Renard, 2018). In general, secondary metabolites or bioactive compounds can be synthetized by shikimic acid pathway, malonic acid pathway, mevalonic acid pathway and non-mevalonate pathway (Tiaz & Zeiger, 2006). Particularly, phenolic compounds are synthesized through shikimic acid pathway and malonic acid pathway (Azmir et al., 2013).

Several types of bioactive compounds can be found in fruit residues, such as polyphenols, carotenoids, vitamins, dietary fibres, and lipids. These compounds vary in polarity, solubility, molecular weight, bioavailability, and metabolic pathways (Banerjee et al., 2017; Sagar et al., 2018; Saini et al., 2019). Such residues, as they contain quantities of bioactive compounds that are sometimes higher than the content present in the pulps, can be effectively used to add value to these by-products (Silva et al., 2014; Sancho et al., 2015; Batista et al., 2017). The highest value-added compounds present are polyphenols with antioxidant and/or antimicrobial activity, demonstrating the potential of these residues for application in various food, pharmaceutical, and cosmetic industries. Some examples are grape pomace, that has various polyphenolic antioxidants as flavonoids, phenolic acids, phenolic alcohols, stilbenes, and lignans (Forbes-Hernández et al., 2014); by-products of citrus juice production that contains essential oils, limonoids, and polyphenols, e.g., flavonoids (Angulo et al., 2012). Citrus peel is particularly rich in flavonoids, carotenoids, dietary fiber, sugars, polyphenols, essential oils, and ascorbic acid (Sharma et al., 2017). Carotenoids can be found in tomato by-products and polyphenols in apple pomace (Renard, 2018), whereas phenolic compounds can be found in guava peels, avocado stones, grape seeds (Kallel et al., 2014), pomegranate peels (Živković et al., 2018).

From the unique characteristics of each group of bioactive compounds found in fruit residues, it is important to assess which compounds or which extraction techniques should be selected to ensure greater yield in the extraction process. For instance, polyphenols can be divided into the following groups according to their chemical complexity and ease of extraction from plant tissues: extractable polyphenols, such as flavonoids, which are characterized by their low molecular weight and ease of extraction by aqueous organic solvent; and polyphenols that are non-extractable due to their structural complexity and low solubility and availability in plant tissue; these include low or high molecular weight compounds (Hernández-Carlos et al., 1989).

A factor to be considered in the use of fruit residues as a source of bioactive compounds is to define which compounds are found in the highest concentration, which facilitates the choice of the most suitable extraction method. Another point to be considered is that concentrations of the target compounds can sometimes over 10-fold, depending on the exact variety (and its maturity stage) (Renard, 2018). In addition to the initial treatment in the product, it is noteworthy to highlight the process to stabilize the co-product that will influence the composition and potential of this residue as a source of bioactive compounds. For instance, the polyphenol composition of apple pomace from juice processing will be different from the original fruit, due to enzymatic oxidation or chemical degradation occurring during juice processing (Renard, 2018).

3 Conventional techniques for extracting bioactive compounds from fruit residues

Conventional or classical techniques such as maceration, Soxhlet extraction, and hydrodistillation are the techniques most often used for extracting bioactive compounds from fruit residues (Azmir et al., 2013). The maceration extraction process consists of mixing the solid material with an organic solvent of appropriate polarity (Galanakis, 2012). This process has been optimized by using agitation to increase the diffusion of compounds into the solvent, thus elevating temperatures to improve dissolution, decreasing solvent viscosity, and decreasing the particle size of the solid material to facilitate mass transfer and consequently increase the extraction speed. However, this type of extraction has some disadvantages such as long extraction times, the use of large amounts of vegetal mass and solvents, and low yield (Azmir et al., 2013; Renard, 2018).

This technique has been used for the extraction of bioactive compounds from residues of pineapple, acerola, guava, passion fruit, tamarind, soursop, papaya, mango, suriname cherry, and sapodilla to obtain Total Phenolic Contents (TPC) between 100.7 and 12,696.0 mg GAE/100g dry basis (Silva et al., 2014) (Table 1). In grape and orange residues, TPC of 67.1 and 146.6 mg GAE/g dry basis were obtained, respectively (Soto et al., 2019). In tangerine peels, the maximum extraction of TPC was obtained (28 mg GAE/g extract) by maceration at a temperature of 40 °C when using 80% methanol as the extracting solvent (Safdar et al., 2017). Suleria et al. (2020) obtained TPC between 0.45 and 25.5 mg GAE/g in peels of apple, apricot, avocado, banana, custard apple, dragon fruit, grapefruit, kiwifruit, mango, lime, melon, nectarine, orange, papaya, passion fruit, peach, pear, pineapple, plum, and pomegranate using maceration extraction with 70% ethanol, agitation in a shaking incubator at 120 rpm and 4 °C. Lim et al. (2019) used maceration extraction with different concentrations of ethanol under agitation to recover TPC of mango seed kernel, and they obtained a maximum value of 107.7 mg GAE/g sample with 50% ethanol solution.

Table 1. Conventional techniques for extracting bioactive compounds in fruit residues.

Extraction methods	Fruit residues	Bioactive compounds	References
Solution of Acetone:hexane (for β-carotene and Lycopene)	- Pineapple (peel and pulp's leftovers)	- Total Phenolic Compounds (TPC), anthocyanins, β-carotene	Silva et al. (2014)
50% ethanol and 70% acetone at room temperature (for TPC)	- Acerola (seed)	TPC, yellow flavonoids, anthocyanins, β-carotene	
1.5 N HCl in 85% ethanol (for yellow flavonoids, anthocyanins)	- cashew apple (peel and pulp's leftovers)	-TPC, yellow flavonoids, anthocyanins, β-carotene	
50% ethanol at 70 °C with stirring (for resveratrol)	- Guava (peel, pulp's leftovers, and seed)	-TPC, yellow flavonoids, anthocyanins, β-carotene, resveratrol, coumarin	
Maceration with 50% ethanol at room temperature (for coumarin)	- Soursop (pulp's leftovers and seed)	- TPC	
	Papaya (peel, pulp's leftovers, and seed)	TPC, yellow flavonoids, anthocyanins, β-carotene, Lycopene	
	Mango (peel and pulp's leftovers)	TPC, yellow flavonoids, anthocyanins, β-carotene, coumarin	
	Passion fruit (seed)	TPC, yellow flavonoids, anthocyanins, β-carotene, coumarin	

Table 1. Continued…

Extraction methods	Fruit residues	Bioactive compounds	References
	Surinam cherry (pulp's leftovers)	TPC, yellow flavonoids, anthocyanins, β-carotene, coumarin, resveratrol	
	Sapodilla (peel, pulp's leftovers and seed)	TPC, anthocyanins, Lycopene	
Deionized water and heated at 60 °C	Orange waste (non-specified) Pomace of grape (*Vitis vinifera* "Cavernet Sauvignon")	Gallic acid, caffeic acid, cumaric acid, chlorogenic acid, protocatechuic acid, rutin, naringenin, Kaempferol, apigenin, resveratrol, vanillin	Soto et al. (2019)
Maceration with ethanol, methanol, acetone, and ethyl acetate (50%, 80%, 100%) at 40 °C	Kinnow mandarin Peel	Total polyphenols, gallic acid, coumaric acid, chlorogenic acid, caffeic, ferulic acid, catechin, epicatechin, hesperdin, naringenin, quercetin, kaempferol	Safdar et al. (2017)
0-100% ethanol under stirring at room temperature.	Mango seed kernel	Gallic acid, caffeic acid, rutin, penta-O-galloyl-b-D-glucose and galotannins	Lim et al. (2019)
Distilled water, ethanol, methanol and acetone solutions (40-80%) under agitation at 30 °C	Granadilla Seeds	Epigallocatechin, caffeic acid, epigallocatechin gallate, epicatechin gallate, Kaempferol-3-glucoside, propyl gallate, hesperedin, coumarin, trans-cinnamic acid, quercetin, luteolin, naringenin	Santos et al. (2019)
70% ethanol	Apple, apricot, avocado, banana, custard apple, dragon fruit, grapefruit, kiwifruit, mango, lime, melon, nectarine, orange, papaya, passionfruit, peach, pear, pineapple, plum and pomegranate Peels	Phenolic acids, flavonoids, lignans, stilbenes	Suleria et al. (2020)
Soxlhet with 100% ethanol	Tamarind (sweet variety) Seeds	TPC	Reis et al. (2016)
Soxlhet with 20% ethanol	Pineapple Skins	TPC	Alias & Abbas (2017)
Hydrodistillation	Pineapple Peels	Glycine, Acetic acid, Dimethylsilanediol, Stigmasterol, Ethyl iso-allocholate, Heptatriacotanol, Oleic acid, 9,12,15-Octadecatrienoic acid	Mohamad et al. (2019)
Hydrodistillation	Pomegranate Peels	Majority compounds ((-)-Borneol Dibutyl phthalate and Oleic acid	Ara & Raofie (2016)

In Soxhlet extraction, the bioactive compounds are extracted from the solid material using a solvent which is heated and condensed in a Soxhlet apparatus, concentrating the compounds of interest. The efficiency of this process depends on parameters such as solubility, mass transfer, and solid material characteristics. The selection of a suitable solvent is one of the most important factors for the extraction of bioactive compounds since it must be based on its ability to extract the target compound. It is also important consider the type of sample and the strength of interactions between analyte and matrix. This technique is often used when the desired compound to be extracted has specific solubility in a certain solvent and the impurities are insoluble in it. However, it is not appropriate for the extraction of heat-labile compounds (Zygler et al., 2012). Reis et al. (2016) used the Soxhlet technique with pure ethanol to extract TPC from sweet variety tamarind seeds and obtained 387.4 GAE/g extract. Alias & Abbas (2017) extracted TPC from pineapple peels by this technique and obtained 28.8 mg GAE/g dry basis.

The hydrodistillation process is based on a mixture of the solid material with water which will be subjected to hydrodistillation through an electric heating mantle and the volatile compounds are condensed and recovered (Chen et al., 2021). In this process, the compounds are extracted by hydrodiffusion, hydrolysis, and decomposition by heat (Azmir et al., 2013). The disadvantage of hydrodistillation is the loss of some components, natural pigments, and heat-labile bioactive compounds, due to the use of high temperatures, above the boiling point of water (Aramrueang et al., 2019). This technique was used to extract essential oil and hydrosol containing important volatile compounds from pineapple peel (Mohamad et al., 2019) and pomegranate peel (Ara & Raofie, 2016). However, there are no reports of using this technique for extracting phenolic compounds.

Considering that the yield of bioactive compounds from fruit residues is influenced mainly by the conditions under which the process is carried out, it is necessary to optimize the sequence of extraction process, for example by removing external waxes and lipids prior to aqueous processing, to increase the selectivity and minimize the economic value of the process. In this context, it is recommended to replace conventional extraction methods with organic solvents for “greener” methods (Banerjee et al., 2017).

One of the major challenges for extracting bioactive compounds from fruit residues is the choice of extraction method. Among the several factors, the perishable nature and the large amount of waste that is normally generated in industries, makes it difficult to apply conventional extraction methods such as extraction with organic solvents or individual extraction of compounds on a large scale (Banerjee et al., 2017). As an alternative, emerging extraction techniques have been widely studied for this purpose over the years.

4 Emerging techniques for extracting bioactive compounds in fruit and vegetable waste

Extraction techniques considered emerging are based in the use of non-thermal concepts to facilitate the extraction without risking the matrix overheating while decreasing energy use (Renard, 2018). These techniques have been studied to minimize the limitations arising from conventional extraction techniques, such as degradation of heat-labile compounds, low extraction yields, high solvent consumption, high energy consumption, long times for extraction, and formation of toxic residues (Azmir et al., 2013; Banerjee et al., 2017). Among the most used emerging techniques for the extraction of bioactive compounds from fruit residues (Table 2), one can cite Ultrasound-Assisted Extraction (UAE), microwave, Supercritical Fluid Extraction (SFE), Pressurized Liquid Extraction (PLE), and Pulsed Electric Field (PEF). In comparison with conventional extraction methods for example, microwave and ultrasonication are easer of applicability for high moisture residues, because internal moisture helps in disruption of the structure when the molecules are subjected to excitation using external energy sources such as microwaves (Attard et al., 2014). In process where water may not be used, the ethanol is the most preferred solvent due to its lower boiling point, quick recovery and the “generally regarded as safe” status as defined by the US FDA. However, this alcohol is not adequate for the extraction of carotenoids (Banerjee et al., 2017).

Table 2. Emerging techniques for extracting bioactive compounds from fruit residues.

Extraction methods	Fruit Residues	Bioactive compounds	References
Ethanol (10-90%) in ultrasonic bath	Pomegranate peel	Total polyphenols (ellagic acid, gallic acid, punicalagin and punicalin)	Živković et al. (2018)
80% methanol in ultrasonic bath	Defatted seed flours (from Five apple cultivars (Fuji Zhen Aztec, Granny Smith, Pink Lady, Super Chief, Jeromine	Ellagic acid, (−)-epicatechin, ferulic acid, (+)-catechin, gallic acid, chlorogenic acid, phloridzin, and caffeic acid	Gunes et al. (2019)
Ultrasound-Assisted Extraction (UAE) with ethanol, methanol, acetone, and ethyl acetate (50%, 80%, 100%) at 40 °C	Kinnow mandarin peel	Total polyphenols, gallic acid, coumaric acid, chlorogenic acid, caffeic, ferulic acid, catechin, epicatechin, hesperdin, naringenin, quercetin, kaempferol	Safdar et al. (2017)
Sub and supercritical CO_2, pure and combined with ethanol.	Tamarind (sweet variety Seeds)	TPC, antioxidants fatty acids (9, 12-octadecadienoic acid, cis- 10-heptadecenoico and n-hexadecanoic acid	Reis et al. (2016)
Supercritical CO_2	Pomegranate Peels	Major compounds oleic acid, palmitic acid and (-)-Borneol	Ara and Raofie (2016)
UAE with ethanol, water and 50% ethanol solution; Supercritical Fluid Extraction (SFE) with CO_2; Pressurized liquid extraction	Feijoa (*Acca sellowiana* (O. B.) Peel	TPC	Santos et al. (2019)
Microwave Assisted Extraction (MAE) with 100% ethanol, water and 50% ethanol solution at 30 °C, 60 °C and 90 °C	Pineapple Skins	TPC	Alias and Abbas (2017)
MAE with 50% ethanol; UAE with water	Pomegranate Peels	TPC, punicalagin	Kaderides et al. (2019)
MAE and UAE with deep eutectic solvents	Grape Skin	TPC	Bubalo et al. (2016)
Extraction assisted by pulsed electric energy	Mango Peels	TPC	Parniakov et al. (2016)
UAE with hydroethanolic solution	waste	TPC, total flavonoid	Silva et al. (2020)

In the UAE technique, there is a cavitation process generated by acoustic waves (>20kHZ), which interact with the solvent and dissolved gas by creating free bubbles that can expand to a maximum size and violently collapse, generating locally extreme heat and pressures. As consequence of this phenomenon, the cell walls can be ruptured, providing channels for solvent access, and mass transfer is improved, facilitating the removal of bioactive compounds (Renard, 2018). The UAE process has the following advantages when compared to conventional extraction techniques: greater solvent penetration into solid material; shorter extraction time; higher yield and reproducibility; lower solvent consumption; extraction of heat-labile components; and reduced maintenance costs and economy of energy. In addition, it is considered to be a “green” and economical process (Aramrueang et al., 2019; Medina-Torres et al., 2017). Safdar et al. (2017) obtained greater extraction of TPC from tangerine peel by the ultrasound technique (32.5 mg GAE/g extract) when compared to that obtained by the maceration technique (28.0 mg GAE/g extract). Gunes et al. (2019) obtained TPC between 2861 and 5141 mg GAE/kg flour in the extracts of defatted seed flours from 5 cultivars of apple using 80% methanol and an ultrasonic bath. Kaderides et al. (2019) obtained 119.8 mg GAE/g dry of pomegranate peel flour after UAE using a solution of acetic acid and acetonitrile. Živković et al. (2018) obtained TPC between 3.58 and 190.9 mg GAE/g dry weight of pomegranate peel after extraction optimized by ultrasound. Santos et al. (2019) used the UAE (68 mg GAE/g) for the extraction of TPC from flour from bean husks and obtained a value close to that obtained by Soxhlet extraction (71 mg GAE/g) using a 50% aqueous solution of ethanol. Silva et al. (2020) obtained higher extraction of TPC in acerola waste using UAE and a hydroethanolic solution (TPC= 931.2 mg/100 g dry) than that obtained when conventional solid-liquid extraction was used (TPC = 211.8 mg/100 g dry).

Microwave-Assisted Extraction (MAE) has the principle of electromagnetic irradiation of the polar solvent and the sample, resulting in superheating the solid material and rupturing it. Rapid heating is generated by ionic conduction and dipole rotation mechanisms. The electromagnetic field occurs in a frequency range from 300 MHz to 300 GHz. This technique has the advantages of high extraction yield, a small temperature gradient, reduced process time, low solvent consumption, and a small extraction unit. However, it is not considered to be an appropriate technique to obtain thermally sensitive compounds. Process efficiency depends on factors such as the proportion of solvent used, microwave power level, irradiation time, extraction time, temperature, and solvent concentration (Azmir et al., 2013). Alias & Abbas (2017) obtained higher TPC (207.7 mg GAE/g dry) and higher antioxidant activity (EC_{50} = 13.2 mg/mL) in pineapple peel after extraction with 50% ethanol in a microwave when compared with that obtained by Soxhlet extraction (TPC and EC_{50} of 28.8 mg GAE/g dw and 2.8 mg/L, respectively). Kaderides et al. (2019) obtained greater extraction of phenolic compounds (199.4 mg GAE/g dry) in pomegranate peel flour by microwave treatment compared to that obtained by ultrasound (119.8 mg GAE/g dry).

The Supercritical Fluid Extraction (SFE) technique is based on increasing the temperature and pressure of a substance or solvent above its critical values. Changes in fluid density in its supercritical state allow for variation in solvency power, resulting in selective extractions of compounds of interest (Silva et al., 2016). Supercritical extraction occurs by passing the solvent through a packed bed containing the solid material whose bioactive compounds will be dissolved and carried away by the solvent. Then, by reducing the pressure and/or increasing the temperature, the solvent-free extract will be obtained (Silva et al., 2016). This technique predominantly uses supercritical fluids of CO_2, ethanol, and water, which are generally recognized as safe (GRAS) by U.S. Food and Drug Administration (FDA) and European Food Safety Authority (EFSA). Particularly, supercritical carbon dioxide is considered an attractive alternative to organic solvents for not being explosive, toxic, expensive, and possesses the ability to solubilize lipophilic substances and can be easily removed from the final products (Ahmad et al., 2019). The use of supercritical solvents with different physicochemical properties such as diffusivity, density, viscosity, and dielectric constant makes the SFE technique advantageous over conventional extraction processes. The low viscosity and high diffusivity make these fluids easily penetrate the solid material, increasing the rate of extraction of compounds of interest. Another advantage of this technique is the possibility of direct coupling with analytical chromatographic techniques, such as gas chromatography and supercritical fluid chromatography (Herrero et al., 2006).

Reis et al. (2016) obtained greater extraction of phenolics (471.0 GAE/g extract) from tamarind seeds by the SFE technique with ethanol when compared to Soxhlet extraction (387.4 GAE/g extract). However, Santos et al. (2019) obtained lower TPC (between 11 and 23 mg GAE/g extract) in feijoa husks through extraction by SFE (CO_2/ethanol) than those obtained by assisted ultrasound, Soxhlet, and pressurized liquid techniques (between 34 and 132 mg GAE/g extract).

The pressurized liquid extraction technique is based on the use of pressure (between 5 and 15 Mpa) to keep the solvent a liquid at temperatures higher than its boiling point (between 40 and 200 °C). The use of high pressures facilitates the penetration of the solvent into the pores of the solid material, and the high temperatures cause better diffusion of the solvent into the solid material in addition to helping to break up plant cells. It results in increased solubility and a higher mass transfer rate. As a consequence, this process promotes a reduced extraction time and lower consumption of solvents (Aramrueang et al., 2019). The possibility of process automation and the use of solvents recognized as safe are also other advantages of this technique. However, as it is a process that applies high temperatures, it is not considered an ideal extraction system for heat-labile compounds. In addition, it is considered an extraction method with high operational cost (Aramrueang et al., 2019). Santos et al. (2019) obtained greater extraction of TPC from bean husk flour (132 mg GAE/g extract) per pressurized liquid (ethanol-water solvent, temperature of 80 °C and pressure of 100 bar) than the values obtained by extraction by supercritical fluid (CO_2/ethanol, 55 °C, 300 bar) (23 mg GAE/g), Soxhlet (ethanol/water) (71.1 mg GAE/g), and ultrasound (ethanol/water) (68.2 mg GAE/g).

Pulsed electric field extraction or electroporation is a non-thermal technique. It is applied as a pre-treatment before using conventional or emerging techniques to facilitate the extraction of compounds. It has the ability to electroporate cell structures (Azmir et al., 2013). In this process, the conductivity and permeability of the plant tissue membrane increases due to the strength of the applied electric field. This promotes membrane softening and rupture in addition to the formation of pores in the cell walls which facilitate the extraction of bioactive compounds present in the solid matrix (Azmir et al., 2013).

This technique has shown advantages over conventional extraction methods, such as process economy, reduced extraction time, little or no heat generation (enabling the extraction of heat-labile compounds), and it poses no danger to the environment (Ricci et al., 2018). Parniakov et al. (2016) obtained higher levels of TPC (approximately 2.3 mg GAE/kg dry) in mango peel flour using pulsed electric field extraction followed by aqueous extraction at 50 °C and pH 6.0 than the maximum obtained using only the aqueous extraction (at 50 °C and pH 11.0) (approximately 1.4 mg GAE/kg dry).

4.1 Emerging techniques using alternative solvents

Regarding techniques for extracting bioactive compounds, it was found that most limitations are related to the right choice of solvents, the polarity of the target compounds, molecular affinity between solvent and solute, mass transfer, use of co-solvent, environmental safety, extraction of heat-labile compounds, human toxicity, and financial viability (Putnik et al., 2018; Aramrueang et al., 2019).

To propose clean and environmentally safe alternatives for the extraction of bioactive compounds, it is also important to choose the most suitable solvent. In this context, alternative solvents that are considered "green", such as Deep Eutectic Solvents (DES), have shown great potential for the extraction of different bioactive compounds from fruit residues (Paiva et al., 2014). DESs are generated from the mixture of two or more components consisting of a hydrogen bond donor (the vitamins, amines, sugars, alcohols, and carboxylic acids) and a hydrogen bond acceptor (as choline chloride), establishing a supramolecular assembly structure with hydrogen bonds (Paiva et al., 2014). These solvents have low vapor pressure, low cost, and greater ability to solubilize secondary plant metabolites. Therefore, they are considered to be excellent alternatives to conventional extraction solvents such as methanol and acetone (Saha et al., 2019; Skarpalezos & Detsi, 2019).

Grillo et al. (2020) used DES (choline chloride:lactic acid) associated with ultrasound and microwave for anthocyanin extraction from the residues of blueberry processing and compared it with a conventional extraction with an acidified hydroalcoholic solution (60% v/v hydroalcoholic solution with 0.8% v/v of hydrochloric acid) at 55 °C and 200 rpm. The authors obtained total anthocyanin contents of approximately 19.4, 15.9, 25.8, and 21.2 mg/g by conventional extraction, DES with conventional heating and stirring for 2 h at 55 °C and at 200 rpm, DES with microwave, and DES with ultrasound, respectively. Jeong et al. (2015) also obtained higher extraction of total anthocyanin (about 63 mg/g) from grape skin, using a deep eutectic solvent composed of citric acid and D-(+)-maltose and ultrasound assisted, than that obtained with conventional extraction methods (between approximately 25 and 34 mg/g) such as heating with a solution of

1.5 N HCl:95% aqueous ethanol, a stirring method with methanol, and ultrasound assistance using a mixture of MeOH:water:trifluoroacetic acid). Bubalo et al. (2016) obtained higher free anthocyanins, (+)-catechin, and quercetin-3-O-glucoside contents when they used DES (choline chloride-oxalic acid) associated with the ultrasound assisted technique than the results obtained with DES in conventional extraction (under stirring) and DES associated with assisted microwave. Ozturk et al. (2018) used solid-liquid extraction with choline chloride-based deep eutectic solvents (DES) to extract polyphenol compounds of orange peel. The authors obtained the highest TPC (3.61 mg GAE/g of orange peel) and antioxidant potential (30.6 µg/mL) with DES than the obtained with aqueous ethanol 30% solution. Pal and Jadeja (2020) used deep eutectic solvents (lactic acid/sodium acetate/water) based on a MAE method to recover polyphenolic compounds from ripe mango peel. Under the optimal conditions (436.45 W, time of 19.66 min, and liquid-to-solid ratio of 59.82 mL/g), the authors obtained 56.17 mg of GAE/g, ferric reducing antioxidant power of 683.27 mmol ascorbic acid equivalent/g and 2,2-diphenyl-1-picrylhydrazyl scavenging activity of 82.64 DPPHsc%.

Also, as an alternative to organic solvents, extraction with cyclodextrins (CDs) has been used. These are cyclic oligosaccharides that occur naturally due to starch degradation. The β-CDs are cheap and the ones most widely used (Pinho et al., 2014). El Darra et al. (2018) obtained the highest TPC (0.72 mg GAE/g) in peach pomace when used 50mg/mL of β-CD assisted extraction when compared with conventional solvent (ethanol) in the same concentration.

From the standpoint of selectivity and yield from the extraction of bioactive compounds in fruit residues, it has been verified that, in general, extraction methods do not allow the complete release of bound phenolics of plant origin (Dey et al., 2016). As an alternative, in recent years, several researchers have used enzyme-assisted extraction techniques or fermentation processes as tools to increase the bioavailability of phenolic compounds present in fruit residues, as will be discussed below (Martins et al., 2011; Dey et al., 2016; Dulf et al., 2015, 2016, 2017, 2018; Sadh et al., 2018; Moccia et al., 2019; Torres-León et al., 2019; Santos et al., 2020a, 2020b).

5 Enzymatic extraction and extraction processes by fermentation of bioactive compounds from fruit residues

Extraction by enzymes is a technique used in order to assist in breaking the complex bonds of the constituents of bioactive compounds that mix with proteins, pectin, starch, and cellulose in the plant matrix. Enzymes provide increased permeability of the cell wall; therefore, higher extraction yields of bioactive compounds are achieved, and they may remove the unnecessary components from cell walls, and the barriers of water solubility and insolubility to improve the transparency of the system, thus promoting high catalytic efficiency and preserving the original efficacy of the natural products (Cheng et al., 2015). However, the elevated costs to produce enzymes and the fact of Enzyme Assisted Extraction (EAE) not to be easy to apply on industrial scale because enzymes behave is limited by environmental conditions (Cheng et al., 2015), there are some disadvantages of using this method for extracting bioactive compounds from fruits. Considering that many microorganisms, through the enzymes produced during the process, are capable to synthesize secondary metabolites or modify the structure of existing compounds, fermentation processes (Submerged Fermentation (SF), and Solid-State Fermentation (SSF)) have been highlighted as alternative for the extraction as well as formation of phenolic compounds (Dey et al., 2016).

In EAE, enzymes can be obtained through biotechnological processes using bacteria and fungi, or they can be extracted from animal organs or parts of vegetables and fruits. Purified enzymes such as cellulase, pectinase, and hemicellulase are then applied directly to the solid material for compound extraction (Cheng et al., 2015). Table 3 shows research related to the enzymatic extraction of bioactive compounds from fruit residues. Wu et al. (2015) performed extraction of bioactive compounds from the residue obtained from the fruit juice *Nitraria tangutorun* Bobr. using commercial cellulase associated with Soxhlet, ultrasound, microwave, and simultaneous microwave and ultrasound. In addition, both methods were used without an enzyme. The authors obtained higher extraction of phenols, flavonoids, and anthocyanin when EAE was associated with simultaneous microwave and when ultrasound was used. The antioxidant capacity of *N. tangutorum* juice by-products extracts was 27.62-190.23%, i.e., higher than those obtained by traditional extraction methods. Teles et al. (2019) used an enzymatic cocktail produced by SSF of grape pomace and a wheat bran mixture with *A. niger* to extract bioactive compounds from grape pomace. The authors obtained increases of total phenolics, proanthocyanidins, and anthocyanins of 79.9%, 121.5%, and 21.0%, respectively, in the treated residue.

Table 3. Enzyme-assisted extraction and fermentation processes for the recovery of bioactive compounds from fruit residues.

Extraction method	Fruit residue	Bioactive compounds	References
Enzymatic extraction (Cellulase) after simultaneous ultrasound and microwave treatment	*Nitraria tangutorum* juice by-products	Total phenols content (157.5 mg GAE/g); total flavonoids (101.3 mg QE/g); total anthocyanins (82.3 mg CGE/g).	Wu et al. (2015)
Soxhlet enzymatic extraction		Total phenols content (88.2 mg GAE/g); total flavonoids (72.5 mg QE/g); total anthocyanins (66.4 mg CGE/g)	
Ultrasound-assisted enzymatic extraction		Total phenols content (126.0 mg GAE/g); total flavonoids (93.0 mg QE/g); total anthocyanins (76.4 mg CGE/g).	
Microwave-assisted enzymatic extraction		Total phenols content (97.5 mg GAE/g); total flavonoids (79.6 mg QE/g); total anthocyanins (74.9 mg CGE/g).	
Enzyme assisted extraction (enzymatic cocktail obtained after SSF of mixture grape pomace and wheat bran with *A. niger*)	Grape pomace	Increases of total phenolics, proanthocyanidins, and anthocyanins of 79.92%, 121.53% and 20.96%, respectively.	Teles et al. (2019)
Pre-extraction of polyphenols with ethanol followed by FS using culture medium + polyphenol extract with *A. fumigatus*	Orange peel	Increases of polyphenols and flavonoids of 29 and 26% after 54 and 12 h fermentation, respectively.	Sepúlveda et al. (2020)
FS with *Lactobacillus* strains (*L. plantarum, L. paracasei, L. fermentum and L. casei*)	Peel and seeds of acerola and guava	Increases of polyphenols of 173 and 28% and flavonoids of 20 and 22% in acerola and guava residues, respectively, after FS for 120 h.	Oliveira et al. (2020)
FS using the fungus *Calocybe indica*.	Mixture of banana peel powder and defatted peanut residue powder	Increases of 100% and 142% in total phenolics and total flavonoid content, respectively.	Kapri et al. (2020)

Table 3. Continued…

Extraction method	Fruit residue	Bioactive compounds	References
SSF with *A. niger* and extraction with ethanol/water solution (56:44, v/v) and heating at 60 °C	Avocado seeds	Increases of polyphenol content of 75% after 314 h of fermentation.	Yepes-Betancur et al. (2021)
SSF *A. niger* and *Saccharomyces cerevisae* and extraction with 7:3 v/v ethanol/water mixture using an ultrasound microwave system	Pomegranate peel	5-fold higher extraction of ellagic acid with *S. cerevisae* than that obtained with *A. niger* and 10-fold higher than that of the unfermented material.	Moccia et al. (2019)
SSF with *A. niger* followed by extraction with solvents (distilled water, aqueous solutions at 40% and 80% acetone, 40% and 80% ethanol)	Granadilla seeds	Increases of polyphenols and flavonoids of 43.6 and 45.8% after 48 and 168 h fermentation, respectively.	Santos et al. (2020a)
SSF with *A. niger* followed by extraction with solvents (distilled water, aqueous solutions at 40% and 80% acetone, 40% and 80% ethanol)	Tamarind peel and mixture of tamarind peel and seeds	For seeds, increases of 524 and 748% of total phenolic and total flavonoid content, respectively. For mixture of seeds and peels, increases of 67% of total phenolic content.	Santos et al. (2020b)
SSF with *A. niger* followed by extraction with a mixture of hydrochloric acid/methanol/water in an ultrasonic bath	*Sambucus nigra* L. and *Sambucus ebulus* L. berry pomace	Increases of polyphenols of 18.82% for *S. ebulus* and 11.11% for *S. nigra.*	Dulf et al. (2015)
SSF with *A. niger* followed extraction with water	Pomegranate peel	Increases of total polyphenols and total flavonoids of 18.5 and 64.5%, respectively.	Bind et al. (2014)
SSF with *A. niger* and *R. oligosporus* followed by extraction with a mixture of hydrochloric acid/methanol/water in an ultrasonic bath	Plum fruit by-products	Total phenolic contents increased by over 30% with *R. oligosporus* and by >21% with *A. niger.*	Dulf et al. (2016)
SSF with *A. niger* and *R. oligosporus* followed by extraction a mixture of hydrochloric acid/methanol/water in an ultrasonic bath	Apricot pomace	With *A. niger*, increases of total phenolics and total flavonoids of 30 and 12%, respectively. With *R. olisgosporus*, increases of total phenolic and total flavonoid of 70 and 38%, respectively.	Dulf et al. (2017)
SSF with *A. niger* and *R. oligosporus* followed by extraction a mixture of hydrochloric acid/methanol/water in an ultrasonic bath	Chokeberry pomace	With *A. niger*, increases of total phenolics and total flavonoids of 79 and 50%, respectively. With *R. olisgosporus*, increases of total phenolics and total flavonoids of 87 and 57%, respectively.	Dulf et al. (2018)

The extraction processes by SF or SSF can be considered as a pre-treatment to increase the availability of bioactive compounds present in the solid material which will be used later in other extraction methods.

Fermentation promotes the bioconversion of conjugated forms of phenolic compounds into their soluble forms, with a consequent change in the profile of bioactive substances and an increase in their biological activities (Sadh et al., 2018). SF is an effective process for the biotransformation of molecules. In this process, microorganisms are grown in a liquid growth medium containing essential nutrients (Dey et al., 2016). Sepúlveda et al. (2020) extracted polyphenolic compounds from orange peel, and the purified extract was used to obtain polyphenols and specifically ellagic acid through SF using *Aspergillus fumigatus.* The authors obtained 29% and 26% increases in TPC and Total Flavonoid Content (TFC), respectively, after 54 and 12 h of fermentation, respectively, and the maximum ellagic acid production was 18.7 mg/g. Kapri et al. (2020) obtained increases of 100% and 142% in TPC and TFC, respectively, after SF of the mixture of banana peel powder and defatted peanut residue powder using the fungus *Calocybe* indica. Oliveira et al. (2020) obtained increases of 173 and 28% in TPC and 20 and 22% of TFC in acerola and guava residues, respectively, after FS for 120 h using a mixture of *Lactobacillus* strains.

The extraction of bioactive compounds by SSF consists of deposition of microorganisms on solid material with sufficient moisture to allow microbial growth and metabolism (Ajila et al., 2011). This process has three main phases: solid; gas and liquid phases. Initially, fungi germinate, and hyphae develop on the substrate surface. Next, aerial hyphae protrude into the gaseous space for fungus reproduction, while vegetative (or penetrative) hyphae penetrate the liquid-filled pores of the solid material. Metabolic activities mainly occur near the substrate surface and within the pores. Enzymes produced by the fungus during fermentation will be responsible for the extraction and/or formation of bioactive compounds present in the solid material (Hölker & Lenz, 2005).

Filamentous fungi have great potential to form bioactive compounds by SSF, as they produce different enzymes when grown on different substrates (Singh nee' Nigam & Pandey, 2009). Researchers have reported improvement in the extraction of bioactive compound contents from apple pomace, pomegranate residues, and cranberry pomace using strains of *Phanerocheate chrysosporium, A. niger, Rhyzopus oligosporus, and Lentinus edodes,* respectively (Vattem & Shetty, 2002, 2003; Ajila et al., 2011; Aguilar-Zárate et al., 2018). In fact, *A. niger* has been widely used in these processes due to its ability to synthesize more than 19 types of enzymes, such as a-rhamnosidase, β-glucosidase, protease, cellulase, α-amylase, xylanase, pectinase, and lipase, among others. These enzymes degrade the cell wall of the solid material and release bioactive compounds present in the residues that were in bound form or they synthesize new compounds, with mobilization for subsequent extraction with solvents (Madeira Junior et al., 2015; Dey et al., 2016; Sadh et al., 2018). *R. oligosporus* has also stood out in the extraction of bioactive compounds by SSF. This is due to its high production of β-glucosidase, which acts in the hydrolysis of phenolic glycosides (Lee et al., 2008). Researchers have reported advantages of SSF over SF for the extraction of bioactive compounds from agro-industrial residues, such as higher productivity, low water and energy consumption, easy aeration, fewer requirements in the sterilization steps, similarity to the natural habitat of microorganisms, and easier recovery of bioproducts, in addition to being an environmentally "friendly" technique (Martins et al., 2011; Dey et al., 2016).

SSF has increased the levels of TPC and TFC in pomegranate, grape, apricot, plum fruit, tamarind, granadilla, avocado, Chokeberry, S*ambucus nigra* L. and *S. ebulus* L. berry residues in values ranging from 11% to 748% in relation to the unfermented residue (Table 3) (Bind et al., 2014; Dulf et al., 2015, 2016, 2017, 2018; Moccia et al., 2019; Santos et al., 2020a, 2020b; Yepes-Betancur et al., 2021). These results may be due to the action of enzymes such as β-glucosidases, cellulases, pectinases, and proteases in the release of compounds bound to the solid matrix or in the transformation of existing compounds into the form of their derivatives (Dulf et al., 2015). However, some researchers have observed that in the final stages of fermentation there is a decrease in bioactive compounds (Dulf et al., 2018; Santos et al., 2020a, 2020b). This phenomenon is associated with the probable degradation of these compounds, caused by the polymerization of phenolics through oxidative enzymes (peroxidases), as a response to the stress induced in the fungus due to the lack of nutrients as sources of carbon and nitrogen (Vattem et al., 2004).

Then, the enzyme-assisted extraction improves yield and quality of compounds obtained and fermentative processes promote increased level of compounds in fermented samples, due to liberation of phenolics from cell wall after colonization of fungus, action of different ligninolytic and hydrolytic enzymes produced by microorganisms during fermentation, as well as some soluble phenolic compounds might be formed by the microorganism. However, the extracts obtained after fermentation contains various unknown complex compounds, requiring expensive purification steps to obtain specific compounds of industrial interest. In addition, different unwanted compounds with toxic effects may be produced during fermentation process, being necessary extensive *in vivo* and toxicological researches before application (Dey et al., 2016).

6 Conclusions

The exploration of fruit residues as a source of bioactive compounds is considered a promising field that requires techniques that provide an efficient extraction of these compounds. In recent years, researchers have been seeking to use emergent methods alone or in association with conventional techniques that result in high yields, extraction of heat-labile compounds, shorter times, and less generation of toxic waste. EAE and fermentation techniques have been highlighted as promising tools for the pre-extraction of bioactive compounds since the concentrations of specific compounds can be increased or new ones can be formed in the fruit residues after this process. As most techniques use organic solvents, it could be noted that, in recent years, the solvents that are considered “green”, such as deep eutectic solvents and cyclodextrins, have been highlighted in the extraction of bioactive compounds from fruit residues. To choose the most suitable extraction technique, factors such as cost, yield, and extraction time are among the most relevant to be considered. The valorization of fruit residues as additives, natural preservatives, or sources of bioactive compounds that can be used in the pharmaceutical, cosmetic and food industries significantly minimize environmental problems caused by the generation of these residues.

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