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Farid Chemat, Anne-Sylvie Fabiano-Tixier, Maryline Vian, Tamara Allaf,
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Farid Chemat [a,*], Anne Sylvie Fabiano-Tixier [a], Maryline Abert Vian [a], Tamara Allaf [b],
Eugene Vorobiev [c]

[a] *Avignon University, INRA, UMR 408, GREEN Extraction Team, F-84000 Avignon, France*
[b] *ABCAR-DIC, F-17100 La Rochelle, France*
[c] *Laboratoire Transformations Intégrées de la Matière Renouvelable, Équipe Technologies Agro-Industrielles, Université de Technologie de Compiègne (UTC),*
F-60205 Compiègne, France

A B S T R A C T

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This review presents useful and green techniques of solvent-free extraction used in ancient times, such as extraction of olive oil and citrus essential oil, and innovative techniques, such as pulsed electric field, microwave, instantaneous controlled pressure drop, and extrusion. We discuss the devices, their applications, mechanisms, and parameters influencing sample preparation prior to analysis of natural products.

Contents

* Corresponding author. Tel.: +33 490 14 44 65/+33 490 14 44 40; Fax: +33 490 14 44 41.
  E-mail address: Farid.Chemat@univ-avignon.fr (F. Chemat).

1. Introduction

An analytical procedure for natural products comprises three steps:

- **extraction** [e.g., solvent extraction in single step or many steps, Soxhlet extraction, Kumagawa extraction, Clevenger extraction, Likens-Nickerson simultaneous distillation-extraction, and solvent extraction intensified by innovative techniques, such as microwave (MW) or ultrasound, pressure liquid solvent extraction, and supercritical solvent extraction];
- **evaporation** of the solvent by distillation for concentration or purification; and,
- **analysis** [e.g., gas chromatography (GC), GC coupled with mass spectrometry (MS), high-performance liquid chromatography (HPLC) coupled to UV or MS, nuclear magnetic resonance (NMR), X-ray diffraction (XRD) or gravimetry].

While the analysis step is complete after only seconds or minutes, extraction and distillation take at least several hours, even days, and are frequently carried out by prolonged heating and stirring in boiling solvent followed by prolonged heating to eliminate the solvent. Thus, the principal limiting step of chemical analysis is the extraction of the metabolites from the matrix into a solvent and then concentration by evaporation or transfer of the desired compounds by liquid-liquid extraction (LLE).

The conventional solvent-extraction procedure represents not only 80% of the total processing time, but also 90% of the required energy, and more than 99% of the solvent used for the whole analysis procedure. With decreasing fossil resources, such as petrol as a source of solvents, increasing energy prices and the drive to reduce CO_2 emissions, researchers are being challenged to find new technologies in order to reduce or to eliminate solvent and energy consumption, to meet legal requirements on emissions, process safety and control, and to reduce cost and to increase quality and functionality. In the past 10 years, trends in analytical chemistry have turned toward the Green Analytical Chemistry (GAC) [1], which endeavors to develop new techniques that reduce the influence of chemicals on the environment. The challenge of the GAC is to develop techniques that meet the need for information while reducing the environmental impact of the analyses. For this reason, petroleum-based solvents have to be avoided (Fig. 1).

Solvent-free extraction of natural products has probably been used since the discovery of fire. Egyptians and Phoenicians, Jews and Arabs, Indians and Chinese, Greeks and Romans, and even Mayas and Aztecs all possessed innovative extraction processes used even for perfume, medicine or food. The first extraction techniques were discovered without using solvents. The most widely known extraction technique is extraction of olive oil using mechanical pressing, which was recognized as a solvent-free technique for millennia.

Virgin olive oils are extracted from olive fruits using only physical methods, which include crushing olives, malaxation of the resulting pastes and separation of the oily phase. Because of its location in mesocarp of cells and the use of purely mechanical apparatus for its extraction, virgin olive oil does not require further treatment before consumption. It therefore preserves a great number of volatile and non-volatile compounds, responsible for the aroma and the presence of antioxidant compounds that inhibit lipid autoxidation (i.e., there is no enzymatic oxidation by oxygen).

Another technique most common for aromas and perfumes is extraction of essential oils using cold pressing without heating and without any solvent. The principle of this mechanical process is based on machine squeezing the citrus pericarps at room temperature for the release of essential oil from citrus fruits, which are washed in cold running water. The essence is then isolated by decantation or centrifugation. The oleaginous cavities on the peel are pressed to
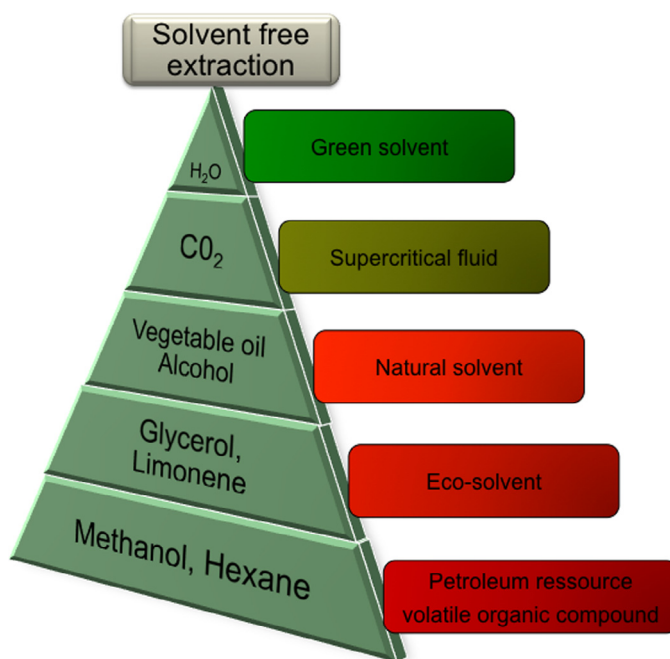

Fig. 1. From alternative solvents to solvent free.

burst by two horizontal ribbed rollers (sfumatrice) or a slow-moving Archimedean screw coupled to an abrasive shell (pelatrice) (Fig. 2), so the essential oil is released. The oil-water emulsion is separated after rinsing with a fine spray of water.

A central objective in extraction of natural products has been to develop green extraction techniques and more economically competitive processes for the efficient extraction of natural substances with potential application in the food, cosmetic or agrochemical industries [2]. Three major solutions have been identified to design and to demonstrate green extraction on laboratory and industrial scales to approach optimal consumption of raw materials, solvents and energy:

(1) improvement and optimization of existing processes;
(2) use of non-dedicated equipment; and,
(3) innovation in processes and procedures that eliminate solvents.

Industry and scientists should view the six principles of green extraction of natural products as a direction to establish an innovative, green label, charter and standard, and to innovate in not only process but also all aspects of chemistry: analysis, synthesis, purification, and extraction (Fig. 3).

In this context, the development of solvent-free techniques is of great interest in order to modernize conventional processes, making them greener, cleaner, safer and easier to perform. Several techniques, such as MW, instantaneous controlled pressure drop [*Détente Instantanée Controlée* (DIC)], pulsed electric field (PEF), and extrusion have been designed and successfully applied for solvent-free extraction of secondary and primary metabolites.

These innovative techniques make it convenient to perform extraction very efficiently in the absence of any organic solvents, so-called dry-media conditions. The advantages of using dry-media conditions range from faster extraction with different selectivity to more economical conditions due to the absence of organic solvents. However, in spite of the possible advantages, there are few applications of solvent-free extraction. The slow evolution can to

Fig. 2. Conventional techniques of solvent-free extraction for olive oil and citrus essential oil.
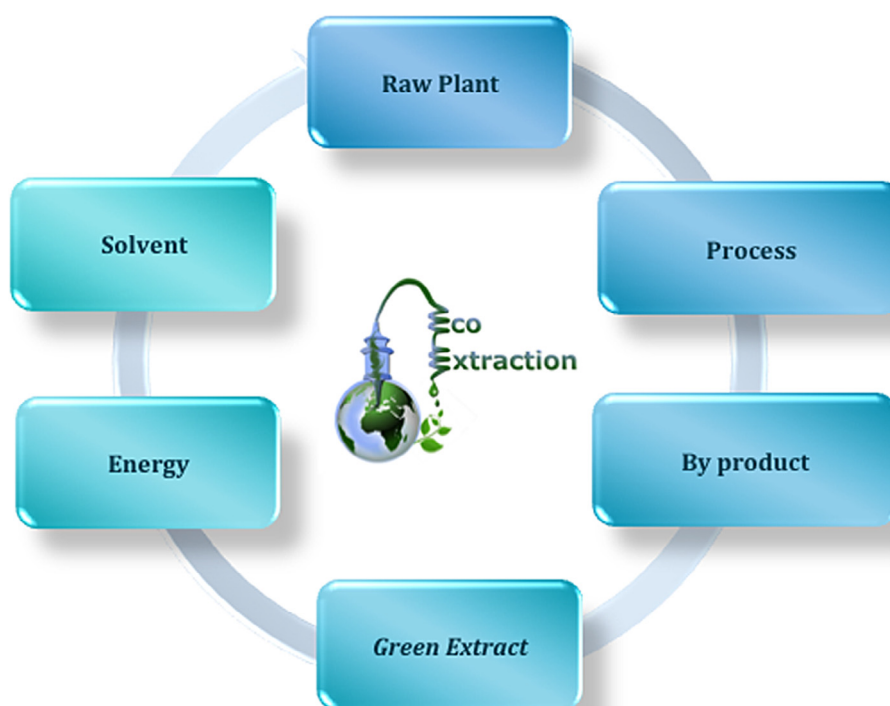

Fig. 3. Green extraction of natural products.

some extent be explained by the required combination of reactor design of the technique (heat transfer, distribution of electric field and temperature) and complexity of plant matrices and target metabolites (chemical classes, and state of plant material).

Using MWs, PEF, extrusion, or DIC, solvent-free extraction can now be completed in minutes instead of hours with high reproducibility, eliminating the consumption of petroleum solvent and at the same time removing a time-consuming step in analytical chemistry: distillation, simplifying manipulation and work-up, increasing purity of the final product, eliminating post-treatment of wastewater and consuming only a fraction of the energy normally needed for a conventional solvent-extraction method. Using solvent-free extraction, several classes of compounds, such as essential oils, aromas, edible oils, anti-oxidants, and other organic compounds, have been extracted efficiently from a variety of matrices (mainly animal tissues, food, and plant materials).

This review presents solvent-free extraction of food and natural products. It provides the necessary theoretical background and some details about techniques [MW, PEF, extrusion, or DIC], some applications and environmental impacts.

2. Pressing – extrusion

2.1. Principle

For the production of sugar, wine and fruit juices, or the dehydration of biological wastes, and in vegetable-oil industries, extraction is realized by pressing. The pressing phase is composed of a compression step to exude a fluid containing the target metabolites from the porous matrix. The cells of fruit and vegetable tissues are surrounded with membranes and closed by a cell wall embedded into a middle lamella. The components of the rigid wall prevent easy damage to the membranes, so they limit the efficiency of extraction by pressing. Two types of press are mainly used: hydraulic and screw. Using hydraulic pressing, the separation of the extract from the matrix is due to the application of a uniaxial force on the material. The pressure applied on the raw material can be very high (above 50 MPa up to 100 MPa for cocoa and up to 40 MPa for olive oil) [3].

Screw presses have progressively replaced hydraulic presses. During pressing, a helical screw, in a barrel toward a restriction, conveys the raw material from the inlet to the outlet of the press. At the stage of the restriction area, shear forces are developed along the screw, and allow expression of oil contained in the seeds.

2.2. Process and procedure

Savoire et al. conducted a large review of influential factors in hydraulic and screw pressing [4]. The use of a piston is necessary to perform expression during hydraulic pressing, which puts a specific pressure on the material. A wide variety of screw presses exist and could be classified in three main categories:

- ***Expellers*** are composed of an horizontal screw rotating in a perforated barrel formed by regularly spaced metal bars to allow flow of the expelled oil. The necessary pressure increase to expel oil and fluids is obtained by a cone partly obstructed at the screw head. In this type of press, pressures rise up to 110 MPa and minimum oil content of the order of 3.5% can be obtained [3].
- ***Expanders*** and twin-screw extruders have dedicated applications. The first is used for pre-treatment or expression of low-oil content material (soybeans) and the second has the major advantage of skipping the pre-treatment steps, depending on the

screw configurations. However, expression by twin-screw extruders has not yet transferred to the scale of industrial processes.
- ***Twin-screw extruders*** play an important role in transforming the material physically and chemically in a single step. This process seems to be most used in preparing samples for analysis, so we present a more precise mode of operation for this technique. A twin-screw extruder is based on: a conveying action realized by forward pitch screw, and radial compression and shearing actions by Monolobe screw. Bilobe, which exerts a significant mixing and shearing action, conveying and axial compression actions in combination with forward pitch screw, and reversed pitch screw, which carries out intensive shearing and considerable mixing, and exerts strong axial compression in combination with a forward pitch screw [4].

The choice of these characteristics of screw elements, in position or spacing (pitch, stagger angle, length), determines the screw profile or configuration (Fig. 4). The performance is mainly influenced by these configuration arrangements (product transformation, residence time distribution, and mechanical energy input) during extrusion processing. In theory, the operation of a screw press and the hydraulic press operating horizontally are comparable. The necessary compression for liquid exudation (metabolites) is due to the formation of the plug, which is generated by gradually reducing the volume available along the press. These pressures are the result of two forces applied on the seed mass:

- radial (thrust on the cage); and,
- axial (taking forward the matter in the press) [5–8].

2.3. Applications

Pressing for solvent-free extraction is used in a great number of industrial applications in the food domain, for example:

- juice extraction from grapes, fruit, and tomatoes;
- oil extraction (olives, cocoa butter, oleaginous seeds);
- concentrate in dry matter (sugar pulps, sludge, wastes from food industry).
- separation of the serum from curd in cheese manufacturing.

The combination of pressing with other processes can be studied in a research context, in order to obtain better extracts (quality and quantity *versus* time) at the stage of process intensification. For example, De Haan et al. [9,10] introduced gas-assisted mechanical expression (GAME) to increase oil-extraction yield and to reduce the effective mechanical pressure applied on the seeds using CO_2 assistance. New research work {e.g., Mhemdi et al. [11]} investigated the pre-treatment of sugar beets with PEF to facilitate the expression process. The feasibility of the pressing process in preparing samples has been investigated on different matrices (Table 1).

Monrad et al. [12] have studied extraction of anthocyanins and flavan-3-ols from red-grape pomace using an expeller process. This work demonstrated that this process allowed extraction of 68% of the monomeric anthocyanins for crude and 41% with dried samples, extraction of total flavan-3-ols are 58% and 38%, respectively, and enhancements of antioxidant activities by ORAC assay were 58% and 38% compared with those obtained with extracts from conventional procedures.

Celhay et al. [13] used a twin-screw extractor to realize extraction of polyphenols from a wood by-product. The extracts, due to their polyphenolic content and their high antioxidant activity, can be used, e.g., as high-value substances for food and health applications.
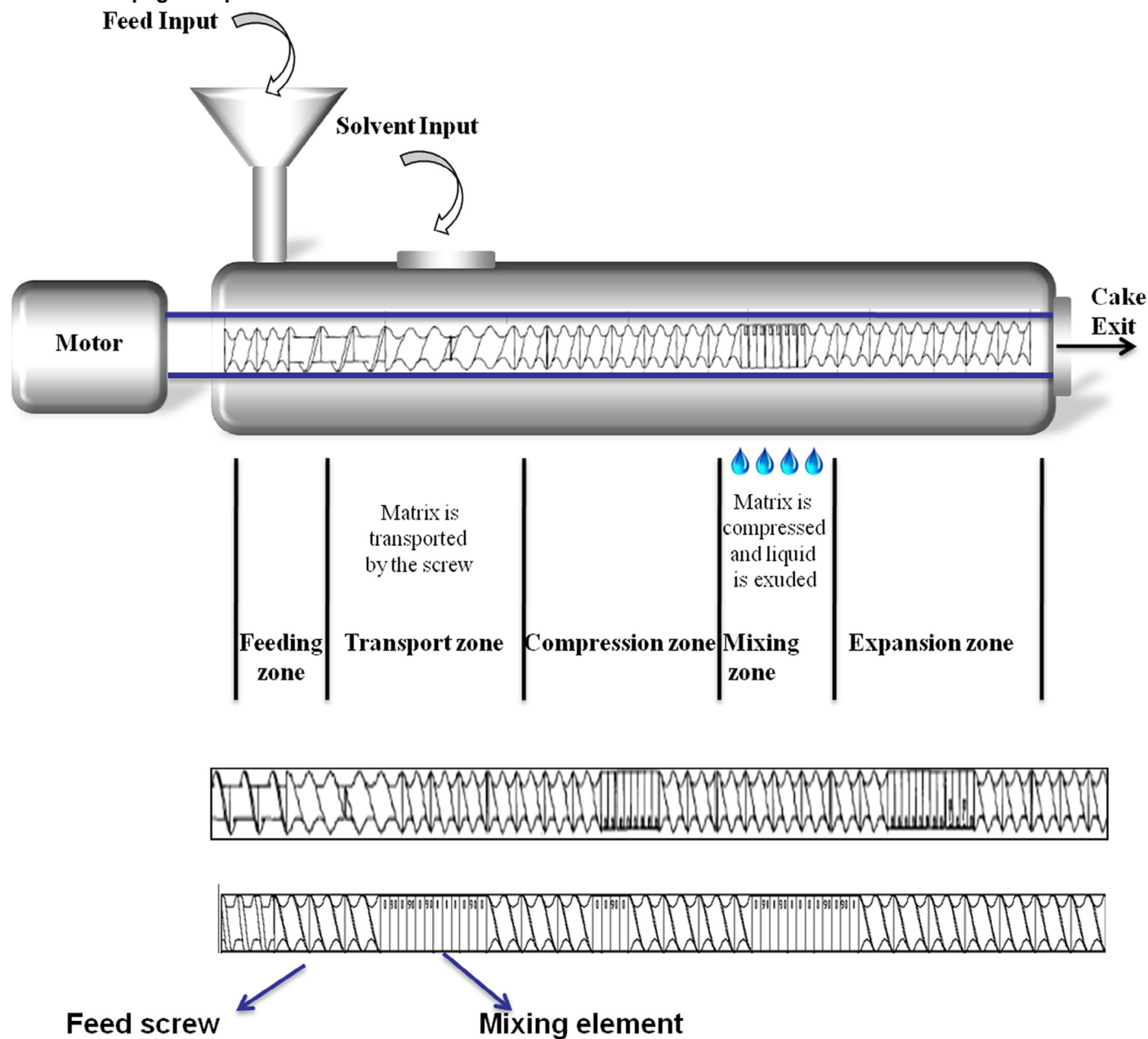
Fig. 4. Screw profile of twin-screw extractor.

Pinto de Abreu et al. [14] studied extraction of carotenoids after pressing of cashew apple. This work has demonstrated that pressing allowed an increase of a factor 10 in total carotenoid content. Pressing is mainly used for the extraction of oil from various plants.

Solvent-free extraction by pressing has been studied on sunflower seeds [15], rice bran [16], jatropha seeds [17], and walnut flour [18]. Industrial oil extraction from oleaginous seeds is commonly realized through mechanical pressing, which gives good-quality oils.

Table 1
Examples of applications and experimental conditions of pressing and extrusion

Matrix	Analytes	Experimental conditions	Analysis and detection	Ref.
Red grape	Anthocyanins	Expeller	UV-visible, HPLC	[12]
Wood	Polyphenols	Twin-screw extruder	UV-visible,	[13]
Cashew apple	Carotenoids	Helical-type press	HPLC-DAD-MS	[14]
Sunflower seed	Vegetable oil	Twin-screw extruder	Iodine and acid value	[15]
Rice bran	Vegetable oil	Screw press	GC-MS	[16]
Jatropha seed	Vegetable oil	Screw press	Iodine and acid value	[17]
Walnut flour	Vegetable oil	Hydraulic press	Spectrophotometer	[18]

3. Instant controlled pressure drop

3.1. Principle

DIC was developed by Allaf et al. [19], based on the main principle of the thermodynamics of instantaneity. DIC treatment can be used to expand different materials by an appropriate auto-vaporization that depends on the amount of vapor generated, the internal pressure constraints, the pressure-drop rate, and the viscoelastic behavior of the material. DIC is classified as a high-temperature short-time (HTST) process and consists of thermo-mechanical processing induced by subjecting the product to a fast transition from high steam pressure to vacuum.

The two main steps of DIC extraction are usually a heating step, followed by an abrupt pressure drop towards a vacuum (about 5 kPa with a rate higher than 0.5 MPa/s). Indeed, the sample, whose water content is generally adjusted to about 30% dry basis, is subjected to heating under high pressure (up to 1 MPa) at high temperature (up to 180°C) for a short time (5–60 s). A pressure drop is applied in the treatment vessel after this pre-conditioning heating and homogenization stage. By abruptly reducing the pressure, instant auto-vaporization of the moisture inside the material will occur. Several physical modifications of the sample are due to this rapid depressurization: auto-vaporization of water and volatile or non-volatile compounds contained in the sample, an instantaneous cooling of the sample, and swelling and even rupture of cells, and secretion of metabolites through cell walls. These physical modifications lead to texture change, which results in higher porosity, increased specific, surface area and reduced diffusion resistance.

3.2. Process and procedure

There are four major components in the DIC reactor (Fig. 5):

(1) processing extraction vessel, which is an autoclave with a heating jacket where samples are placed and treated;
(2) instantaneous pressure valve, which ensures a controlled liberation of pressure contained in the extraction vessel to the vacuum tank;
(3) vacuum system, which consists mainly of a vacuum tank with a volume 50 times greater than the processing vessel; the vacuum tank is cooled through a double jacket for condensation of the extracts; and,
(4) condensate is recuperated through a trap; a water ring pump maintains the tank pressure at about 5 kPa.

When steam is used as heating fluid, the heat transfer in vegetal material is enhanced by the initial vacuum, which assures closer contact of heat with the exchange surface. After closing the valve, the autoclave is filled with steam up to a processing pressure. An abrupt pressure drop within the autoclave is obtained after a certain time at a fixed processing pressure by instantaneously opening the pneumatic valve (less than 0.2 s). Just after the vacuum is reached, the instant valve is closed and the atmospheric pressure is reached. A specific vessel placed under the vacuum tank retains the emulsion of condensed steam and extracted metabolites.

A multi-cycle DIC process comprises a number of cycles, each containing repetition of the stages of heating/homogenization and pressure-drop towards a vacuum. Each following cycle (i > 1) is performed by directly injecting steam after the vacuum stage of the
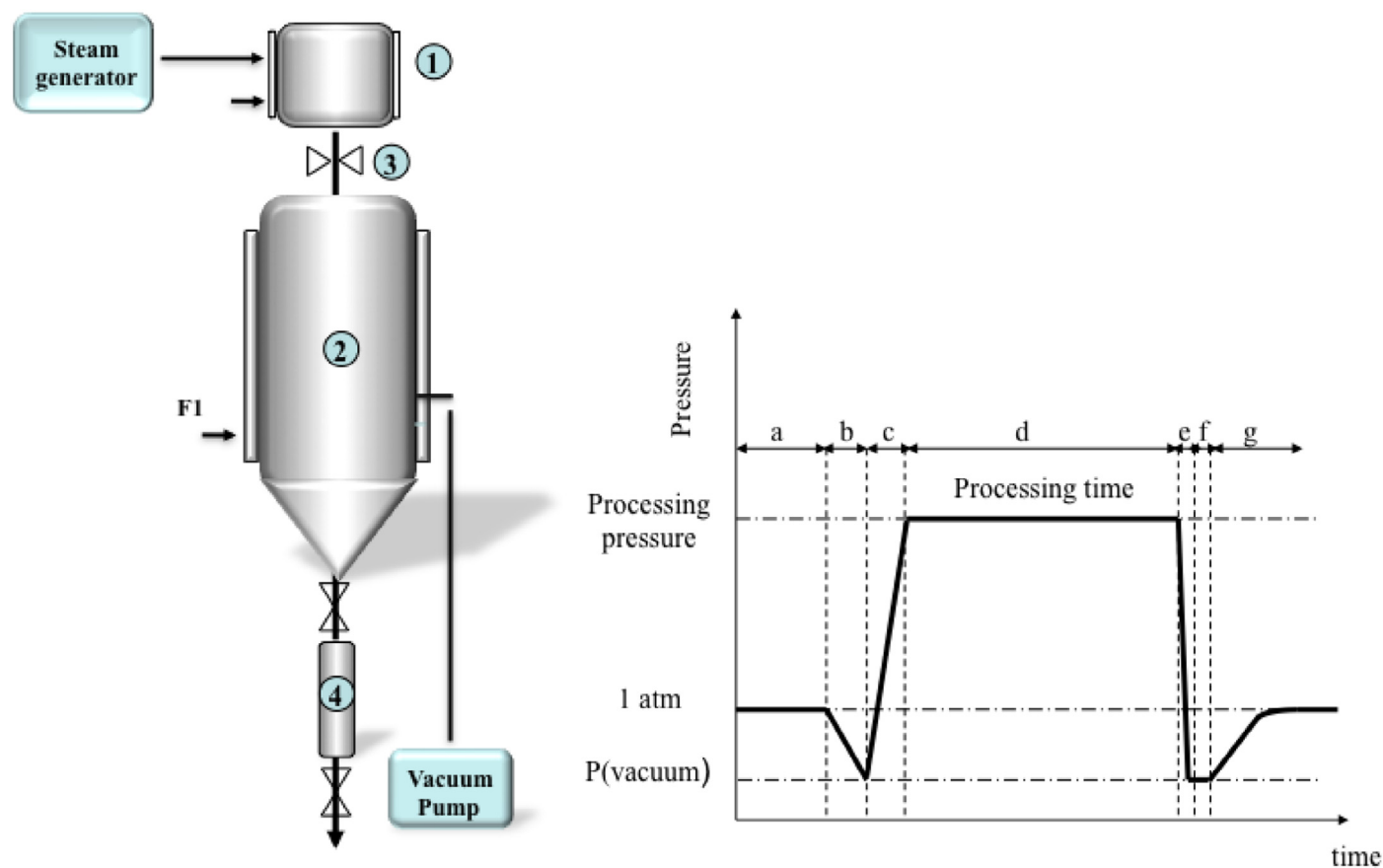

Fig. 5. DIC reactor and pressure-time profile: (1) autoclave with heating jacket; (2) vacuum tank with cooling water jacket; (3) controlled instant pressure-drop valve; and, (4) extract container.

previous cycle (i–1) without release towards the atmospheric pressure. The last cycle (i = n) is finished by the release-to-atmosphere stage. The total heating time is the heating time of all cycles (n x d), where d is the heating time of each cycle, which is slightly less than the total processing time.

3.3. Industrial applications

The preparation of different samples by the DIC process was investigated on different matrices (Table 2). DIC-assisted extraction of anthocyanins from Roselle calyces was studied by Benamor et al. [25], who demonstrated that extraction kinetics and yields of these compounds were increased by the DIC process. They also noticed the impact of DIC treatment on extraction of oligosaccharides (stachyose and ciceritol) from the seeds of the Indian *Tephrosia purpurea* plant [27]. Compared to extraction where the sample was not pre-treated by DIC, an improvement of solid-fluid extraction of the compound of interest was shown in these studies. The DIC process can also be used for the extraction of polyphenols, such as diterpens (carnosic acid) and phenolic acids (rosmarinic acid) from rosemary leaves, to illustrate the bio-refinery concept [24].

Indeed, since DIC treatment is a perfectly controlled process, its operating parameters can be optimized in order to modify, partly or completely, the organization of the raw material tissue, depending on solvent-extraction requirements. DIC has also been used for the extraction of essential oils from various plants, such as orange peel [20], myrtle leaves [21], and lavender flowers [22]. The main advantages are:

(1) no solvent is used;
(2) short time of contact of essential oil and secondary metabolites with heating zones to avoid thermal degradation; and,
(3) an abrupt pressure variation, which allows a rapid release of essential oils by instant autovaporization coupled to dramatically effective Darcy-type internal transfer of essential oil vapors.

In some cases, DIC can trigger the rupture of the oil-containing glands. These aspects together allow increasing kinetics, yields and final product quality compared to other processes for extracting essential oils [23]. The effectiveness of this operation is mainly due to the number of DIC cycles, and the processing temperature. It slightly depends on processing time. By optimizing DIC operating parameters, the duration required to reach maximum extraction yield really is better [60–240 s, whereas it takes more than 3 h to reach similar yield with hydro-distillation (HD)].

In solvent extraction [e.g., water, or supercritical CO_2 (SC-CO_2)], authors used DIC as a pre-treatment process. For example, DIC pre-treatment prior to supercritical CO_2 extraction was used by Rochova et al. [26]. It increased the extraction rate by reducing the process time (several minutes *versus* several hours), and possibly increased yield, which was comparable to or even higher than conventional SC-CO_2 extraction. In terms of quality, DIC systematically obtained higher quality essential oils, as revealed by the higher content of oxygenated compounds. Finally, DIC use implies reducing the amount of solvent (about 25% of that required with material without DIC).

3.3.1. Scaling up industrial units for food
DIC treatment is usually defined as a short-time operation, requiring a processing time of 5–60 s, depending on the raw material and the objectives. Thus, large-capacity industrial DIC equipment has been defined as normally implying a relatively small-volume treatment vessel.

The second specificity of DIC treatment is the similarity of the operating parameters (i.e., pressure, temperature, heating time, vacuum level and pressure drop rate, initial water content, etc.) whatever the scale.

The third specificity of DIC treatment concerns the vacuum level, usually 3–5 kPa, which implies using low-cost equipment, such as a liquid-ring vacuum pump. Other aspects enable the whole equipment manufacturing to be simplified. Thus, the vacuum tank can have various shapes and forms and be positioned at some distance from the treatment vessel. The whole operation can be completely automated.

3.3.2. Scaling down with cosmetic or pharmaceutical units
Cosmetic and pharmaceutical ingredients are increasingly required to be based on natural plants instead of chemical compounds manufactured synthetically, so various green extraction processes [e.g., solvent-free processes, SC-CO_2 extraction, and ultrasound (US)-assisted extraction] were defined in order to reduce both energy consumption and environmental impact. For all these extraction processes, particle size has a great impact on yields and kinetics. The lower the granulometry, the easier the extraction process in terms of both yields and kinetics.

However, natural-plant tissues and structures are well known as inadequate for easily expelling or extracting internal compounds. Cell membranes and cell walls are too hard to allow transport of these compounds towards the surrounding medium. By using adequate operating parameters, DIC allows the plant to attain higher porosity and lower tortuosity, and can even lead to disruption of cell membranes and cell walls. Thus, appropriately optimized DIC treatment permits dramatic intensification of each of these conventional or innovative extraction processes in terms of process performance and extract quality. These shorter time/higher yield operations allow small-sized, high-capacity DIC units, which are very convenient and useful in the cosmetic and pharmaceutical industries.

3.3.3. Scaling down and biochemical assessment through micro-DIC units
As explained in the previous paragraph, it is normally too difficult to assess the composition of any plant organ, such as fruits, leaves, roots, and vegetables. Although various operations of grinding are systematically used as pretreatment for different spectrometric or chromatographic measurements, the amount of compound required would systematically be underestimated, and this limits the assessment efficiency, whatever the method being considered. Some pretreatment methods, such as French-Press, can shear the cells and disrupt the cell-wall structure. Such an operation

Table 2
Examples of applications and experimental conditions of DIC

| Matrix	Analytes	Experimental conditions	Analysis and detection	Ref.
Orange peel	Essential oil	0.6 mPa , 152 s, 11 cycles	GC-FID, GC-MS	[20]
Myrtle leaves	Essential oil	0.6 mPa , 120 s, 4 cycles	GC-FID, GC-MS	[21]
Lavender flowers	Essential oil	0.6 mPa, 240 s, 6 cycles	GC-FID, GC-MS	[22]
Angelica seeds	Essential oil	0.6 mPa, 120 s, 4 cycles	GC-FID, GC-MS	[21]
Rosemary leaves	Essential oil	0.6 mPa, 300 s, 1 cycles	GC-FID, GC-MS	[23]
Rosemary leaves	Polyphenol	0.6 mPa, 180 s, 11 cycles	UV-visible, HPLC	[24]
Roselle	Anthocyanins	0.18 mPa, 20 s, 1 cycles	UV-Visible, HPLC	[25]
Soybeans	Edible oil	0.3 mPa, 60 s, 1 cycles	GC-FID, GC-MS	[26]
Tephrosia seeds	Ciceritol	0.6 mPa, 240 s, 1 cycles	HPLC-DAD	[27]
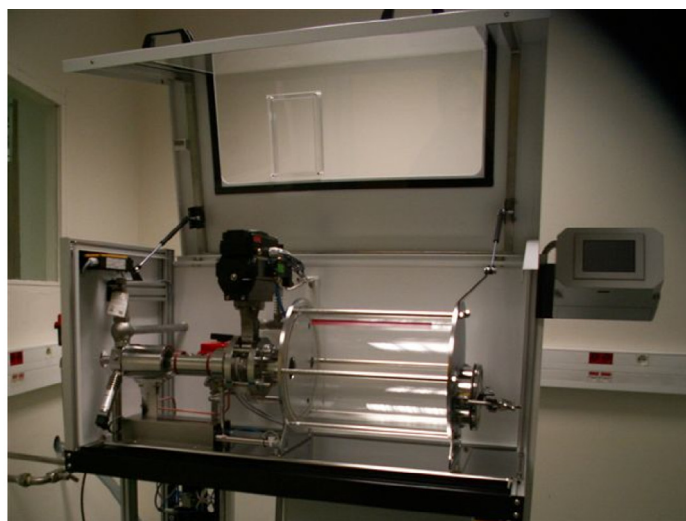
Fig. 6. Downscaling of DIC.

used as pretreatment can open these strongboxes of cells to make their contents more available and make the determination of the composition more realistic. A similar situation can be achieved using DIC treatment. However, DIC can be applied for almost all plant organs, while the French-Press system is appropriate for only pumpable systems (e.g., liquid solution).

A micro-DIC unit with a 30-mL treatment vessel was designed and manufactured by ABCAR-DIC Process (La Rochelle, France). It could be inserted within an suitable spectrometer or chromatograph (Fig. 6).

4. Solvent -free microwave extraction

4.1. Principle

MWs are electromagnetic waves with a frequency range of 0.1–3 GHz. MWs comprise electric and magnetic field components in propagating electromagnetic energy, which acts as non-ionizing radiation that causes molecular motions of ions and rotation of the dipoles, but does not affect molecular structure. When dielectric materials containing permanent or induced dipoles are placed in a MW field, the rotation of the dipoles in the alternating field produces heat. More precisely, the applied MW field causes the molecules, on average, to spend slightly more time orienting themselves in the direction of the electric field rather than in other directions. When the electric field is removed, thermal agitation returns the molecules to a disordered state in the relaxation time, and thermal energy is released. Thus, MW heating results from the dissipation of the electromagnetic waves in the irradiated medium. The power dissipated in the medium depends on the complex permittivity of the material and the local time-averaged electric-field strength.

MWs provide a non-contact heat source, which can not only make heating more effective and selective, but also help to accelerate energy transfer, start-up and response to heating control, and to reduce thermal gradient, equipment size and operation units. Solvent-free MW extraction (SFME) is a key sustainable technology in achieving the objective of green chemistry. It has been rapidly developed as one of the attractive techniques to extract and to separate interesting high added-value compounds from solid samples. With the help of MWs, extraction can now be completed without solvents in minutes instead of hours with various advantages (e.g., high reproducibility, less energy consumption, shorter

procedures, and higher purity of the final product). Several classes of compounds, such as essential oils, antioxidants, pigments, aromas, and other organic compounds, have been separated efficiently from a variety of raw materials, in particular from natural-plant resources [28].

The simplest, most innovative SFME technique is MW hydrodiffusion and gravity (MHG), which is an original "upside down" alembic combining MW heating and Earth gravity at atmospheric pressure [29]. MHG was conceived for laboratory applications in the extraction of pigments, aroma components, and antioxidants from different kinds of plant. The simple principle of this technique consists of placing plant material inside a MW reactor, without adding any solvent or water. Heating the internal water within the plant material allows the destruction of plant cells containing bioactive components. All the possible extracts, including the internal water of the plant, will be released and transferred from inside to outside the plant material. This is the physical hydrodiffusion phenomenon, which allows the extracts to drop out of the MW reactor under the effect of Earth gravity and to fall into a cooling system outside the MW oven through the perforated Pyrex disc, where the extracts are continuously condensed. The crude extracts are collected in a receiving flask for further analysis (Fig. 6).

The MHG system was modified by introducing vacuum created by a vacuum pump, which is fitted between the condenser and the flask used to collect the natural-plant extracts. The crude extracts are collected and freeze-dried before further analysis [30]. It is important to note that this green method allows extraction of bioactive components without distillation and evaporation, which are the most energy-consuming processes of the unit operations. MHG is not a modified MW-assisted extraction that uses organic solvents, an SFME that evaporates the bioactive extracts with *in situ* water, or a modified hydrodistillation that uses a large quantity of water and energy consumption.

4.2. Process and procedure

An open vessel multimode MW extractor (NEOS-GR, Milestone Srl, Italy) operating at 2.45 GHz and maximum power of 900 W, with a 1.5-L Pyrex extraction vessel was used to perform MHG extraction (Fig. 7). During the solvent-free extraction experiments, temperature was monitored by an external infrared (IR) sensor.
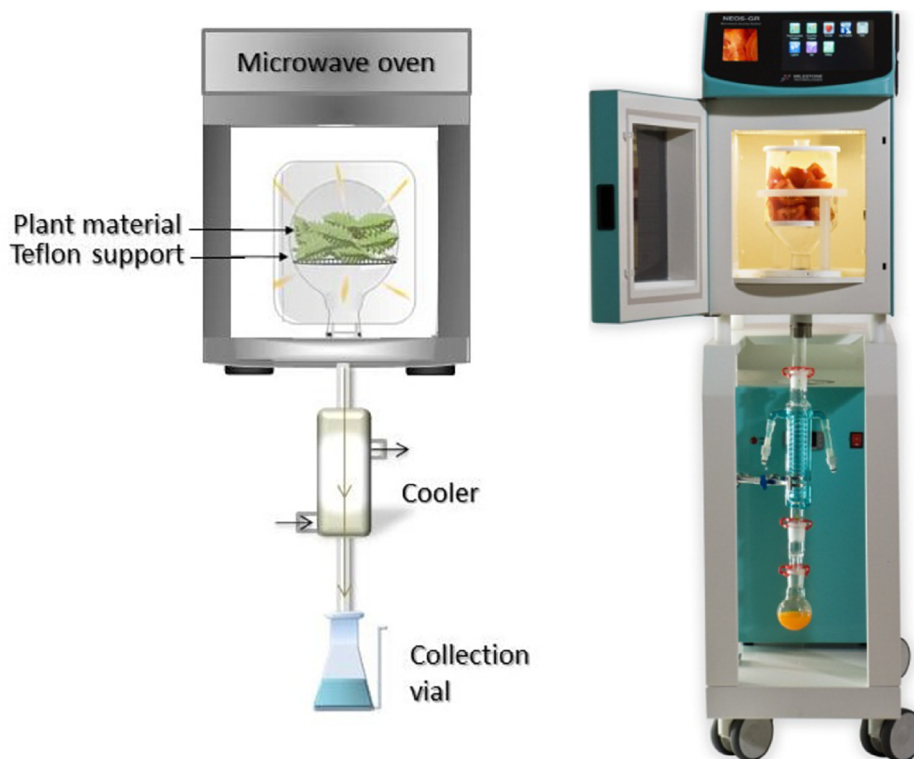
Fig. 7. Solvent-free microwave extraction using microwave hydro-diffusion and gravity.

4.3. Applications

The feasibility of the MHG process in preparing samples was investigated for different matrices, as shown in Table 3. This process was applied to many kinds of plants, such as aromatic plants and citrus plants for an essential-oil extraction [30–32].

The first example is *Mentha pulegium* L. extraction [30], where 0.95% of essential oil was obtained by heating 500 g of matrix at 500 W for 20 min at atmospheric pressure. The essential oils of *M. spicata* L. and *M. pulegium* L. extracted by MHG or HD were rather similar in their composition. The same number of volatile secondary metabolites was found in the essential oils isolated by MHG or HD, with similar yields. Identification of the EO components was carried out by GC-MS and quantitated by a GC coupled to a flame-ionization detection (FID) system.

For *Citrus limon* (L.) [31], a MW irradiation power of 500 W for 500 g of plant material was chosen as an optimum for only 15 min to extract the essential oil completely. MHG is clearly quicker than conventional HD; an extraction time of 15 min with MHG provided yields comparable to those obtained after 180 min by HD, which is the one of the reference methods in essential-oil extraction. The overall yield of essential oil obtained from lime peels was $1.0 \pm 0.1\%$ and $1.1 \pm 0.1\%$ by MHG and HD, respectively. In this application, MW irradiation greatly accelerated the extraction process, but without causing considerable changes in the volatile-oil composition.

Another example with *Rosmarinus officinalis* L. [32] was tested, and the yields of rosemary essential oil with the different isolation methods were $0.35 \pm 0.07\%$ and $0.33 \pm 0.09\%$ for HD and MHG, respectively. In this case, slightly greater amounts of oxygenated compounds were present in the essential oils of the aromatic plant isolated by MHG than by HD, so MHG offers the possibility for a better reproduction of natural aroma of the essential oil from rosemary leaves than the HD. Moreover, the MW procedure yielded essential oils that could be analyzed or used directly without any clean-up, solvent-exchange or centrifugation steps.

This innovative technique was also applied to the extraction of antioxidant compounds. Zill-e-Huma et al. [33,34] reported MHG for extracting flavonoids from onion. The plant tissues were strongly disrupted by MW irradiation, as seen through the microscopic observation of extracts, so that target compounds could be efficiently extracted and detected by HPLC and other

Table 3
Examples of applications and experimental conditions of SFME

Matrix	Analytes	Experimental conditions	Analysis and detection	Ref.
Mint *Mentha spicata L., Mentha pulegium L.*	Essential oil	P.atm, 500 W, 20 min	GC-MS/GC-FID	[30]
Rosemary leaves *Rosmarinus officinalis L.*	Essential oil	P.atm, 500 W, 15 min	GC-MS/GC-FID	[31]
Citrus peel	Essential oil	P.atm, 500 W, 15 min	GC-MS/GC-FID	[32]
Onion *Allium cepa L.*	Polyphenols	P.atm, 500 W, 30 min	HPLC-DAD	[33]
Red, yellow, white and grelot onion	Polyphenols	P.atm, 500 W, 30 min	HPLC-DAD	[34]
Sea buckthorn *Hippophae rhamnoides*	Polyphenols	P.atm, 400 W, 15 min	HPLC-DAD	[35]

contents by Folin-Ciocalteu method and reducing power by the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.

5. Pulsed electric field

5.1. Principle

Interest in the application of electric field to treatment of food and agricultural raw materials has increased in the past two decades. In the 1940s, electric fields were used in food processing for purposes other than inactivation of microorganisms. PEF increases the permeability of plant tissues, facilitating extraction of cellular fluid. Then, it was used as a pre-treatment step prior to juice pressing or as an intermediate treatment after producing the juice. Recently, the benefits of the PEF technology were demonstrated in extracting valuable compounds from a large number of fruits and vegetables, and agricultural waste [36].

PEF technology involves the application of short duration pulses (μs) of moderate or high electric field strengths (0.1–50 kV/cm) to products placed between two electrodes at room temperature. Application of PEFs of high intensity and duration from μs to ms to biological cells (plant, animal and microbial) may cause temporary or permanent permeabilization of cell membranes [37] (Fig. 8). The cell membranes are charged and pores are formed in the membranes favoring the extraction. Generally, terms describing this phenomenon are "electroporation" or "electropermeabilization". The degree of electroporation depends on many factors, including plant-material compounds, electric-field intensity, type of pulse waveform, treatment time and number of pulses. Depending on treatment intensity, electro-permeabilization of membranes leads to reversible or irreversible pore formation and cell disintegration, which is often a key processing step in food and bioengineering operations.

5.2. Process and procedure

PEF equipment consists of a high-voltage pulse generator, a treatment chamber and necessary control devices (Fig. 9). The treatment chamber, wherein the product is exposed to the electric field pulses. consists of at least two electrodes, one on high voltage and the other at ground potential, separated by insulating material in different geometric configurations. The product experiences a force per unit charge (the electric field), which is responsible for the irreversible breakdown of the cell membrane [36].

5.3. Applications

Recently, different examples and advantages of PEF treatment for enhancing extraction from plant tissues were reported. PEF was demonstrated to improve mass-transfer processes, resulting in greater extraction of different hydrophilic intracellular materials
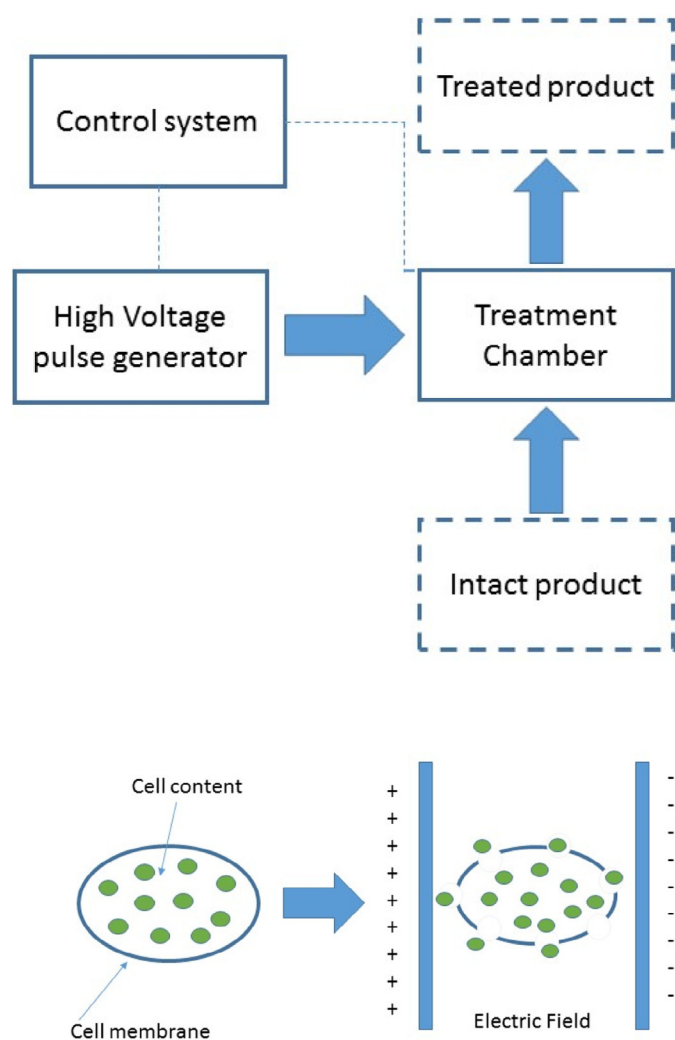

Fig. 8. PEF system and its impact in generating electroporation.

analysis. MHG was also applied to sea-buckthorn by-products to extract specific antioxidants, producing a little lower yield of flavonol in a very short time (15 min) in comparison to classical methods but with a higher content of reducing compounds in MHG extracts [35]. Extracts obtained using the MHG technique and conventional solvent extraction (CSE) method were analyzed with HPLC for quantification of flavonoids along with evaluating their phenolic
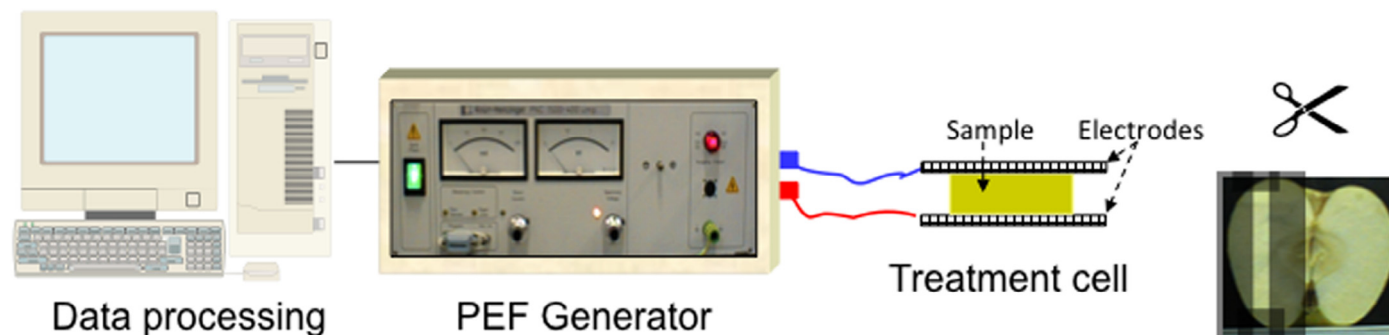

Fig. 9. PEF equipment of a high-voltage pulse generator and a treatment chamber.

Table 4
Examples of applications and experimental conditions of PEF

Matrix	Analytes	Experimental conditions	Analysis and detection	Ref.
Grape	Anthocyanins	3 kV/cm, 30 pulses, 70°C, ethanol and water	LC-DAD/ESI-MS	[38]
Red cabbage	Anthocyanins	2.5 kV/cm, 50 pulses	HPLC-MS	[39]
Purple-fleshed potato	Anthocyanins	3.4 kV/cm, 35 pulses, 10–40°C, ethanol and water	HPLC-DAD	[40]
Grape seeds	Polyphenols	8–20 kV/cm, 0–20ms, 50°C, ethanol	UV-Visible spectroscopy	[41]
Sugar beet	Sucrose	7 kV/cm, 20 pulses, 40°C, 60 min	Refractometry	[42]
Red beet	Betalains	0.375–1.5 kV/cm, 100-µs pulses, 30–80°C, water	UV-Visible spectroscopy	[43]

{e.g., polyphenols from a large number of fruits, vegetables and agricultural waste [38–41], sucrose from the sugar beet [42], and betalains from red beets [43]}. These references provide a brief overview of the many applications of PEF-assisted extraction of valuable compounds from natural products (Table 4).

PEF has mainly been used to extract polyphenols {e.g., recovery from grape seeds [41]}. The PEF efficiency was improved when the treatment was performed at 50°C in the presence of ethanol. PEF pre-treatment increased both the extraction kinetics and the maximum yield of polyphenols. The final content of polyphenols was 9 g GAE/100 g DM after 15 min extraction.

Corrales [38] compared extraction of anthocyanins from grape by-products using ultrasonics, high hydrostatic pressure (HHP) and PEF, and reported an improvement of 10% in extraction compared to HHP and 17% more compared to conventional extraction, with the least increase being observed with ultrasonics. They used an electric-field strength of 3 kV/cm and 30 pulses and further extraction was carried out at 70°C using ethanol and water solvents.

Anthocyanins were also extracted from red cabbage by PEF treatment [39]. These authors showed that PEF treatment enhanced total extraction of anthocyanins in water 2.15 times with a higher proportion of non-acylated forms than control.

In a recent study [40], PEF treatment was used for anthocyanin extraction from purple-fleshed potato at different extraction times (60–480 min) and temperatures (10–40°C) using water and ethanol as solvents (48% and 96%, respectively). A PEF treatment of 3.4 kV/cm and 105 µs (35 pulses of 3 µs) resulted in the highest cell-disintegration index at the lowest specific energy requirements (8.92 kJ/kg). This PEF treatment increased the anthocyanin extraction yield, the effect being higher at the lower extraction temperature with water as solvent.

Lopez et al. [41] showed PEF as a suitable pre-treatment before the extraction of sugar beets in order to reduce the extracting temperature, the time and the energy requirements over traditional thermal extraction. A treatment of 20 pulses at 7 kV/cm (3.9 kJ/kg) reduced the temperature for 80% extraction of sucrose from 70°C to 40°C in a 60-min extraction process.

PEF treatments proved to enhance betalain extraction efficiency with relatively low levels of tissue damage and low energy consumption. PEF treatment, using trains of monopolar rectangular 100 µs pulses with electric-field strength E = 375–1500 V/cm, and total treatment time $t_{PEF} = 0$–0.2 s, was found effective for accelerating the extraction of betalains and reducing the time of extraction [43].

6. Conclusion and future trends

In this review, we discussed how the concept of solvent-free extraction has become an important issue in the chemistry of natural products. Detailed analysis of past and present literature explicitly confirms the usefulness of "original" methods of solvent-free extraction. The understanding, on the molecular scale, of processes relevant to solvent-free extraction techniques has not yet reached the degree of maturity of other topics in analytical chemistry. Such a challenge is somewhat ambitious and requires a special approach. We hope that this review will widen the scope of laboratory and commercial success for the potential applications of solvent-free extraction in food and natural-product chemistry.

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