



Pulsed Electric Fields Technology for Healthy Food Products

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Abstract

A great number of research works have demonstrated the feasibility of pulsed electric fields (PEF) technology as a novel treatment to obtain safe and high-quality foods with significant concentration of health-related compounds. PEF can be applied either as a preservation process with the potential to substitute conventional heat treatments or as a way to assist common industrial food processes with the aim of enhancing the characteristics of the final product and improving their efficiency in terms of processing time and yield. PEF application provides an excellent advantage to retain significant amounts of valuable compounds, due to its non-thermal nature, thus resulting in safe high-quality foods from both sensory and nutritional points of view, which is greatly appreciated by current consumers. This review gathers the most relevant information related to the application of PEF, using either high or moderate intensities of processing conditions, to different food matrices with the aim of enhancing their health-related attributes and improve the efficiency of some processes. The main challenges and opportunities for being implemented at industrial level are highlighted.

Keywords Pulsed electric fields · Non-thermal food preservation · Assisting process · Bioactive compounds · Extraction yield

Introduction

Nowadays consumers are more conscious about what they eat, claiming for safe, natural, fresh, or minimally processed foods with high-quality standards and, furthermore, health-promoting substances that contribute to their well-being. Hence, new food product development involves processes that are focused on not only obtaining innocuous products but also achieving high concentration of health-related compounds. During several decades, thermal pasteurization and sterilization have been the most used technologies for food preservation by the successful inactivation of pathogenic and spoilage microorganisms as well as deteriorative enzymes. However,

the high temperatures achieved during processing lead to nutrient losses and detrimental effects in the physicochemical and sensorial characteristics of treated products. As a result, the overall quality of the product is degraded, thus jeopardizing the acceptance by consumers. In order to satisfy current consumers' demands, the advances in food science and research have given rise to non-thermal food processing technologies, able to render added value products by retaining, or even enhancing, the concentration of their bioactive compounds and nutritional properties without losing the sight of their microbial safety [1].

Among non-thermal technologies, pulsed electric fields (PEF) has demonstrated a great potential to be applied for food preservation purposes and as a treatment to assist different processes in the food industry [2]. Usually, PEF is conducted by applying pulses of high voltage (1–45 kV/cm) to foods placed between two electrodes at ambient, sub-ambient, or slightly above ambient temperature for short periods of time (μ s). Depending on the voltage applied, PEF technology can be classified in high intensity (20–45 kV/cm) or moderate/low intensity (0.1–20 kV/cm) treatments [3, 4]. Most of the currently available scientific evidence is related to the effectiveness of high-intensity pulsed electric fields (HIPEF) for the cold pasteurization of liquid foods with a minimal impact in their nutritional, functional, and

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sensory attributes [5–7]. Nonetheless, during the last decade, different research groups have evaluated the application of moderate-intensity pulsed electric fields (MIPEF) as pretreatment to improve oil and juice extraction yields as well to enhance their bioactive compound profile [2, 5, 6]. In addition, the application of MIPEF at the lowest intensities (0.1–3 kV/cm) is currently being researched to prospect its potential as an abiotic elicitor, stimulating the biosynthesis of secondary metabolites in fruit and vegetables [8–10].

On the other hand, interesting applications of MIPEF process have been also focused to assist and improve drying, freezing, frying, or osmotic dehydration (OD) treatments. Namely, [11–13] agreed that not only the electroporation mechanism but also a loss of key electrolytes involved in the active transport of the cell membrane such as Ca^{+2} and Na^{+2} , results in an enhancement of mass transfer and decrease of sugar content in OD-kiwifruits. Moreover, apoplastic and symplastic transport phenomena occurring in MIPEF-treated kiwifruit increased the dehydration rate during OD as reported by [12]. According to [13] the MIPEF-OD combination improved water loss in strawberry and kiwifruit treated at low intensities (0.2 kV/cm) regardless of the amount of sugar employed for OD process. The water distribution and water-solid exchange of MIPEF-treated sliced apples prior to freeze-drying was studied by [14]. The authors observed a significant reduction in the initial freezing temperature and freezable water content of MIPEF-treated apples relating it to an increment of cell disintegration index (z) which increased along with specific energy input (W).

The aim of this review is to cover the most recent applications of PEF technology at high intensities for food preservation and at moderate intensities for extraction purposes, as well to identify challenges and opportunities for being implemented in the food industry.

Pulsed Electric Fields Processing: Basic Principles

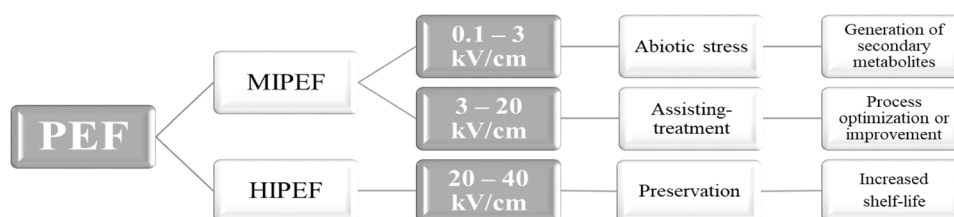
PEF processing involves the application of short pulses (μs) of electric fields intensity (E) varying from 0.1 to 40 kV/cm, depending on the pursued objective. As seen in Fig. 1, the increase of product shelf-life by the inactivation of microorganisms and enzymes is usually achieved with HIPEF (20–40 kV/cm). On the other hand, MIPEF

(1–20 kV/cm) is commonly applied as pretreatments for the improvement/optimization of drying, freezing, and extraction, among other processes. Likewise, MIPEF processing at low E (0.1–3 kV/cm) has demonstrated able to induce stress reactions in horticultural crops generating secondary metabolites [3, 4]. Due to the high intensities achieved during HIPEF processing, its application must be conducted in homogeneous liquid products free of solid particles or gas bubbles in order to prevent dielectric breakdown [15], while MIPEF could be used in solid and liquid foods. Regardless of the treatment intensity, the temperature achieved during the process is far below of that commonly used in conventional treatments [16]. This represents a great advantage in front of other technologies, since most heat-sensitive bioactive compounds associated to health benefits are well preserved or even enhanced. Furthermore, total treatment time is very short, which results in saving energy and operational costs.

The main mechanisms associated to PEF technology are related to electroporation phenomena and electrical breakdown. According to [2], the phospholipid bilayer and proteins of the cell membrane get unstable under the applied electric fields, causing the formation of holes and increase of cell membrane permeability. As a result, different effects in PEF-treated products could occur, e.g., damage to cellular structural integrity of microorganisms leading to microbial death [17], disruption of plant cell wall improving extraction yield of diverse target compounds [18–20] or even, a promotion of the antioxidant content in fruits [10]. Furthermore, [2] clearly describe that the application of electric fields can cause modification in electrically-sensitive components, thus accelerating mass and heat transfer in some processes such as drying, freezing, and OD, among other. Hence, some biological effects such as molecular modification, and polarization or realignment of molecules with dipole moments could occur during processing. Depending on treatment conditions, these changes may end up producing reversible or irreversible damage.

In this sense, it has been proved that the effectiveness of PEF processing is related to (a) operating processing parameters: electric fields intensity (kV/cm), pulse shape (quadratic or exponential), pulse polarity (monopolar or bipolar), frequency (Hz) and treatment time (μs –ms) and (b) food matrix properties such as electrical conductivity, pH, and composition [16]. Since foods are complex organic

Fig. 1 Pulsed electric fields at different electric fields intensities for food application



materials, their study under the application of PEF must be conducted specifically in each product to define the optimum processing conditions able to attain the highest quality standards related to safety and health attributes. In this line, a large number of studies conducted over the last decades have demonstrated the feasibility of PEF application for different purposes in the food industry: microbial or enzyme inactivation, functionality enhancement, extractability increase, and recovery of nutritionally valuable compounds in a diverse variety of foods.

Applications

HIPEF for the Improvement of Nutritional and Functional Properties of Liquid Foods

HIPEF processing constitutes a potential alternative to traditional thermal treatments to obtain safe products with a long shelf-life and high stability. Since its application is conducted at low or moderate temperatures, usually below 40 °C, most thermolabile compounds, partially degraded

when thermal pasteurization or sterilization are applied, are much better retained [5]. At the same time, it has been corroborated that physicochemical and sensorial properties of HIPEF-treated products are minimally affected, resulting in liquid foods with high quality attributes.

Many research efforts have been done to evaluate the influence of HIPEF on different bioactive compounds, such as hydro- and liposoluble vitamins, phenolic compounds, carotenoids, glucosinolates, chlorophyll, amino acids, fatty acids, among others, in diverse liquid matrices (Table 1). In most cases, available results evidence that the concentration of phytochemicals is well retained, minimally modified, or even increased, immediately after processing and during shelf-life. However, these effects correlate well with the HIPEF process intensity and food matrix characteristics such as physicochemical properties and composition, as well as on the nature of the studied compound. Comprehensive information regarding the potential applications of HIPEF to preserve and promote bioactive compounds in different foods can be found in [6, 7, 9, 16]. Some of the most relevant findings related to the effects of HIPEF on health-related compounds are summarized below.

Table 1 Effect of HIPEF processing on bioactive compounds contained in liquid foods

Bioactive compound	Food matrix	HIPEF processing	Effect	Reference
Vitamin C	Apple juice	30 kV/cm	NSC	[21]
	Apple cider	28, 32, 36, and 40 kV/cm, 25, 50, 100 μ s	NSC	[22]
	Grape juice	65 and 80 kV/cm using 20–40 pulses	NSC	[23]
B-complex vitamins	Milk	18.3, 22.6, and 27.1 kV/cm; 47–400 μ s	NSC	[24]
	Milk	15–35 kV/cm, 12.5–75 μ s	NSC	[25]
	Fruit juice-milk beverage	35 kV/cm, 1800 μ s, 200 Hz, bipolar 4- μ s pulses	NSC	[26]
Total phenolic compounds	Fruit juice-milk beverage	35 kV/cm, 1400 μ s, 200 Hz, bipolar 4- μ s pulses	NSC	[27]
	Kombucha	281.6 kJ/kg	twofold significant increase	[28]
	Date juice	35 kV/cm, 1400 μ s, 100 Hz bipolar 4- μ s pulses	Increase (18%)	[29]
Total carotenoids	Mango juice	35 kV/cm, 1400 μ s, 200 Hz, bipolar 4- μ s pulses	Decrease (13%)	[30]
	Fruit juice-milk beverage	35 kV/cm, 1400 μ s, 200 Hz, bipolar 4- μ s pulses	Decrease (10%)	[31]
Glucosinolates	Broccoli juice	15–35 kV/cm, 2000 μ s, 200 Hz, monopolar or bipolar 4- μ s pulses	threefold increase	[32]
Betacyanins	Prickly pear juice	35 kV/cm, 45 Hz, 3 μ s-monopolar pulses	NSC	[33]
Chlorophylls	Broccoli juice	25–35 kV/cm, 1250 μ s	Increase (16–20.7%)	[34]
Iron	Fruit juice-milk beverage	35 kV/cm, 1400 μ s, 200 Hz, bipolar 4- μ s pulses	Increase (300%)	[35]

Effects on Vitamins

It is well known that vitamins are vital organic molecules that have an essential role in human metabolism. Furthermore, some of them are well known for their high antioxidant capacity, having a significant impact in food quality due to their reducing nature, as well as in consumers' health. Hence, their retention in processed foods is of high importance. Since vitamin C is highly sensitive to processing and storage, scientists have studied the impact of HIPEF treatment in vitamin C retention from different products to find the optimal conditions to maintain its highest concentration (Fig. 2), usually comparing the obtained results with the effects of conventional treatments. A complete investigation on vitamin C structure as affected by HIPEF was conducted by [36] using a vitamin C solution to perform the experiments. Their results indicated that HIPEF treatment did not cause any damage to vitamin C and was able to slow down the oxidation process under the experimental conditions (5–35 kV/cm, for 800 μ s). Besides, the authors observed that HIPEF affected the conformation of vitamin C, promoting transformation of the vitamin C isomer enol-form into keto-form. Interestingly, available data in literature show a greater vitamin C retention

in HIPEF-treated fruit juices and mixed beverages compared with those treated by heat-pasteurization [6, 31, 37–39, 41]. Processing parameters such as E, pulse shape (σ), treatment time (t), and pulse polarity play an important role in vitamin C retention. Sánchez-Vega et al. (2015) observed that the residual content of vitamin C in broccoli juice treated by HIPEF ranged from 67.0–90.1 %; achieving the maximum concentration at 35 kV/cm and 500 μ s with monopolar pulses. Similarly, 98.0 % retention of vitamin C after HIPEF processing (35 kV/cm, 3 μ s monopolar pulses) of *Opuntia dillenii* juice was reported by [33]. In a recent study [43] corroborated that ascorbic acid concentration of a mixed mandarin and Hallabong tanger juice treated at 16 kV/cm and 100 kJ/L was not degraded compared to the untreated juice. The best retention of vitamin C in HIPEF-treated products in monopolar mode could be related to the inactivation of the enzymes involved in vitamin C oxidation [6]. According to [36], HIPEF processing slows down the involved oxidative reactions. Different authors have attempted to describe the depletion of vitamin C content using exponential response models. Sánchez-Vega et al. [42] indicated that a second-order polynomial model accurately described ($p < 0.0001$, $R^2 = 0.83$) the changes in vitamin C concentration of HIPEF-processed broccoli juice. Likewise

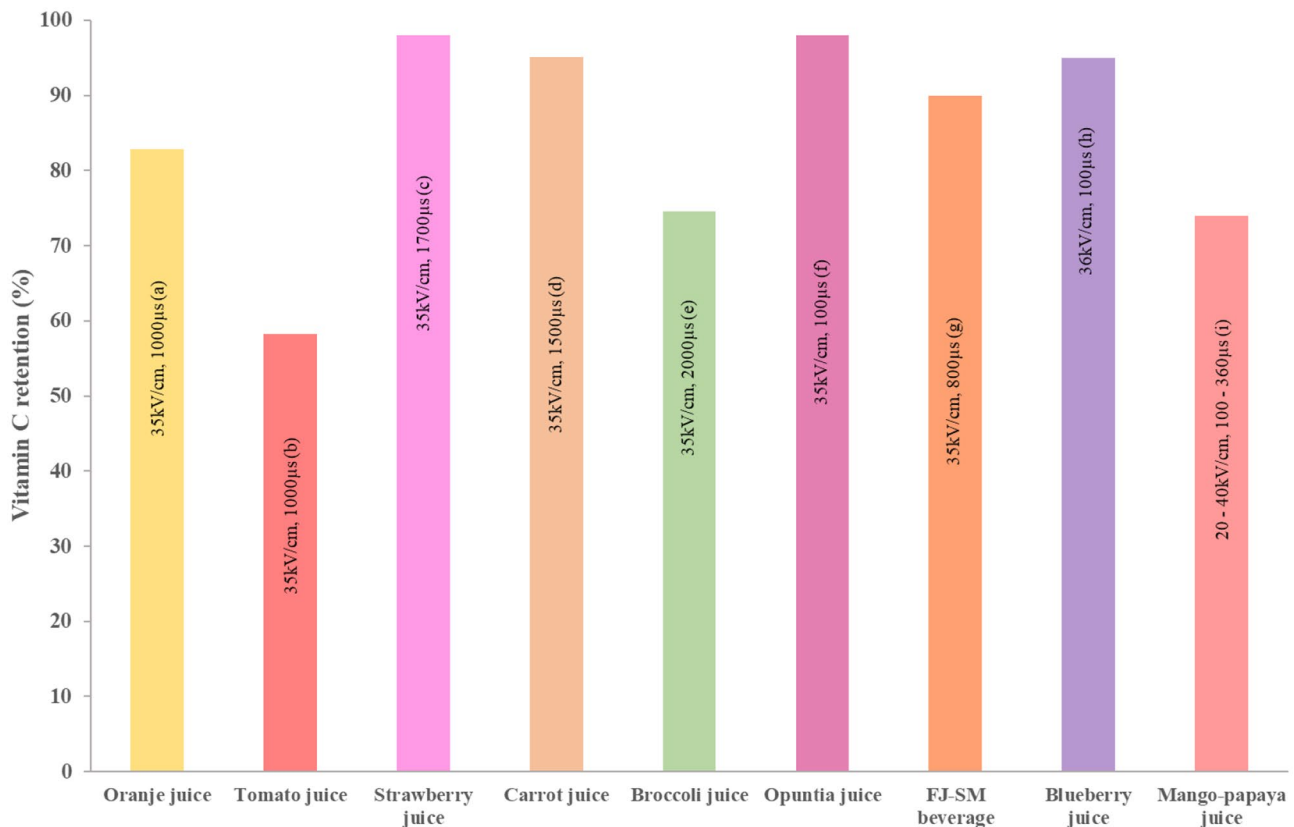


Fig. 2 Effects of HIPEF on vitamin C retention in different liquid foods. (a) [44]. (b) [45]. (c) [39, 40]. (d) [41]. (e) [42]. (f) [33]. (g) [38]. (h) [46]. (i) [47]. *FJ-SM fruit juice-soymilk

[39] indicated that a Weibull model ($R^2 \text{ adj} \geq 0.84$) was able to describe the kinetic changes of vitamin C in tomato and strawberry juices as affected by E and t , demonstrating an inverse relationship between these parameters and vitamin C retention. Also [26] stated that the Weibull model accurately described (R^2 from 95.5–99.2, A_f from 1.01–1.11) the degradation kinetics of vitamin C during refrigerated storage of HIPEF processed (35 kV/cm, 1800 μ s, 200 Hz and 4 μ s-bipolar pulses) fruit juices-milk mixed beverages

The impact of HIPEF processing on vitamin B complex and fat-soluble vitamins has been mainly studied in milk or milk-based products. Riener et al. [25] stated that HIPEF treatment ranging from 15 to 35 kV/cm during 12.5 to 75 μ s did not affect the concentration of thiamine, riboflavin, retinol, or α -tocopherol of milk immediately after processing. The same effect was reported by [24] when applying HIPEF treatments at different E (18.3–27.1 kV/cm) in skim milk and simulated milk ultrafiltrate (SMUF). These authors reported that no significant differences in thiamine, riboflavin, cholecalciferol, and tocopherol concentration were observed between HIPEF-processed milks and those untreated. Interestingly, [26, 48] observed that pantothenic acid, biotin, and riboflavin levels in HIPEF-treated mixed beverages, containing fruit juices and milk, were higher than those in heated beverages.

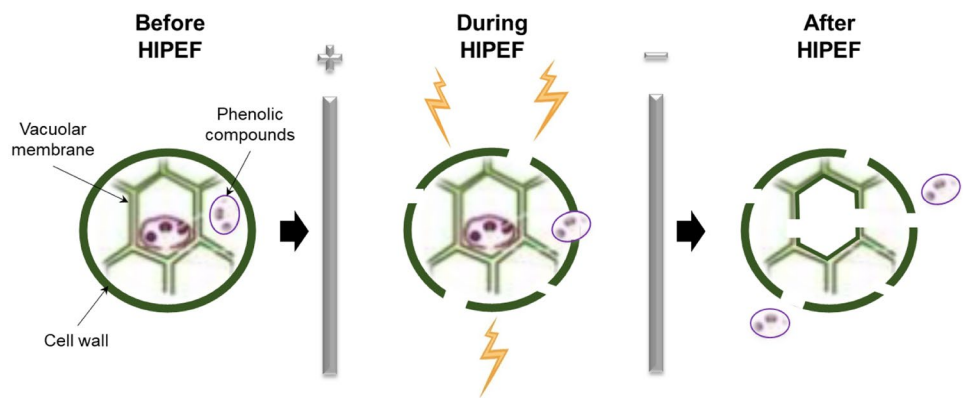
Otherwise, few research works have been conducted regarding vitamin A as affected by HIPEF. As reported for other vitamins, vitamin A changes after HIPEF processing are minimal compared with those presented in conventionally pasteurized foods. Cortés et al. [49] and Torregrosa et al. [50] found that HIPEF-processed orange-carrot and orange juices had higher pro-vitamin A compound content than heat-treated juices. In view of the existing scientific evidence, it could be stated that these compounds are more heat-sensitive than HIPEF-sensitive, indicating that HIPEF process can be used to preserve the original concentration of vitamins in liquid products.

Effects on Phenolic Compounds

Phenolic compounds (PCs) are the largest group of secondary metabolites distributed in the plant kingdom. Due to their high antioxidant capacity, they act as inhibitors of oxidative enzymes, as metal chelators, or as free radical scavengers [51–53]. Their retention in processed foods is of great interest since these molecules have demonstrated beneficial effects in human health, either as preventive or even as therapeutic agents for degenerative and chronic diseases [29]. Due to their high importance, several researchers have evaluated the impact of HIPEF, applied as preservation treatment, on different plant-based beverages including fruit and vegetable juices, mixed beverages and, recently, Kombucha [6, 28].

Interestingly, there is no consensus in the available literature regarding the effects of HIPEF on PC. Different changes in the concentration of phenolic acids (ferulic, *p*-coumaric, caffeic, ellagic, and chlorogenic) of tomato, orange, and strawberry juices have been observed immediately after HIPEF processing; however, they were always better retained in comparison with heat-processed juices. In addition, observed changes were mainly related to their molecular structure [40, 54, 55]. In accordance to these results, [27] highlighted that the application of HIPEF at 35 kV/cm during 1800 μ s in a fruit juice-milk beverage led to a higher concentration of individual PC than the use of a conventional thermal processing. On the other hand, [42] reported that the relative content of total PC of broccoli juice varied from 80.0 to 96.1% after HIPEF processing at different E (15, 25, and 35 kV/cm) in monopolar and bipolar mode. Similarly, 18.0 and 40.0% decreases in polyphenol concentrations were detected in apple and mango juices, respectively, after HIPEF processing [30, 56]. Sánchez-Vega et al. [42] suggested that the residual polyphenol oxidase activity might be associated with the degradation of PC during HIPEF processing. By contrast, the concentration of PC in date juice was significantly higher after the HIPEF process (569.55 mg/L) in comparison with untreated (483.32 mg/L) and thermally treated (494.35 mg/L) juices [29]. Likewise, [28] demonstrated that lower W (246 kJ/kg) renders Kombucha beverage with higher polyphenolic content compared with untreated samples. Moussa-Ayoub et al. [33] also indicated that HIPEF treatment caused a significant increase in isorhamnetin-3-O-rutinoside, the predominant flavonol of prickly pear juice, regarding the untreated juice. Díaz-Ribas et al. [57] investigated the effects of HIPEF processing on herbal infusions and demonstrated that HIPEF application (800 kJ/kg) increased the amount of free phenolic acids (222%) showing that HIPEF may be a feasible process for herbal infusions. The influence of HIPEF on the phenolic content of apricot juice was also evaluated by [58]. Authors identified that HIPEF processing caused a slight increase of 3–8% on the phenolic content of treated juice compared with that without any processing. Raham et al. [58] suggested that the augment of total phenolic concentration might have happened due to the breakage of the cell wall and release of bound phenolic content due to the effects of the HIPEF treatment. According to [54], permeabilization of plant cells and vacuolar membranes is likely to occur during HIPEF processing, thus leading to an increase of PC (Fig. 3). Furthermore, biochemical or enzymatic reactions occurring as consequence of processing could also lead to the formation of new compounds, increasing the accumulation of phenolics in HIPEF-treated products [5, 29].

Fig. 3 Cell wall permeabilization effect during HIPEF treatment and release of phenolic compounds



Effects on Carotenoids

Regular consumption of carotenoids is well correlated to human health because of their antioxidant capacity and pro-vitamin A activity, with β -carotene having the highest activity [59]. These compounds represent a vast group of unsaturated molecules with a large conjugate double-bonds structure, which makes them highly susceptible to chemical reactions such as oxidation and isomerization during processing and storage. Finding the optimal processing conditions in order to better preserve carotenoids in HIPEF-treated products has been a great challenge for scientists. Different matrices such as fruit and vegetable juices as well as mixed beverages containing fruit juices and milk or soymilk have been evaluated considering the impact of HIPEF on their individual or total carotenoid concentration. Obtained results are controversial since not all carotenoids appear to react equally under HIPEF processing. Although the reason for these effects is not well known up today, it could be speculated that carotenoid conversions are triggered by HIPEF treatments. It has been demonstrated that processing parameters such as E , σ , pulse frequency (f), t , and pulse polarity have a significant impact on carotenoid concentration. Namely, [39, 45] indicated that bipolar pulses at the highest E , σ , and f raised lycopene concentration in tomato juices. Likewise, [41, 42, 49, 50, 60] concluded that more intensive HIPEF processes lead to a significant increase in the carotenoid content of plant-based products such as tomato, orange, carrot, orange-carrot, grape, and broccoli juices. Accordingly, [61] observed a significant increase in total carotenoid in a papaya-mango juice blend after HIPEF processing at 35 kV/cm. It might be possible that HIPEF process causes a disruption of the chloroplast and chromoplast membrane structures and of the protein-carotenoid complex, making carotenoids more accessible for extraction [62]. Otherwise, no significant changes in carotenoid content were observed in a HIPEF-treated orange juice or orange juice-milk beverage in comparison with the untreated samples [63, 64]. In line with this, [5] stated that individual carotenoids with antioxidant activity such as β -carotene, β -cryptoxanthin, zeaxanthin, and lutein were highly stable under HIPEF treatment.

Contrarily, [31, 65] indicated that the initial concentration of most individual carotenoids in fruit juice-mixed beverages containing milk or soymilk was significantly diminished immediately after HIPEF processing at 35 kV/cm, 200 Hz, and 1800 μ s; however, the carotenoid content in HIPEF-processed beverages was higher than that in thermally treated blends. Morales de la Peña et al. [31] proposed that different biochemical reactions may take place during HIPEF resulting in an enhancement or a loss of the individual carotenoids in plant-based foods. Interestingly, [42] observed a significant reduction of 34.5% and 49.3% in lutein and β -carotene concentration, respectively, in HIPEF-processed broccoli juice at 15 kV/cm for 500 μ s. The authors inferred that at these processing conditions, microorganisms and oxidative enzymes present in vegetables are not completely eliminated, hence eventually causing a decrease in carotenoid concentrations in the final product.

The effects observed during shelf-life studies indicate that different reactions such as isomerization or oxidation could occur throughout the time in HIPEF-treated products leading to different changes in carotenoid content. According to [66], the content of *trans*-lycopene significantly depleted as a result of isomerization phenomena, since *trans*-lycopene can be converted to 13-*cis*-lycopene, which can be transformed in other *cis*-isomers. In agreement, [63] indicated that the xanthophyll lutein is more susceptible to isomerization or oxidation process than other carotenoids. In this sense, the authors observed that the content of this molecule was considerably diminished in a HIPEF-treated orange juice after 40 days of refrigerated storage. Nonetheless, higher stability of carotenoid compounds in HIPEF-treated products in comparison with thermally treated foods was reported by [7]. In this line, [65] showed that the concentration of lutein, zeaxanthin, and β -cryptoxanthin of a HIPEF-treated fruit juice-soymilk mixed beverage significantly declined during 56 days of storage, although the degradation was lower in comparison with that observed in heated beverages. First-order kinetic models ($R^2 \geq 0.866$) successfully fit carotenoid degradation as a function of storage time for HIPEF-processed tomato, carrot, and orange

juices, as well as in a mixed beverage prepared with orange juice and milk, with rate constants between 1.4×10^{-2} and $2.3 \times 10^{-2} \text{ days}^{-1}$ [6]. Also, [41] indicated that degradation of β -carotene of HIPEF-treated carrot juice (35 kV/cm for 1500 μs and frequency of 100 Hz) followed an exponential trend ($R^2 = 0.8007\text{--}0.9603$) with degradation rates varying from 1.8×10^{-2} to $2.2 \times 10^{-2} \text{ days}^{-1}$.

Effects on Amino Acids and Fatty Acids

Amino acids and fatty acids have been also evaluated as affected by HIPEF treatment conditions. Available information is though scarce compared with data reported for other previously described bioactive compounds. It has been reported that the application of HIPEF may cause denaturation of the primary structure of proteins, leading to some effects in the concentration of free amino acids [67]. [68] reported insignificant changes on the free amino acid profile of grape must after HIPEF processing at 35 kV/cm with 4 μs -bipolar pulses at 1000 Hz. The only exceptions were lauric acid, which diminished after processing and histidine, tryptophan, asparagine, and ornithine, which slightly increased. Similarly, [69] observed that valine concentration significantly augmented in a mixed beverage processed at 35 kV/cm with 4 μs -bipolar pulses at 200 Hz during 800 μs . Nonetheless, by increasing the treatment time to 1400 μs , the concentrations of glutamic acid, glycine, tyrosine, valine, leucine, and lysine considerably decreased. The authors suggested that some chemical reactions such as desulfurization, deamination, and isomerization resulting in the degradation of some amino acids could occur during processing of the beverages. In a recent study, [70] observed that a HIPEF processing with monopolar and bipolar pulses at 15–35 kV/cm during 500–2000 μs had a significant influence on the free amino acid concentration of broccoli juice. It was reported that the highest content of free amino acids was obtained when broccoli juice was HIPEF treated at 15 kV/cm and 2000 μs in monopolar mode. The authors related the increment of histidine (159.3%) and lysine (157.8%) to the increase of enzyme activity associated to their biosynthesis.

The concentration of unsaturated fatty acids in foods is of high importance from both the nutritional and the technological point of view. Hence, the impact of HIPEF treatments on fatty acids has been assessed by different researchers. Zeng et al. [71] evaluated the effects of HIPEF treatments (20–50 kV/cm for 40 μs) in peanut oil and observed slight changes in fatty acids composition. Most studies conducted up today concur that HIPEF processes caused no major modification of most fatty acids in different products such as grape juice, whole milk, and mixed beverages containing fruit juices and milk, or soymilk [35, 65, 68, 71, 73]. Only [65] observed that polyunsaturated fatty acids contents, namely linoleic, eicosapentanoic, and docosahexanoic acids, were found to

decrease after HIPEF process, as it happened in the thermally treated samples. In addition, fatty acids concentrations in HIPEF treated milk and fruit juice-soymilk beverages tended to increase throughout the storage; this effect could be related to the presence of spoilage microorganisms that contribute to fat degradation or to biochemical changes of volatile compounds occurring during processing [65, 73].

MIPEF as an Assisting Process

During the last decades, food applications of PEF have evolved from cold pasteurization treatments to assisting treatments of well-established processes in the food industry. Different research studies have demonstrated that the application of MIPEF as a pretreatment may enhance process efficiency by reducing operation times and increasing extraction yields as well as nutritional and nutraceutical properties of extracted food products [11, 75]. It has been observed that under the effects of MIPEF treatments, cell membranes may be irreversibly or reversibly permeabilized [5]. According to [9], MIPEF application has been well associated to a great recovery of high-added value compounds from different matrices; the improvement of mass transfer rates; the enhancement of osmotic dehydration, drying, and freezing efficiency; and the increase in oil, juice, and bioactive compound extraction yields from different media. In this sense, MIPEF processing has the capability of being implemented to assist diverse unit operations, thus allowing the development of high-quality products. Relevant information regarding MIPEF-assisted processing in OD, extraction, drying, freezing, and acceleration of winemaking treatment are available in complete reviews conducted by [2, 5].

Juice Extraction

Bobinaite et al. [76] applied MIPEF processing of different intensity (1.0, 3.0, and 5.0 kV/cm) to improve juice extraction yield (EY) from blueberries. Their results indicated an increment of z along with E but independent of W . The highest juice EY increase, 31.8%, was achieved at 3.0 kV/cm while the lowest juice EY increase, 23.9%, was obtained after applying a treatment of 5.0 kV/cm. This lower increase at 5.0 kV/cm was associated with a loss of firmness, which augmented compaction of the press cake closing its capillaries. Recently, [77] determined juice EY of orange, pomelo, and lemon pretreated at 3.0 kV/cm and observed that the EY incremented by 25.0, 37.0, and 59.0%, respectively, compared with the juice EY from untreated citrus fruits. In a different research, [78] applied thermal, enzymatic, and MIPEF pretreatments to compare their effect in the juice EY of prickly pears. The processes increased the EY by 85.9% (thermal), 97.0% (enzymatic), and 27.9% (MIPEF) compared with the juice EY from untreated prickly pears. Even though

the lowest EY was achieved using MIPEF processing, this technology maintained similar physicochemical characteristics to the juice extracted from untreated prickly pear. Indeed, the combined use of MIPEF with mild thermal or enzymatic treatments presents some interesting applications that are worth of investigation.

In addition to the enhancement of the mechanical extraction process increasing the juice EY from different fruits, it has been observed that MIPEF processing increases the total phenolic content (TPC) and total anthocyanin content (TAC) of the extracted juices compared with juices from untreated samples, leading to an improvement of their antioxidant capacity (AC); some interesting results could be consulted in Table 2.

Oil Extraction

MIPEF have also been used as a pretreatment to increase the oil EY from different products. Abenoza et al. [79] evaluated oil EY from olive paste treated at E of 0–2.0 kV/cm with and without malaxation (15–26 °C during 0, 15, and 30 min). They reported an increment of 54.5% in the oil EY of olive paste treated by MIPEF without malaxation, while the MIPEF processed followed by malaxation at 15 °C during 30 min improved it by 14.1%. Furthermore, the oils extracted from MIPEF-treated olives maintained without significant changes their sensory and physicochemical properties. Puértolas and Martínez de Marañón [80] incorporated MIPEF at a pilot scale on olive oil production to investigate its effect on oil EY and oil properties. The application of 2.0 kV/cm increased the oil EY and acidity values by 13.3 and 15.8%, respectively. These results were related to a double effect generated by electroporation of the cell membrane and oil release from the oil-water emulsion produced during oil extraction. Andreou et al. [81] obtained different oil EY from Amfissis, Manaki, and Tsounati olives treated at a W of 20 kJ/kg; the highest increment was reported for Manaki olives (19.4%) while the lowest for Tsounati olives (3.0%). This effect was associated with the physical characteristics of each variety.

Peroxide value, K270, and K232 were similar in all olive oils except for acidity, which was higher in oils extracted from MIPEF-treated olives. Likewise, [82] reported an increment ranging from 2.3 to 6.0% in oil EY of Carolea, Ottobratica, and Coratina olives pretreated at 17 kJ/kg with no significant differences in oil physicochemical properties and oxidative stability within varieties. The authors concluded that besides olive physical characteristics, oil EY depended on specific composition and agronomic factors, which might interfere in the electrical diffusion.

Similarly, MIPEF has been applied as pretreatments to enhance the oil EY from different oilseeds. Namely, [83] applied MIPEF at different W (40–240 kJ/kg) as a pretreatment to the oil extraction process in sesame seeds. The authors observed that the application of MIPEF at 40 kJ/kg increased oil EY by 4.9% in comparison with the oil extracted from untreated seeds and, interestingly, the increment of W did not cause any z increase nor improve oil EY. Recently, [84] compared ultrasound (US), MIPEF, and US-MIPEF combination to increase oil extraction from sunflower seeds. According to the authors, MIPEF treatment at 1.1 kV/cm was the most suitable technology to enhance the oil EY, increasing it from 30.5 to 47.7% which was related to a significant increment in z . However, similar to the effects observed in oil extracted from MIPEF-treated olives, the MIPEF processing also increased the acidity value of sunflower oil, with no changes in other physicochemical properties. The authors attributed MIPEF effectiveness to a homogeneous application of the electric fields causing pore formation confirmed by scanning electron microscopy (SEM). In another research, [85] studied the effect of MIPEF on hulled and dehulled rapeseed previous to oil extraction by Soxhlet and mechanical pressing. The authors reported an increment in Soxhlet oil EY of hulled rapeseeds pretreated at 84 kJ/kg (39.1%), while no improvement was observed for dehulled seeds. In contrast, it was observed that the application of MIPEF previous to mechanical pressing did not improve the oil EY from hulled or dehulled rapeseeds. Acidity value was the only parameter that significantly increased in oil extracted

Table 2 Extraction yield and bioactive compounds profile of juices extracted from fruits treated by MIPEF

Food matrix	MIPEF processing	EY	TPC	TAC	AC	Comparison to untreated fruits	References
Blueberry	E, 3.0 kV/cm	56.3	109.1	50.2	162.2	Increase of TPC, TAC, and AC	[76]
Orange	E, 3.0 kV/cm	60	95.9	–	–	Increase of TPC	[77]
Pomelo		74	132.8				
Lemon		63	136.3				
Prickly pear	W, 5.0 kJ/kg	42.6	–	–	–	Increase of isorhamnetin-3-O-rutinoside content Reduction of betaxanthins content and AC	[78]

EY juice extraction yield is expressed as g of juice per 100 g of fruit, TPC total phenolic content (mg gallic acid equivalents/100 mL), TAC total anthocyanin content (mg cyanidin-3-glucoside equivalents/100 mL), AC antioxidant capacity (mg trolox equivalents/100 mL), E electric field strength, W specific energy input

from MIPEF-treated rapeseeds. According to [85], triglyceride degradation, and a consequent increase in free fatty acid concentrations, is likely to occur after MIPEF by the release of lipase enzymes. It is important to emphasize that improvements in oil EY of different matrix pretreated by MIPEF have been associated with the electroporation mechanism, mainly based on the determination of z . Likewise, increments in acidity values have been reported in the extracted oils being related to an increase in lipase activity [85]. However, there is still a gap of knowledge concerning the MIPEF mechanism on fruits and seeds that could be fulfilled with complementary microscopy and enzymatic activity analysis.

Some authors have employed different staining and microscopy techniques to propose a mechanism that relates MIPEF processing to the enhancement of oil EY from microalgae. Han et al. [86] applied a MIPEF process at 20 kV/cm followed by chloroform/methanol oil extraction to a *Chlorella pyrenoidosa* suspension, leading to a 31.9% increase in the oil EY in comparison with the oil extracted from the untreated microalga. Moreover, fluorescence and SEM microscopy demonstrated the loss of cell integrity by electroporation in microalgae treated by MIPEF. The authors stated that rather than release oil from intracellular space, MIPEF process leads to a loss of water and hydrophilic compounds causing oil fusion within the cell facilitating contact between oil and solvents (Fig. 4). In another work, [87] evaluated the application of MIPEF, mechanical stressing, and its combination to enhance oil extraction from *Chlamydomonas reinhardtii*. Interestingly, the MIPEF processing at 5.5 kV/cm did not improve the oil extraction, but the combination of MIPEF and mechanical stress caused a 50.5% increase compared with untreated microalgae. These results were associated with an increase in cell permeability provoked by loss of cell viability detected by flow cytometer analysis. Cell lysis was suggested to occur as a result of cell wall rearrangements induced by mechanical stress, while electrical stress contributed through irreversible electroporation and fusion of oil droplets.

In addition to the effects of MIPEF observed in the oil EY of olives and oilseeds, the impact in the bioactive compound profile of the extracted oil has been also evaluated. Interestingly, different studies have reported that TPC, chlorophyll, carotenoids, and α -tocopherol content increased in

oils extracted from PEF-treated products maintaining, at the same time, their fatty acid profile, γ -tocopherol concentration, and lignan content with no significant changes, as reported in Table 3. In this sense, the use of MIPEF as pretreatment of the oil extraction process led to higher yields and, furthermore, to oils with significant contents of bioactive compounds that could be related to health-related benefits. Nonetheless, further research should be conducted in order to evaluate the bioavailability of these compounds immediately after processing and during storage.

Bioactive Compound Extraction

The application of MIPEF to improve phytochemicals extraction has been investigated in different food matrices, such as whole fruits, vegetables, and industrial processing by-products with different purposes. Firstly, MIPEF has been applied on fruits and vegetables with the aim of increasing their bioactive compound concentration and thus their functional properties. Secondly, by-products from the food industry could be processed by MIPEF to recover their principal bioactive compounds, develop functional ingredients, and incorporate them into cosmetic or food formulations.

Saldaña et al. [88] attempted to incorporate MIPEF processing into wine production by investigating the effect of E , pulse number (n), and t on total PC, color, and maceration time of grapes from different varieties. An overall increase in total PC (50.7%) and color intensity (64.0%) of grapes from different varieties pretreated with 1 pulse at 5.0 kV/cm during 100 μ s was observed; however, the maceration time differed among varieties. The combined effect of E and t was suggested to improve electroporation by effectively reaching the maximum transmembrane voltage at MIPEF-treated conditions. Similarly, [89] explored MIPEF application as a pretreatment to improve the industrial-scale production of Garnacha wine by increasing its phenolic compound content and quality characteristics. It was concluded that a MIPEF pretreatment at 4.0 kV/cm caused a significant increase in total PC, total AC, and condensed tannins (CTs) of 29.5, 18.2, and 42.6%, respectively, after only 3 days of maceration, compared with untreated grapes. Concerning wine quality characteristics, the authors reported that color parameters of wine produced from MIPEF-treated grapes significantly

Fig. 4 Improvement of oil extraction yield (EY) by MIPEF: **a** electroporation induces the release of hydrophilic compounds and intracellular water, **b** reducing cell size, oil vacuoles fusion, and facilitating solvent penetration. Adapted from [86]

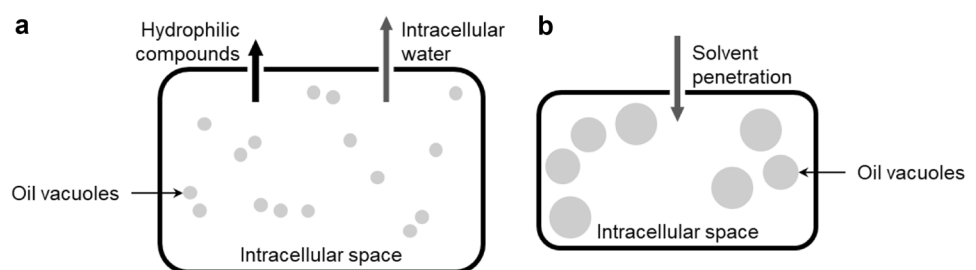


Table 3 Extraction yield and bioactive compounds profile of oils extracted from olives and oilseeds treated by MIPEF

Food matrix	MIPEF processing	EY	CC	α -T	TPC	Comparison to untreated samples	References
Olive	E, 2.0 kV/cm w/malaxation	14.1	4.4	247.8	148.9	Increase of α -T Reduction of CC, carotenoid content, and TPC	[79]
Olive	E, 2.0 kV/cm	22.7	–	143	451 ^a	Increase of α -T, TPC, and phytosterols content	[80]
Olive	W, 20 kJ/kg	13.2	–	–	153.5	Increase of TPC	[81]
Amfissis		11.9			341		
Manaki		25.2			928.5		
Tsounati							
Olive	W, 17 kJ/kg	15.9	–	–	–	Increase of total phenols and oleuropein derivatives	[82]
Carolea		13.1					
Ottobratica		16.6					
Coratina							
Sesame seeds	W, 40 kJ/kg	71.6	–	–	–	–	[83]
Sunflower seed	E, 1.1 kV/cm	47.7	–	602.2	–	Increase of α -T	[84]
Rapeseed	W, 84 kJ/kg	42.9	28.6	185.7	185.0 ^a	Increase of CC, α -T, TPC, AC, and phytosterols content	[85]
Hulled		36.5	268.2	242.9	–		
Dehulled							

EY extraction yield (g of oil/100 g of food), CC chlorophyll content (mg pheophitin/kg), α -T α -tocopherol concentration (mg/kg), TPC total phenolic content (mg gallic acid equivalents/kg), AC antioxidant capacity, E electric field strength, W specific energy input

^aTPC was expressed as mg of caffeic acid equivalents per kg of oil

improved, and no negative effects on aroma, pH, alcohol content, and total acidity were detected. Maza et al. [89] stated that color improvement was related to the extraction of condensed tannins from grape skin rather than seeds. Luengo et al. [90] compared the impact of MIPEF processes at different *W* (0–70 kJ/kg), on *z* and betanine extraction from red beet. The authors observed that up to 20 kJ/kg, *z* depended on *W* rather than *E* or *t*, while comparable increments in betanine extraction yields of 660.0% and 720.0% were reported when MIPEF treatment was applied at 29 and 43 kJ/kg, respectively. [91] compared MIPEF processing and drying as pretreatments to enhance phenolic compound extraction from whole tea leaves and observed that MIPEF at *W* of 22 kJ/kg increased 100.0% and 215.4% catechin extraction rate in comparison with drying and fresh leaves, respectively, with no differences in the PC profile. The authors attributed the increase in catechin contents to the electroporation phenomena, which was confirmed by SEM images. In another research, a MIPEF process at 2.0 kV/cm and 20 pulses was applied to enhance the bioactive compound content of sliced pumpkin [92]. Lutein, α -carotene, and β -carotene concentrations raised 47.1, 38.9, and 34.2% compared with untreated pumpkins. Interestingly, pumpkin treated by MIPEF maintained its concentration of total PC, but antioxidant activity was significantly reduced. [93] applied PEF processing combined with thermal treatment to increase PC, proteins, and polysaccharide extraction from white button mushrooms. They reported that the combination of PEF (*E*, 38.4 kV/cm; *t*, 272 μ s) and thermal treatment (85 °C, 2.6 min) enhanced PC, proteins, and polysaccharide EY by 23.1, 3.8, and 23.4%, respectively, compared with a conventional aqueous extraction. This improvement was

associated to the electroporation phenomena and phospholipid rearrangement in the cell membrane provoked by the thermal and MIPEF treatments.

Recently, different researchers have focused their attention in the effects of PEF at low intensities (0.1–3.0 kV/cm) to induce the generation of secondary metabolism as a stress response. Namely, [94] employed MIPEF processing to induce carotenoid accumulation on whole tomatoes stored at different conditions. Application of 0.02 kJ/kg incremented total carotenoids and lycopene content by 58.0 and 150.0%, respectively, in tomatoes stored at 12 °C for 150 h without affecting its physicochemical properties. Contrarily, the MIPEF treatment applied at higher *W* (0.4 kJ/kg) enhanced the concentration of β -carotene (77.0%), γ -carotene (200.0%), and lutein (238.0%) of whole tomatoes stored at 12 °C for 24 h, but compromised tomato pH and hardness. In another research, same authors evaluated MIPEF processes at different *E* (0.4, 1.2, and 2.0 kV/cm) along with 5, 18, and 30 pulses to increase the carotenoid content of whole tomatoes before puree preparation. Total carotenoids and lycopene concentrations in tomato puree improved by 50.0 and 53.0%, respectively, when MIPEF-treated tomatoes at 2.0 kV/cm and 30 pulses were used, suggesting that this treatment elicited a stress response in the whole tomato which was corroborated by an increment of acetaldehyde production and a reduction of ethylene concentration. However, despite the highest increases were obtained under these conditions, a greater bioaccessibility was achieved when lesser pulses were applied (*E*, 2.0 kV/cm; *n*, 5) to whole tomatoes. Hence, lycopene, β -carotene, γ -carotene, and

lutein bioaccessibility was increased by 132.0, 53.0, 527.0, and 125.0%, respectively, compared with purees prepared from untreated tomatoes. These results were related to competitive inhibition among carotenoids and its interaction with cell wall components, both produced by the application of more intense MIPEF treatments [95, 96]. In a recent study, [97] assessed total PC of whole carrots MIPEF-treated at different W (0.1–3.9 kJ/kg). The authors reported no significant changes in TPC immediately after MIPEF treatment, but TPC increased by 39.5 and 40.1% in samples treated at 0.6 and 0.9 kJ/kg, respectively, after 24 h of refrigerated storage (4 °C). Also, MIPEF processes applied at highest W values were directly related to an increment in conductivity and a loss of cell viability. After the obtained evidence from these results, the authors concluded that rather than a release of PC, electroporation of whole carrots induced ions lost drifting in the biosynthesis of PC as a result of the stress response.

Due to the large number of by-products generated by the food industry during the production of fruits, vegetables, grains, and seeds, the application of MIPEF has been resulted of great interest for researchers in order to extract bioactive compounds from shells, peels, bagasse, leaves, and barks, among others, and to develop functional ingredients, having positive impact in the environment. Recent studies have been conducted during the last decade obtaining interesting results; however the available information is scarce compared with other MIPEF applications. Lohani and Muthukumarappan [98] used MIPEF to enhance total PC and AC of sorghum flour and apple pomace. After a MIPEF process at 2.0 kV/cm and 875 μ s, total PC and AC of sorghum flour increased 24.8 and 33.9%, respectively. Likewise, in the apple pomace treated at 2.0 kV/cm for 500 μ s, a higher increase was observed; the total PC and AC were boosted by 37.4 and 86.0%, respectively, compared with untreated samples. The authors confirmed through light microscopy and SEM analysis of MIPEF-treated by-products that the observed changes were related to electroporation phenomena. Recently, [99] investigated the MIPEF impact on bioactive compound extraction from brewers' spent grain. MIPEF treatment at 2.8 kV/cm and 3000 pulses caused an increase in protein and reducing sugar concentrations by 5.8 and 4.9% in light samples along with 37.0 and 53.4% in dark samples, respectively, while total PC and AC of both grains were not affected by MIPEF. Barbosa-Pereira et al. [100] used MIPEF to enhance the extraction of PC from cocoa and coffee by-products of several varieties, geographical origin, and industrial processing. The authors observed an overall improvement in the concentration of total PC of cocoa and coffee samples (75.0 and 83.0%, respectively) pretreated at 1.4 kV/cm and 1000

pulses. However, the MIPEF impact on PC profile differed among samples being associated to particular characteristics of each analyzed by-product. Similarly, PC concentration and AC of yellow onion by-products treated by MIPEF were evaluated [101]. Results indicated that at 4.1 kV/cm and 51 pulses, the total PC, and quercetin content along with DPPH and FRAP antioxidant capacities improved by 130.3, 71.1, 89.7, and 16.9%, respectively, compared with a conventional aqueous extraction.

Conclusions and Future Trends

Scientific data available in literature evidence the potential of PEF technology to retain health-related compounds that are commonly highly sensitive to heat, thus providing safe, healthy, and high-quality products. In this sense, being applied with a preservation or assisting purpose, PEF process is capable of maintaining, or even enhancing, the concentration of bioactive compounds in liquid food products immediately after processing and during storage, while extending their shelf-life. PEF can be also applied to enhance the juice and oil extraction yield from different fruits and oilseeds, along with the improvement of their bioactive concentration, which is mainly attributed to electroporation phenomena, increasing health-related attributes of PEF-treated products. Furthermore, the use of PEF as an abiotic stressor might be a potential strategy for increasing the concentration of bioactive compounds in a natural way without the necessity of supplementing with synthetic compounds, which is very valued by current conscious consumers. In addition, PEF could be industrially implemented to develop energy-efficient processes and decrease operational costs without affecting the quality of the final products, and even enhancing their functional properties. Although great progresses have been made, mainly on the application of PEF at high intensities for liquid food preservation by assuring microbial inactivation, further research is required to better understand the main mechanisms involved during PEF processing at moderate intensities, as well as to fully optimize and apply the developed processes at industrial levels to obtain high-quality products satisfying current consumer claims.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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