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RECEIVED 13 February 2024

ACCEPTED 18 April 2024

PUBLISHED 02 May 2024

CITATION

Su W, Wang Q, Li J, Qiu Z and Qiu Y (2024)
Effects of pulsed electric field technology on
the nutritional value and biological function
of plant food.
Front. Sustain. Food Syst. 8:1385533.
doi: 10.3389/fsufs.2024.1385533

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Effects of pulsed electric field technology on the nutritional value and biological function of plant food

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Pulsed Electric Fields (PEF) technology is an emerging non-thermal food processing technology that is widely used because of its low damage to food quality and its ability to kill harmful microorganisms in food. The oxidative stress caused by PEF results in the synthesis and accumulation of nutrients, and plant foods stimulated by PEF produce large amounts of Reactive Oxygen Species (ROS), which activate the metabolite synthesis pathway and eventually synthesize proteins, polyphenols, thioglucosides and carotenoids; ROS accumulate in the cell membrane of microorganisms and cause protein expression. The accumulation of ROS in the cell membrane of microorganisms can cause abnormal protein expression, damage the lipid layer and Deoxyribonucleic Acid (DNA), and eventually lead to microbial inactivation. PEF technology in food processing are multifaceted, enhancing both the nutritional value of food through the augmented presence of health-beneficial compounds and ensuring food safety by effectively inactivating harmful microorganisms. This innovative technology stands at the forefront of food processing solutions, promising to fulfill the growing consumer demand for foods that are not only safe and of high quality but also rich in nutritional value. PEF's role as a cornerstone technology in the food industry heralds a new era of food processing, where nutrition and safety converge to meet the sophisticated needs of today's consumers.

KEYWORDS

non-thermal processing, nutrient enhancement, reactive oxygen species (ROS) generation, nutritional bioenhancement, microbial safety, non-thermal pasteurization alternatives

1 Introduction

Traditional food processing techniques, while essential for nutrient extraction, food storage, and drying, often negatively impact the nutritional value and safety of food. These techniques, including large-scale industrial processes like heat treatment, can lead to nutrient loss and the formation of hazardous substances, ultimately posing risks to human health (Vorobiev and Lebovka, 2006; Aghamirzaei et al., 2015; Pan et al., 2017). The primary drawback of these traditional methods is their tendency to compromise the nutritional quality and flavor of food in pursuit of processing efficiency (Golshan Tafti et al., 2013; Tafti et al., 2013). Specifically, thermal processing methods can irreversibly damage thermally unstable

nutrients or those present in trace amounts, thereby hindering nutrient accumulation in foods (Lee et al., 2011).

Furthermore, the reduction in nutrient levels is exacerbated by the excessive use of chemical additives in some traditional processing techniques, raising concerns about their long-term implications for food quality and safety (Sanchez-Moreno et al., 2009). This context highlights the urgent need for innovative food processing technologies. Such technologies aim to not only minimize nutrient loss but also enhance the biosynthesis of vital compounds like phenols, carotenoids, and proteins through the stimulation of metabolic pathways (Cisneros-Zevallos, 2003; Jacobo-Velazquez and Cisneros-Zevallos, 2012; Jacobo-Velazquez and Cisneros-Zevallos, 2013). Emerging techniques, including high static pressure, pulsed electric fields (PEF), ultrasound, and ultraviolet light, represent promising alternatives to traditional methods by offering the potential to improve both the nutritional quality and safety of food products (Chen et al., 2010; Kumar and Han, 2012; Xu et al., 2017; Asaithambi et al., 2021; Chen et al., 2022; Liu et al., 2023).

With advantages such as requiring low temperatures, rapid processing time, and being environmentally friendly, PEF is rapidly gaining attention as a promising food processing technology (Manzoor et al., 2019a,b; Rahaman et al., 2020; Lal et al., 2021). PEF causes negligible damage to food nutrients compared to traditional food processing techniques and can eliminate some hazardous microorganisms to extend the preservation time of food (Soliva-Fortuny et al., 2009; Napotnik et al., 2016; Peng et al., 2016; Liu C. Y. et al., 2021; Nabilah et al., 2022; Preetha et al., 2023). Most research in PEF has focused on active substance extraction, pasteurization, and the drying and thawing of food products, and there is relatively little research on the influence of PEF on biometabolic pathways that induce the synthesis of nutrients in food products (Boussetta et al., 2011, 2012; Rahaman et al., 2019; Ahmed et al., 2020; Preetha et al., 2021).

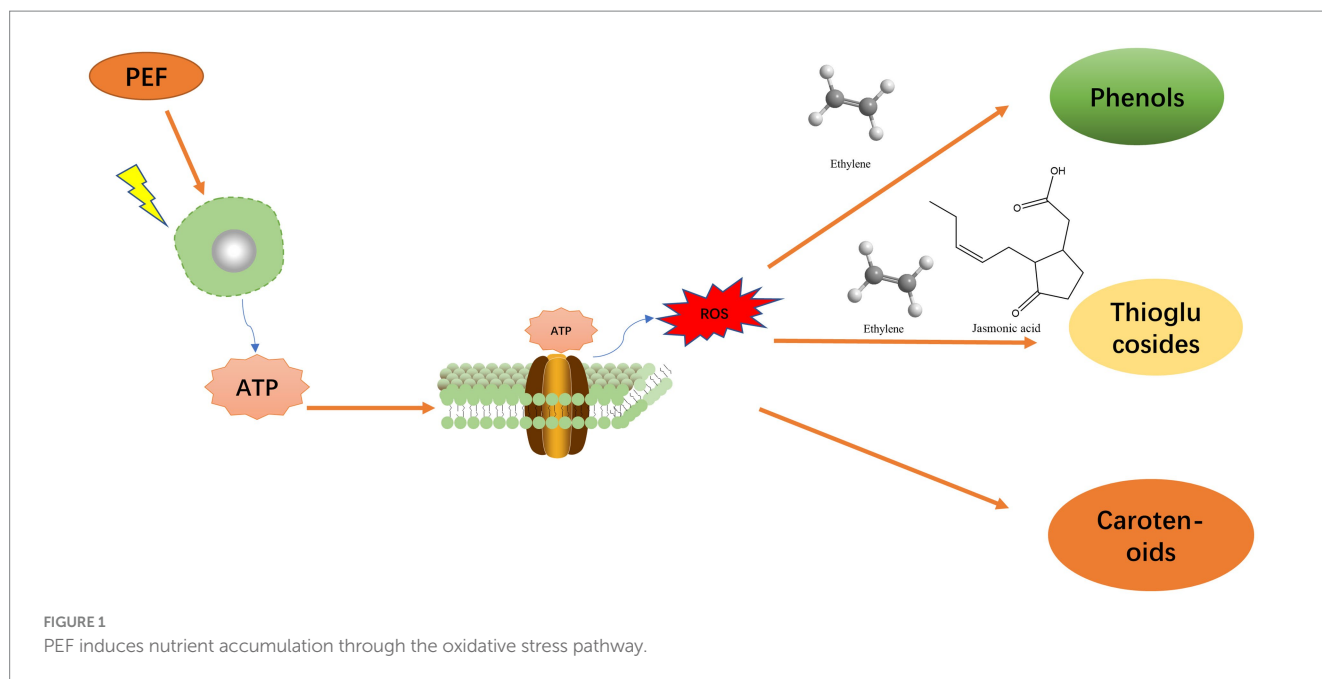
PEF causes serious stimulation and damage to plants, resulting in the production of reactive oxygen species (ROS), which can further damage plant cells. Plants secrete many metabolites to protect themselves by reducing this damage, and some of these metabolites are also essential nutrients for the human body (Kris-Etherton et al., 2002; Liu J. H. et al., 2021). These nutrients include primary and secondary metabolites, such as polyphenolic compounds and proteins, which have a strong protective effect on human cells and reduce the damage caused by oxidative stress in the human body (Kris-Etherton et al., 2002; Alegret, 2007). In response to external stimuli, plant cells undergo an immediate response, releasing stress signaling factors to activate signaling networks, which then participate in the synthesis of transcription factors and eventually generate metabolites. In the stress physiology of plant foods after treatment with PEF (Pereira et al., 2009; Vallverdu-Queralt et al., 2012; Banks et al., 2015; Kasprzycka et al., 2021), this process causes an increase in nutrients (Vallverdu-Queralt et al., 2013). PEF increases cell membrane permeability and even causes cell membrane rupture, which triggers a stress response in cells, similar to injury stress (Bussler et al., 2015). The cells then secrete several substances to protect themselves from damage. When PEFs are applied to microorganisms, they also produce large amounts of ROS, which will eventually cause structural damage to DNA and proteins, lipid peroxidation, and even cell membrane rupture, leading to the death of microorganisms and achieving sterilization (Sierra and McComb, 2008; Takatsuji et al., 2017; Wang et al., 2022).

This paper reviews the synthesis of plant nutrients through the oxidative stress pathway in PEF to enhance the extraction rate of active ingredients. The mechanism of microbial killing by PEF through the oxidative stress pathway is also summarized to provide future research directions for the effects of PEF technology on food quality via the oxidative stress pathway.

2 PEF induces nutrient synthesis in plant foods

Previous studies have shown that PEF stimulates cells and thus, greatly increases intracellular ROS generation (Gabriel and Teissie, 1994; Pereira et al., 2009). It has also been shown that the level of ROS is closely related to the health status of cells and is usually a common indicator of plant cell response to stress owing to damage caused by external stimuli. Specifically, when plant cell membrane is damaged or ruptured, ATP released from the damaged cell binds to its cell membrane receptor resulting in ROS production (Vallverdu-Queralt et al., 2012, 2013). Thus, when plants are exposed to external stimuli, their stress response is triggered. It has also been shown that ethylene production involves the conversion of methionine, generation of aminoethoxyvinylglycine, and release of ethylene. Further, the synthesis of jasmonic acid primarily involves the lipid metabolism pathway, wherein lipid molecules, such as phospholipids undergo oxidation and degradation to produce precursors of jasmonic acid after which enzymes, such as lipoxygenases, play a pivotal role in the synthesis of jasmonic acid from these precursors by catalyzing lipid oxidation reactions (Vallverdu-Queralt et al., 2012). These secondary stress signaling molecules diffuse through the cell membrane and initiate signal transduction networks leading to the activation of transcription factors that promote nutrient synthesis in plants (Jacobo-Velazquez et al., 2017). The ROS signaling pathway typically involves the activation of specific transcription factors, such as those belonging to the MYB (Myeloblastosis) and bHLH (basic Helix–Loop–Helix) families. These factors play crucial regulatory roles in controlling the synthesis of phenolic compounds. Further, activated transcription factors stimulate the expression of relevant genes that encode the key enzymes involved in the biosynthesis pathways of phenolic compounds, such as phenoloxidases, peroxidases, and polyphenoloxidases. Under induction by ROS and ethylene, plants initiate the synthesis and accumulation of various phenolic compounds, including flavonoids, anthocyanins, phenolic acids, and polyphenols. These compounds then exhibit diverse biological activities, such as antioxidant activity, resist pathogen infection, and act as a defense against oxidative stress (Jacobo-Velazquez et al., 2015). Conversely however, ethylene and jasmonic acid induce the synthesis of thioglucosidic substances (Villarreal-Garcia et al., 2016). ROS also induce the synthesis of compounds, such as carotenoids (Bouvier et al., 1998; Figure 1).

PEF technology is lauded for its ability to enhance the synthesis of secondary metabolites in plant cells through significant stimulation, yet it also poses potential collateral damage to plant cells, potentially impacting plant metabolism and overall quality (Attri et al., 2022). To mitigate these adverse effects, optimizing PEF parameters—such as intensity, duration, and frequency—is crucial to minimize cellular damage while maximizing the production of desired metabolites. By fine-tuning the PEF process parameters, we can ensure that they



contribute positively to food quality and safety, aligning with the overarching goal of improving the healthfulness and sustainability of our food systems (Pipliya et al., 2023; Trusinska et al., 2023; Negi et al., 2024).

2.1 Phenols and carotenoids

Galindo et al. (2009) treated potatoes with PEF (100–400 kv/cm, 1 ms) to produce tissue trauma and then observed a series of metabolic reactions. First, a significant amount of ROS was generated, peaking at 30–60 min (Bolwell, 1996). Second, crosslinking of hydroxyproline-rich glycoproteins in the cell wall of potatoes occurred within 1 min after treatment. Over time, biosynthesis of suberin occurred at the cut surface (Bernards et al., 1999). This was accompanied by a significant increase in chlorogenic acid, which improves the protection of plant tissues against oxidative stress (Matsuda et al., 2003). Levels of cell membrane-associated metabolites, such as sterols and galactosylceramide, were also significantly increased after injury (Piironen et al., 2000). After 24 h, the potatoes synthesized peptides to increase protein content in response to oxidative stress damage (von Wieren et al., 1999).

Leong et al. investigated the effect of PEF treatment on the protective responses in different carrot species to resist H_2O_2 -induced oxidative damage (Table 1). Purple Haze and Nutri Red under 303 kJ/kg treatment improved the resistance of Caco-2 cells to oxidative damage by restoring cell viability and inhibiting NO production (Leong et al., 2016a). The total carotenoid content in purée was higher than that of the untreated variety under treatment conditions of 0.8 kV/cm. Total carotenoids increased significantly when the electric field intensity was increased from 0.3 kV/cm to 0.8 kV/cm. The total phenolic content increased significantly when the energy input was increased from 35 to 303 kJ/kg compared to the untreated control. The greatest increase in total phenolic content was observed after the PEF treatment at 303 kJ/kg compared to that after PEF treatment under

different conditions. These findings suggest that greater recovery of total phenols from carrots may require more intense treatment, either by increasing the electric field intensity to induce higher cell damage or by supplying more energy to the cells to make cell damage last longer and to damage more cells. The Yellow Solar purée extract obtained at 0.8 kV/cm significantly increased Caco-2 cell viability and inhibited lactate dehydrogenase leakage and NO production. PEF-treated carrot extracts exhibited a good protective effect on cell viability and were able to restore the number of viable cells and the percentage of NO production in oxidatively stressed (H_2O_2 -containing) cells to levels similar to those in non-stressed (H_2O_2 -free) cells. Furthermore, the cytoprotective capacity of PEF extracts was linearly related to total carotenoid content and total anthocyanin content. For Yellow Solar carrots, which had a low total carotenoid content, PEF significantly increased the carotenoid content (Leong et al., 2016a; Rezaeinezhad et al., 2022). Leong et al. (2016b) also found that PEF treatment shortened the immersion time and improved the protection against H_2O_2 oxidative stress in *Vitis Vinifera*. Leong et al. also evaluated the effect of PEFs on the release of anthocyanins and the health effects of Pinot noir grape juice; Pinot noir grapes treated with PEF were able to produce substances with stronger antioxidant activity and exhibited greater protection against oxidative stress (Leong et al., 2016c; Chen et al., 2023).

PEF-processed tomatoes contained much higher concentrations of lutein, carotene, trans-lycopene, and cis-lycopene than heat-processed tomatoes. This was not only because the low-temperature treatment reduced nutrient decomposition but also because tomato cells damaged by PEF released ATP. This ATP diffused between cellular spaces as a trauma signal, thereby stimulating a stress response, enhancing the metabolic activity of tomatoes, and synthesizing a large number of nutrients (Basu et al., 2001; Perveen et al., 2015; Desmarchelier et al., 2018; Ali et al., 2021). Application of PEF treatment leads to the production of more nutrients with high levels of cis-isomers and higher bioavailability of cis-isomeric nutrients in the human body. Vallverdú-Queralt et al. applied medium

TABLE 1 Effect of PEF on the synthesis of bioactive substances and bacterial apoptosis through the oxidative stress pathway.

Category	Materials	Induction pathway	Results	References
Phenols and carotenoids	Potato	Oxidative stress	Large amounts of chlorogenic acid and proteins are produced	Matsuda et al. (2003)
	Carrot	Oxidative stress	Total carotenoid content and total anthocyanin content increased significantly	Leong et al. (2016a) and Rezaeinezhad et al. (2022)
	Pinot noir grape	Oxidative stress	Exhibits better phytochemicals and can protect cells from oxidative stress	Leong et al. (2016a)
	Tomato	Oxidative stress	Production of more nutrients with high levels of cis-isomers and higher bioavailability of cis-isomers in humans	Vallverdu-Queralt et al. (2013)
Saponin	Ginseng	Oxidative stress	PEF-derived ginsenosides maintain a more intact surface morphology of the cells	Liu et al. (2017)
Thioglucosides	Broccoli	Oxidative stress	Significant enhancement in glucosin, thioglucomanan, mustard, and neomustard levels in broccoli florets and stems	Aguilo-Aguayo et al. (2015)
Bacteria	<i>Staphylococcus aureus</i>	Oxidative stress	Excess ROS induce severe oxidative stress, ultimately leading to ROS-mediated apoptosis in bacterial cells	Qi et al. (2022)
	<i>E. coli</i>	Oxidative stress	Excess ROS cause abnormal protein expression and bacterial apoptosis	Liu Z. Y. et al. (2019)

intensity (1 kV/cm, 16 monopolar pulses of 4 μ s, 0.1 Hz) PEF to raw tomatoes, which increased the content of 15-cis lycopene in tomato juice by 63%–65% (Vallverdu-Queralt et al., 2013). Martinez et al. (2020) used PEF (15 kV/cm, 6.8 μ s pulse width) to aid the extraction of carotenoids from dried *Rhodotorula glutinis* yeast and improved the extraction rate of carotenoids through PEF by optimizing the experimental conditions. The extraction rate was 20% without PEF treatment but increased to 80% after PEF treatment. Kokkali et al. (2020) found that the antioxidant capacity of PEF-treated microalgae bio extracts was significantly enhanced. PEF can increase the number of nutrients synthesized in plant-based foods, which can be utilized to customize extraction parameters for low levels of active substances and synthesize more of the nutrients required by humans (Leong et al., 2016a; Rezaeinezhad et al., 2022).

2.2 Thioglucosides

Thioglucoside is widely used in the pharmaceutical and food industries for its antibacterial, neuroprotective, and anticancer effects (Watson et al., 2013; Choudhury et al., 2022; Hoffmann et al., 2022; Marcinkowska and Jelen, 2022; Youseif et al., 2022; Zhou et al., 2022). Thioglucoside is water-soluble and heat-sensitive; therefore, heating causes a large loss of thioglucosides, and heat treatment should be avoided when extracting these components (Hoffmann et al., 2022; Marcinkowska and Jelen, 2022). Thioglucoside maintains stable chemical properties and biological activity in the subcellular space of plant tissues. When cells rupture, black mustard enzymes come into contact with and hydrolyze thioglucoside, and the main product, isothiocyanate, is responsible for the strong taste of mustard, radish, and broccoli sprouts. The loss of thioglucoside is also closely related to the inactivation of black mustard enzymes (Hanschen and Schreiner, 2017). Whether PEF can inactivate black mustard enzymes for the purpose of retaining thioglucosides has been a focus of research (Yen and Wei, 1993; Ghawi et al., 2012; Cebeci et al., 2022).

PEF injury triggers the production of ROS, which activates plant primary and secondary metabolism (Jacobo-Velazquez et al.,

2015). In addition, other signaling molecules, such as ethylene and jasmonic acid, are produced after injury and play a key role as regulators of ROS levels and the expression of secondary metabolic genes, triggering the accumulation of specific secondary metabolites (Torres-Contreras et al., 2014a,b). In the case of broccoli, wounding triggers the biosynthesis and accumulation of indolic glucosinolates (Torres-Contreras et al., 2014b). The black mustard enzyme in broccoli catalyzes the enzymatic reaction of thioglucosinolates to produce other bioactive products, the content of which is the main factor affecting the quality of broccoli products. Frandsen et al. found that PEF treatment at 35 kv/cm inactivated the black mustard enzyme and that most thioglucosins were degraded prior to PEF treatment. If PEF can successfully extract thioglucoside, then the inactivation of the black mustard enzyme by high pressure treatment is required (Frandsen et al., 2014).

Extraction of thioglucosides from broccoli flower stems using PEF technology was performed by Aguilo-Aguayo et al. (2015). The samples were subjected to an electric field intensity of 1–4 kV/cm at 5 Hz and a treatment time of 50 to 1,000 μ s. The results of the experiments revealed that glucosinolates, thioglucosinolates, mustardolates, and neo mustardolates increased by approximately two-fold in broccoli florets after PEF pretreatment, while black mustard enzymes were found to remain active. This experiment found that PEF can increase the extraction efficiency of thioglucosides without enzyme inactivation, which may become a new research direction.

3 PEF induces bacterial apoptosis

PEF technologies are potentially the most important cold pasteurization and sterilization food preservation technique to replace or partially substitute thermal processes. During the PEF process, lysis of microorganisms is caused by irreversible structural changes in the membranes, leading to pore formation and destruction of the semipermeable barrier of the membrane (Qin et al., 1996; Lasekan

et al., 2017; Pimentel et al., 2023; Wei et al., 2023; Wu et al., 2023; Zhu et al., 2023; Wang et al., 2024; Figure 2).

Qi et al. (2022) analyzed the mechanism of PEF action on *Staphylococcus aureus* from the perspective of ROS-mediated oxidative stress. After PEF treatment, the level of ROS in *S. aureus* increased, and the excess ROS induced severe oxidative stress, leading to lipid peroxidation, DNA oxidation, cytosolic fatty acid damage, structural damage to proteins and DNA, and significant abnormalities in the expression of oxidative stress-related genes, ultimately leading to ROS-mediated apoptosis in bacterial cells (Qi et al., 2022), as shown in Figure 2. Liu Z. Y. et al. (2019) compared the changes in the protein expression of *E. coli* before and after PEF treatment using mass spectrometry. Excessive accumulation of ROS has been shown to significantly reduce AcnB protein levels in superoxide dismutase-deficient *Salmonella enterica* (Thorgersen and Downs, 2009). Another upregulated enzyme was the bifunctional proline utilization enzyme A (PutA; A0A0G3K113), the deletion of which in *E. coli* has been shown to increase cellular susceptibility to oxidative damage (Zhang et al., 2015). Membrane permeability increases after applying PEF to *E. coli*, which not only increases the entry of harmful substances into *E. coli* cells but also causes osmotic imbalance (Aronsson et al., 2005). Particularly strong electric fields can cause irreparable perforations in the cell membrane, leading to irreversible cell damage, destruction, and eventual death (Locke et al., 2006). To ensure the inactivation of microorganisms, an electric field with a field strength of 25 kV/cm or more is required in food processing (Toepfl et al., 2007).

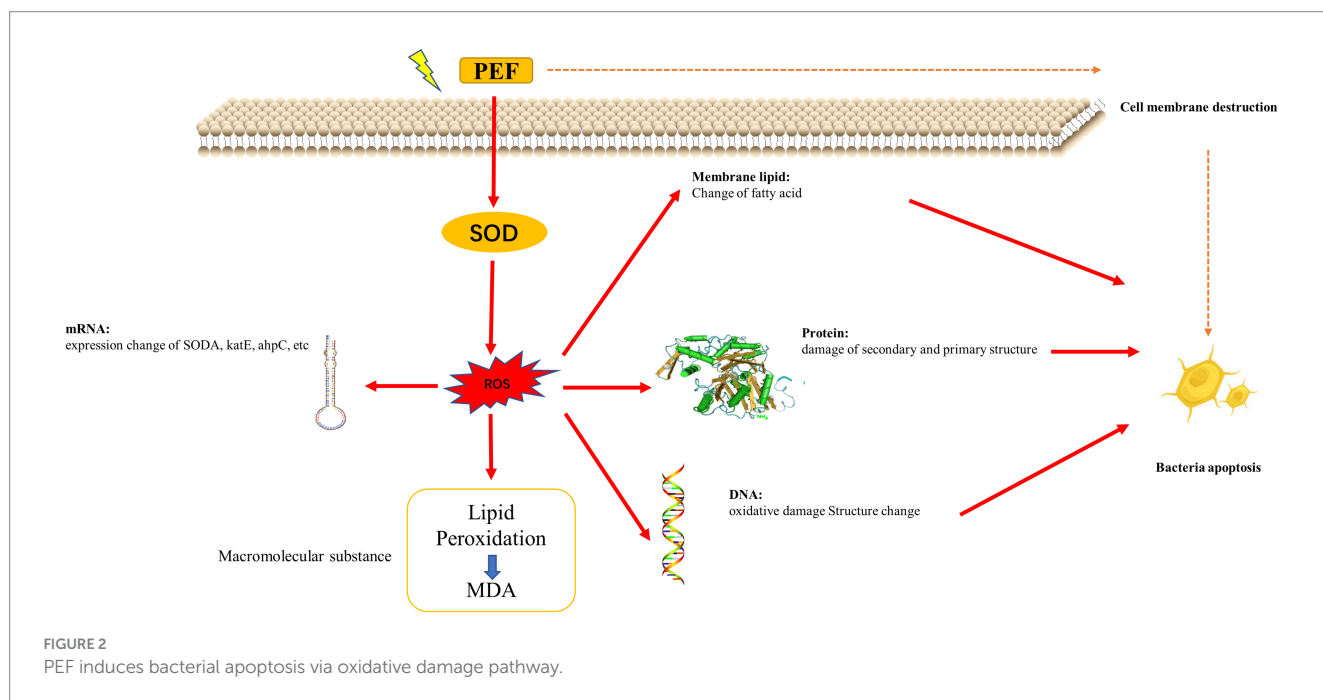
et al., 2022). PEF stimulates plants to secrete metabolites by causing damage to plant cells, thereby increasing the accumulation of nutrients (Ye et al., 2004; Liu Z. B. et al., 2019). To elucidate the conditions for the optimal accumulation of nutrients in food caused by PEF, future research should be based on “omics” approaches (genomics, proteomics, and metabolomics) to elucidate the mechanisms in terms of genetic material and proteins, allowing the design of optimized biological processes to obtain the highest accumulation of bioactive molecules in plant foods.

PEF technology has a high extraction efficiency compared to traditional extraction techniques and can extract nutrients more efficiently (Minamitani et al., 2015; Qi et al., 2022). The nutrients extracted from PEF have a more pronounced health effect on the human body (Wang et al., 2013; Zandalinas and Mittler, 2018; Jin et al., 2021). The cis-isomeric metabolites produced by PEF stimulation are better absorbed by the body, and PEF extracts significantly increase cell viability (During and Harrison, 2004; Unlu et al., 2007). We can use this theory to customize extraction methods for nutrients that have strong health effects but are low in content.

In addition to increasing nutrient accumulation, oxidative stress damage after PEF stimulation can also kill microorganisms. In addition to membrane damage, which is the main cause of microbial inactivation, PEF affects the metabolism of microorganisms through the oxidative stress pathway and kills microorganisms by affecting their protein expression and damaging their genetic material. The process of killing microorganisms does not cause a thermal effect, does not affect the taste of food, and even increases the accumulation of nutrients (Morales-de la Pena et al., 2011; Khan et al., 2017). Thus, PEF treatment results in foods with increased nutrient content and low microbial content, with no adverse effects on food quality parameters such as taste and color, meaning the produced foods can be marketed as commodities or raw materials for further processing (Ortega-Rivas and Salmeron-Ochoa, 2014; Misra et al., 2017; Skowron and Wantuch, 2020).

4 Conclusions future research

As an emerging non-thermal processing technology for food, PEF has become a hot new research topic due to its ability to increase nutrient content and kill microorganisms through oxidative stress pathways (Zulueta et al., 2013; Jacobo-Velazquez et al., 2017; Andreou



5 Future research

In the field of food processing, the PEF extraction technique only allows the treatment of a limited volume of food owing to the dual limitations of field strength and electrode spacing, which are particularly prominent in industrial-scale applications. Under such conditions, a small flux makes industrial scale-up challenging. Further, even though PEF shows potential for highly efficient, safe, and environmental friendly food processing, the small volume of its treatment chamber and the lack of flexibility limit its industrial scale application. Therefore, strategies for obtaining a sufficiently large treatment chamber volume for large-scale industrialized food processing are necessary.

Finally, owing to its high maintenance cost, the PEF extraction technique is more intensive than other extraction technologies. Therefore, the design of a food processing unit that is suitable for large-scale industrialized applications is key for advancing this field. Until these technical and cost-related challenges are overcome, maximizing the potential of this extraction technology in the food processing field will remain challenging.

Author contributions

WS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. QW: Investigation, Project

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Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was supported financially by the Education Department of Jilin Province (JJKH20210994KJ).

Conflict of interest

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