Review

Outcomes of Pulsed Electric Fields and Nonthermal Plasma Treatments on Seed Germination and Protein Functions

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Abstract: To meet the needs of the hungry population, it is critical to boost agricultural product production while minimizing contaminated waste. The use of two nonthermal technologies, pulsed electric field (PEF) and nonthermal plasma (NTP), is increasing every day. As both PEF and NTP are relatively newer areas, there is limited knowledge about these two technologies and their modes of action. Studies showed that PEF treatment on the plant seeds helps germination and seedling growth. The positive impact of PEF intensity is highly dependent on the seed coat type and plant species. Another nonthermal technology, NTP, affects seed germination, seedling growth, yield, and resilience to abiotic stress when generated at varying pressures with and without different feed gases. Early germination, germination rate, and germination percentage were all improved when the seedlings were treated with NTP. Similarly to the PEF treatment, NTP had a negative or no effect on germination. This review examined the effects of PEF and NTP on seed germination and analyzed the situation and mechanism behind the positive or negative effect. Deactivation of proteins and enzymes to extend the shelf life of beverages is another prominent application of PEF and NTP. The interaction of PEF and NTP with proteins aids in understanding the microscopic mechanism of these technologies. Therefore, we covered in this review the potential structural and functional changes in proteins/enzymes as a result of PEF and NTP, as well as a comparison of the benefits and drawbacks of these two technologies.

Keywords: pulsed electric field; nonthermal plasma; seed germination; protein function

1. Introduction

The electric field affects plants in both soil and air, as the Earth has an electrified environment maintained by a global circuit. Plant cells contain membrane potential because of ion channels or pumps that allow electric current to flow through them [1]. Various studies have been conducted to study the electric current effect on plants [2]. According to Kotaka et al., the action of ions (positive or negative) in the air can affect variations in plant respiration [3]. Song et al. [4] and Lee et al. [5] showed the impact of air anions on the growth of lettuce and kale, respectively. Seed germination starts with water uptake by the seed and ends with embryonic axis extension. This can take 24–36 h depending on the germination conditions, followed by radicle elongation [6]. Frequently, plants are facing

multiple stress that results in decreasing crop yields. Different types of seed treatment techniques were used to increase crop yield under regularly changing environmental stress [7].

Traditional methods, such as cooking, boiling, frying, drying, etc., are frequently used to increase the shelf life of food products [8]. As the world population increases, there is a drastic increase in the consumption of processed foodstuffs that increases chemical additives and thermal processing. This results in inadvertent outcomes such as the formation of toxic compounds, nutrient losses, adverse effects on color and texture, etc. [9]. Nonthermal technologies such as pulsed electric field (PEF) and nonthermal plasma (NTP) have been used to solve these problems.

PEF has shown its importance in the food industry by assisting in different processes such as improvement in oil and juice extraction yields [10,11], secondary metabolites' biosynthesis stimulation [12,13], cold pasteurization [14], improvement of nutritional and functional properties of liquid foods [15], seed germination [16], inactivating microorganisms [17], nutrient solution for hydroponic system [18], etc. In general, high-voltage pulses are placed between two electrodes for a brief time; depending upon the voltage supplied, the PEF is categorized into low/moderate-intensity PEF (10–2000 kV/m) and high-intensity PEF (2000–4500 kV/m) [19]. PEF works on the electrical breakdown and electroporation process (formation of holes in the cell membrane and phospholipid bilayers) [15,20]. PEF treatment can result in an interruption in the plant cell wall that can help in improving the extraction process of target compounds [21]. Additionally, the applied electric field can modify the electrical sensitive components and accelerate heat and mass transfer in drying and freezing processes [10]. PEF treatment also alters the proteins'/enzymes' structure [20] and the polarization of molecules with dipole moments (changes can be reversible or irreversible depending upon the molecules and treatment conditions) [15]. Additionally, the effectiveness of PEF treatment depends upon the food matrix (pH, composition, electrical conductivity) and operating parameters (electric field intensity, pulse polarity, pulse shape, treatment time, and frequency) [22].

Nonthermal plasma (NTP) is a complicated combination of electrons, positive and radicals, negatively charged ions, neutral atoms, and molecules [23]. NTP can produce both ROS and reactive nitrogen species (RNS), and their concentration depends upon the feed gas and plasma parameters. Depending on the concentration of reactive species and the nature of biological species, they have positive and negative impacts on biological systems. The utilization of plasma in agriculture has increased promptly in both pre-harvest and post-harvest cases [7,24,25]. Our group recently increased soil fertility by producing NH_4NO_3 using NTP that helped in increased germination percentage and yield of radish sprouts [26]. NTP treatments such as direct or indirect treatment showed bactericidal effects through protein oxidation, DNA and cell membrane damage, etc. [27–29]. In contrast, the NTP treatment could activate the plant growth-promoting bacteria [30]. NTP properties make it an ideal technology to treat beverages to increase their shelf life [31].

This review focused on the germination rate/percentage and structural and functional changes in proteins/enzymes after PEF and NTP treatments to elaborate their roles in seed treatment and beverages' preservation.

2. Germination Rate and Seedling Growth in Plants after the Pulsed Electric Field Treatment

There are limited reports on the impact of PEF on seed germination and seedling growth, as shown in Table 1. Through this review, we focused on some of the reported work by the various groups on the diverse plant species such as Leaf Lettuce, Barley, *Arabidopsis*, Kale, Wheat, Chickpea, Mung bean, Bitter gourd, Tomato, *Medicago Sativa*, Chili, Smallflower Morningglory, and Green Foxtail.

Table 1. Changes in the growth parameters of plants after PEF treatment.

Plant species	Treatment Condition	Results	Ref.
Leaf Lettuce (<i>Early Impulse</i>)	Electric field intensity (20–200 kV/m); pulse repetition was fixed to 1 pps.	Growth stimulation for 20–100 kV/m and growth inhibition >100 kV/m electric field strength.	[32]
<i>Arabidopsis</i>	Electric field intensity (500–5000 kV/m); pulse duration 10–100 ns.	Growth stimulating and increased leaf area observed at 500 kV/m at all pulse durations. The 5000 kV/m with a 10-ns pulse showed no lethal effect on the seedlings, while growth inhibition was observed at 5000 kV/m for 100-ns pulse.	[33]
Wheat (<i>Tritium aestivum</i>)	Electric field intensity (50–200 kV/m); pulse width (20 µs); frequency (5 Hz); number of pulses 100	Increased seedling growth at 140 kV/m treatment, while no increase in seedling growth was observed at 50 kV/m. PEF-treated seeds treated at 200 kV/m showed high glutathione content and enhanced antioxidant activity.	[34]
Wheat (<i>Tritium aestivum</i>)	Electric field intensity (200–600 kV/m); pulse width (100 µs); frequency (1 Hz); number of pulses (25 and 50)	Increased seed germination and seedling growth parameters were observed for 600 kV/m at 50-pulse treatment. The increase in soluble proteins, carotenoids, chlorophylls, total phenolic contents, etc., for treated seeds' plantlets' juice, compared to the untreated seeds' plantlets' juice.	[35]
Mung bean (<i>Vigna radiata</i>)	Electric field intensity (0.8–2.4 kV/m); exposed time (0.5–2.5 h)	Increased seed germination percentage at static electric field 1.2 kV/m for 0.5-h treatment. Treatment for 1–2.5 h at 2.0-kV/m static electric field leads to enhancement of roots' length (0–89%), stems (4.8–77%), and leaves (6.5–59%) than control	[36]
Barley	Electric field intensity (100 kV/m)	The first fraction of the electric separator treatment changes the viability and germination difference from the control seed.	[37]
Barley	2–9.5 kV; exposure time (35 min)	An increase in seed germination, germination index, germination potential, and vigor index was observed for 3.5- and 5-kV treatment.	[38]
Bitter melon (<i>Momordica charantia</i>)	Electric field intensity (30–100 kV/m); exposed time (20 min)	Seeds treated at 30- and 50-kV/cm showed a positive effect on germination, while higher intensity treatment (100 kV/m) caused the decrease in germination.	[39]
Chickpea (<i>Cicer arietinum</i>)	Electric field intensity (27–107 kV/m); treatment time (15 min)	Seeds' treatment at ~47 kV/m for 15 min (at 13 °C) caused increased mean germination time and improved root and shoot length.	[40]
Chickpea (<i>Cicer arietinum</i>)	3–12 V; induced for 10 min for 100 days	3-V and 6-V treatment results in early seed germination, while no difference for control and 12 V. The increase in the plant heights observed for 3, 6, 9, and 12 V as 25.5, 30.5, 11.8, and 17.1%, respectively. The increase in root length by 28.6, 24.0, and 3.0% were under treatment at 3, 6, and 12 V, respectively. Leaf numbers were higher under 3-, 6-, 9-, and 12-V treatment as 25.3, 25.2, 15, and 19.3%, respectively.	[41]
Tomato	Electric field intensity (1000–3000 kV/m); frequency (50 Hz); exposure time (10–30 s).	2000-kV/m treatment for 20 s caused 100% germination, whereas 76% for control. The weight of tomato seedlings was increased for treated seeds than untreated ones.	[42]

Table 1. Cont.

Plant species	Treatment Condition	Results	Ref.
<i>Medicago Sativa</i>	1–7 V; exposure time (2.5 and 5 h)	The highest germination rate was observed after treatment of seeds for 5 h with 3 V. The lowest germination rate was observed for 4-V treatment for 2.5 h. 1 V at 2.5- and 5-h treatment caused no significant effect than control samples. The maximum average root length was obtained for 1-h treatment at 4 V. The 7-V administration could effectively enhance the germination process with increased crop yield.	[43]
Chili (<i>Capsicum annuum</i>)	Combination of 250–1000 G and 100–400 mA	The highest germination was observed during the combination seed treatment (magnetic field (1000 G) for 30 min + electric field (100 mA) for 1 min), and separate treatment of 300-mA electric field for 1 min. A high magnetic field enhanced germination capacity and seedling characteristics.	[44]
Smallflower Morningglory [<i>Jacquemontia tamnifolia</i> (L.) Briseb.] and Green Foxtail [<i>Setaria viridis</i> (L.) Beauv.].	Single electric pulse of 4–6-kV and 60–80-kV repetitive pulse.	Seedling emergence was higher (99%) in Smallflower Morningglory seeds treated with a 10-Hz repetitive pulse for 5 s than in seeds exposed to 10 repeating single pulses (87%) or untreated seeds (91%). There were no variations in Green Foxtail seeds treated with any intensity of PEF.	[45]
Two kale cultivars (<i>Brassica oleracea</i> var. acephala cv. TBC and Abundance)	Electric field intensity (8.6–58.8 V/m); electric currents (10–100 mA).	PEF treatment results in higher fresh and dry weights than the control for 50-mA current. After treatment, there was an increase in calcium, total phenolic compounds, and antioxidant level.	[16]

Wheat seeds treated with PEF at 140 kV/m (100 pulses) [34] and 600 kV/m (50 pulses) [35] results in increased seedling growth. PEF treatment at 200 kV/m (100 pulses) decreases coleoptile and primary leaf growth [34]. The increase in glutathione content and antioxidant enzyme activity was observed at 200 kV/m (100 pulses), while no significant change was observed at 140-kV/m (100 pulses) treatment [34]; 600-kV/m (50 pulses) treatment resulted in a significant increase in soluble proteins, carotenoids, chlorophylls, total phenolic contents, etc. for treated seeds' plantlets' juice, compared to the untreated seeds' plantlets' juice [35]. This shows that low-intensity PEF with fewer pulses or high-intensity PEF with fewer pulses significantly contributed to wheat germination and seedling growth than high-intensity PEF with high pulses.

Barley seeds' treatment with electric field 100 kV/m resulted in a significant increase in germination for the first fraction of treatment while it declined for the second fraction of the electric field [37]. In another study, barley seeds, when treated with a high-voltage electric field (3.5 and 5 kV) for 35 min continued up to 10 days, resulted in significant increase in seed germination. At the same time, no positive effect was observed for 0, 2, 6.5, 8, and 9.5 kV. This shows that medium voltage is better for seed germination, and high or low voltage is ineffective to promote germination [38]. However, barley seeds treated with 120-kV/m electric fields result in radicle elongation without affecting the gross metabolic activity and α -amylase concentration decreased for treated seeds [46]. Chickpea seeds' treatment of ~47-kV/m PEF for 15 min caused increased mean germination time [40]. In another study, Chickpea seeds treated with an electric field (3 V and 6 V induced for 10 min for 100 days) showed the enhanced rate of seed germination, while no change was observed for 12 V, although a decrease in seedling root and shoot length was observed for 12-V treatment as compared to 3 V and 6 V treatment [41].

The above data showed that low-intensity electric field has no significant effect on seed germination, seedling growth, and biochemical effect. In contrast, high electric field intensity causes a negative impact on seed germination, but it significantly affects the

biochemical changes in the plants. At the same time, the medium electric field strength is best for germination and seedling growth. However, the electric field strength value for medium and high can depend upon the seed coat thickness. The increased seedling emergence of seeds under particular PEF treatments can be attributed to hard seed coats. Seeds can absorb water when the seed coat is damaged by PEF treatment, allowing for water imbibition, whereas seeds lacking solid seed coats result in the embryo's damage, which causes decreased seedling emergence with the same amount of PEF intensity [45]. Another possibility is that the electric field can activate the ion transport that helps in improving the nutrient uptake [16]. It is worth noting that significant changes in the biochemical properties were observed (mainly at high electric field strength) that showed no direct correlation with the germination of seeds, although the change in biochemical properties, such as protein level, antioxidant level, etc., can influence plant growth and yield. Protein/enzyme plays an essential role in plant growth, and the deactivation of enzymes can improve the shelf life and quality of beverages such as juices, which are mentioned in the following section.

3. Impact of the PEF on the Proteins'/Enzymes' Conformation and Activity

Changes in the conformation and function of proteins/enzymes are shown in Table 2.

Table 2. Conformational and functional changes in proteins/enzymes after PEF treatment.

Protein/Enzymes	Treatment Condition	Results	Ref.
Soybean protein isolate	Electric field intensity (3000 kV/m); pulse width (2 μ s); pulse cycle (0.001–0.009 s); frequency (400 Hz).	Increased random coils and β -sheets and a decrease in α -helix after PEF treatment were obtained.	[47]
Soybean protein isolate	Electric field intensity (0–5000 kV/m); pulse width (40 μ s); frequency (1.0 kHz).	At 3500-kV/m intensity, the conversion of β -turn to α -helix happened. There was an increase and decrease in anti-parallel β -sheets and β -sheets' contents at high pulse intensity, respectively.	[48]
Soybean lipoxygenase	Electric field intensity (2000–4200 kV/m); pulse width (1–5 s); frequency (100–600 Hz); treatment time (1036 μ s).	The maximum inactivation of 88% was achieved at 4200-kV/m electric fields at a pulse frequency of 400 Hz and pulse width of 2 μ s treated for 1036 μ s at 25 °C.	[49]
Soybean lipoxygenase	Electric field intensity (2000–4200 kV/m); treatment time (25–100 μ s); pre-treatment temperature (23–50 °C).	The highest inactivation level of 84.5% was observed at 4000 kV/m with preheating to 50 °C, and treatment for 100 μ s.	[50]
Horseradish peroxidase	Electric field intensity (500–2500 kV/m); pulse width (1.5 μ s); frequency (10 Hz); pulses (207–1449).	Loss of α -helix content at 2200 kV/m for 87 pulses and the relative fluorescence intensity increased with increased electric field strength from 2000 to 2500 kV/m. The relative activity reduced to 16.7 and 34.7% for electric field strength of 2500 kV/m (207 pulses) and 2200 kV/m (1214 pulses), respectively	[51]
Lysozyme	Electric field intensity (2500–3500 kV/m); pulse width (2 μ s); frequency (1000 Hz); treatment time (0–1200 μ s).	The unfolding of lysozyme tertiary structure induced by 3500-kV/m treatment for 1200 μ s. The lysozyme activity was reduced by 19.2, 27.7, and 38.1% for 25, 30, and 35 kV/cm, respectively, for 1200 μ s.	[52]
β -lactoglobulin	Electric field intensity (1250 kV/m); number of pulses (1–10); time between pulses (15 s).	The peak temperature of β -lactoglobulin decreased to 71.0 ± 2.0 from 75.4 ± 0.7 (control) at 10 pulses.	[53]

Table 2. Cont.

Protein/Enzymes	Treatment Condition	Results	Ref.
Ovalbumin	Electric field intensity (2500–3500 kV/m); pulse width (5 μ s); frequency (20 Hz); treatment time (60–240 μ s).	The free sulphhydryl (-SH) protein content was significantly increased after treatment in the range of 2000–2500 kV/m. The increase and decrease in free SH content at 3500 kV/m for 0–60 μ s and 120–240 μ s, respectively. The decrease in α -helix and slightly increased β -sheet contents for a long treatment time of 120–180 μ s at 3500 kV/m. However, at 3500 kV/m, treatment for 60 μ s did not significantly alter the secondary structure of the protein.	[54]
Pepsin	Electric field intensity (3420 kV/m); pulse width (2 μ s); pulse repetition rate (800 pps for 0–603 μ s).	The aggregation and inactivation of pepsin were reported.	[55]
Pepsin	Electric field intensity (2520–3560 kV/m); pulse width (2 μ s); pulse repetition (200 Hz); treatment time (0–500 μ s).	Self-aggregation at 3560 kV/m for 300 μ s and increased as the treatment increases. The decreased activity was observed as treatment time increased.	[56]
Canola protein	10–35 kV: frequency (600 Hz); pulse width (8 μ s); treatment time (180 s).	Decrease in α -helices and β -turns, but increase in the number of random coils and also induced protein aggregation	[57]
Peroxidase in Tomato juice	Electric field intensity (3500 kV/m); frequency (50–250 Hz); pulse width (1–7 μ s); treatment time (1000–2000 μ s).	Reduced peroxidase activity was most effective for prolonging the treatment time of 2000 μ s at 200 Hz. The bipolar mode showed more effectiveness than monopolar mode for enzyme inactivation.	[58]
Peroxidase and Polyphenoloxidase in apple juice	Electric field intensity (2000–4000 kV/m); treatment time (25–100 μ s); pre-treatment temperatures (23–50 $^{\circ}$ C).	The decreased residual activity with increased treatment time and electric field strength.	[59]
Polyphenoloxidase in grape juice	Electric field intensity (2500–3500 kV/m); pulse width (4 μ s); frequency (200–1000 Hz); treatment time (1–5 ms).	At 3500 kV/m and 630 Hz no polyphenoloxidase activity was detected, while 58.13% of depletion was achieved at 3020 kV/m and 1000 Hz for 5-ms treatment.	[60]
Pectin methyl esterase in orange juice	Electric field intensity (500–3500 kV/m); pulse width (4 μ s); frequency (200 Hz); treatment time (100–1500 μ s).	The maximum inactivation reached about 80% when juice samples were processed at 3500 kV/m for 1500 μ s at 200 Hz without exceeding 37.5 $^{\circ}$ C.	[61]
Pectin methyl esterase in red grapefruit juice	Electric field intensity (2000–4000 kV/m); treatment time (25–100 μ s); pre-treatment temperatures (23–50 $^{\circ}$ C).	96.8% inactivation of pectin methyl esterase in red grapefruit juice was obtained with a 50- $^{\circ}$ C preheating and 4000-kV/m PEF treatment time for 100 μ s.	[62]
α -amylase	Electric field intensity (250–1250 kV/m); pulse width (10 μ s); input energy into the reactor per pulse (0.02–0.6 J).	The fluorescence peak showed redshift with decreased intensity for PEF intensity higher than 750 kV/m. The reduction in α -amylase activity increased at a higher electric field.	[63]
α -amylase, glucoamylase, and acid carboxypeptidase	Electric field intensity (4000 kV/m); voltage (4 kV); pulse width (10 μ s); frequency (1 Hz); the current was approximately 9.5 A and 27.5 A at 4 $^{\circ}$ C and 25 $^{\circ}$ C, respectively.	Residual activities of α -amylase, glucoamylase, and acid carboxypeptidase decreased at 4 $^{\circ}$ C for 300 pulses but were not affected for 900 pulses. The α -amylase activity decreased as the number of pulses increases at 25 $^{\circ}$ C, but no change was obtained at 35 $^{\circ}$ C. Glucoamylase activity showed significant decreases for 300 pulses at 25 $^{\circ}$ C compared to 4 $^{\circ}$ C. Acid carboxypeptidase activity significantly increased by 300-pulse treatment at 25 $^{\circ}$ C.	[64]

Soybean protein treated with a 3000-kV/m electric field increases random coils and β -sheets and decreases in α -helix [47]. The PEF treatment changes the vibrational peaks of S-S and C-C, as indicated by the disturbance in sulfhydryl's and disulfide bonds. PEF treatments influenced the hydrophobicity of protein by changing the tyrosine vibration frequency [47]. Another study showed 3500-kV/m intensity of PEF treatment on soybean protein resulted in conversion of β -turn to α -helix and induced a change in the orientation of the α -helix dipole moment. Additionally, at high pulse intensity, there was an increase and decrease in anti-parallel β -sheets' and β -sheets' contents, respectively [48]. These structural changes are due to a change in the bond vibration of C-O, C-O-H, C-O-C, C-H, and N-H by PEF treatment [48]. Soybean lipoxygenase protein treated with 4200 kV/m caused maximum inactivation of 88% after 1036 μ s [49]. Another group achieved the 84.5% inactivation of soybean lipoxygenase after a 4000-kV/m treatment with preheating to 50 °C after 100 μ s [50]. This shows that preheating can speed up the PEF-induced enzyme deactivation rate.

Inactivation of polyphenol oxidase was stronger than peroxidase in apple juice, showing that inactivation strongly depends upon the protein structure [59]. Some studies revealed the extent of deactivation of peroxidase in tomato juice [58] and polyphenol oxidase in grape juice [60] after PEF treatment, depending upon the intensity of the electric field and treatment time. Pectin methyl esterase protein in orange juice and red grapefruit juice treated with PEF gives rise to inactivation [61,62]. Pectin methyl esterase protein in orange juice showed a maximum inactivation of about 80% when juice samples were processed at 3500 kV/m without exceeding temperature to 37.5 °C [61]. Conversely, 96.8% inactivation of pectin methyl esterase in red grapefruit juice was obtained with a 50-°C preheating and 4000-kV/m PEF treatment [62]. Hence, pectin methyl esterase inactivation increases as the electric field strength, treatment times, and preheating temperatures increase. The distortion of the α -amylase structure and activity was obtained when treated for the higher electric field (>750 kV/m). These changes are not the result of the increase in temperature by the Joule's effect but due to modification in the tryptophan residues [63]. Another study supported the above statement as α -amylase activity decreased as pulses increased at 25 °C [64].

The above studies clearly showed that a higher electric field is required to deactivate the enzymes. Still, the extent of the deactivation depends upon the enzyme structure and treatment time while also being influenced by the preheating treatment.

NTP is another nonthermal treatment that has gained attention in seed germination and enzyme deactivation.

4. Effect of NTP on Seed Germination

NTP treatment on the plant seeds affects seed germination, seedling growth, and yield of the plant. Our previous reviews described the plasma action on seedling growth and probable mechanism [7,24]. The current study focused on the latest results on seed germination using NTP, as shown in Table 3.

Table 3. Effect of NTP on seed germination.

Plant Species	Plasma Device	Seed Germination	Ref.
Rice (<i>Oryza sativa</i>)	Scalable dielectric barrier discharge plasma; pressure (101,325 Pa); voltage (7.96 kV); frequency (9.2 kHz); power (2.17 W); treatment time (10 s or 60 s for the total 3 min).	The heat-stressed rice seeds treated with plasma showed a higher germination rate than without plasma heat-stressed seeds.	[65]
Rice (<i>Oryza Sativa</i>)	Low-pressure dielectric barrier discharge plasma; voltage (3–5 kV); frequency (3–8 kHz); pressure (1333 Pa); power (~45 W); gas (Ar + Air).	After 8-min plasma treatment, the maximum seed germination was 9.72% higher than control seeds.	[66]

Table 3. Cont.

Plant Species	Plasma Device	Seed Germination	Ref.
Rice (<i>Oryza Sativa</i>)	Hybrid cold plasma (HCP) (array of 10 × 70 electrodes (~1400 tips in total) covering ~30 cm ²); frequency (~ 700 Hz); voltage (~ 14 kV); gas (Ar).	The final germination percentage of the treated rice seeds was ~98%, whereas that of the nontreated seeds was ~90%.	[67]
Rice (<i>Oryza sativa</i>)	Atmospheric pressure multi-pin plasma generator; pressure (101,325 Pa); voltage (18, 22, 26, and 30 kV)	Germination rate increased by 6–22% for plasma-treated samples than control. The average length of root, shoot, seedling, and dry weight was enhanced by 2, 1.49, 1.41, and 1.08 times, respectively, than control.	[68]
Taikeng 9 (TK9) brown rice	Low-pressure plasma; pressure (800 Pa); voltage (1–3 kV); discharge gas (Air); treatment time (10 min)	Increased germination rate, water uptake, seedling length, and GABA levels at 3 kV for 10-min plasma treatment.	[69]
Radish (<i>Raphanus sativus</i>)	Scalable dielectric barrier discharge plasma; pressure (101,325 Pa); frequency (14.4 kHz); power density (3.05 W/cm ²); treatment time (3 min).	Maximal germination percentage increased by 8% for gray-colored seeds while not influencing the brown seeds harvested in the same year.	[70]
Radish (<i>Raphanus sativus</i>)	Scalable dielectric barrier discharge plasma: frequency (10 kHz); pressure (101,325 Pa); voltage (9.2 kV); current (0.2 A); treatment time (180 s).	The seeds' treatment at x = 5 and y = 3 mm resulted in 250% growth enhancement.	[71]
Radish (<i>Raphanus sativus</i>)	Scalable dielectric barrier discharge plasma; frequency (10 kHz); pressure (101,325 Pa); voltage (9.2 kV); current (0.2 A); gases (NO (10%) + N ₂ , Air, O ₂ , N ₂ , and He); treatment time (180 s).	The growth enhancement was observed for O ₂ , Air, and NO (10%) + N ₂ gases' plasma, but no considerable contribution was noticed for N ₂ , He, and Ar plasma. Moreover, humid air plasma irradiation was more effective in growth enhancement than dry.	[72]
Radish (<i>Raphanus sativus</i>)	Plasma torch; voltage (7–10 kV); frequency (12 kHz); gas (Air and O ₂); treatment time (60 min).	The total length (stem and root length) increased by 1.6 and 1.2 times for O ₂ and Air feed gas plasma treatment than control.	[73]
Radish (<i>Raphanus sativus</i>)	Plasma jet; power (90 and 140 W); pressure (101,325 Pa); treatment time (2, 4, and 6 min); gas (Ar).	Plasma treatment had a positive effect on germination enhancement. An increase in total mass observed at 140 W than 90 W. Plasma treatment at 140-W power increased total mass by 9–12% and average length by 3 cm compared to untreated seeds.	[74]
Radish (<i>Raphanus sativus</i>)	Radio-frequency low-pressure plasma; pressure (100 Pa); power (50 W); frequency (13.56 MHz); feed gases (O ₂ and N ₂); treatment time (30 min).	The average length of the sprouts increased by 60% for O ₂ plasma than control, while no change was observed for N ₂ plasma compared to control.	[75]
Radish (<i>Raphanus sativus</i>)	Radio-frequency low-pressure plasma; pressure (40 Pa); power (50 W); frequency (13.56 MHz); feed gases (Air); treatment time (10–90 min).	Plasma treatment increased germination rate and growth enhancement.	[76]
Centipedegrass (<i>Eremochloa ophiuroides</i>)	Radio-frequency discharge HD-2N cold plasma apparatus; pressure (150 Pa); frequency (13.56 MHz); feed gas (He + Air (8:1, v/v)); power (0–210 W); treatment time (15 s).	The germination rate was increased by 13.95, 20.93, and 12.39%, by 90-, 120-, and 180-W treatments, respectively, compared to control. However, no substantial difference was observed when seeds were treated at 30-, 60-, 150-, or 210-W plasma compared to control.	[77]
Bambara groundnut (<i>Vigna subterranean</i>)	Radiofrequency low-pressure plasma; pressure (~46 Pa); frequency (13.56 MHz); treatment time (10–30 s).	Germination was improved by 22% when seeds were treated at 10 W for 10 s. Treatment at 10 W for 20 and 30 s caused enhanced germination percentage by 15 and 3%, and 30-W treatment for 10 s showed increments in germination percentage by 7%.	[78]

Table 3. Cont.

Plant Species	Plasma Device	Seed Germination	Ref.
Barley (<i>Hordeum vulgare</i>), Rape (<i>Brassica napus</i>), Lupine (<i>Lupinus angustifolius</i>)	Microwave-driven discharge plasma; feed gas (Air); frequency (2.45 GHz); supplied power (4 kW).	There were negative impacts on maximum germination at certain treatment conditions, depending on the tested plant species.	[79]
Barley (<i>Hordeum vulgare</i>)	Surface dielectric barrier discharge (SDBD) plasma; frequency (14.4 kHz); pressure (101,325 Pa); voltage (8 kV); average power (51.7 W); treatment time (6 min).	It accelerated the early growth of barley sprouts. The fresh weight of barley seedlings increased by 137.5% on the 9th day after the plasma treatment. The treatment enhanced the primary metabolites' (soluble sugars and free amino acids) and secondary metabolites' contents (saponarin, GABA, and policosanols).	[80]
Barley (<i>Hordeum vulgare</i>)	Low-pressure plasma (26 Pa); discharge current (40 mA); treatment time (4 min); pulse duration (150–200 ms).	There was no significant change in germinated seeds' quantity and plant length for control or plasma-treated samples.	[81]
Soybean (<i>Glycine max</i>)	Diffuse coplanar surface barrier discharge plasma; voltage (20 kV); pressure (101,325 Pa); frequency (14 kHz); power (400 W); gas (Air, O ₂ , and N ₂); treatment time (30–120 s).	All treatments (except N ₂ plasma for 90 and 120 s) positively affected germination percentage. For 60-s O ₂ plasma treatment, seed germination increased up to 80% compared to control (60%).	[82]
Soybean (<i>Glycine max</i>)	DBD plasma; voltage (0–25 kV); pressure (101,325 Pa); frequency (50 Hz); feed gases (N ₂ and O ₂); treatment time (2 and 3 min).	3- and 2-min of N ₂ and O ₂ plasma treatment resulted in 1.2-fold increment in total fresh weight than control. The full length of soybean plant increased to 4–10% after plasma treatment.	[83]
Soybean (<i>Glycine max</i>)	HD-2N low-pressure plasma; pressure (150 Pa); variable power (60–120 W); frequency (13.56 MHz).	The improvement in the seed germination and seedling growth was obtained at 80-W power. Shoot length, shoot dry weight, root length, and root dry weight increased by 13.77, 21.95, 21.42, and 27.51%, respectively, after plasma treatment with respect to control.	[84]
Pine (<i>Pinus sylvestris</i>), black pine (<i>Pinus nigra Arnold</i>), and mountain pine (<i>Pinus mugo Turra</i>)	Diffuse coplanar surface barrier discharge plasma; voltage (20 kV); pressure (101,325 Pa); frequency (14 kHz); power (400 W); treatment time (1–60 s).	The highest <i>P. sylvestris</i> seed germinations of 16.7% after 3-s treatment and 50.7 and 59.3% after 5-s treatment. In <i>P. mugo</i> seed germinations of 29.3 and 44.7% after 1- and 5-s exposure and <i>P. nigra</i> germination after 1-, 3-, and 5-s plasma treatment were 59.3, 80.7, and 86.0%, respectively. There was no significant increase in germination rate and germination index in all tested pines, except germination rate in <i>P. sylvestris</i> on the 4th day was 45.4% after 3 s ($p < 0.05$) and control (17.7%).	[85]
Maize (<i>Zea mays</i>)	Low-frequency glow discharge plasma; pressure (~53,328.9 Pa); power (~45 W); voltage (5 kV); frequency (5 kHz); gas (Ar + O ₂); treated time 30–120 s.	The highest seed germination rate of the control was $75.81 \pm 1.47\%$, and after 90-s treatment with plasma, germination was $91.71 \pm 1.11\%$. The extended exposure time >120 s resulted in a declined germination rate.	[86]
Maize (<i>Zea mays</i>)	Diffuse Coplanar Surface Barrier Discharge (DCSBD) plasma; frequency (14 kHz); pressure (101,325 Pa); voltage (10 kV); plasma density (100 W. cm ⁻³); gas (Air); treatment time (60 and 120 s)	60-s treatment positively influenced germination and early growth of seedlings. The length, fresh and dry weight increased by 21%, 10%, and 14%, respectively, while increased plasma exposure for 120 s significantly showed inhibition for all parameters compared with control.	[87]
Wheat (<i>Triticum spp.</i>)	Low-pressure plasma; voltage (600–850 V); gas (Ar).	Increased germination by 57–60% than control.	[88]

Table 3. Cont.

Plant Species	Plasma Device	Seed Germination	Ref.
Wheat (<i>Triticum</i> spp.)	Low-pressure plasma; He gas; pressure (150 Pa); variable power (60–100 W).	The improved germination rate and germination potential compared with control.	[89]
Wheat (<i>Triticum aestivum</i>)	Surface discharge plasma; voltage (15 kV); pressure (101,325 Pa); frequency (50 Hz); average power (2.7 W); treatment time (15 min).	Slightly affected the germination rate.	[90]
Wheat (<i>Triticum aestivum</i>)	Glow discharge plasma; pressure (~1333.22 Pa); frequency (3–5 kHz); gas (Ar + O ₂); treatment time (60 s)	Enhanced seed germination of 12.38% and 7.14% were obtained for Ar + O ₂ gas mixture and Air, respectively	[91]
Wheat (<i>Triticum aestivum</i>)	Atmospheric pressure dielectric barrier discharge plasma; pressure (101,325 Pa); frequency (16 kHz); voltage (20 kV); power (30 W); treatment time (10–900 s).	Plasma treatment increased the germination during the first 48 h after seeding, for short treatment times (<120 s). Germination percentage up to 40% for 120 s compared to control (20%).	[92]
Wheat (<i>Triticum aestivum</i>)	Glow discharge plasma; pressure (1333 Pa); frequency (3–5 kHz); feed gas (mixture of O ₂ and Air); treatment time (3–9 min)	6-min treatment resulted in 95–100% seed germination and a 20% increase in wheat yield.	[93]
Wheat (<i>Triticum aestivum</i>)	Low-pressure Plasonic AR-550-M plasma; pressure (140 Pa); power (500 W); feed gas (Air); treatment time (180–2400 s).	Plasma treatment inhibited the germinating acceleration of wheat in first days, but enhancement of footstalk was observed on plants grown from seeds treated for medium time. 3-min treatment caused heavier shoots (dry weight 5.4 g) than samples treated by 10-, 20-, and 40-min plasma exposure.	[94]
Wheat (Xiaoyan 22)	Low-pressure dielectric barrier discharge plasma; pressure (1333 Pa); voltage (0–50 kV); frequency (50 Hz); working gases (Air, N ₂ , Ar, and O ₂)	Increased germination potential to 35.5, 28, and 24% for Ar, N ₂ , and Air feed gases' plasma, respectively.	[95]
Wheat (Xiaoyan 22)	Dielectric barrier discharge plasma; pressure (101,325 Pa); working gas (Air); power (1.50 W); voltage (13.0 kV).	After treatment, the increased germination rate, germination potential, and germination index were 9.1, 26.7, and 16.9%, respectively.	[96]
Wheat (Xiaoyan 22)	DBD plasma; voltage (13 kV); pressure (101,325 Pa); power (1.50 W); feed gas (Air); treatment time (7 min).	The germination potential and germination rate were increased to 27.2 and 27.6%, respectively, after plasma treatment. Additionally, root and shoot length increased after treatment.	[97]
Wheat (Giza 168)	Plasma jet; voltage (2.6 kV); pressure (101,325 Pa); current (38.1 mA); feed gas (N ₂); treatment time (4 min)	Enhanced germination rate and germination percentage were obtained after plasma treatment. Plasma treatment for 4 min resulted in 54.3% higher mean dry weight than control samples.	[98]
Winter Wheat	Dielectric barrier discharge plasma reactor with coaxial geometry; working gases He and Ar.	He and Ar feed showed faster germination for Ar plasma than He plasma and control	[99]
Winter Wheat	RF pressure plasma; frequency (13.56 MHz); feed gas (Air); treatment time (180 s).	After plasma treatment, the grain and spike yields were enhanced to 58 and 75%, respectively, compared to control in the presence of haze stress.	[100]
Pea (<i>Pisum sativum</i>)	Dielectric surface barrier discharge plasma; pressure (101,325 Pa); frequency (14 kHz); voltage (10 kV); average power density (2.3 W/cm ²); treatment time (60–300 s).	Significant increase in the total percentage of seed germination to 95% after 120-s treatment, while 77.5% for control.	[101]

Table 3. Cont.

Plant Species	Plasma Device	Seed Germination	Ref.
Pea (<i>Pisum sativum</i>)	FSG plasma (a semi-automatic device) system; voltage (15 kV); gas (Air); treatment time (30 s and 60 s).	80% and 74% of seeds were germinated after 30-s and 60-s treatment, respectively.	[102]
Pea (<i>Pisum sativum</i>)	Diffuse coplanar surface barrier discharge; voltage (20 kV); frequency (14 kHz); power (400 W); gas (N ₂ , O ₂ and mixture of N ₂ :O ₂); treatment time (60–300 s).	The least seed DNA was damaged in the presence of Air plasma; however, DNA damage increased with increasing the N ₂ content and treatment time. The improved germination and growth were observed for 120-s treatment, and air plasma showed improved germination with the least DNA damage.	[103]
Mung bean (<i>Vigna radiata</i>)	Microplasma array plasma; pressure (101,325 Pa); frequency (9.0 kHz); gases (Air, O ₂ , N ₂ , and He); treatment time (10 min).	The germination index increased by 58.3 and 41.7% for Air and O ₂ treatments, respectively. He or N ₂ plasma resulted in no notable disparity than control.	[104]
Ajwain	RF capacitively coupled plasma; frequency (13.56 MHz); pressure (9.9 Pa); power (50–100 W); treatment time (2 min).	The germination percentage increased by 11.1% at 50-W power, although higher RF plasma power damaged the seeds.	[105]
Poppy (<i>Papaver somniferum</i>)	Plasonic AR-550-M; power (500 W); low-pressure; gas (mixture of O ₂ and Ar); treatment time (0–5400 s).	Major differences in seed germination rate were recorded for 3-min, 5-min, and 10-min plasma treatment than vacuum and reference samples. Short treatment time showed significantly higher seedling growth on the 6th day of cultivation for short treatment time. The treated samples were 137%, 125%, and 131% longer for 3 min, 5 min, and 10 min, respectively, than reference samples.	[106]
Oilseed rape (<i>Brassica napus</i>)	Capacitively coupled plasma (CCP) was generated by radio frequency discharge; frequency (13.56 MHz); frequency; power (100 W); pressure (150 Pa); treatment time (15 s); gas (He).	Under drought stress, germination was improved by 6.25% and 4.44% for Zhongshuang 7 and Zhongshuang 11, respectively. Seedling growth characteristics such as root and shoot lengths, lateral root number, and dry weight were improved significantly after plasma treatment. Plasma treatment increased the superoxide dismutase activity by 17.71% and 13.00% for Zhongshuang 7 and 11, respectively. Catalase activity increased by 16.52% and 13.21% for Zhongshuang 7 and 11, respectively. An increase in soluble sugar and protein contents but a decrease in the malondialdehyde content for the plasma-treated samples occurred.	[107]
Sweet basil (<i>Ocimum basilicum</i>)	RF plasma; frequency (13.56 MHz); frequency; power (30–270 W); pressure (40 Pa); treatment time (10 min); gas (mixture of O ₂ and Ar).	The 150-W plasma treatment had the highest stimulatory effect on germination and vigor among all the studied doses of treatments.	[108]
Black gram (<i>Vigna mungo</i>)	Dielectric surface barrier discharge plasma; pressure (53,328 Pa); power (45 W); voltage (5 kV); frequency (4.5 kHz); gas (Air); treatment time (20–180 s).	Air-DBD for 120-s treatment increased the seed germination rate by 14 ± 0.6% than control. The maximum root and shoot length and dry weight were also observed for the same treatment time. Air-DBD treatment enhanced the nitrogen content in the seeds that contributed to an enhanced germination rate. Plasma-treated seed revealed the enhanced superoxide dismutase and Ascorbate peroxidases' (APXs') activities.	[109]

Recently, heat-stressed Rice (*Oryza sativa*) seeds treated with Air-scalar DBD plasma with 2.17-W discharge power showed a higher germination rate than without plasma heat-stressed seeds throughout the germination time course [65]. Rice seeds with low-pressure (~1333 Pa) DBD plasma working with Ar + Air gas mixture [66], hybrid cold plasma (HCP) with Ar feed gas [67], and Air-atmospheric pressure multi-pin plasma generator [68] showed an increase in seed germination and germination rate. The percentage increase in seed germination and germination rate depends on the plasma device and treatment time and it is independent of the pressure (positive effect shown in low-pressure and atmospheric pressure).

In plasma agriculture, another most-studied plant species was radish (*Raphanus sativus*). Radish treated with Air-scalar DBD [70], Ar-plasma jet [74], and Air-radio-frequency (RF) low-pressure (40 Pa) plasma [76] showed a positive effect in the seed germination. Our recent work reported that an increase in maximal germination percentage depends upon the harvest year and seed coat color [70]. The maximal germination percentage increased by 8% for gray-color seeds for the 2017 harvest while not influencing the brown seeds. The plasma effect had no significant effect on seed germination for the 2018 harvest for brown and gray radish seeds [70]. This might be the reason that, in other studies, growth enhancement was reported after the various plasma treatment (scalar DBD, plasma torch, and RF-low pressure (100 Pa plasma) with O₂ and N₂ feed gases) but not the germination rate or percentage [71–73,75]. RF-low pressure (100 Pa) plasma with O₂ feed gas showed increased sprouts' length, but no change was observed on seed germination [75], while RF-low pressure (40 Pa) plasma with Air feed gas showed increased seed germination rate and growth enhancement [76]. Some authors reported that radish seeds treated with O₂- RF-low pressure (100 Pa) plasma showed no morphological changes on seed coat using SEM analysis and no oxides were formed (seed surface not modified) by FTIR-ATR spectra [75], while no surface analysis was performed using Air- RF-low pressure (40 Pa) plasma [76]. There might be seed surface modification that occurred at 40 Pa, or, as we discussed above, the harvest year and seed coat color also play an essential role in the germination of radish sprouts.

Barley (*Hordeum vulgare*) seeds' treatment with microwave-driven Air plasma showed a negative effect on maximum germination [79], while barley seeds treated with low-pressure (26 Pa) plasma had no effect on the germination [81], whereas treatment with surface dielectric barrier discharge (SDBD) plasma resulted in the early growth of barley sprouts [80]. The increase in early growth and fresh weight of barley sprouts was due to improved levels of the primary metabolites (soluble sugars and free amino acids) and secondary metabolites (saponarin, GABA, and policosanols) [80]. Soybean (*Glycine max*) seeds treated with O₂-diffuse coplanar surface barrier discharge (DCSBD) plasma [82] and HD-2N low-pressure (150 Pa) plasma [84] resulted in a positive effect on seed germination. The reduced germination of control soybean seeds was due to seeds' age. The increased germination after DCSBD was due to a positive impact on succinate dehydrogenase activity, while inhibition of germination at high DCSBD doses caused by significant oxidative stress resulted in lipid peroxidation, stimulated lactate, and alcohol dehydrogenase activities and inhibited succinate dehydrogenase activity [82]. Maize seeds (*Zea mays*) treated with low-frequency glow discharge plasma (LFGD) at ~53,328.9 Pa [86] and Air-DCSBD caused a positive influence in germination [87]. LFGD treatment enhances enzymatic activities related to seed germination and stimulates the decomposition of the essential nutrients that trigger compounds to increase the consumption of seed reserves and promote seedling [86]. Dehydrogenase activity was significantly higher in embryos after the seed was treated with Air-DCSBD that might affect early germination [87].

We observed that wheat was the most studied plant species using NTP at low pressure and atmospheric pressure, and, in the majority of cases, it showed a positive impact on germination. Wheat (*Triticum* spp.) seeds treated with low-pressure plasma [88,89] revealed an improved germination rate and germination potential. Similarly, wheat (*Triticum aestivum*) seeds treated with surface discharge plasma [90], low-frequency glow discharge

plasma at ~1333 Pa [91,93], and atmospheric pressure DBD [92] showed increased germination percentage and germination rate, while low-pressure (140 Pa) Plasmonic AR-550-M plasma [94] demonstrated inhibited germination but enhanced footstalk. Wheat (Xiaoyan 22) seeds treated with DBD plasma working with various working gases showed an increase in germination rate and germination potential [95–97]. Additionally, wheat (Giza 168) [98] and winter wheat [99] seeds treated with plasma jet and DBD reactor also showed enhanced germination potential, respectively.

The impact of feed gases on the wheat germination was also reported, as the moderate-intensity DBD plasma in the presence of Air, N₂, and Ar plasmas showed positive impacts on the seed germination, while no significant effect was obtained for the O₂ plasma treatment. This was because the etching effect was stronger for Ar and N₂ plasma than O₂ plasma. Additionally, the relative electroconductivity of wheat increased for Air, N₂, and Ar plasmas that remained consistent with the change in water uptake and germination. Moreover, soluble protein production was also improved after plasma treatment; hence, plasma treatment can accelerate the physiological reactions that increase the germination index [95]. It was also mentioned that plasma treatment helps in penetrating active compounds, which further results in an accelerated wheat seed germination process [98]. H₂O₂ plays a key role in the plant life cycle, such as the release of dormancy during seed germination [110]. It was also reported that external H₂O₂ treatment helps in seed germination [111]. Thus, H₂O₂ is produced during the plasma discharge taken up by seeds during the imbibition process and regulates the metabolic processes that helps in germination [99].

Almost all the researchers found that NTP treatment assists the penetration of RONS to the seeds that benefit from increasing protein and antioxidant levels and accelerates the physiological reactions. During these processes, the reaction of RONS with proteins may have both positive and negative impacts on the plants. Additionally, NTP is used to deactivate enzymes in beverages such as fruit juices to maintain the quality of food products. To consider this, we focus on the NTP-induced modification in proteins'/enzymes' structures and functions in the next section.

5. NTP Effect on the Structural and Functional Changes of Proteins/Enzymes at Atmospheric Pressure

Like the PEF effect on proteins/enzymes, plasma treatment also modified the proteins'/enzymes' structure and function, as described in Table 4.

Table 4. Conformational and functional changes of proteins/enzymes after NTP treatment.

Proteins/Enzymes	Treatment Condition	Results	Ref
Lipase	Radiofrequency glow discharge Jet; power (180 W); gas (He); temperature (~57 °C).	The relative lipase activity was increased by 1.4 times after 50-s treatment. The increase in fluorescence emission intensity was compared to control.	[112]
Lysozyme	Low-frequency plasma; gas (He + O ₂); voltage (−3.5 to +5.0 kV); frequency (13.9 kHz).	This resulted in decreased residual activity after the 30-min plasma treatment. Additionally, the secondary structure of lysozyme changed slightly after plasma exposure. The quenching in fluorescence intensity was observed with plasma treatment.	[113]
Lysozyme	Dielectric surface barrier discharge plasma; voltage (1.23 kV); frequency (16 kHz); gas (N ₂ and Air). Plasma Jet; voltage (0.6 kV); frequency (24 kHz) 24 kHz frequency; gas (N ₂ and Air).	DBD and Jet for 8 and 12 min with Air and N ₂ as feed gases decreased activity. The structure of lysozyme was more disturbed with N ₂ feed gas than Air feed gas plasma.	[114]

Table 4. Cont.

Proteins/Enzymes	Treatment Condition	Results	Ref
α -Chymotrypsin	Plasma Jet; power supply: Neon light trans-operated at 60 Hz; treatment time (5 min); gas (Air).	Decrease in thermodynamic properties of α -chymotrypsin after plasma treatment. Additionally, there was an increase in α -helix and a reduction of β -sheet content and quenching in fluorescence spectra after plasma treatment.	[115]
Hemoglobin and Myoglobin	Plasma Jet; power supply: Neon light trans-operated at 60 Hz; gases (Air, N ₂ and Ar); treatment time (3 min).	The maximum decrease in α -helix and the highest level of protein oxidation for N ₂ plasma than Air and Ar feed gases' plasma.	[116]
Hemoglobin	Dielectric surface barrier discharge plasma; frequency (10 kHz); voltage (9.2 kV); current (0.2 A); discharge power density (1.49 W/cm ²); gases (Air, Ar, He, NO (10%) + N ₂ and N ₂); treatment time (10 min).	The 10-min DBD treatment with O ₂ , N ₂ , and Air feed gases' plasma resulted in minor changes in α -helix and β -sheet content, while changes were insignificant for Ar, He, and NO (10%) + N ₂ gases' plasma. Fluorescence spectroscopy showed the maximum redshift Air plasma compared to other feed gases.	[117]
Hemoglobin and Myoglobin	DBD plasma Jet; voltage (0.7 kV); current (3 mA); frequency (16 kHz); gases (Ar, Ar + O ₂ (0.2%, 0.3%, and 0.4%), Ar + N ₂ (0.2%, 0.3%, and 0.4%)).	The CD and Fourier transform infrared spectroscopy data showed as the treatment time increased, the percentage of α -helical decreased, and the percentage of β -sheet increased for pure Ar plasma and Ar mixtures (Ar + O ₂ and Ar + N ₂).	[118]
Alkaline phosphatase	Dielectric surface barrier discharge plasma; gas (Air); voltage (40–60 kV); operation time (15–300 s).	The decreased α -helix and β -sheets' content as plasma treatment time increased. The reduced activity of alkaline phosphatase was obtained after the 180-s treatment.	[119]
MTH1880	Dielectric surface barrier discharge plasma; gas (Air); voltage (0.9 kV); treatment time (10–20 min).	The decrease and increase in α -helix and β -sheets' contents, respectively, after plasma treatment. The quenching in fluorescence spectra increased with treatment time due to changes in the Tyrosine residue environment or its modification. The melting temperature of MTH was not significantly changed after plasma treatment for 10 and 15 min, whereas it dropped by 5 °C for 20-min treatment. ¹ H- ¹⁵ N NMR spectroscopy showed peak shifts for Histidine 52 and Tyrosine 59 after 20-min plasma treatment.	[120]
Lactate dehydrogenase	Dielectric surface barrier discharge plasma; gas (He); voltage (12 kV); frequency (24 kHz); power density (0.9 W/cm ²).	The α -helix content decreased, whereas β -sheet content increased by direct plasma treatment. Indirect plasma treatment caused decreased α -helix content, and β -sheet content increased. The activity was reduced to 67.5% after direct plasma exposure and 71.14% after the indirect plasma treatment for 300 s.	[121]
NADPH oxidases 1 (NOX1)	Dielectric surface barrier discharge plasma; gas (Air); voltage (1.08 kV); treatment time (1–5 min). Plasma Jet; gas (Air); voltage (1.6 kV); treatment time (1–3 min).	¹ H- ¹⁵ N NMR spectroscopy showed the peak shifts for glutamine 413, aspartic acid 427, cysteine 430 and 441, valine 432, glycine 443 and 446, and arginine 444 after plasma treatment.	[122]
Tyrosinase	Dielectric surface barrier discharge plasma; gas (He and He + O ₂); voltage (0.85 or 1.1 kV _{rms}); frequency (20 kHz); treatment time (10–60 min).	Tyrosinase enzyme treated with plasma with He and He + O ₂ feed gases showed decreased activity. The activity was significantly reduced after He + O ₂ plasma treatment than pure He plasma after 30 min.	[123]

Table 4. Cont.

Proteins/Enzymes	Treatment Condition	Results	Ref
Peroxidase (POD) and Polyphenol oxidase (PPO)	Plasma Jet; voltage (2–6 kV); frequency (1.1 MHz); gas (Ar and Ar + O ₂ (0.01–0.1%)); treatment time (0–360 s).	The combination of Ar + 0.05% O ₂ plasma for 350 s resulted in 90% inactivated polyphenol oxidase. At the same time, Ar + 0.05% O ₂ resulted in an 85% decrease in the peroxidase activity. For polyphenol oxidase and peroxidase, the α -helix content decreased, while β -sheet content increased after plasma treatment.	[124]
Horseradish Peroxidase	Dielectric surface barrier discharge plasma Jet; gas (Ar + O ₂); voltage (7 kV).	Peroxidase activity decreased to 17% after 10-min plasma treatment. Moreover, the α -helix content decreased and β -sheet increased after 10-min plasma exposure, and no remarkable change was observed for the random coil.	[125]
Peroxidase in tomato extract	Dielectric surface barrier discharge plasma; gas (He and Ar); voltage (10 kV); power (20 W); operation time (1–6 min). Gliding Arc; gas (Air); voltage (14 kV); frequency (20 kHz); power (50 W).	The decrease in peroxidase activity to 7.32% after plasma treatment.	[126]
Pectin Methylesterase, lipoxygenase, Polyphenol Oxidase, and Peroxidase in carrot juice	Dielectric surface barrier discharge plasma; gas (Air); voltage (60–80 kV); operation time (4 min).	The minimum residual activities of pectin-methylesterase after plasma treatment. The residual activity, lipoxygenase, polyphenol oxidase, and peroxidase were 10.21, 13.42, 11.20, and 15.73% observed, respectively, at 70 kV for 4-min treatment.	[127]
α -amylase (dry brown rice)	Dielectric surface barrier discharge plasma; power (250 W); frequency (15 kHz); treatment time (5–20 min).	The α -amylase activity significantly increased after plasma treatment and the maximum increase after 5-min treatment of around 1.21-fold growth than the control.	[128]

The lysozyme treated with a low-frequency (LF) plasma jet using He + O₂ feed gas decreases the residual activity and alters the secondary structure. After 30 min of plasma treatment, the lysozyme's random coil structure formed was nearly the same as that treated with 6.0 M guanidine HCl. Quenching was observed without change in maximum wavelength in fluorescence spectra, and this was due to the chemical modification of tryptophan by NTP treatment. Some authors suggested that the change in lysozyme structure was due to reactive species such as $\cdot\text{OH}$, $\text{O}_2^{\cdot-}$, $\text{HO}_2\cdot$, and $\text{NO}\cdot$ that cause modification in the side chain of the amino acids such as tryptophan, tyrosine, cysteine, and phenylalanine [113]. In another study, the lysozyme was treated with DBD and Jet for 8 min, 12 min with Air and N₂ as feed gases, resulting in decreased activity and structural changes. Circular dichroism (CD), fluorescence, and thermodynamic analysis revealed that the structure of lysozyme is more disturbed with N₂ feed gas plasma than Air feed gas plasma. X-crystallographic data of lysozyme after NTP treatment showed that structural changes occurred at loop 3 and loop 6 along with the substrate-binding sites Tryptophan 62 and 108 and Aspartic acid 101.

Additionally, similar to the above work, the authors observed quenching in the fluorescence intensity without changing the wavelength after the plasma treatment. More structural and functional changes were observed in N₂ plasma than Air plasma because N₂ plasma generates more $\cdot\text{OH}$ and H₂O₂ than Air plasma. The N₂ metastable level (N₂(A₃ Σ u⁺)) can dissociate H₂O to produce $\cdot\text{OH}$ and $\cdot\text{H}$. In addition, the exciting 2N atom also generates two OH radicals, while the presence of O₂ in Air plasma reduced the OH density, affecting the H₂O₂ concentration [114]. Both studies showed that, independent of the plasma devices and feed gases, the decrease in residual activity and a change in the secondary structure was observed; no wavelength shift in the fluorescence spectra was noticed, while the quenching of the peak was observed in both studies [113,114]. Moreover, both

studies concluded that the structural changes and functional changes in lysozyme were due to RONS generated by plasma, especially OH radical [113,114], rather than plasma-induced UV [113].

Peroxidase and polyphenol oxidase treated with Ar + O₂ gas mixtures' plasma Jet caused structural and functional changes. The α -helix content decreased, and β -sheet content increased after plasma treatment for both peroxidase and polyphenol oxidase proteins. In comparison, activity was reduced to 85% for peroxidase and 90% for polyphenol oxidase after plasma treatment [124]. In a separate study, horseradish peroxidase enzyme treated with Ar + O₂ DBD Jet decreased to 17% after a 10-min plasma treatment. Structural changes were similar to the above study in that the α -helix content decreased and β -sheet increased [125]. Peroxidase tomato extract treated with He and Ar DBD plasma and Air gliding arc results in decreased peroxidase activity to 7.32% after plasma treatment [126]. Further, the residual activity of peroxidase and polyphenol oxidase in carrot juice treated with Air DBD plasma reduced to 15.73 and 11.20%, respectively [127]. These studies showed that peroxidase and polyphenol oxidase activity could be reduced after the NTP treatment, independent of plasma sources and feeding gases. Although peroxidase showed more resistance to NTP treatment than polyphenol oxidase in all the cases, the structural changes were similar for peroxidase and polyphenol oxidase (α -helix content decreased and β -sheet increased).

6. Conclusions

PEF and NTP have great potential in agriculture (improved seed germination, seedling growth, high yield, and stress tolerance) and food processing (inactivation of enzymes in beverages such as fruit juices). Both technologies are environmentally friendly and easy to use. Understanding the mechanism behind improved seed germination after PEF and NTP treatments, on the other hand, is critical for future technological investigation and standardization. Different degrees of electric field strength were employed on different plant seeds in these studies, making it difficult to determine the particular electric field intensity that promotes seed germination. Nonetheless, we inferred from the research that moderate PEF intensity causes seed coat degradation, which increases water imbibition and allows an embryo to stretch, which aids seed germination. In contrast, seeds treated with a high intensity of PEF results in delayed seed germination that might damage the embryo, resulting in decreased seedling emergence. Therefore, it is important to use the same electric field intensity on different plant seeds under controlled atmospheric conditions to standardize the PEF treatment that helps in promoting germination. One of the mechanisms behind the PEF treatment is the modification of biomolecule structures (secondary and tertiary) and functional properties. It was noticed that PEF-induced structural changes also alter the physicochemical and functional properties of proteins/enzymes. In general, a decrease in the α -helix content and an increase in the β -sheet were observed after moderate PEF treatment, while high-intensity PEF treatment caused aggregation. Furthermore, after PEF treatment, enzyme activity dropped, which was substantially influenced by treatment time, enzyme native structure, PEF intensity, and preheating temperature. However, more research at consistent NTP treatment circumstances and with numerous plant seed types is still needed. According to the investigations, there is no universal rule that assists seed germination in terms of pressure, power density, working gas composition, or treatment time. Furthermore, there was no clear answer to whether NTP enhanced physiological reactions by modifying the seed surface or not. In the maximum studies, no significant change in the morphology of the seed coat was reported. However, it was known that reactive species (RONS) and UV/VUS generated by NTP initiate the reactions in seeds despite the fact that PEF induced seed coat damage that helped in nutrients' uptake.

NTP's role in preserving fruit juices by deactivating enzymes and proteins showed both an increase and decrease in activity depending upon the treatment time and NTP source. Like PEF, the reduction in the α -helix content and increase in β -sheet were observed in most cases after NTP treatment. Additionally, it was clearly shown in the NTP

treatment the reactive species produced by NTP oxidized the amino acids of the proteins. Indeed, the oxidation of amino acids depends upon the treatment conditions, reactive species' concentration, and the oxidative property of amino acids (some are more prone to oxidation).

The overall result is that PEF and NTP treatment aid seed germination and seedling growth but also alter the structure and function of proteins and enzymes. Additionally, there is a need to develop the standard protocol using PEF or NTP parameters, treatment time, various plant seeds, seed coat color, harvest year, seed morphology, feeding gases composition, etc. to use these technologies at an industrial scale.

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