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Ozone and cold plasma: Emerging oxidation technologies for inactivation of enzymes in fruits, vegetables, and fruit juices

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ABSTRACT

Inactivation of deteriorative enzymes preserves the quality of fruits and vegetables and extends the shelf-life of fruit juices. Ozonation and cold plasma treatment are recent additions to the thermal and non-thermal methods for enzyme inactivation. However, these techniques stand out in their effectiveness and versatility for the treatment of a wide range of fruits and vegetables. This article appraises the mode of action and applications of ozone and cold plasma for the inactivation of enzymes in fruits, vegetables, and fruit juices. Further, a comprehensive discussion is presented on the influential parameters of enzyme inactivation effectiveness of ozonation and cold plasma processing. Besides, the latter sections of this article highlight the challenges that impose hurdles in the commercial applications of these unconventional techniques and the way forward in improving their efficacy and industrial applications.

1. Introduction

'Enzymes', the biological catalysts are a class of proteins that accelerate chemical reactions in foods, which can be either beneficial or undesirable. For instance, *'enzymatic browning'*, an oxidative reaction that occurs in foods, is favorable to flavor development in coffee, cocoa, tea, figs, and raisins, but not acceptable in cut fruits and vegetables such as apples, potatoes, and avocadoes. It is the result of interaction between oxygen in the air and the phenolic compounds in fruits, in the presence of enzymes such as phenolase, peroxidase (PO) and polyphenol oxidase (PPO) entrapped in the cells of the fruits. These enzymes catalyze the conversion of phenols into melanin – a pigment that imparts brown coloration (Lattanzio et al., 1989). Consumer preference for fruits and vegetables is impacted by brown discoloration. Further, enzymes embedded within the tissues of fruits and vegetables turn active during the postharvest, processing, and storage stages to hasten metabolic changes and alter physical characteristics such as color and softness. Specifically, in climacteric fruits, wherein ripening happens during the storage period, uncontrolled enzyme activity can lead to adverse changes in their quality characteristics such as color, texture, flavor, and nutritional properties (Lamikanra, 2002).

Similar to the role of PO and PPO in the browning of fruits, softening of plant tissues is catalyzed by enzymes such as cellulase, pectinase, hemicellulase, polygalacturonase, β-xylosidase, glucanase, and xylanase. These enzymes accelerate cell wall degradation by promoting the depolymerization or dissolution of constituent polysaccharides such as pectin, cellulose, and hemicellulose (Chea et al., 2019; Pan et al., 2021).

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List of abbreviations

AC	Alternating Current
ACC	Amino cyclo-propane carboxylate
CAT	Catalase
CD	Circular dichroism
DBD	Dielectric barrier discharge
DLS	Dynamic Light Scattering
DSC	Differential Scanning calorimetry
FAO	Food and Agricultural Organization
FTIR	Fourier Transform Infrared
GRAS	Generally Recognized as Safe
HPP	High Pressure Processing
NMR	Nuclear Magnetic Resonance
PEF	Pulsed Electric Field
PG	Polygalacturonase
PME	Pectin Methyl Esterase
PO	Peroxidase
PPA	Plasma Processed Air
PPO	Polyphenol oxidase
ROS	Reactive Oxygen Species
SAXS	Small Angle X-ray scattering
SOD	Superoxide dismutase

Besides the above, enzymes such as lipoxygenase and ascorbic acid oxidase cause off-flavors and loss of nutrients (ex. vitamin C) in fruits and vegetables (Pandiselvam et al., 2020). On the other hand, rancidity in oils, milk, and meat and textural and nutrient losses in eggs, marine, and meat products are other deteriorative reactions catalyzed by enzymes such as lipase and thiaminase (Khanashyam et al., 2021; Thirumdas & Annapure, 2020). Thus, enzymatic reactions jeopardize the nutritional and organoleptic quality of foods and reduce the shelf-life of agro-produces, thereby leading to wastage and economic loss. Denaturation or inactivation of enzymes is the one-stop solution to the challenges mentioned above. Therefore, devising effective methods for enzyme inactivation has always remained an active area of research amongst food scientists and technologists.

Controlling enzymatic reactions in fruits, vegetables, and their value-added products gain importance due to the following reasons. Fruits and vegetables constitute about 22% of global food production (FAO, World Food and Agriculture – Statistical Yearbook, 2020), and 50% of fresh fruits are lost due to enzyme-mediated discoloration. For many years, thermal processing techniques such as heating, blanching, sterilization, and pasteurization have been widely used for enzyme inactivation in fruits and vegetables. Though thermal processing is effective in the inactivation of targeted enzymes, extreme treatment conditions were found to have adverse effects on the product quality, nutritional and sensory properties, and stability (Terefe, Buckow, & Versteeg, 2014).

Concerning the enzyme inactivation in fruits and vegetables, non-thermal processing techniques have an edge over their thermal processing counterparts, as they can preserve the flavor, aroma, and nutritional qualities by applying lower temperature and shorter processing times (Kaavya et al., 2019; Niveditha et al., 2021). Enzyme inactivation has been achieved by various non-thermal techniques such as Pulsed Electric Field (PEF), High Pressure Processing (HPP), irradiation, and ultrasonication. Nevertheless, each of these techniques has pros and cons concerning effective enzyme inactivation and product quality. For instance, PEF treatment inactivates enzymes by instigating conformational changes in their structure upon applying a high voltage (kV/cm) in short pulses (s or ms) (Jadhav et al., 2021; Zhang, Tian, Du, & Fang, 2017). The ambient, sub-ambient, and above-ambient temperature encountered during PEF processing is much lower than that applied in the thermal processing methods. However, it suffers from limitations such as leaking metal ions from the electrode into food products (Nowosad et al., 2021), high processing costs, and the inability to inactivate enzymes in products that require low storage temperatures. Likewise, the enzyme inactivation mechanism of HPP (>600 MPa) involves the disruption of their non-covalent hydrogen bonds (Lou et al., 2022) and electrostatic and hydrophobic interactions that modify their secondary and tertiary molecular structures. Majority of the deteriorative enzymes in foods are pressure resistant, affecting the inactivation efficiency of HPP technique (Podolak et al., 2020).

On the other hand, during ultrasound processing, the sound waves that traverse through a fluid medium create alternating pressure waves that result in cavitation, a physical phenomenon that results in high pressure and temperature in the order of 50 MPa and 5000 K, respectively (Anaya-Esparza et al., 2017). Combined with the above physical stress, the generated free radicals cause modifications in the enzyme structure/conformation to inactivate them eventually (Nahidul Islam et al., 2014). Ultrasound processing has been found successful in inactivating peroxidase, polyphenol oxidase, and ascorbate peroxidase in melon juice (Fonteles et al., 2012) and pectin methylesterase in blackberry juice (Cervantes-Elizarrarás et al., 2017). But, the free radicals generated during ultrasound processing can catalyze undesirable reactions and damage the proteins, amino acids, and lipids in food substances (Arvanitoyannis et al., 2017). Irradiation deals with enzyme inactivation by generating free radicals generated from the chemical reactions when electromagnetic energy is deposited on the molecules in the food product. But, food enzymes are more resistant than microbes to irradiation, and hence complete inactivation of an enzyme needs about 5–10 times higher irradiation dosage than that required for microbial inactivation (Yannam et al., 2020). Moreover, the development of off-flavors at higher irradiation dosages is also reported (Pinto et al., 2020). Thus, the limitations of the above processes instigated the research fraternity to explore more effective non-thermal approaches for enzyme inactivation. This led to the advent of using ozone and cold plasma for enzyme inactivation, which are relatively recent additions to the list of non-thermal technologies.

Ozone is a triatomic form of oxygen (O_3) and a naturally available gas in the atmosphere. It is produced by passing oxygen (O_2) gas through a high-voltage electrical discharge or using ultraviolet light irradiation (Mahapatra et al., 2005). It is used either in the gaseous state or after dissolving in water (Kaavya et al., 2021; Sarron et al., 2021). Ozone is a high-permeability oxidant with a proven ability to inhibit enzymes that are responsible for softening and loss of firmness (ex. methyl esterase) in fruits during storage (Chen et al., 2020; Pandiselvam et al., 2020). It is categorized as GRAS (Generally Recognized as Safe) for food contact applications by the United States Food and Drug Administration (FDA, 2001). Ozone is a permitted antimicrobial agent for the treatment, storage, and processing of foods. Initially, the applications of ozone were limited to wastewater treatment and drinking water disinfection. Later, the effectiveness of ozone against catalase (CAT), an antioxidant enzyme, was realized. Studies showed the involvement of oxyradicals in the ozone-mediated inactivation of catalase (Whiteside & Hassan, 1988). Since then, the applications of ozone for enzyme inactivation have been investigated in different food products, including fruits and vegetables.

Strikingly, ozone is known to preserve the firmness of fruits during storage by retarding the activity of glycosidases and esterases and decelerating the solubility of neutral sugars in the cell wall. The oxidative nature of ozone is responsible for inactivating fruit softening enzymes such as methylesterase (El-Eryan & Tarabih, 2020). The optimum concentration of ozone, treatment time, and other conditions has been standardized for different commodities to enable effective and safe usage of ozone for enzyme inactivation applications. Various researchers have demonstrated the effectiveness of ozone treatment in deactivating different enzymes in varied fruits, vegetables, and fruit juices (Table 1). Studies have revealed that ozone prevents the degradation of post-harvest quality in crops by controlling the activity of enzymes responsible for the decomposition of antioxidants and chlorophyll. In addition, ozone suppresses the deterioration of chlorophyll by acting upon the chlorophyll damaging enzymes such as pheophytinase, chlorophyllase, and chlorophyll degrading peroxidase.

a) *Non-residual nature:*

Plasma is the fourth state of matter in addition to the solid, liquid, and gaseous states. Plasma is formed when the temperature of a gas is increased to a point where ionization occurs. The energy required to increase the gas temperature can be derived from different sources such as electromagnetic radiation (microwave, radiofrequency), laser light, heat, and electricity. Thermal plasmaexists at an extremely high temperature of the order of 10^7 K and is hence not suitable for food processing applications. However, the temperature of the cold plasma is close to ambient temperature (below 40 °C), even if the electron temperature is ten thousand kelvin (Mastwijk & Nierop Groot, 2010). Being a rapid non-thermal sterilization technique, cold plasma is used for the surface decontamination of fresh produce without altering its nutrient quality (Sruthi et al., 2021). Several studies have established the effectiveness of cold plasma (Table 2) for enzyme inactivation in fresh fruits and vegetables (apples, melon, potato, mushroom) and fruit juices (apple juice, carrot juice, orange juice) (Kovačević et al., 2016; Lacombe et al., 2015; Misra et al., 2015; Pankaj et al., 2017; Sarangapani et al., 2017; Wang et al., 2012; Xu et al., 2017). For instance, Xu et al. (2017) reported a 74% reduction in the activity of pectin methylesterase (PME) in orange juice when air was used as the plasma generation source.

This review intends to present the state-of-the-art mechanism of enzyme inactivation and applications of ozone and cold plasma techniques for the same in fruits, vegetables, and fruit juices. The recent innovations and challenges pertaining to these two techniques will be emphasized. Insights will be provided on the influential factors involved in ozone and cold plasma processing and their effects on the degree of enzyme inactivation in different fruits and vegetables and their products. The information presented in this review would be useful to researchers in optimizing and designing their ozone and cold plasma processing trials for enzyme inactivation and characterizing the products to determine the treatment efficacy.

2. Ozone-mediated inactivation of enzymes in fruits, vegetables, and fruit juices

2.1. Mechanism of enzyme inactivation by ozone

An earlier study attributed the enzyme inactivation mechanism of ozone to the oxidation of sulfhydryl groups in the cysteine residues of enzymes (Chang, 1971). Later, in 1988, Whiteside and Hassan presented a clear elucidation of the mechanism, by considering catalase (CAT) as the model enzyme. CAT is a metalloprotein oxidoreductase enzyme capable of scavenging hydrogen peroxide at high concentrations (Cao et al., 2003; Day, 2009). It is a tetrameric heme protein having a monomeric molecular weight of 64 kDa, which cleaves H_2O_2 into oxygen and water (Lee et al., 2003). Each subunit of CAT has a b-type heme with its absorption maximum at 405 nm when the enzyme is in its inactive state. Hence, the integrity of heme in CAT can be evaluated by measuring the absorbance at 405 nm. In accordance with the above, the gaseous ozone-mediated CAT inactivation was ascribed to the irreversible oxidation and/or the loss of its heme functional groups. Evidence was also observed on the involvement of oxyradicals as mediators in the ozone-mediated inactivation of catalase (Fig. 1). The rate-determining factors of enzyme inactivation by ozone were found to be the ozone concentration, length of exposure time, composition of the exposure medium, and pH (Whiteside & Hassan, 1988).

A later study by Lee et al. (2003) revised the enzyme inactivation mechanism with ozone by using ozonized water as a substitute for gaseous ozone. Ozone gas from the generator was bubbled through ice-chilled water at pH 2. Two antioxidant enzymes (Cu/Zn superoxide dismutase [CuZnSOD] and CAT) were prepared at a concentration of 10 μM in 100 mM phosphate buffer (pH 7.4). The enzyme solutions were treated with ozonized water containing 500 μM of ozone and incubated at room temperature for 30 min. Subsequently, their activities were measured in terms of heme absorption at 405 nm. Results showed that the degree of enzyme inactivation by ozone was a function of the concentration of both ozone and the enzymes. CAT was relatively more resistant to ozone than CuZn-SOD. While the extent of inactivation was ~50% for CuZnSOD with 45 μM ozone, the same level of enzyme inactivation was achieved only at ~500 μM of [ozone] for CAT.

Further, it was observed that the loss of heme in CAT increased with the increase in ozone concentration. But, the degree of heme loss was substantially less than the corresponding activity loss (Fig. 2[a]), which signified that the ozone-mediated inactivation of CAT was mainly due to damages in protein moiety that eventually led to the heme release. Based on the observations from gel electrophoresis, it was ascertained that ozone did not cause site-specific fragmentation of CAT. Instead, a high molecular weight (>200 kD) aggregate was noticed, for which the intensity increased with ozone concentration. Thus, it is evident that ozone causes the aggregation of CAT without site-specific fragmentation. Also, a constant ozone concentration (500 μM) and greater inactivation (>80%) were achieved at a lower concentration of CAT (5 μM) (Fig. 2[b]). A similar trend was observed for the other antioxidant enzymes (CuZnSOD and glutathione peroxidase) as well.

An enzyme namely, Antisense ACC oxidase present in melons displays a clear reduction in softening. In another study, it was reported that in kiwifruits after exposure to ozone the enzymes ACC oxidase and ACC synthase continued their activity only at smaller concentrations compared to ozone untreated fruits indicating the role of ozone in controlling the maturation level (Minas et al., 2014). Certain pectin backbones in the side chains, arabinogalactans, and galactans present in the cell wall were degraded by α-arabinase and β-galactosidase enzymes, resulting in augmented solubility of pectin and developed cell wall polysaccharides further available to various enzymes which are involved in pectin degradation.

2.2. Applications of ozone for enzyme inactivation in fruits, fruit juices, and vegetables

2.2.1. Ozone-mediated enzyme inactivation in fruits

The efficacy of ozone treatment for enzyme inactivation depends on factors such as the physical state (gaseous or aqueous) and concentration of ozone and treatment time. Gaseous ozone proved effective in reducing softening of cantaloupe melons at a concentration of 0.15 ppm during the day and 0.3 ppm overnight for a storage period of 13-days at 6 °C. The same was achieved by the inhibitory action of ozone on cell wall enzymes such as α-arabinopyranosidase (α-Ara), β-galactopyranosidase (β-Gal), polygalacturonase (PG), and pectinmethylesterase (PME). Consequently, the solubility of neutral sugars of the cell wall was decelerated and the fruit firmness was retained. Compared to control fruits, ozone-treated fruits showed reduced activity (measured in μmol g^{-1} (fresh weight) min^{-1}) of the aforementioned cell wall enzymes. On the contrary, gaseous ozone treatment did not have any influence on the enzymatic activity of PME in melons during 13 days of storage (Fig. 3) (Toti et al., 2018). Thus, apart from ozone concentration and exposure time, the resistance of an enzyme to ozone treatment and the influence of food matrices can also influence the efficacy of ozonation in enzyme inactivation.

CK: Control melon fruits; O^3: Ozone-treated melon fruits.

Different from the above study, Nayak et al. (2020) applied aqueous

Table 1
Effect of ozone treatment on different enzymes in fruits, vegetables and juices.

Product	Treatment Condition	Result	Effect on other quality parameters	Reference
Fruits				
Cantaloupe Melon	Cold storage and treated with 0.15 ppm gaseous ozone during the day and 0.3 ppm overnight.	Reduced activities α-arabinopyranosidase, β-galactopyranoside and polygalactoronase. No effect on Pectin methyl esterase activity.	In comparison to control, ozone treated fruits had a higher firmness and reduced ethylene content. During storage, ozone also lowered the bacteria count. Furthermore, total carotenoids were found to be greater in the treated fruits, with no significant differences between the control and treated fruits. Ascorbic acid, color, total soluble solids concentration, and acidity did not show any significant differences.	Toti et al. (2018)
Kiwi	Cold storage (0 °C, 95% RH) with gaseous ozone (0.3 ppm) for 1, 3, and 5 months and then storage at 20 °C for up to 12 days.	ACC oxidase and ACC synthase remained at very low concentrations at maturation stage, inhibitory action on glycosidases.	The fruits showed delayed ripening and flesh softening after ozone exposure.	Minas et al. (2014)
Lime	Fruits treated with 0.3 ppm aqueous ozone for 5 and 10 min, edible coating with gum arabic at 10% and combination of aqueous ozone and gum arabic. Fruits were stored at 7 °C ± 1 and 90–95% relative humidity (RH) for 60 days.	Reduced chlorophyllase activity.	Ozone treatment significantly reduced the weight loss, respiratory rate and decay percentage of the fruits.	El-Eryan and Tarabih (2020)
Longan	Ozone exposure (200 μL/L) alone or combined with citric, ascorbic or oxalic acid for 0, 15, 30, 60 and 120 min and then storage at 25 °C.	Fruit treated with ozone in combination with oxalic or citric acid had less browning and a reduction of PPO activity.	The fruits treated with ozone showed less browning, enzyme activity and microbial decay.	Whangchai et al. (2006)
Strawberries	Strawberries were treated with 0 (control), 2, 4, and 8 ppm ozone for 30 min at 0 °C and stored at 0 ± 1 °C and 90% ± 5% RH.	Inhibition of POD and CAT enzyme activity.	The ozone treatment inhibited the decrease in ascorbic acid. It also reduced the weight loss, respiration rate and senescence of strawberry.	Zhang et al. (2011)
Tangerine	Ozone exposure (200 ppm) for 0, 2, 4 or 6 h and storage for 3 days at 25 °C with 75–80% RH.	The antioxidant enzyme activities increased after ozone fumigation and remained significantly higher through storage.	Fruit that was exposed to ozone for 4 or 6 h had a lower incidence and severity of illness. SEM analysis indicated that exposing fruit to ozone for 4 and 6 h reduced fungus development on the fruit skin.	Boonkorn et al. (2012)
Vegetables				
Asparagus (green)	Dipping in water containing ozone (1 ppm) for 30 min and storage in modified atmosphere packaging (MAP) at 3 °C for 25 days.	The enzyme activities including PAL, SOD, APX and GR were inhibited by aqueous ozone treatment.	The ozone treatment controlled the increase in cellulose, and hemicellulose fraction in asparagus, and maintained the textural property of the food.	An et al. (2007)
Carrot	Dipping in water containing ozone (10 ppm) for 10 min and storage in air or in modified atmosphere packaging (MAP).	Decrease in activities of PPO and POD.	Due to ozonation and CA storage, there was a considerable decrease in ascorbic acid, carotenoids, and oxidative enzymes such polyphenol oxidase (PPO) and peroxidase (POD).	Chauhan et al. (2011)
Celery	Dipping into water containing ozone at 0.03, 0.08 and 0.18 ppm for 5 min and storage for 9 days at 4 °C.	Polyphenol oxidase (PPO) activity inhibition.	The ozone treatment reduced the respiration rate of fresh-cut celery and maintained the sensory quality. However, there is no significant difference between Vitamin C and total content before and after treatment	Zhang et al. (2005)
Coriander	0.68 mg/L ozone has been used in the treatment of coriander before storing at 20 °C.	Reduced activity of polyphenol oxidase, chlorophyllase, chlorophyll-degrading peroxidase, Mg-dechelatase, and pheophytinase. Increased activity of peroxidase, catalase, and ascorbate peroxidase enzymes.	Ozone preserved the harvested coriander's color while suppressing respiration and polyphenol oxidase activity.	Xu et al. (2019)
Lettuce	Washing with water containing ozone at 10, 20 and 10 ppm activated by ultraviolet C and storage in modified atmosphere for 13 days at 4 °C.	Controlled browning and maintenance of initial texture and appearance.	The ozone treatment controlled the browning, reduced the microbial activity and had no detrimental effect on antioxidant compounds.	Beltrán et al. (2005a)
Lettuce	Lettuce samples (A) immersed in water containing ozone at 1.0 ppm for 1 min and dried for 5 min, and (B) immersed in aqueous ozone (1.0 ppm, 1 min), dried for 5 min.	Reduction of PPO, POD and PME activity	Even though ozone was able to reduce the enzyme activity and browning reactions, a negative effect on texture was reported which resulted in a lowed sensory score.	Rico et al. (2006)
Lettuce	Prewashing in water containing ozone (1 ppm, 4 °C, 120 s) or chlorine (100–200 ppm) and subsequent washing in tap water (4 °C, 90 s), and storage at 4 °C for up to 9 days	PAL, PPO and POD Activity significantly reduced. PAL activity and the associated rise of 3,5-di-O-caffeoylquinic acid concentrations.	Even though ozonated water treatment was able to reduce the enzyme activity, it showed a poor visual quality as compared to chlorine water.	Baur et al. (2004)
Tomato plant	Spraying tomato plants with ozonated water at concentrations below 10 mg/L for 25 days.	SOD and CAT activity substantially increased in response to treatment with ozonated water.	Significant decreases in plant pathogen were reported.	Guo et al. (2019)

(*continued on next page*)

Table 1 (*continued*)

Product	Treatment Condition	Result	Effect on other quality parameters	Reference
Tomato fruit	Exposure to 10 ppm ozone for 5, 10, or 20 min, and storage for 9 days at 20 °C with 90% RH.	Reduction in PME activity.	The ozone treatments had no effect on fruit color, sugar content, acidity, or antioxidant capacity, but they did minimize fruit damage and weight loss, as well as induce phenolic compound accumulation.	Rodoni et al. (2010)
Juices				
Peach juice	Two ozone levels at the inlet gas flow were used as 0.11 and 0.20 mg/min.mL. Ozone treatment temperature and time were given at 20 °C and 12 min.	POD and PPO activities decreased as ozone concentration and treatment time increased. After 12 min of application, the maximum activity reductions ranged from 99.5% to 99.8% for POD and between 93.9% and 97.3% for PPO, depending on the ozone concentration.	During ozonation, there were no substantial or minor changes in pH, °Brix, and titratable acidity. In all treated samples, lightness (L*) fell slightly in the first minute of Ozone exposure and thereafter stayed nearly constant, while the a* parameter increased slightly. For ozonized juices, significant reductions in apparent viscosity and a trend toward Newtonian flow were seen with respect to ozone treatment time increased.	Jaramillo-Sánchez et al. (2018)
Sugarcane Juice	Oxygen concentrator flow rate and ozone feed rate have been maintained at 2 L/min and 1.2 g/h, respectively. Ozone treatment temperature and time were given at 25 °C and 20 min.	The use of nonthermal preservation treatment was effective in reducing the activity of browning enzymes (PPO as well as POD) while maintaining overall product acceptability during storage at refrigerated conditions.	The ozone treatment reduced the microbial count and pH of the sugarcane juice during storage.	Garud et al. (2017)

PPO- Polyphenol oxidase; POD-peroxidase; PAL-phenylalanine ammonia lyase; SOD-superoxide dismutase; APX-ascorbate peroxidase; GR-glutathione reductase; PME -Pectin methylesterase.

ozone at a concentration of 0.1 ppm for 1–4 min to protect strawberry fruits against softening. The O_3-treated fruits were air-dried and stored under both ambient (25 °C and 45–50% relative humidity [RH]) and low temperature (2 °C and 90% RH) conditions. The influence of ozone treatment time on fruit quality was assessed in this study. Results showed that the firmness was maximum (1.36 N/1.20 N) in aqueous ozone-treated strawberries fruits for 2 min under both ambient and cold storage conditions. However, when the treatment time was increased to 4 min, firmness dropped by 4.4% (1.30 N) relative to the values of fruits treated for a shorter duration. The positive effect of ozone on fruit firmness was attributed to its inactivation effect on pectin methylesterase and its ability to suppress ethylene gas production. The reduced firmness in fruits exposed to ozonefor a longtime was explained by the possible degradation of the cuticular components of strawberries at a faster rate (Zhang et al., 2011).

Yet another study investigated the synergistic effects of aqueous ozone and ultrasound on the storage equality of strawberry fruits. Accordingly, strawberry fruits were treated by a combination of both ozone and ultrasound for different durations: 1, 2, or 3 min. After ozone treatment, the fruits were stored at 2 °C and 95% RH for 12 days. Treatment time of 3 min was found to be optimal under cold storage, as it retained 62%, 52%, and 70% higher catalase (CAT), peroxidase (PO), and superoxide dismutase (SOD) activities, respectively, in treated strawberries relative to control (Fig. 3). This is relevant as the above antioxidant enzymes scavenge reactive oxygen species (ROS) such as superoxide and hydrogen peroxide and reduce them into oxygen and water. As a result, the fruit cells are protected against oxidative damage during stress conditions and natural senescence (Misra et al., 2015). In the absence of ozone treatment, the activity of these enzymes increases until a specific time point and then diminishes gradually as the fruits progress towards senescence (Zhang et al., 2011). But, the treatment with ozone and ultrasound postponed the peak attainment and controlled the reduction in enzyme activity (Fig. 4), thus enhancing the ability of the treated strawberry to catalyze ROS. In a nutshell, this study showed that the combined treatment of ozone and ultrasound for 3 min increased the shelf life of strawberry fruits for up to 12 days under cold storage conditions (Maryam et al., 2021).

2.2.2. *Ozonation of fruit juices for enzyme inactivation*

Different types of fruit juices have been subjected to ozone treatment and the resultant changes in enzyme activity and product quality have been studied by many researchers. Jaramillo-Sánchez et al. (2018) assessed the efficacy of ozone in reducing the activity of enzymes (PO and PPO) in peach juice. In this study, a bubble column was used to apply ozone in doses at different concentrations ranging from 0.06 to 2.48 g/L. Results revealed a non-linear pattern in the reduction of PPO and PO activities with an increase in the treatment time. For example, peach juice ozonated for 1 min showed a substantial reduction in the PPO and PO residual activity at 50% and 20.4% of the initial value, respectively. However, these values decreased to 97.3% and 99.8%, when the treatment time was prolonged to 12 min. This signifies that the enzymatic activity causes the browning of peach juice during the initial stages of processing (Jaramillo-Sánchez et al., 2018).

Another study was performed to evaluate the effect of gaseous ozone on the pectin methylesterase (PME) activity of clarified and unclarified watermelon juice. This is of significance as PME catalyzes undesirable changes in the viscosity, cloudiness, flavor, and color of watermelon juice. Thus, reducing PME activity can restrain the degradation of pectic substances and reduce the loss in viscosity and cloud stability of watermelon juice (Aguiló-Aguayo et al., 2010). Ozone was injected into watermelon juice for 25 min in a sealed compartment. Strikingly, it was observed that the PME activity of both unclarified and clarified ozone-treated watermelon juices increased significantly from 0.359 to 0.57 and from 0.408 to 0.635, respectively. Therefore, it is evident that the ozone treatment is not capable of inactivating PME in the watermelon juices through the increasing ozone processing time. Consequently, after the ozone treatment, a substantial reduction was observed in the cloudiness of watermelon juice, which was ascribed to the increase in PME activity (Lee et al., 2021).

The above observation is consistent with the observation reported in the previous section on the inability of ozone to reduce PME activity in cantaloupe melons. Similar observations as above were also noted in pummelo juice, wherein gaseous ozone at 600 mg/h was dosed into unfiltered and filtered juices for 50 min (Shah et al., 2019). The PME activity of unfiltered juice ranged between 0.02 and 0.10 PME μ/mL and increased with the ozone treatment time. On the contrary, the effect of ozone on the PME activity in filtered pummelo juice was trivial and varied from 0.04 to 0.07 PME μ/mL. Abiotic stress activity and the resulting increase in oxidation due to ozone concentration were stated as plausible reasons for the refuting intensification of PME activity in unfiltered pummelo juice. Ozone competes with organic substances in the fruit pulp to prevent the PME reaction of pectin forming calcium

Table 2
Effect of cold plasma treatment on different enzymes in fruits, vegetablesand juices.

Product	Plasma source	Treatment Condition	Result	Effect on other quality parameters	Reference
Fruits					
Fresh cut Apples	Dielectric barrier discharge	Voltage: 15 kV (peak to peak) from DC source; Frequency: 12.7 kHz; Time: 10, 20 and 30 min; Gas: Air; Flowrate: 1.5 m/s	Decrease in PPO activity; Residual activity of 88, 68 and 42% after 10, 20 and 30 min of treatment.	Plasma treatment did not show any significant effect on Soluble Solids, titratable acidity and color.	Tappi et al. (2014)
Fresh cut Apples	Microwave plasma torch	Frequency: 2.45 GHz, ~1.2 kW power input, 20 L/min gas flow rate	PPO and POD activity reduced around 62% and 65% respectively.	The apple cubes after plasma treatment were found to be softer than control samples.	Buβler et al. (2017)
Fresh cut Melon	Dielectric barrier discharge	Voltage: 15 kVp-p from DC source; Frequency: 12.5 kHz; Time: 15 + 15, 30 + 30 min; Gas: air.	POD Residual activity was 91% and 82% after 15 + 15- and 30 + 30-min treatment respectively. PME residual activity was 94% after 30 + 30 mintreatment.	Plasma treatment did not show any significant effect on TSS, titratable acidity, color or dry matter content.	Tappi et al. (2016)
Vegetables					
Fresh Cut Potato	Microwave plasma torch	Operating at 2.45 GHz, 1.2 kW power input, 20 L/min gas flow rate	PPO and POD activity reduced around 77% and 89%, respectively.	A lower browning index were reported for potato cubes after plasma treatment	Buβler et al. (2017)
Horseradish	Cold plasma jet	Frequency: 1.1 MHz; Operation time:0–360 s; Gas: argon, argon + oxygen(0.01–0.1%); flow rate:5 L/min.	POD activity reduced by 90% and 85% after 180 and 240 s, respectively.	Not given	Surowsky et al. (2013)
Mushroom	Cold plasma jet	Frequency: 1.1 MHz; time:0–360 s; Gas: argon, argon + oxygen (0.01–0.1%); flow rate:5 L/min; Operation time: 0–360 s	PPO was reported to be more stable than POD. A reduction in PPO activity by 90% and POD activity by 85% was reported after 180 and 240 s respectively.	Not given	Surowsky et al. (2013)
Mushroom	Atmospheric plasma jet	18 kVp-p, 10 kHz, 98% Argon+2% Oxygen; flow rate: 5 L/min.	Even though there was an increase in SOD activity with respect to plasma treatment, the treated samples showed a reduced microbial and physicochemical activity resulting in delayed softening and increased shelf life.	The plasma treatment significantly reduced the fungal and bacterial count. No significant effect was observed on color, antioxidant activity or pH of the product.	Xu et al. (2016)
Tomato extract	Dielectric barrier discharge	Voltage: 30–50 kV; Frequency: 50 Hz; time: 0–5 min; Gas: Air	The enzyme activity was found to decrease in a voltage and time dependent manner. Kinetic model of POD inactivation was best described by Sigmoidal logistic function.	Not given	Pankaj et al. (2013)
Juices					
Apple juice	Direct plasma exposure	Voltage: 20 kV; frequency: 20–65 kHz; time: 1–5 min; Gas: air.	The PPO enzyme inactivation was significantly higher for spark discharge plasma treatment than glow discharge plasma at the end of 5 min treatment. A residual activity of 16.6% and 55.5% were respectively observed at the end of each treatment.	There was an enhanced retention of green color and polyphenol content after plasma treatment.	Illera et al. (2019)
Apple juice	Dielectric barrier discharge	Frequency: 50, 200, 400, 600 and 900 Hz; voltage: 20 kV; time: 15 min.	The frequency of 50 Hz has provided a decrease in both PPO (−50%) and POD (−56%) in apple juice.	The plasma treatment increased the total phenolic content of apple slice and apple juice. The antioxidant capacity significantly increased for apple juice but not for sliced apples.	Farias et al. (2020)
Carrot juice	Dielectric barrier discharge	Voltage: 60, 70 and 80 kV; two dielectric quartz plates; time: 3 and 4 min.	The maximum inactivation of enzymes (POD, PPO, LOX and PME) was achieved at 70 kV for 4 min. A residual activity of 11.20, 15.73, 10.21 and 13.42 were reported forPPO, POD, PME and LOX respectively after 70 kV for 4 min treatment.	Plasma treatment have the maximum retention of color, ascorbic acid, total phenols, flavonoids, and tannins as compared to thermal treatment.	Umair et al. (2019)

PPO- Polyphenol oxidase; POD-peroxidase; PAL-phenylalanine ammonia lyase; SOD-superoxide dismutase; APX-ascorbate peroxidase; GR-glutathione reductase; PME -Pectin methylesterase.

pectate complexes (Croak & Corredig, 2006).

2.2.3. Ozone-mediated enzyme inactivation in vegetables

The resourcefulness of ozone in extending the shelf life of horticultural produce is well-established. While the influential parameters are the same as those discussed in the case of fruits and fruit juices, the effects of ozone treatment on enzyme inactivation vary with the vegetable type. Generally, in vegetables, the positive influence of ozone treatment has been observed in increasing the activity of antioxidant enzymes such as SOD and CAT. The roles of these antioxidant enzymes in plants are to establish an antioxidant defense system, eradicate the accumulation of ROS, prevent membrane lipid peroxidation and eventually improve the stress resistance of plants. SOD is most defensive against ROS oxidative damage and eliminates the excessive accumulation of O_2 (Moyo et al., 2012). CAT is capable of decomposing H_2O_2 and reducing its toxicity (Liu et al., 2015). Various vegetables such as tomatoes, cabbage, lettuce, and eggplant have been subjected to ozonation for the control of enzyme activity, of which, some of the recent and pertinent case studies are presented in this section.

Tomatoes (*Solanum lycopersicum*) were sprayed with ozonated water at different concentrations (2–14 mg/L) for 25 days. Compared to the control, the SOD activity in tomatoes increased with the concentration of ozonated water of up to 8 mg/L. Post ozone treatment, the SOD activities in the tomato leaves were 18.7%, 35.4%, 33.0%, 37.0% and 27.4%, respectively, under 2–8 mg/L of ozonated water concentration. Further, the change in the activity of CAT matched with that of SOD (Fig. 5) (Guo et al., 2019). A positive correlation between the activities of SOD and CAT protects the plant against *Meloidogyne incognita* infection by controlling the oxidative stress defense during tomato development (Veronico et al., 2017; Zacheo et al., 1995).

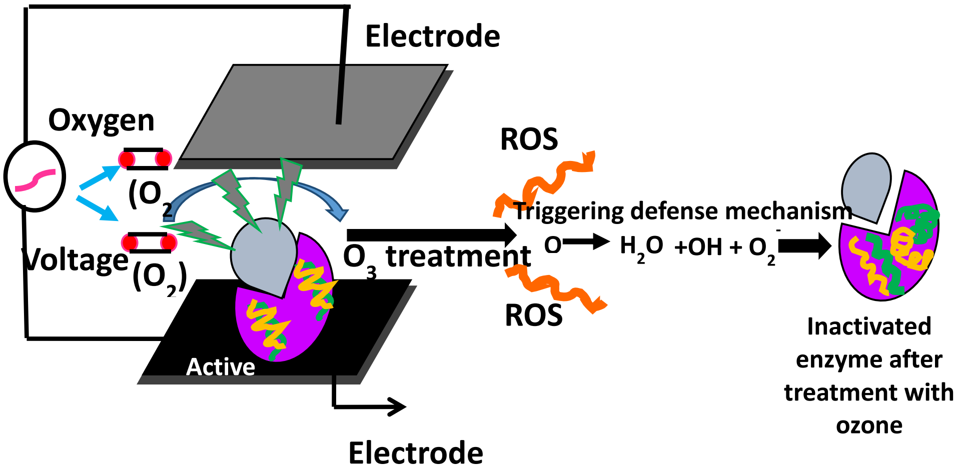

Fig. 1. A schematic diagram of ozone processing system for inactivation of enzyme in fruits and vegetables.

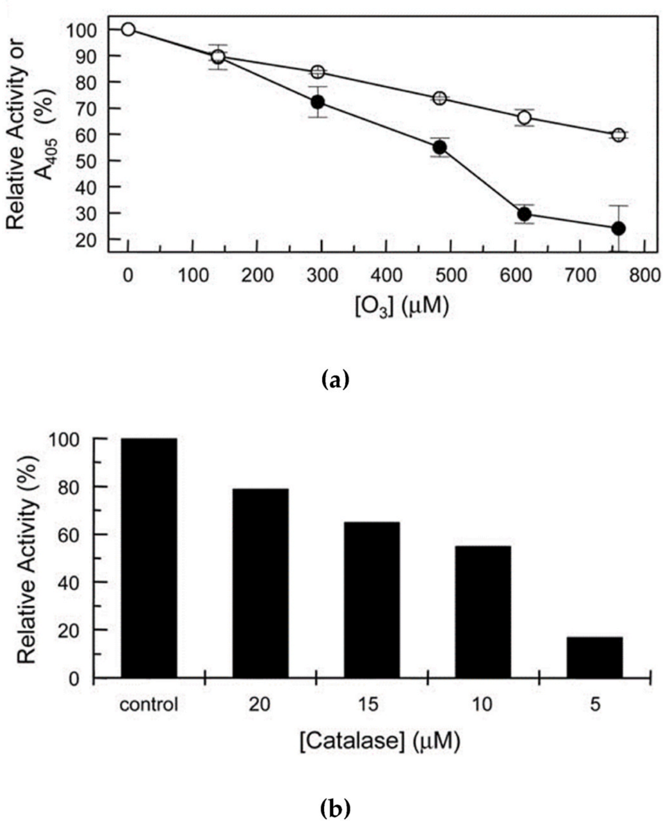

Fig. 2. Ozone-mediated inactivation of catalase: (a) Enzyme activity of catalase after ozone treatment (closed circles) and the hemeabsorption at 405 nm (open circles); (b) Relationship between enzyme activity and catalase concentration at a constant concentration of ozone at 500 μM (Adopted with permission from Lee et al., 2003).

Coriander is prone to quality depletion while storage. Ozone at a concentration of about 0.68 mg/L was used in the treatment of coriander before storing at 20 °C to enhance its postharvest storage quality. Results showed that ozone treatment was effective in retaining the external attributes of coriander, whilst exerting good control over the respiration level and PPO activity. Also, the ozone treatment was capable of preventing the decline in chlorophyll content by inhibiting the activity of chlorophyll degrading enzymes such as chlorophyllase, pheophytinase, and Mg-dechelatase. The findings also suggested that ozone inhibited hydrogen peroxide accumulation and superoxide anion production effectively, thus enhancing the power of ascorbate peroxidase, catalase, and peroxidase enzymes with respect to control coriander samples (Xu et al., 2019).

The influence of interim ozone processing in a gaseous state (10 μL/L; 10 min) on the cell wall quality of tomatoes was assessed by Rodoni et al. (2010). The ozone treatment did not alter the acidity, color, sugar percent, and antioxidant ability, but decreased the fruit degradation and weight loss and caused phenolic compounds to accumulate. Furthermore, softening was delayed in the ozone-treated fruits. Analysis of the cell wall revealed that ozone exposure reduced the solubility of pectin, but not hemicellulose. In ozone-treated fruit, polyuronic depolymerization was also reduced. Although the function of the enzyme that initiates the degradation of pectin namely β-Gal and PG was not altered by the treatments, a strong reduction in PME activity was observed. Findings indicated that short-range processing methods with ozone aided in reducing damage to fruit and excess bruising, which are the two chief attribute that restricts the storage period of tomatoes generally. Reduced disintegration (depolymerization and solubilization) of pectic polysaccharides may be related to the effect of the treatments on fruit softening.

On the other hand, higher SOD activity was detected in eggplant treated with ozone at a concentration of 110 nmol mol^{-1} for 25 days within a phytotron (a greenhouse enclosure). SOD activities improved in the eggplant leaves after 15 days of ozone treatment. In response to the above, the CAT activity also increased during the initial 10 days of ozone exposure, but reduced after 15 days and then improved again untilthe 25th day, which is the end of the storage period. A substantial variation in enzyme activity was observed between the control and treated samples (Fig. 6). Enhanced SOD activity in ozone-treated eggplant is favorable with respect to scavenging ROS (Li et al., 2019). As discussed earlier, the increased activities of SOD and CAT were attributed to theplant's response to oxidative stress. As the superoxide anion is quite unstable, it readily decomposes into H_2O_2 in the presence of SOD (Rao & Davis, 1999).

2.2.4. *Advantages, challenges, and way forward in using ozone for enzyme inactivation in fruits and vegetables*

Ozone treatment has its pros and cons. Effective ozone treatment of horticultural produce is certainly a trade-off achieved between its advantages and limitations. The advantages of ozone based enzyme inactivation include:

a) *Non-residual nature:* When a molecule of ozone deactivates enzymes through the oxidation process, the resultant residual ozone molecule splits into O_2which is harmless. This renders ozone processing as an

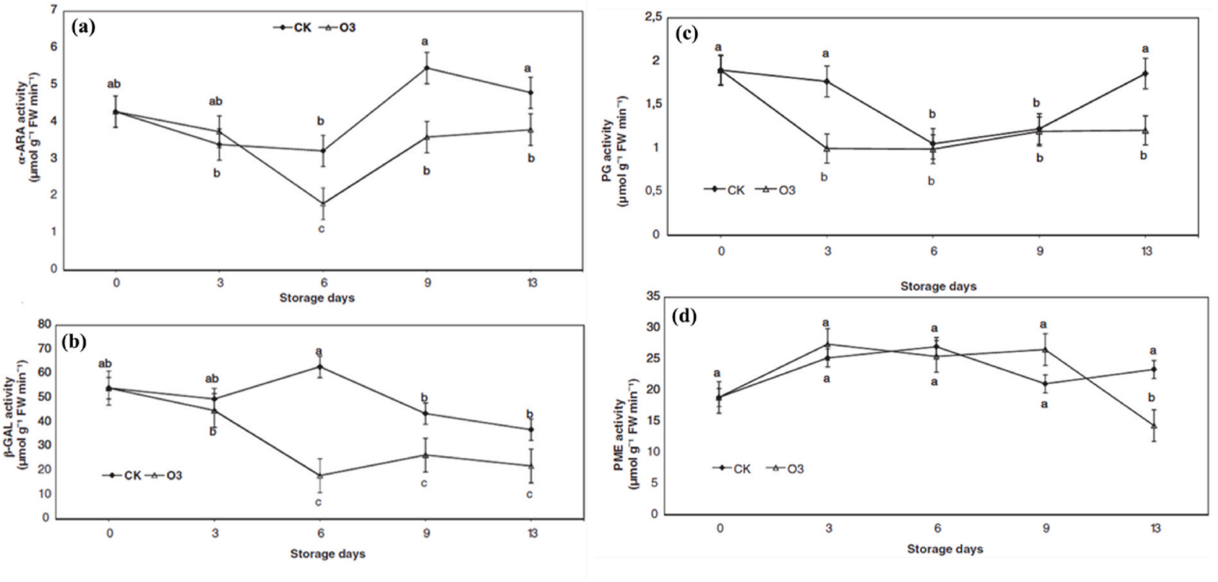

Fig. 3. Effect of ozone treatment on the enzyme activities in melon fruit during cold storage at 6 °C: (a) α-arabinopyranosidase; (b) β-galactopyranosidase; (c) polygalacturonase and (d) pectin methyl esterase activities (Adapted with permission from Toti et al., 2018).

eco-friendly approach for industrial application (Pandiselvam et al., 2019). Thus, the disadvantages associated with free radicals in other non-thermal technologies are not encountered during ozone processing.

b) *Diffusion* capacity through a biological membrane.
c) *Process economics*: Advanced ozone generators can yield substantial ozone concentration at low power consumption. Moreover, these generators are exempted from maintenance and the emission of metallic dust. The above fact promotes an economic output of ozone.

Irrespective of the above advantages, many factors strongly influence the efficacy of ozone treatment and pose limitations when choosing the effective dose of ozone. For instance, the instability of ozone in water containing ozone-resistant molecules such as chlorides or pesticides can lead to incomplete oxidation (Mukherjee et al., 2020). Another downside of the use of ozone in food is its low consumer preference owing to prevailing opinions on its detrimental characteristics. Products formed after degradation by ozonation have not been precisely defined, which is perceived as a major challenge in this technique. Viable and in-vitro epidemiological analyses are often used to monitor the degradation impact on the health of humans and animals. Despite the popularity of ozonation, it is still not as prominent as other widely used technologies because of its high production cost. More studies must be conducted regarding ozone generation in a cost-effective way. For a specific fruit or vegetable, the validation and modelling of ozone treatment process parameters need to be determined. Quality and safety are the major factors that determine the value and acceptance of any fresh produces. The personnel health and safety aspects of ozonation need a comprehensive understanding of the entire range of concentration and exposure time used for food applications. Therefore, the future scope of work concerning ozone treatment is to evaluate the process more technically by applying experimental approaches and innovations along with advancements in the generation of ozone and application units.

3. Cold plasma for the inactivation of enzymes in fruits, vegetables, and fruit juices

3.1. Mechanism of enzyme inactivation by cold plasma

Two different schools of thought exist on the mechanism of enzyme inactivation by cold plasma, related to the amino acids and secondary (2°) structure of the enzymes, respectively. Amino acids are the fundamental units of enzymes, containing amine (-NH$_2$) and carboxyl (-COOH) functional groups with an aliphatic, acyclic, or aromatic side chain (R group). During cold plasma processing, an enzyme is inactivated by the interaction between its constituent amino acids and the plasma-induced reactive oxygen species (ROS) or free radicals (OH, O$_2^-$, HO$_2$,and NO) (Fig. 7). The resultant chemical modifications in the amino acids include oxidation, sulfonation, hydroxylation, amidation and ring opening (Takai et al., 2012). The reactive side chain groups of amino acids are more susceptible to chemical modification by the hydroxyl radicals, superoxide anion radicals, hydroperoxy radicals, and nitric oxide generated from the plasma sources. Particularly, sulfhydryl groups are more susceptible to the reactive species formed during plasma exposure, which leads to the formation of disulfide bonds (Ke & Huang, 2013). In addition, the occurrence of ion bombardment and/or irradiation with radicals and photons, which are taking place during the plasma exposure can lead to chemical degradation of the enzyme constituents (Setsuhara et al., 2013). The ultimate consequence of the above reaction is the loss of enzyme activity (Misra et al., 2016). Specifically, among the different amino acids, the sulfur-containing carbon-chain amino acids are more sensitive to plasma treatment, followed by the aromatic amino acids (ex. tryptophan), five-membered ring amino acids, and basic carbon-chain amino acids (Zhou et al., 2016).

- **Rupture of CNH$_2$**
- **Deprivation of carboxyl group**
- **Catalytic oxidation**

Apart from the chemical modifications mentioned above, *'surface etching'* by reactive species has also been stated as an auxiliary mechanism forthe plasma-induced molecular degradation of amino acids (Setsuhara et al., 2013). Etching occurs due to the adsorption of reactive species (ex. O, molecular radicals (O$_3$), or excited molecules in the metastable state: $^1O_2$ singlet state) on the target entity, here, enzyme, and the consequent formation of volatile compounds such as carbon dioxide and water (Anandharamakrishnan & Ishwarya, 2019). In addition, the etching mechanism may be boosted by UV photons, wherein the photons act in combination with the reactive species to accelerate the inactivation rate (Philip et al., 2002). On the other hand, enzyme inactivation by cold plasma can also be brought about by the disruption of the α-helix secondary structure in the presence of external electric

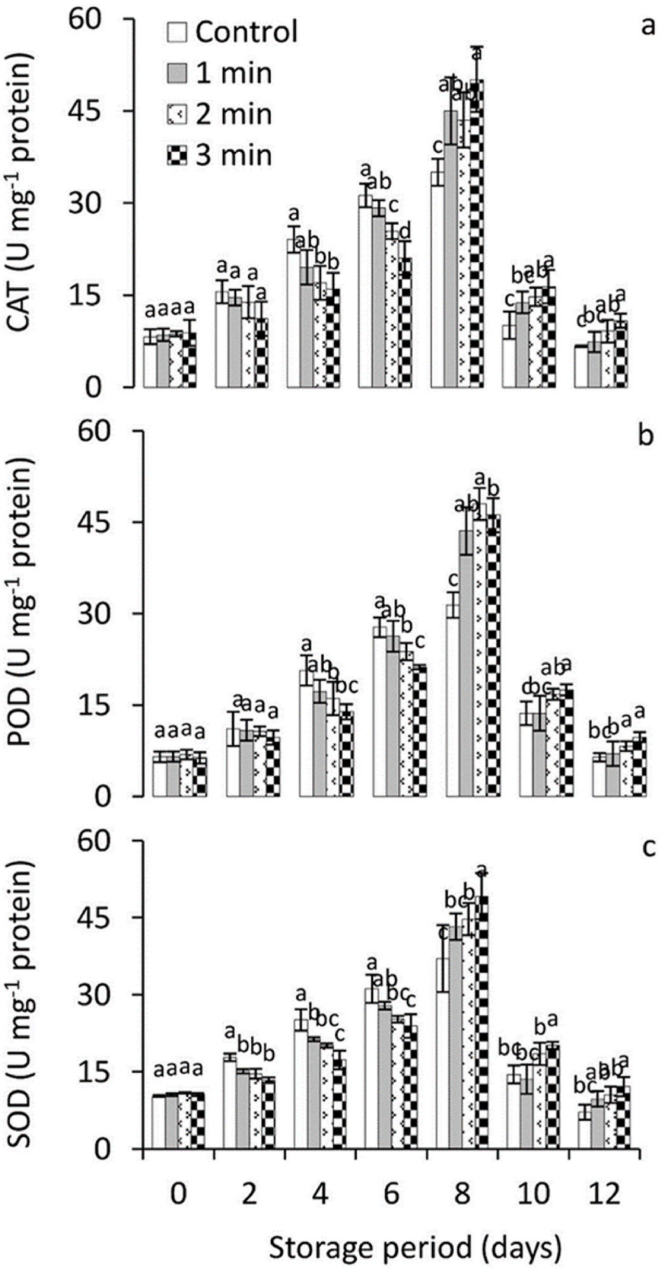

Fig. 4. Synergistic effect of ozone and ultrasound on superoxidedismutase, peroxidase, and catalase activities in strawberry fruits stored at 2 °C (Adopted with permission from Maryam et al., 2021).

fields. This is because proteins exhibit strong dipole moment owing to their α-helix nature (Takaki et al., 2021). In enzymes, cold plasma targets the α-helix and β pleated sheets that constitute the 2° structure. Plasma treatment suppressed the activity of PPO and POD – the key enzymes involved in the browning of fruits, by disrupting their secondary structure. After a treatment time of 360 s, the α-helix content of PPO and POD decreased from 36.9% to 17.8% and 34.9%–5%, respectively. But, the β-sheet content increased from 15.2% to 29.4% and 15.6%–39.9%, respectively (Surowsky et al., 2013). The plasma-induced modifications in the secondary structure of enzymes resulted from the interaction between the protein polymer and the reactive species of plasma (Misra et al., 2016). Analytical techniques such as circular dichroism (CD) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), small-angleX-ray scattering (SAXS), dynamic light scattering (DLS), andnuclear magnetic resonance (NMR) can be employed to study the modifications in the secondary structures of enzymes due to the plasma application (Matsuo et al., 2012).

3.2. *Cold plasma-mediated enzyme inactivation in fruits and vegetables*

Cold plasma can be generated using either of the following devices:

i. Atmospheric pressure plasma jet: In this system, the distance between a needle electrode and a ring or grounded electrode is maintained in the order of millimeters (mm). Voltage is applied between the two electrodes to generate plasmafrom noble gases such as argon or neon. The plasma flame thus generated is in the radiofrequency range with a smaller dimension and greater penetration in narrow gaps.
ii. Dielectric barrier discharge (DBD):This system includes a dielectric material sandwiched between two electrodes. The plasma is generated between the electrodes. The purpose of the dielectric barrier between the electrodes is to avoid the events of electrode etching and corrosion. DBD uses a particular type of alternating current (AC) discharge, which generates cold plasma atatmospheric pressure. The typical AC voltage amplitude and frequency used in this configuration are 1–100 kV and several megahertz (MHz), respectively (Becker et al., 2006; Chirokov et al., 2005).

The other systems for plasma generation include corona discharge and microwave-driven discharge (Anandharamakrishnan & Ishwarya, 2019), which are seldom encountered in the enzyme inactivation applications in fruits and vegetables and hence are not discussed here.As discussed in the previous section on ozone treatment, cold plasma processing for enzyme inactivation is also influenced by multiple factors such as gas composition used for plasma generation, driving voltage, frequency, and the distance between the electrodes. Indeed, plasma chemistry is relatively more complex than the ozone environment.

Zhou et al. (2019) described that cold plasma was more effective than aqueous ozone and UV-Cin increasing the activity of CAT and POD in treated blueberries. Cold plasma increased the peak value of CAT activity to 4.08 U/g on 2nd day, after which it maintained high activity. Moreover, the flesh firmness of cold plasma-treated fruits (51.4%) was much higher than the UV-C (36.1%), and AO-treated counterparts (30.5%), which were attributed to the ability of the former to inhibit the activity of cell wall hydrolyzing enzymes. Suppressed hydrolysis of pectin, hemicellulose, cellulose and the synthesis of lignin postpone the deterioration of fruit firmness (Critzer et al., 2007).

Bußler et al. (2017) applied plasma processed air (PPA) to avoid detrimental browning and nutritional loss in fresh-cut apples and potato tissue. Results of this research indicated that plasma exposure was capable of inhibiting PO and PPO in freshly-cut potatoes and apples. Application of plasma treatment for 10 min decreased the PPO activity of both potato and fresh-cut apple reduced by around 77% and 62%, respectively. Though PO is a highly stable enzyme, it lost its stability after 10 min of exposure to plasma by lowering its activity by 89% and 65% in the tissues of potatoes and fresh-cut apples, respectively.

The plasma treatment of fresh-cut apples using a dielectric barrier discharge (DBD) creator was evaluated by Tappi et al. (2014). Soon after the treatment and the one-day storage period, the key quality attributes and metabolic changes were measured. It is seen that increasing the treatment time from 10 to 30 min linearly decreased the activity of PPO enzymes by around 42%. The authors also observed that a notable decrease in terms of browned areas in treated samples compared to untreated ones (up to about 65% for 30 min and after 4 h of storage). Besides, a substantial drop in browned areas was found in treated samples relative to control samples. By increasing the treatment time, PPO residual activity decreased linearly. Particularly, the exposure to cold plasma tended to decelerate the metabolic processin tissues and thereby mildly impacting the different qualitative attributes.

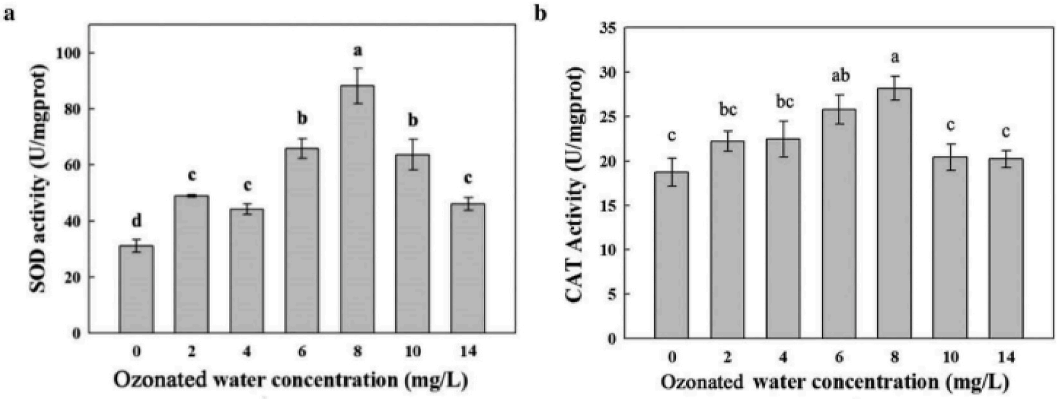

Fig. 5. Effect of treatment with increasing concentrations of ozonated water on the activity of antioxidant enzymes in *Lycopersiconesculentum* leaves: (a) SOD; (b) CAT (Adopted with permission from Guo et al., 2019).

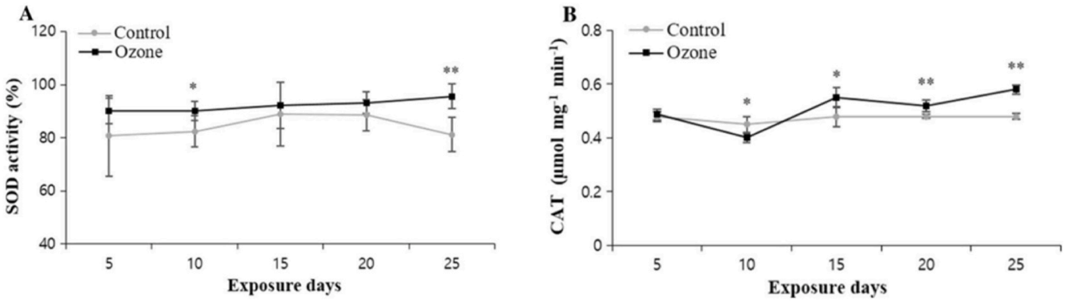

Fig. 6. Effect of ozone treatment on the antioxidant activity of eggplant, measured by the activity of: (a) SOD; (b) CAT (Adopted with permission from Li et al., 2019).

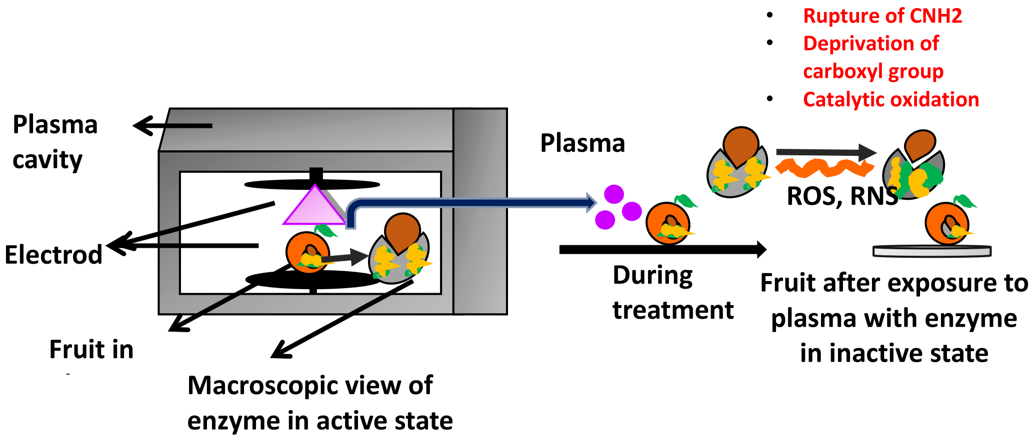

Fig. 7. A schematic diagram of cold plasma processing system for inactivation of enzyme in fruits and vegetables.

Ripened bananas (cut-pieces) were treated with cold plasma for a duration of 1, 1.5, and 2 min to study their effect on PPO activity present in bananas (Gu et al., 2021). Results showed that the activity of PPO in bananas reduced steeply with the extension of plasma exposure time. The PPO activity of bananas was estimated to be 207.40 U (1 min treatment), 185.87 U (1.5 min treatment), and 169.80 (2 min treatment), compared to the untreated sample (271.43 U). Authors reported that during the exposure of bananas to plasma, ROS molecules liberated from plasma could lead to the destruction of reactive amino acid chains present in the enzyme and could result in the destruction of enzyme secondary structures.

A work conducted by Khani et al. (2017) studied the effect of enzyme peroxidases in the extract of fresh-cut tomatoes using DBD. The analyses showed a 90% decrease in the residual activity of peroxidase in tomatoesafter 60 s of air plasma treatment. An overall reduction in enzyme activity of 96.2% was recorded at the end of 6 min. In plasma-treated fresh-cut tomato slices, a similar reduction in enzyme activity was observed. No major color change was detected in the extract of tomato following treatment. The researchers observed that the chemical structure of amino acids may have been altered by the reactive plasma, resulting in decreased enzyme activity.

Another research conducted revealed that exposure to PPA (Plasma Processed Air) was effective in reducing the PO and PPO activity in cut apples and potatoes (Bußler et al., 2017). The authors note that by plasma treatment, browning in potato pieces can be adequately inhibited, whereas browning that occurred during the PPA treatment of apple slices was different from the usual enzymatic browning. The value of pH on the surface of tissue was reduced by 1.5 in both cases, whereas inactivation of the peroxidase enzyme was not seriously affected by dry matter percentage and cell structure in cut apples treated by using a plasma-processed air produced from microwave-assisted plasma discharge.

A reduction by 50% of the PO residual activity in PPA-treated fresh-cut apples was observed after 600 s of exposure owing to structural enzyme alterations. At initial treatment times, however, the authors reported a rise in the browning index (30–75) that finally attained a stable value. The authors also suggested that the rise in the browning index could be responsible for plasma-assisted oxidative deprivation or polymerization of substances before the development of secondary metabolites resulting from exposure to plasma. Similarly, after 6 min of treatment, a decline in PO by 85% had been recorded in a food model unit (Surowsky et al., 2013). The researchers used a plasma jet to analyze the mushroom extracted PO behavior using argon and a combination of argon and O_2 as inlet gases (Xu et al., 2016). A sharp reduction in the peroxidase inactivation was also observed using a diffuse coplanar barrier discharge AAP system (Henselová et al., 2012). The investigators found a 25% reduction in the PO activity in the extract from maize roots after plasmatreatment for 60 s. Arc discharge argon plasma was used to determine the function of the horseradish enzyme in the saline solution prepared with phosphate (Ke & Huang, 2013).

Another work was performed to monitor the impact of plasma on the activity of PO and PME in fresh-cut melons (Tappi et al., 2016). Researchers identified that plasma exposure for 15 + 15 and 30 + 30 min inhibited the activity of PO and PME by 17 and 7%, respectively. Besides, considering the qualitative characteristics of fresh-cut melon, it was observed that plasma treatment had an insignificant effect on the attributes such as titratable acidity, soluble solid content, dry matter, color, and texture. The heat generated during tissue metabolism was reduced in proportion to the period of treatment, whereas a moderate transition to anaerobic metabolism was seen. The activity of PME in freshly sliced melon pieces was lowered using DBD plasma. The researchers highlighted that degradation of the ionic species by plasma compounds is a crucial parameter for the function of enzymes and is the important reason for enzyme inactivation.

3.3. *Cold plasma treatment of fruit juices for enzyme inactivation*

It was found that the enzyme kinetics of inactivation was better defined by the sigmoidal logistic function. Direct plasma exposure had been studied as an alternative non-thermal method to enhance the quality of fresh apple juice by PPO inactivation (Illera et al., 2019). Plasma exposure was carried out at 10.5 kV for 5 min, which achieved about 64–69% of PPO inactivation. Through this study, it was also reported that exposure to plasma enhanced key value attributes of Golden delicious apple juice, with preservation of major quality factors all through prolonged storage trials. Consequently, the above work-established that cold plasma is an effective alternative to conventional thermal methods to retain the quality of fresh cloudy apple juice during its entire shelf-life period. In another study, Umair et al. (2019) reported that the effect of high voltage cold plasma application on enzyme inactivation is significant for carrot juice. In summary, the best conditions for maximum inactivation of PPO, POD, and PME enzymes are a voltage of 70 kV and a treatment time of 4 min.

3.4. *Challenges and way forward in using cold plasma for enzyme inactivation in fruits and vegetables*

Irrespective of the effectiveness of cold plasma in enzyme inactivation as discussed above, certain processing limitations have been identified, such as increased lipid oxidation, decreased color, reduced fruit firmness, and increased acidity. The color values of strawberries were reduced on exposure to atmospheric cold plasma (Misra et al., 2014b). After treatment with atmospheric non-equilibrium plasma for 5 min, the discoloration and wilting reaction in spinach leaves were noticed (Klockow & Keener, 2009). Another significant disadvantage of this process is that, since the plasma effect is a surface phenomenon, using it for the inactivation of enzymes present withinan intact and entire fruit or vegetable is impractical. The other downside is that the use of direct plasma reduces fruit firmness (Misra et al., 2014a). Although studies on the cold plasma-enzyme interactions in selected fruits, vegetables, and model food systems have proved the effectiveness of cold plasma in promoting quality attributes and shelf life extension, the findings cannot be generalized to all types of fresh produces. This is owing to the reason that enzymes are usually bound to cellular organelles in food and biological systems and are abundant in tissues below the food surface (Misra et al., 2016). Furthermore, findings for the inactivation model need to be checked in the actual food environment before making large generalizations. The potential recovery of enzyme activity following treatments is yet another factor that remains unattended and will offer a signal of efficacy with respect to plasma exposure in long term. Thus, it is apparent that the chemistry of plasma largely determines the degree of enzyme activity upon exposure.

The applications of cold plasma have been realized in various areas of the food processing sector. The quantity and stability of energy usage depend on the type of discharge used for cold plasma treatment. Full performance at low operating costs should be optimized based on these criteria for the plasma treatment. Researchers have successfully used plasma to inactivate enzymes in many foods. However, the future focus on obtaining the GRAS status must pertain to the use of plasma in foods. However, extensive research and relevant *in vivo* and *in vitro* testing procedures are mandatory to achieve the above status.

4. Conclusions

From the discussions presented in this paper, it is evident that the studies of yesteryears and recent times have validated the effectiveness of ozone and cold plasma technologies with respect to enzyme inactivation in fruits and vegetables. Yet, the effective inactivation of enzymes in fruits and vegetables using these innovative non-thermal approaches must be tuned further to transform them into effective alternatives for conventional thermal processing methods. Within the scope of this review, we presented the mechanism of action, salient applications, pros, cons, and future direction with respect to the ozonation and cold plasma processing approaches. But, the literature also points to the lacunae that persist in achieving microscopic and molecular level scientific evidence for the different behaviors and degrees of enzyme inactivation in different fresh produce. While the activities of some enzymes are enhanced, those of some are reduced with both ozone and cold plasma, which needs further understanding. The influence of fruit/fruit juice/vegetable matrices concerning the effect of these non-thermal treatments on enzyme activity should be explored in greater detail. In this review, we attempted to emphasize the increasing interest and effectiveness of using cold plasma and ozone technologies for the inactivation of enzymes in fruits, fruit juices, and vegetables. The state of the art discussed in the work is envisaged to instigate the researchers to obtain further technical insights about transforming these two technologies as commercially viable and industrially relevant approaches to extend the shelf-life of horticultural produces. A science-based approach to resolving the challenges involved in these techniques would aid in reducing the postharvest losses of fruits and vegetables, which is of paramount importance to developing economies.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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