Review

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Effects of nonthermal preservation technologies on antioxidant activity of fruits and vegetables: A review

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Abstract

Consumer demand for safe and nutritious fruits and vegetables has given rise to the development of a number of nonthermal food preservation techniques. Recent studies have highlighted that antioxidant activity of fruits and vegetables plays an important role in human health. In this paper, the influences of nonthermal preservation technologies, including pulsed electric field, radiation processing, dense phase carbon dioxide, ozone processing, and edible coatings, on the antioxidant capacity and related compounds in fruits and vegetables are reviewed. The proposed mechanisms and future trends are also discussed to accelerate the further commercialization and exploration of these novel technologies, which will, in turn, help to promote human health.

Keywords

Nonthermal preservation technologies, antioxidant activity, nutrition, mechanism, fruits and vegetables

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INTRODUCTION

The production, consumption, and trade of fruits and vegetables have increased significantly in the past few years. This increase is not only related to their attractive sensorial properties but also because they supply an optimal mixture of biologically active components, such as natural antioxidants, fiber, and other phytochemicals (Yahia, 2010). Intake of fruits and vegetables is positively correlated with a low incidence of chronic disease, which is attributed, in part, to the abundant antioxidants present in edible plants (Ames et al., 1993; Borguini and Ferraz Da Silva Torres, 2009; Giampieri et al., 2012). Antioxidants, such as vitamins, flavonoids, phenolics, and antioxidant enzymes, play a vital role in preventing multiple diseases, including cancers, diabetes, atherosclerosis, and many other autoimmune disorders, in addition to aging by scavenging excrescent free radicals in the body. Thus, great importance should be conferred to these components.

However, the majority of antioxidants in fruits and vegetables undergo a gradual loss during storage, together with the deterioration of sensory and nutritional values after harvest. Appropriate postharvest treatments are needed to preserve the quality and antioxidant compounds of fresh produce.

Although certain thermal postharvest treatments perform well in inactivating microorganisms and spoilage enzymes, which are useful for prolonging the postharvest life of fruits and vegetables, these treatments will often alter their sensorial properties and bioactive compounds. For example, heating processing, especially under severe conditions, may induce some physical and chemical changes that may impair the

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organoleptic properties and reduce the bioavailability or content of several bioactive compounds (de Carvalho et al., 2013; García-Martínez et al., 2013; Kamiloglu and Capanoglu, 2015; Karaman et al., 2014; Ornelas-Paz et al., 2013; Patras et al., 2010). As a consequence of these negative aspects, new preservation methods based on nonthermal techniques, such as pulsed electric fields (PEFs), radiation processing, dense phase carbon dioxide (DPCD), ozone processing, and edible coatings are needed to ensure the safety of fruits and vegetables with "fresh-like" characteristics and to simultaneously meet a desire for improved nutrition and functional characteristics.

Many investigators have examined the influence of nonthermal postharvest treatments on their color, texture, and flavor. However, few studies pertaining to the effects of nonthermal treatments have been performed to study their antioxidant functions (Table 1). This paper provides a critical and detailed summary of the latest advances in correlations between several nonthermal treatments and the antioxidant activity of fruits and vegetables, the possible mechanisms, and the prospective trends in preservation technologies.

PEFs

PEFs have been proposed in recent years as a feasible alternative to conventional food preservation techniques. PEF employs short bursts of electricity, providing safe, fresh-like foods in addition to reducing the loss in quality that can be triggered after thermal processing (Dunn, 2001; Morris et al., 2007). The major parameters of this technology include the electric field strength, treatment time, pulse width, frequency, and polarity. Evidence supports the idea that PEF treatments modify enzymatic activities and reduce microbial populations without excessively degrading the quality attributes and taste of products (Mosqueda-Melgar et al., 2008; Salvia-Trujillo et al., 2011), such as tomatoes, oranges, longans, and apple cider (Azhuvalappil et al., 2010; Zhang et al., 2010). Recent studies paid more attention to the effects of PEF treatment on trophic function improvement, especially the antioxidant capacity and nutrient components (Morales-de la Peña et al., 2010, 2011; Wang et al., 2014).

Antioxidant compound effects

Vitamin C is an essential vitamin that is found in various fruits and vegetables. Its antioxidant properties are helpful in preventing free radical-induced damage to DNA, thereby overcoming related diseases (Fraga et al., 1991; Mares-Perlman, 1997). However, vitamin C decomposes easily under less desirable conditions because of its unstable nature. PEF-treated products exhibited higher vitamin C contents than thermally processed juices during storage in different juices, such as strawberry, tomato, and orange juice (Cortés et al., 2008; Odriozola-Serrano et al., 2008b). This finding might be explained by the mild processing temperatures ($\leq$40 °C) attained during PEF treatment. Moreover, vitamin C retention depended on processing factors. In general, the lower the treatment time, the electric field strength, the pulse width, and the frequency, the better the vitamin C retention in juices (Elez-Martinez and Martin-Belloso, 2007; Odriozola-Serrano et al., 2007).

Phenolic compounds are a large class of natural phytochemicals that can be found in many fruits and vegetables products with powerful antioxidant activities. No significant differences in the total phenolics were observed between PEF-treated and untreated products such as spinach puree, carrot, and tomato juices. Changes in the amounts of individual phenolic acids have also been studied in PEF-treated (35 kV/cm for 1500 µs with 4 µs bipolar pulses at 100 Hz) tomato juices and compared with thermally processed juices (90 °C for 30 or 60 s). Both treated juices underwent a substantial loss of flavonols (kaempferol and quercetin) and phenolic acids (ferulic, chlorogenic, and *p*-coumaric acid) over their storage time at 4 °C. However, their retention in PEF-treated juices was significantly higher than their retention in thermally treated juices (Odriozola-Serrano et al., 2009a). In strawberry juices, no changes in flavonols (quercetin, kaempferol, and myricetin) were observed between treated and fresh juices, while the *p*-hydroxybenzoic content was enhanced significantly and slightly with the same processing (Odriozola-Serrano et al., 2008c).

Anthocyanins are a widely distributed group of phenolics. The pigments are unstable and can be degraded and decolorized by many factors such as the pH, temperature, enzymes, oxygen, and light. In PEF-treated strawberry juice, the anthocyanin content significantly depended on the electric field strength and treatment time (Odriozola-Serrano et al., 2008a). Altuntas et al. (2010) demonstrated that the total anthocyanin stability in sour cherries was retained well after PEF treatments (17–30 kV/cm for 131 µs). Whereas, PEF induced a significant loss of cyanidin-3-glucoside in blood oranges and blackberries, and the degradation increased as the electric field strength increased (Zhang et al., 2007). These changes in anthocyanin contents over the storage time of PEF-treated juices were probably related to the presence of residual enzyme activities such as that of β-glucosidase (Aguiló-Aguayo et al., 2008).

Carotenoids are widespread in a great number of fruits and vegetables. Recent studies showed a higher carotenoid stability in PEF-treated fruit products than

Table 1. Effects of nonthermal technologies (PEF, radiation, DPCD, ozone, edible coatings) on the antioxidant activity of fruits and their products

SNO	Technology	Influencing parameters	Product	Treatment conditions	Major findings	References
1	PEF	• Electric field strength • Pulse (width, frequency, polarity) • Treatment time • Temperature	Watermelon juice	35 kV/cm at 200 Hz for 50 μs with 7 μs biopolar pulses	Lycopene increased by 13%, antioxidant capacity was unchanged, and vitamin C decreased by 28%	(Oms-Oliu et al., 2009)
			Tomatoes	1 kV/cm at 0.1 Hz for 16 μs with 4 μs monopolar pulses	Total polyphenol, lycopene, and antioxidant capacity increased by 36.58, 20.10, and 20%, respectively	(Vallverdu-Queralt et al., 2012)
			Fruit juice-soymilk beverage	35 kV/cm at 200 Hz for 1400 μs with 4 μs biopolar pulses	No changes in total isoflavones but phenolic contents increased by 12.21%	(Morales-de La Peña et al., 2011)
			Sour cherry juice	17–30 kV/cm, 131 μs	Total anthocyanin stability was retained	(Altuntas et al., 2010)
			Strawberry juice	35 kV/cm at 100 Hz for 1700 μs with 4 μs biopolar pulses	No changes in flavonols (quercetin, kaempferol, and myricetin) were observed, whereas p-hydroxybenzoic was enhanced by 13.15%	(Odriozola-Serrano et al., 2008c)
2	Radiation processing	• Irradiation source • Dose and wavelength • Distance of product from radiation source • Exposure time • Temperature	Apple	γ-rays (300 Gy, 600 Gy)	Increased amounts of phenolic content and antioxidant activity were found	(Mostafavi et al., 2012)
			Carrot	UV-B (1.3–12 kJ/m^{-2})	Increased antioxidant capacity (3.2-fold), total soluble phenolics (6.6-fold), and PAL activity (4.9-fold) were observed	(Du et al., 2012)
			Mango	γ-rays (500 Gy) or UV-C (30 min)	Percent loss in phenolic and antioxidant activity during storage was reduced	(Chatha et al., 2013)
			Pineapple, banana, guava	UV-C (2.158 J/m^2, 30 min)	Flavonoid content increased by in pineapple (92.9%), guava (25.2%), and banana (56.9%). No change in phenol content was obtained in pineapple, whereas it was enhanced in guava (51.9%) and banana (86.4%). Antioxidant activity increased in all three fruits	(Alothman et al., 2009b)
			Tomato	UV-B (6.08 kJ/m^2, 60 min)	Phenolic, flavonoid, flavonol concentration increased by 11, 19, 21%. Enhanced antioxidant activity (18%) was observed	(Castagna et al., 2013)
			Mango	Electron beam irradiation (1–3.1 kGy)	No changes in total phenolics and carotenoids but increased flavonol (5.4-fold) contents were observed	(Reyes and Cisneros-Zevallos, 2007)

(continued)

Table 1. Continued

SNO	Technology	Influencing parameters	Product	Treatment conditions	Major findings	References
3	DPCD	• Pressure • Treatment time • Temperature • Product type and penetration	Apple juice	15 MPa, 35 °C, 15 min	Antioxidant activity increased significantly (2.4-fold)	(Porto et al., 2010)
			Red grapefruit juice	13.8, 24.1, and 34.5 MPa, 5.7% CO_2, 40 °C for 5, 7, and 9 min	Losses of antioxidant capacity and ascorbic acid content during storage decreased to 10.3% from 12.8%, and to 9.4% from 15.3%, no changes on total phenolic contents were detected	(Ferrentino et al., 2009b)
			Hami melon juice	35 MPa, 55 °C, 60 min	Ascorbic acid content was 6.4 times than it in untreated fruit after storage	(Zhang et al., 2010)
			Grape juice	34.5 MPa, 8 and 16% CO_2, 6.25 min, 30 °C	Enhanced anthocyanin stability, soluble phenolics, and antioxidant capacity were observed	(Del Pozo-Insfran et al., 2006a)
			Guava puree	30.6 MPa, 6.8 min, 35 °C	No changes on total phenolic contents were detected, whereas antioxidant capacity and ascorbic acid content were enhanced	(Plaza et al., 2010)
4	Ozone processing	• pH • Treatment time • Temperature • Concentration	Pineapple, banana	8±0.2 ml/s, 20 min	Polyphenol and flavonoid content increased by 15.7 and 32% in pineapple, by 8.2% and 14.7% in banana	(Alothman et al., 2010)
			Papaya	3.5 ppm, 96 h, 25±2 °C	Increased ascorbic acid (28.4%), total phenolic (14.3%), beta-carotene (82.2%), and lycopene (52.8%) contents and enhanced antioxidant activity (21.9%) were observed	(Ali et al., 2014)
			Kiwifruit	0.3 ppm, 8, 24, 72, and 144 h	Total carotenoid increase by 2.1, 2.5, 2.8, and 6.8% according to the exposure time of 8, 24, 72, and 144 h	(Minas et al., 2010)
			Tomato	10 ppm, 10 min, 20 °C	The accumulation of phenolic compounds increased by 50%	(Rodoni et al., 2010)
			Strawberry	0.35 ppm, three days, 2 °C	Ascorbic acid content was three times than that of control fruits at the end of storage	(Perez et al., 1999)
5	Edible coatings	• Encapsulating material • Bioactive compound • Temperature	Mango	Alginate + ascorbic and citric acid	Enhanced ascorbic acid (3-fold) was observed, phenols content and antioxidant potential increased by 66 and 61%	(Robles-Sánchez et al., 2013)

(continued)

Table 1. Continued

SNO	Technology	Influencing parameters	Product	Treatment conditions	Major findings	References
			Navel oranges	Carboxymethyl cellulose + *impatiens balsamina* extraction	SOD, CHI, GLU activities, and ascorbic acid content were upgraded by 62.5, 28.6, 102, and 22.2%, respectively	(Zeng et al., 2013)
			Papaya	Polysaccharide + trans-cinnamaldehyde	Losses of vitamin C and total carotenoids were decreased	(Brasil et al., 2012)
			Longon	Chitosan + nano-silica	POD activity decreased by 32.75%, vitamin C loss rates decreased from 22.9 to 14.2%	(Shi et al., 2013)
			Pear	N-acetylcysteine and glutathione + gellan, alginate, or pectin	Total phenolic content increased by 25%, vitamin C loss rates decreased from 47.8 to 15.9%	(Oms-Oliu et al., 2008a)

DPCD: dense phase carbon dioxide; PEF: pulsed electric fields.

in their heat-treated equivalents (Zulueta et al., 2010). Higher concentrations of lycopene and β-carotene have been consistently found in PEF-processed strawberry and tomato juices, respectively, than in untreated juices (Odriozola-Serrano et al., 2008a, 2009a). However, some of the results obtained by different authors are controversial. For instance, a significant decrease was reported in the total carotenoids of orange juice when a bipolar treatment of 30 kV/cm for 100 μs was applied (Cortés et al., 2006). The major cause of carotenoid losses in fruit products could be the oxidation of highly unsaturated structures with an extensive conjugated double-bond system.

Antioxidant capacity effects

Few studies have examined the effects of PEF technology on the total antioxidant activity of fresh products. The antioxidant capacity of watermelon juice was not influenced by treatment conditions consisting of 35 kV/cm field strength at 250 Hz for 2050 μs with a 7 μs pulse width. However, it was significantly reduced when the frequency was decreased to 50 Hz and the pulse width was 1 μs (Oms-Oliu et al., 2009). This finding indicated that the antioxidant capacity retention rose when both the pulse width and frequency increased. This result was contrary to the result described for vitamin C retention above. This contradiction may be explained by the fact that the antioxidant activity is not only related to vitamin C content but also to other compounds such as polyphenols and carotenoids. In addition, bipolar treatments were substantially more helpful than monopolar treatments in maintaining the antioxidant activity of watermelon juice, which was consistent with other studies that were performed in strawberry and tomato juices (Odriozola-Serrano et al., 2009b). In addition, a correlation analysis between the total antioxidant capacity and various related components indicated that the antioxidant activity of PEF-treated watermelon juice was primarily attributed to the lycopene content ($R^2 = 0.964$). By contrast, significant reductions in the antioxidant capacity were observed in PEF-treated (36 kV/cm for 100 μs in 3 μs pulses at 400 Hz) blueberry juices (Barba et al., 2012). These differences in antioxidant capacity retention may be explained by the diverse parameters of PEF treatments, and food properties, such as the not-quite-identical pH values of various fruits and vegetables.

Apart from using PEF for preservation purposes, it is also used to increase metabolite extraction yields from plant foods (López et al., 2009; Puértolas et al., 2013). However, the application of PEF is still limited and the focus is always confined to a limited number of products. Hence, this field still requires further

investigation, particularly with regards to its impacts on the nutritive values of fruits and vegetables.

RADIATION PROCESSING

Irradiation treatment is a valid storage method in which food is exposed to ionizing or nonionizing radiation. The source of ionizing radiation could be γ-rays, X-rays, or high-energy electrons, which carry higher energy to ionize molecules or atoms. Nonionizing radiation is represented primarily by microwaves, infrared, visible light, and ultraviolet rays (UV-A, UA-B, and UA-C).

Prolonging the postharvest life

The effects of radiation depend on many factors, such as the specific wavelength, irradiation dose, process time, temperature, and the peculiarities of the basic raw material (Bhat and Sridhar, 2008; Bhat et al., 2007; Hadjok et al., 2008). An appropriate combination of these factors contributes to a delay in the senescence process and ripening (González-Aguilar et al., 2007), reducing or eliminating food-borne pathogens (Pala and Toklucu, 2011; Sommers et al., 2010b), inducing natural defenses, and maintaining the food quality in terms of its color, flavor, nutrition, and taste (Alothman et al., 2009a).

Applying UV radiation to apples, oranges, guava-and-pineapple juice and applying γ-rays to pomegranates reportedly have destructive effects on microorganisms (Caminiti et al., 2010; Keyser et al., 2008). This technology would be more effective when combined with other technologies and antimicrobials (Sommers et al., 2010a, 2010b). Inhibition was primarily caused by the induction of plant defense mechanisms and DNA damage (Charles et al., 2009), which destroyed the reproductive capabilities and other functions of the cell (DeRuiter and Dwyer, 2002). In addition, membrane and photophysical destruction were hypothesized as the reasons for microbial death (Fine and Gervais, 2004; Krishnamurthy, 2006).

Effects of the antioxidant activity of fruits and vegetables

Radiation influences the antioxidant status by changing the accumulation of antioxidant bioactive compounds (polyphenolics, vitamin C, flavonoids, etc.) or the activity of antioxidant-related enzymes. These effects vary with the energy carried by radiation, the time of exposure, the treated product, and other factors.

Ultraviolet is the most frequently used radiation for preservation of fruits and vegetables. Alothman et al. (2009b) found a significant increase in the flavonoids and phenols of guavas and bananas after 30 min of exposure to UV-C (at an average dose of 2158 kJ/m^2) in a time-dependent manner, which was contrary to the decrement of vitamin C. Similarly, fresh-cut mangoes treated with UV-C for 0, 10, 20, and 30 min exhibited a rise in their phenolic contents and flavonoid compounds with increasing treatment time, while both ascorbic acid and β-carotene were decreased (González-Aguilar et al., 2007). Avena-Bustillos et al. (2012) investigated the effects of UV-B treatment (1.3–12 kJ/m^2) on the total soluble phenolics and antioxidant capacity of carrots. In their study, the antioxidant capacity increased significantly and differed with both the styles of cut carrots and the UV-B dose level. This finding was correlated directly with higher phenolic contents, which indicated that the enhancements were caused by an increase in phenolic contents. There are still many studies that have demonstrated that applications of UV-B irradiation to fruits and vegetables, such as tomatoes (Castagna et al., 2013; Liu et al., 2011), blueberries (Eichholz et al., 2011), carrots (Du et al., 2012), and blackcurrants (Huyskens-Keil et al., 2012), could enhance the total soluble phenolics in treated produce. Ultraviolet radiation generally induces negligible or subtle losses of bioactive compounds because it does not substantially raise the temperature during processing.

γ-rays appeared to be better at controlling the antioxidant activity of mangoes in comparison with UV-C, even though the decline in polyphenolic substances during storage can be controlled by both types of radiation (Chatha et al., 2013). Increases in phenolic and antioxidant activity were observed in apples that were treated with γ-rays at suitable doses, but a dose higher than 900 Gy had the opposite effect (Mostafavi et al., 2012). In addition, the anthocyanin content of grape pomace increased with the γ-ray irradiation dose, with an optimum level of 6 kGy (Ayed et al., 1999). Conversely, a significant reduction in the individual and total anthocyanin contents was observed in pomegranate juice after γ-ray treatments at doses ranging from 3.5 to 10 kGy (Alighourchi et al., 2008). The irradiation effects on anthocyanin pigments depend to some extent on the nature of anthocyanin. For instance, diglycosides are relatively stable in comparison with monoglycosides toward irradiation (Arjeh et al., 2015).

Electron beam irradiation was also used to maintain the sensory quality and for enhancing the antioxidant capacity of fruits and vegetables. This treatment was beneficial for retaining high levels of ascorbic acid, β-carotene, and total sugars without any significant influence on the sensory properties of sun-dried apricots, at 1–3 kGy doses (Wei et al., 2014). Electron beam irradiation (1–3.1 kGy) varied in effectiveness toward different bioactive compounds in mango

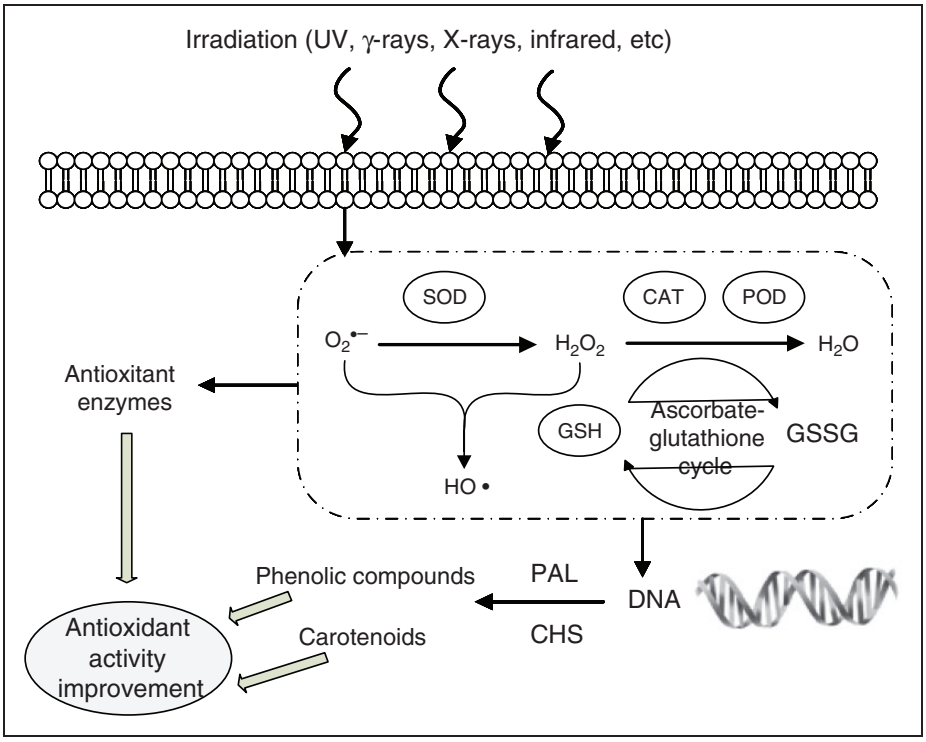

Figure 1. Hypothetical model representing possible mechanism of antioxidant potential improvement by radiation.

(Reyes and Cisneros-Zevallos, 2007). The total phenolics were not affected by the irradiation dose, and the flavonols in treated fruit (at 3.1 kGy) increased significantly after an 18-day storage period. By contrast, the ascorbate content decreased when the dose exceeded 1.5 kGy. In addition, no major change in the carotenoid content was recorded.

It is quite evident that, apart from the application of radiation processing for microbial safety, this novel technology has some potential in enhancing health-promoting compounds. The mechanism through which radiation improves antioxidant potential has been established by several authors (Figure 1). First, radiation exposure acts as an abiotic stress, and it can affect the pathways involved in secondary metabolite biosynthesis, which principally contain phenolic compounds, and thus the cells are protected from environmental stimuli (Cisneros-Zevallos, 2003; Mercier et al., 1994; Stevens et al., 1996). Second, when an adequate dose of irradiation is absorbed by biological tissue, it can interact with atoms or molecules, and then produce primary reactive oxygen species (ROS) (mainly $O_2\bullet^-$). These ROS then trigger a cascade of reactions that result in the formation of a variety of ROS and the accumulation of antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH), maintaining redox homeostasis (Kovacs and Keresztes, 2002). In addition, the ascorbic acid degradation that occurs during radiation may be caused by free radical formation. Third, a hormetic dose of radiation may inflict repairable damage to DNA, and this slight trauma will activate repair mechanisms for DNA damage, which are correlated with the gene expression of enzymes, such as phenylalanine ammonia lyase (PAL), chalcone synthase, and stilbene synthase. These enzymes are helpful in the accumulation of phenolics, carotenoids, and procyanidin contents (Cantos et al., 2000; El Ghaouth et al., 2003; Shama and Alderson, 2005). However, antioxidant compound degradation may be induced by radiation at the same time. Thus, the change in antioxidant activity in treated fruits and vegetables is a result of the above-mentioned synthetic action.

DPCD

DPCD is a collective term for liquid CO_2 and supercritical CO_2 or highly pressurized carbon dioxide. As a nonthermal alternative to heat pasteurization, and as a continuous processing system, DPCD employs pressure in combination with carbon dioxide (CO_2) to maintain food quality, and it has attracted more and more interest in food industries around the world. The pressure commonly used here is less than 90 MPa, which is far from that used for high pressure processing applications (400–600 MPa) (Damar and Balaban, 2006). The unique properties of CO_2 make it an appealing medium for preservation. It is nontoxic, inexpensive, readily

available, nonflammable, and is generally recognized as safe. Moreover, it denotes phases of matter that remain fluid and are still dense with respect to gaseous CO_2. Finally, the low viscosity ($3–7 \times 10^{-5}$ Pa s) and zero surface tension of CO_2 in its supercritical state can help it to penetrate complex structures and porous materials quickly (Zhang et al., 2006).

Microbial inactivation

DPCD works well for inactivating different microorganisms in liquid food, which allows it to retain fresh-like physical, sensory, and nutritional properties without being exposed to the adverse effects of heat. This finding was demonstrated for the first time by Fraser (1951). When compared with many other gases, such as N_2, C_2H_4, Ar, and tetrafluoroethane for its microbial reduction capability, CO_2 was found to be more effective in terms of gas sorption and desorption by cells (Debs-Louka et al., 1999; Dillow et al., 1999). Garcia-Gonzalez et al. (2007) reviewed the DPCD influences on microbial inactivation. The first fresh product study using DPCD was performed on fresh honeydew melons, strawberries, and cucumbers in 1989, to inhibit surface mold growth (Haas et al., 1989). However, the severe tissue damage caused in solid food, even at low pressures, limits its application primarily to liquid products.

Microbial inactivation by DPCD has been reported for various fruit products such as grape juice (Del Pozo-Insfran et al., 2006b; Gunes et al., 2005), apple cider (Ferrentino et al., 2009a; Gunes et al., 2006; Liao et al., 2007), orange (Kincal et al., 2005), and mandarin juice (Lim et al., 2006). The mechanism of action was studied by researchers and a number of hypotheses have been proposed to explain its effects, including a decrease in the cytoplasmic pH, cell membrane modification, explosive cell rupture from internal pressure, the inactivation of key enzymes for cell metabolism, and the extraction of cell wall lipids and intracellular substances. None of these changes have been shown to be responsible for the microbicidal effects of dense phase CO_2 alone, but it may be synergistic for many of these effects (Garcia-Gonzalez et al., 2007; Spilimbergo and Bertucco, 2003). Significant lethal effects on microorganisms are elicited by many factors, such as the water content within the cell, the water activity of the food, pressure, temperature, and the cell growth phase (Calix et al., 2008; Kumagai et al., 1997; Spilimbergo et al., 2002).

Effects on antioxidant compounds

Because CO_2 and its highly reactive constituent species have a profound influence on antioxidant components and enzymes in food system, it is necessary to consider them alongside microbial inactivation. However, there are only limited studies available in the literature regarding DPCD effect on the antioxidant activity of fruits, which should be evaluated on a case-by-case basis for different foods.

Porto et al. (2010) investigated the effects of DPCD treatments at 15 and 25 MPa for 15 min at 35 °C on the antioxidant activity of apple juice. The results showed a statistically significant increase in comparison with untreated samples. In addition, the antioxidant activity of the 25 MPa DPCD sample was significantly higher than that of 15 MPa DPCD. This finding could be explained by the fact that the higher pressure resulted in higher polyphenol oxidase (PPO) inactivation when dense phase CO_2 is applied to apple juice, which means that a larger amount of reduced polyphenols is conserved (Gui et al., 2006b). Meanwhile, DPCD has been showed to change the isoelectric profiles and protein patterns of PPOs and cause conformational changes in their secondary structures (Chen et al., 1992, 1993). In addition, high pressure has also been reported to cause conformational changes in proteins, such as enzyme molecules (Suzuki and Taniguchi, 1972).

Ferrentino et al. (2009b) reported the influence of DPCD (13.8, 24.1, and 34.5 MPa, 5.7% CO_2, 40 °C for 5, 7, and 9 min) treatments on the total antioxidant activity, ascorbic acid, and phenolic contents of red grapefruit juice over six weeks of storage at 4 °C. The antioxidant capacity and ascorbic acid content were retained better after treatment than in the untreated samples regardless of the storage time, and the total phenolic content was not affected by treatment. Similar results were observed by Plaza et al. (2010), when guava puree was exposed to a DPCD treatment of 30.6 MPa for 6.8 min at 35 °C. Furthermore, Zhang et al. (2010) achieved a satisfactory retention of ascorbic acid in DPCD-treated Hami melon juice in comparison with untreated samples at the end of refrigerated storage, even though the concentration always decreased over time. This decrease could be explained by the fact that ascorbic acid is easily oxidized when oxygen is present in the environment; it has a higher stability at lower pH values resulting from the dissolution of CO_2 in the aqueous part of food. In addition, the same authors performed a separate study comparing DPCD with conventional thermal processing, and they concluded that high temperature short time pasteurization (90 °C for 60 s) could also inactivate microorganisms in melon juice, but it caused significant losses in ascorbic acid contents (Chen et al., 2009).

DPCD also appeared to prevent losses in other potential antioxidant compounds such as anthocyanin and β-carotene. An enhanced anthocyanin stability and

better retention of β-carotene were observed in DPCD-treated grape (Del Pozo-Insfran et al., 2006a) and melon juices (Chen et al., 2009), respectively. The underlying mechanism is difficult to establish. However, their stability has been demonstrably governed by the extrinsic control parameters of the pressure and CO_2 concentration gradient and is dependent on the PPO inactivation potential of the treatment (Gui et al., 2006a; Pozo-Insfran et al., 2007).

To date, the literature about antioxidative influences from DPCD has been focused on vitamin C, carotenoids, and total antioxidant activity, and there is a lack of information on the behavior of other phytochemicals or individual polyphenols. In addition, in comparison with individual applications, the combined effects of DPCD with other emerging technologies were better (Ortuño et al., 2013). Limited recent reports about these effects may be explained by practical constraints, such as high costs and engineering problems.

OZONE PROCESSING

Ozone (O_3) is formed from the rearrangement of atoms when oxygen molecules are subjected to UV light or high-voltage electric discharge (Kim et al., 1999). This product inhibits microorganisms through oxidization, and residual ozone spontaneously decomposes to nontoxic products (oxygen), making it an environmentally friendly antimicrobial agent to use in the food industry. For this reason and because of its high biocidal efficacy and wide antimicrobial spectrum, ozone can be a promising candidate for eliminating the practical concerns caused by traditional chemical treatments because of increasingly poor control over a spectrum of spoilage organisms and potentially harmful by-products and residues (Tzortzakis et al., 2007). The recent FDA approval of ozone as a direct food additive has also facilitated its applications to various fruits and vegetables (Miller et al., 2013). Ozone can be applied as either an aqueous or a gaseous phase and has favorable effects that prolong storage life and delay ripening and senescence in various fruit and vegetable products (Mukhopadhyay and Ramaswamy, 2012; Zhao et al., 2013).

Ozone is believed to cause physiological injuries or the loss of antioxidant compounds because of its strong oxidizing activity. However, the overall situation seems complicated and its impacts on the antioxidant and antioxidant capacity vary in a different manner because of broad variations in test conditions and the physicochemical nature of food.

Effects on antioxidant compounds

The effects on ascorbic acid were as follows: A variety of interactions between ascorbic acid and ozone have been reported. Tzortzakis et al. (2007) found that ozone enrichment treatment (0.05 or 1.0 ppm O_3, 13 °C for 12 days) resulted in no significant changes in the ascorbic acid content of tomato. Ali et al. (2014) observed a significant increase in the ascorbic acid in papaya after 10 days of storage upon exposure to 3.5 ppm ozone for 96 h at 25 ± 2 °C in comparison with a control. The increased ascorbic acid content in ozone-treated papaya was also observed in ozone-treated strawberries. Perez et al. (1999) reported that the ascorbic acid contents of strawberries increased significantly after exposure to 0.35 ppm ozone for three days at 2 °C. This increased ascorbic acid under ozone treatment is presumably caused by the activity inhibition that was caused by ozone in several enzymes such as ascorbate peroxidase (POD) and ascorbate oxidase. Ascorbate oxidase is a copper-containing enzyme and is responsible for the enzymatic degradation of ascorbic acid, and ascorbate oxidase oxidizes ascorbic acid to dehydroascorbic acid. Moreover, Perez et al. (1999) have also suggested that ozone stress may lead to the biosynthesis of ascorbic acid by employing carbohydrate reserves. By contrast, Alothman et al. (2010) detected a decrease in the ascorbic acid contents of pineapples, bananas, and guavas that were treated with ozone (8 ± 0.2 ml/s, exposures for 0, 10, 20, and 30 min at an ozone generation time of 1 min). Zhang et al. (2005) concluded that the higher ozone concentrations allowed for lower ascorbic acid retention. Therefore, the decreased ascorbic acid may be caused by the induction of ascorbate oxidase activity caused by high ozone at injurious concentrations and the scavenging of the free radicals that formed during ozone decomposition. The ascorbic acid content depends on the efficiency ratio of its biosynthesis and oxidation, which may be related to the different reactions of various plants under ozone stress.

The effects on carotenoids were as follows: No significant changes in beta-carotene, lutein, and lycopene contents were observed in tomatoes following ozone treatment for 12 days at 13 °C (0.05 or 1.0 ppm O_3) by Tzortzakis et al. (2007). Interestingly, a significant increase in the carotenoid content (including lycopene) after 24 h of exposure to ozone-enriched air was noted in their research. Ali et al. (2014) also reported an increase in the beta-carotene and lycopene contents of 2.5 ppm ozone-preconditioned (96 h) papaya fruits at day 10 in comparison with the control. Similar results were obtained that the total carotenoid contents of kiwifruits were increased by 2.1, 2.5, 2.8, and 6.8% upon exposure to 0.3 ppm of gaseous ozone for 8, 24, 72, and 144 h, respectively (Minas et al., 2010). One possible explanation for this finding is that a specific ozone concentration may induce carotenoid synthesis through the activation or inhibition of a

related enzyme. Chauhan et al. (2011) reported that the total carotenoid content of ozone-treated sliced carrots decreased by 1.7% when compared with that of the control. (The samples were ozonized in water (1:2 w/v; 200 mg O_3/h) for 10 min and stored under controlled atmospheric conditions (2% O_2, 5% CO_2 and 93% N_2) at 6 ± 1 °C and 85% RH for up to 30 days.) Ali et al. (2014) suggested that the reduced beta-carotene and lycopene subjected to high concentrations and long exposures of ozone may be explained by the oxidative cleavage of carotenoids leading to the production of abscisic acid and their antioxidant mechanisms to scavenge free radicals.

The effects on polyphenols were as follows: Published results on the impact of ozone on polyphenol contents are still contradictory. Ali et al. (2014) detected a significant increase in the total phenolic contents of all treated papaya after four days in response to ozone treatments (1.5, 2.5, 3.5, and 5 ppm) for up to 10 days of ambient storage. Similarly, applying 10 ppm ozone for 10 min induced the accumulation of phenolic compounds with a 50% increase relative to the control after six days of storage at 20 °C in tomato (Rodoni et al., 2010). Alothman et al. (2010) also revealed that the polyphenol contents of both bananas and pineapples increased significantly in response to up to 20 min of ozone treatment whereas it decreased inversely in guava fruit with the treatment time. This reaction to the ozone treatment could be attributed to inhibited PPO activity, which was indirectly shown by Zhao et al. (2013). Activated PAL is one of the key enzymes that are involved in synthesizing phenol compounds in plant tissues, and this finding has been indirectly validated by other research (Gonzalez-Aguilar et al., 2007). The increased phenolic contents might also be caused by a cell wall modification that occurred during ozone exposure, which may release some of the conjugated phenolic compounds in the cell wall. The phenolic compounds can scavenge the by-products of ozone decomposition (hydroperoxyl, hydroxyl, and superoxide radicals) in fruits and vegetables to different extents, and it may lead to a reduction in phenolic contents after long-lasting or high concentrations of ozone exposures.

The effects on anthocyanins were as follows: Reports regarding ozone's effects on anthocyanin contents are still limited and controversial. Perez et al. (1999) revealed that the anthocyanin contents of ozonated strawberries (0.35 ppm at 2 °C for three days) were significantly lower than the contents of untreated fruits. Similar results were found in blackberry fruits that were stored for 12 days at 2 °C in a 0.3 ppm ozone atmosphere (Barth et al., 1995); a sharp decrease in the anthocyanin levels of ozonated blackberries was determined after four days of storage. Anthocyanin degradation is the result of the strong oxidizing potential of ozone. By contrast, Alexandre et al. (2012) observed that after 13 days of storage at 4 ± 1 °C, ozonated strawberries (0.3 ppm in aqueous phase at 15 ± 2 °C) preserved 82% of the anthocyanins on average (when compared with fresh samples), while untreated and water-washed samples only retained 55% under refrigerated storage.

Effects on the antioxidant capacity

The impact of the antioxidant activity depends on the composition and various changes in the antioxidant compounds as induced by ozone, which may be related to different product properties. Ali et al. (2014) attributed the enhanced antioxidant activity of papaya fruits to increased phenolic compounds from ozone exposure (1.5, 2.5, 3.5, and 5 ppm) for 96 h from day 4 until day 8. Alothman et al. (2010) observed an increased antioxidant activity in both pineapples and bananas after ozone exposure (8 ± 0.2 ml/s) for up to 20 min, but the activity tended to decrease after 30 min of treatment. It is proposed that the increased antioxidant capacity was at an expected level for the increase in total phenolic contents in the two fruits. For guava, the antioxidant capacity of the fruits decreased with the increased treatment time, which was explained by the sharp decrease in the vitamin C contents of guava fruits.

Ozone induces oxidant stress in fresh product when it comes in contact with plant tissue and induces various physiological responses, including the synthesis of antioxidants, polyamines, ethylene, phenolic compounds, and other secondary metabolites (Forney, 2003). Because of wide variations in test conditions and the physicochemical nature of foods, controversial results relating to antioxidants and antioxidant activity were demonstrated. In summary, ozone treatment can prolong storage life and delay the ripening and senescence of various fruit and vegetable products. The impact of ozone on the physiology and quality of fruits and vegetables varies according to the chemical composition of food, the ozone dose, and the application type and time (Rawson et al., 2011), and thus require further investigations to ascertain a proper application.

EDIBLE COATINGS

Edible coatings play an important role in the distribution, marketing, and conservation of fruits and vegetables, depending on their potential to improve shelf life and maintain sensory, microbiological, and nutritional qualities. This envelope can efficiently delay the senescence of produce by creating a modified atmosphere between the fresh product and the surrounding atmosphere. This coating acts as a semipermeable barrier to

retard solute and moisture migration; to reduce gas exchange, respiration, and oxidative reaction rates; and to suppress physiological disorders in fruits and vegetables (Falguera et al., 2011; Guillén et al., 2013).

Traditional edible coatings change the antioxidant activity of fruits and vegetables

The traditional materials that are used to make edible coatings are based on polysaccharides (such as chitosan, starch, alginate, pullulan, pections, and cellulose derivatives), proteins (such as soybean protein, zein, caseinate, gelatin, and collagen), lipids (such as natural wax and acetylated monoglycerides), or their combinations, which can take advantage of each compound. Applying this film can be helpful for extending shelf life and preserving quality during the postharvest life of fruits and vegetables. Among them, chitosan and its derivatives show the broadest applications on account of their physicochemical properties such as null toxicity, biodegradability, biocompatibility with human tissues and particularly their antifungal and antimicrobial properties (Aider, 2010). These materials also have the potential to induce defense-related enzymes, such as POD and PAL. Table 2 summarizes some of these compounds and their effects on food quality, especially antioxidant activity changes.

A new type of edible coatings change the antioxidant activity of fruits and vegetables

In recent years, bioactive compounds have been incorporated into edible coating technology to optimize fresh food quality by enhancing the shelf life and nutritional quality, increasing consumer acceptance and other factors (Tajkarimi et al., 2010). Among the most often-used bioactive compounds are antimicrobials, antioxidants, flavors, probiotics, and nutraceutical substances (Ayala-Zavala et al., 2011) (Table 3), which can be found in a large number of animals, plants, microorganisms, and marine organism species, and they are obtained by extraction and biotechnological methods (Kris-Etherton et al., 2002). The incorporation of nutraceuticals into coating material provides many advantages in fruits and vegetables preservation, for example, by offering protection against high temperatures, bright lights, reducing the risk of pathogenic microorganism growth on food surfaces, and providing functional products with health benefits to consumers (Azarakhsh et al., 2014; Kayaci and Uyar, 2012; Mantilla et al., 2013; Oliveira et al., 2012; Perdones et al., 2012; Wu and Chen, 2013).

Some studies have concentrated on the influence of edible coatings as carriers of bioactive compounds on the antioxidant activity of fruits and vegetables, and there have been some positive results. Robles-Sánchez et al. (2013) reported that the incorporation of ascorbic and citric acid into alginate-based coating formulations was effective in enhancing the nutritional quality, preserving the color of fresh-cut mangoes, and increasing the antioxidant potential of fruit cubes. Zeng et al. (2013) amended carboxymethyl cellulose coating with an extract from *impatiens balsamina* stems, and they found that the novel coating had a beneficial impact on the overall quality of navel oranges by reducing fruit spoilage and moisture loss and maintaining the ascorbic acid content and titratable acidity. In addition, the coating could effectively upgrade the SOD, chitinase, and β-1,3-glucanase activities, which were important in the antioxidant activity of the fruit. Brasil et al. (2012) observed a decline in the vitamin C and total carotenoids losses of fresh-cut papaya when using polysaccharide-based edible coating enriched with trans-cinnamaldehyde. Similarly, Shi et al. (2013) revealed that there were also significant decreases in the POD activity and vitamin C loss rates of longon coated with chitosan/nano-silica film, which could extend shelf life, reduce the browning index, and retard weight loss. Same results were also showed in jujube preservation using chitosan film with nano-silica (Yu et al., 2012) and using a combination of chitosan and 1-methylcyclopropene coating (Zhong and Xia, 2007). Oms-Oliu et al. (2008a) used the coating formulation as an N-acetylcysteine and GSH carrier to prevent fresh-cut pears from browning for two weeks without affecting the firmness of the fruit wedges. Simultaneously, the increased vitamin C and total phenolic content observed in pears coated with gellan, alginate, and pectin including antibrowning contributed to their improved antioxidant potential. These studies represent promising advances in the search for new coating material and novel applications of edible films as carriers of different bioactive compounds, in addition to new possibilities for developing functional foods with antioxidant activity.

Future trends

Although the functions of edible coatings have been well supported by many research groups, more studies are still needed to increase the shelf life and improve the nutritional quality of fruits and vegetables, particularly their antioxidant status. For example, we can start with the following aspects:

1. Additional research on bioactive compounds. As we know, many bioactive compounds that were incorporated into edible coatings were effective in enhancing food quality, and some of them were obtained from plant extracts and other natural sources.

Table 2. Quality improvement and antioxidant activity changes in fruits with edible coatings

Fruit	Edible material	Quality improvement	Antioxidant activity change	References
Pear	Chitosan, calcium chloride, pullulan	Prolong postharvest life	Loss of antioxidant capacity, phenolic content, SOD and CAT activity during storage decreased from 31.6 to 15.8%, 80 to 33.3%, 57.1 to 42.9%, 50 to 31.6%	(Kou et al., 2013)
Tomato	Gum arabic	Delay ripening process	Total phenolic content and antioxidant capacity increased by 38.3 and 133%	(Ali et al., 2013)
Melon	Alginate, pectin, gellan	Increased water vapor resistance, prevent dehydration, maintain firmness	Loss of vitamin C during storage decreased from 23 to 15%, phenolic content and antioxidant capacity increased by 13.6 and 57.1%	(Oms-Oliu et al., 2008b)
Sweet cherry	Sodium alginate	Reduces color changes, acidity, and firmness losses and respiration rate	Total phenolic content and antioxidant capacity increased by 48 and 31.3%	(Díaz-Mula et al., 2012)
Apple	Calcium ascorbate	Improves sensory quality	Antioxidant capacity and content of vitamin C were 16 times and 20 times more than untreated fruit	(Aguayo et al., 2010)
Pomegranate	Aloe vera gel	Improves sensory quality, maintains firmness, is antimicrobial (mesophilic aerobics, yeast, and molds)	Total phenolic and anthocyanins increased by 54.1 and 69.8%	(Martínez-Romero et al., 2013)
Guava	Chitosan	Reduces firmness and weight loss; decreases chlorophyll activity, soluble solid contents, titratable acidity, and MDA changes	Losses of vitamin C, SOD, and CAT activity during storage decreased from 20.5 to 10.7%, 15.3 to 6.6%, 35.3 to 17.6%, $O_2^{\bullet -}$ production decreased by 18.2%	(Hong et al., 2012)
Litchi	Chitosan	Restrains respiration, reduces moisture and weight loss, lowers the heat of respiration	Activity of PPO decreased by 32.6%	(Lin et al., 2011)
Strawberry	Carboxymethyl cellulose, hydroxypropylmethylcellulose	Delay weight loss, decay percentage, pH, titratable acidity, total soluble solid contents	Losses of total phenolics and ascorbic acid decreased from 84.2 to 62.1%, 76.9 to 38.5%	(Gol et al., 2013)

Therefore, a principal component analysis, structural identification, and characterization are required to investigate further applications for these compounds in fresh produce.

2. Combinations with other technologies. Some postharvest treatments, such as heat, ozone, and irradiation, performed well when used together with edible coatings to achieve better preserved products (Feng et al., 2012; Pandey et al., 2013). However, there is still a lack of data regarding their effects on the antioxidant activity at present. Elbarbary and Mostafa (2014) enhanced the antioxidant activity of carboxymethyl chitosan film with γ-rays, which also had a positive influence on delaying spoilage and decreasing the malondialdehyde contents of peach fruits. Xiao et al. (2010) investigated the effects of pure oxygen pretreatment plus chitosan coating containing 0.03% rosemary extracts on the quality of fresh-cut pears, and they found that the fruits not only retained higher firmness, polyphenols,

Table 3. List of bioactive compounds incorporated into edible coating technology

Bioactive compounds	Examples
Antimicrobials	Cinnamon, palmarosa, and lemongrass oils; lemongrass, oregano oil, and vanillin; thyme essential oil; green tea extract; oregano oil; cinnamon leaf and garlic oil
Antioxidants	N-acetylcysteine and glutathione; essential oils; Vitamin C and tea polyphenols; Vitamin E; ellagic acid; ascorbic and citric acid
Flavors	Pandan leaf extract; peppermint; thymol and geraniol; limonene and β-unsaturated aldehydes; n-hexanal and D-limonene; linoleic acid and isoleucine
Probiotics	*Bifidobacterium lactis* Bb-12; *Lactobacillus acidophilus*; *Lactobacillus rhamnosus* GG

vitamin C, and soluble solids, but they also inhibited PPO activity, weight loss, and softening. Perhaps these changes occurred because the treatments can increase the cohesive strength of coatings through the formation of cross-links, but the actual mechanisms of action still remain unknown.

3. Lucubrate mechanism of action. A preliminary study showed that the low O_2 ($\leq$2.5%) and high CO_2 ($\geq$7%) atmospheres caused by edible coatings are helpful in encouraging a greater production of phenolic compounds, which are related to oxidative stress (Oms-Oliu et al., 2008b). Besides, activated PAL under stress conditions plays an important role in promoting the accumulation of phenolic compounds. Additionally, the production of volatile compounds such as ethanol and acetaldehyde can also improve the antioxidant status of fruits and vegetables. The improvement is related to the induction of ROS-scavenging enzymes such as POD, SOD, CAT, and antifungal compounds such as phytoalexins and limonene (Chanjirakul et al., 2006; Fisher and Phillips, 2008). Nevertheless, there are still many questions that require further research, such as the acting site of related enzymes, influence factors, and synergistic effects with other treatments.

CONCLUSIONS

Nonthermal preservation technologies may meet the growing consumer demand for healthier foods with minor changes or improvements in nutrition. Because the combination of various treatments has been shown to be more effective for obtaining antioxidant-enhanced products, additional studies regarding the selection of appropriate systems and the optimization of processing conditions might bring the combined approaches closer to commercial applications. In addition, few studies that assessed the effects of nonthermal treatments on the bioavailability of antioxidant compounds have reported an increase in plasma vitamin C levels and a decrease in the levels of oxidative stress and inflammation biomarkers in healthy humans (Sánchez-Moreno et al., 2004, 2009). Thus, new applications for combined nonthermal systems should be further explored to stabilize not only the contents of antioxidant-related substances but also their bioavailability in humans.

Although the mechanisms of antioxidant activity improvement as induced by postharvest treatments are relatively well known to be related to stress tolerance, there are still many questions, such as what leads to stress tolerance and what factors modulate stress response and the probable interaction between different stresses and the response. In addition, a better understanding of the complex physicochemical mechanisms through which bioactive compounds are degraded could provide a basis for increasing the antioxidant capacity of fruits and vegetables, thereby promoting improved consumer health.

AUTHORS' CONTRIBUTION

XZ and LJ made equal contributions to this manuscript.

DECLARATION OF CONFLICTING INTERESTS

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