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## Effects of Nonthermal Plasma Technology on Functional Food Components

Aliyu Idris Muhammad

*Zhejiang University, Department of Food Science and Nutrition, Hangzhou, China*

Xinyu Liao

*Zhejiang University, Department of Food Science and Nutrition, Hangzhou, China*

Patrick Cullen

*Technological University Dublin, [pj.cullen@tudublin.ie](mailto:pj.cullen@tudublin.ie)*

*See next page for additional authors*

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## Authors

Aliyu Idris Muhammad, Xinyu Liao, Patrick Cullen, Donghong Liu, Qisen Xiang, Jun Wang, Shiguo Chen, Xingqian Ye, and Tian Ding

# Effects of Nonthermal Plasma Technology on Functional Food Components

Aliyu Idris Muhammad , Xinyu Liao, Patrick J. Cullen, Donghong Liu , Qisen Xiang, Jun Wang, Shiguo Chen, Xingqian Ye, and Tian Ding 

**Abstract:** Understanding the impact of nonthermal plasma (NTP) technology on key nutritional and functional food components is of paramount importance for the successful adoption of the technology by industry. NTP technology (NTPPT) has demonstrated marked antimicrobial efficacies with good retention of important physical, chemical, sensory, and nutritional parameters for an array of food products. This paper presents the influence of NTPPT on selected functional food components with a focus on low-molecular-weight bioactive compounds and vitamins. We discuss the mechanisms of bioactive compound alteration by plasma-reactive species and classify their influence on vitamins and their antioxidant capacities. The impact of NTP on specific bioactive compounds depends both on plasma properties and the food matrix. Induced changes are mainly associated with oxidative degradation and cleavage of double bonds in organic compounds. The effects reported to date are mainly time-dependent increases in the concentrations of polyphenols, vitamin C, or increases in antioxidant activity. Also, improvement in the extraction efficiency of polyphenols is observed. The review highlights future research needs regarding the complex mechanisms of interaction with plasma species. NTP is a novel technology that can both negatively and positively affect the functional components in food.

**Keywords:** antimicrobial peptides, antioxidant activity, ascorbic acid, bioactive compounds, cold plasma, polyphenols

## Introduction

Fruits and vegetables (F&V) are known for their health benefits. However, given the complexity of the modern food supply chain, most foods are subjected to some degree of processing to preserve their freshness. Such intervention may modify the functional components of F&V or their juices (Bevilacqua et al., 2017; Gironés-Vilaplana, Huertas, Moreno, Periago, & García-Viguera, 2016). The term “functional components” refers to particular biomolecules found in foods which, apart from their basic nutritional properties, have the ability to protect human vital organs from diseases (Abuajah, Ogbonna, & Osuji, 2015). Although functional components are not regarded as medicine, their importance in a “healthy” diet for disease prevention is now universally accepted. Functional components include nontoxic phytochemicals which are derived from plant-based foods like fruits, vegetables,

and whole grains (Craig, 1997; Idehen, Tang, & Sang, 2016; Liu, 2007).

Bioactive compounds are not nutritious but are considered as antioxidants that work alongside fiber, minerals, and vitamins to boost human health (Gironés-Vilaplana et al., 2016; Kongkachuichai, Charoensiri, Yakoh, Kringkasemsee, & Insung, 2015; Liu, 2013; Mann, 2011). Phytochemicals include phenolic compounds, carotenoids, sulfides, phytosterols, glucosinolates, lycopene, isoflavones,  $\beta$ -glucan, and lignans (Idehen et al., 2016; Liu, 2013; Mann, 2011; Noomhorm, Ahmad, & Anal, 2014; Schreiner & Huyskens-Keil, 2006). Approximately 900 phytochemicals have been identified in foods. A serving of F&V may contain nearly 100 different phytochemicals (Srividya, Venkatesh, & Vishnuvarthan, 2010). Bioactive compounds are also found in animals and include long-chain omega-3 polyunsaturated fatty acids, bioactive peptides, linolenic acid conjugates, and probiotic microorganisms. They are derived from animal products such as fish, milk, and fermented milk products (Abuajah et al., 2015). All the aforementioned bioactive components have been linked with dual-purpose effects of providing nourishment and lowering or preventing diseases, such cardiovascular disease, breast cancer, diabetes, and obesity (Idehen et al., 2016; Zhao et al., 2016). In view of their significance, there is a need to study how processing techniques may affect their functionality. Likewise, it is noteworthy that conventional thermal processing may have significant adverse effects on such food components. Sensitive food components such as volatiles, governing aroma, may be destroyed leading to lower-value products (Galanakis, 2017). Functional components

CRF3-2018-0094 Submitted 4/19/2018, Accepted 6/14/2018. Authors Muhammad, Liao, Liu, Chen, Ye, and Ding are with Dept. of Food Science and Nutrition, Zhejiang Key Laboratory for Agro-Food Processing, Zhejiang Univ., Hangzhou, Zhejiang, 310058, China. Author Muhammad is with Dept. of Agricultural and Environmental Engineering, Faculty of Engineering, Bayero Univ. Kano, Nigeria. Author Cullen is with BioPlasma Research Group, Dublin Inst. of Technology, Dublin, Ireland. Author Cullen is with Dept. of Chemical and Environmental Engineering, Univ. of Nottingham, Nottingham, NG7 2RD, United Kingdom. Author Xiang is with College of Food and Biological Engineering, Zhengzhou Univ. of Light Industry, Zhengzhou, 450002, P.R. China. Author Wang is with College of Food Science and Engineering, Qingdao Agricultural Univ., Chengyang, Qingdao, China. Direct inquiries to author Liu (E-mail: [dhliu@zju.edu.cn](mailto:dhliu@zju.edu.cn); [tding@zju.edu.cn](mailto:tding@zju.edu.cn)).

in different food matrices are also found to be impaired due to their thermolabile characteristics (Howard, Jeffery, Wallig, & Klein, 1997; Song & Milner, 2001), and significant amounts of bioactive compounds in thermally preserved F&V are lost along with their freshness and succulent nature (Bahram-Parvar & Lim, 2018; Bevilacqua et al., 2017; Gironés-Vilaplana et al., 2016). In a study on phenolic compound degradation due to heating, a model solution of flavonols, rutin, and quercetin was found to be degraded after cooking, and their radical-scavenging activity was reduced. The new products formed from the quercetin degradation retained about 20% scavenging activity (Buchner, Krumbein, Rohn, & Kroh, 2006). Consequently, the demand for whole and fresh-cut ready-to-eat F&V by consumers over the years has led to a surge in demand for nonthermal treatments of high-value food products (Coutinho et al., 2018; Filho et al., 2016). Nonthermal technologies are typically found to be superior treatment techniques due to their reduced quality deterioration and beneficial influence on functional activity enhancement. Foods subjected to innovative technologies like ultrasound, gamma irradiation, high-hydrostatic-pressure processing, pulsed electric field, ultraviolet irradiation (UV-C), ozone, plasma-activated water (PAW), and cold atmospheric plasma have been reported to improve the retention of key nutrients, quality, and functional properties (Aguiló-Aguayo, Gangopadhyay, Lyng, Brunton, & Rai, 2017; Barba et al., 2017; Cano, Hernandez, & Ancos, 1997; Galanakis, 2017; Ma et al., 2015; Thirumdas, Sarangapani, & Annapure, 2014; Thirumdas, Trimukhe, Deshmukh, & Annapure, 2016; Zhang et al., 2017; Zhang, Chen, Li, Li, & Zhang, 2015). The antimicrobial effects of these technologies has since been established, and many comprehensive review and research articles have been published (Aguiló-Aguayo, Charles, Renard, Page, & Carlin, 2013; Fernández, Noriega, & Thompson, 2013; Liao, Liu, Xiang, Ahn, Chen, Ye, & Ding, 2017a; Liao, Muhammad, Chen, Hu, Ye, Liu, & Ding (2018) Niemira, 2012; Pignata, Angelo, Fea, & Gilli, 2017; Scholtz, Pazlarova, Souskova, Khun, & Julak, 2015; Weltmann et al., 2008; Ziuzina, Patil, Cullen, Keener, & Bourke, 2014). Apart from their antimicrobial effects, processing with some of the aforementioned technologies has the capability of inducing functional modification of higher-molecular-weight biomolecules like starch and protein. This may come with undesired effects from the formation of short-chain aldehydes with toxic metabolites (Dong, Gao, Xu, & Chen, 2017; Liao et al., 2018; Liao et al., 2017a; Muhammad, Xiang, Liao, Liu, & Ding, 2018; Pankaj, Bueno-ferrer, Misra, Bourke, & Cullen, 2014; Sarangapani et al., 2016; Thirumdas et al., 2016; Zhu, 2017). In general, nonthermal plasma technology (NTP) is an innovative technology with a diverse range of applications across different industries, such as improving the adhesion, functional, and surface energy properties of polymers and electronics, treatments of textile materials and waste water, wound healing, and sterilization of medical equipment (Harry, 2010; Joubert et al., 2013; Lotfy, 2017; Muhammad et al., 2018; Pankaj & Keener, 2018; Roth, 1995; Takai, Kitano, Kuwabara, & Shiraki, 2012; Xinpei Lu et al., 2008; Yildirim et al., 2008). In food applications, the antimicrobial effect of NTP has been demonstrated for many products. A rapidly expanding body of literature can be found regarding the potent plasma efficacy of plasma-reactive species (RS), such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) and their interactions with microorganisms on different food matrices (Ekezie, Sun, & Cheng, 2017; Fernández, Shearer, Wilson, & Thompson, 2012; Fridman et al., 2007; Kostov et al., 2010; Laroussi, Mendis, & Rosenberg, 2003; Liao, et al., 2017a;

Liao, Xiang, Liu, Chen, Ye, & Ding, 2017b; Mir, Shah, & Mir, 2016; Misra, Keener, Bourke, Mosnier, & Cullen, 2014; Misra, Tiwari, Raghavarao, & Cullen, 2011; Niemira, 2012; Pankaj, Misra, & Cullen, 2013; Smet et al., 2017; Surowsky, Schlüter, & Knorr, 2014; Ziuzina et al., 2014). However, the effects of NTP treatment on lower-molecular-weight bioactive compounds have been studied to a lesser degree. Consequently, this review presents an overview of recent studies on the application of NTP on functional components, vitamins, and their antioxidant potentials. The review also discusses the possible plasma mechanisms of degradation or enhancement of functional components.

## Nonthermal Plasma (NTP) Generation

Plasma exists as the fourth state of matter after solid, liquid, and gas. There are two key classifications of plasma, thermal (equilibrium) and nonthermal (nonequilibrium) plasma. A thermal plasma is generated when a gas is heated at a high-temperature range of about 20 000 K to achieve the ionization of the gas. At this condition, all the ions, electrons, and chemical species are in thermodynamic equilibrium (Harry, 2010; Misra, Schlüter, & Cullen, 2016). In the NTP, the applied energy leads to an elastic collision of the gas particles, atoms, and electrons. This results in the transfer of some kinetic energy to other particles in such a way that the cooling of the uncharged particles and neutral ions is more rapid than the energy transfer from the electrons. At this point, the electrons are at a higher temperature of between 1 and 10 eV, while the neutrons, ions, and radicals remain close to room temperature. This allows the gas bulk to remain at a low temperature, hence the plasma is referred as NTP (Fridman, 2008; Harry, 2010; Misra et al., 2016; Scholtz et al., 2015). Such conditions enable the treatment of thermolabile food materials. The NTP can be generated through ionization of gases, such as N<sub>2</sub>, O<sub>2</sub>, or noble gases (He, Ar, or Ne) or combinations thereof that could either be at a reduced or atmospheric pressure (Ekezie et al., 2017; Niemira, 2012; Pinela & Ferreira, 2017; Scholtz et al., 2015). NTP can be generated by any type of energy, such as electrical, photoionization, optical (UV light), heat radiation, radio frequency, and microwave energy. The most prominent are electrical or electromagnetic energy (Fridman, 2008; Liao et al., 2017a; Pankaj & Keener, 2017). The key NTP species for biological treatments are often found to be ROS, such as superoxide anion (O<sub>2</sub><sup>-</sup>), atomic oxygen (O), singlet oxygen (<sup>1</sup>O<sub>2</sub>), hydroxyl radical (OH•), and ozone (O<sub>3</sub>); RNS, such as excited nitrogen N<sub>2</sub>, atomic nitrogen N, nitric oxide NO•; and also UV photons, positive and negative ions, and free electrons (Laroussi & Leipold, 2004; Liao et al., 2018; Ni, Lynch, Modic, Whalley, & Walsh, 2016; Schlüter & Fröhling, 2014; Scholtz et al., 2015).

In recent years, there has been an increasing number of studies in the literature on the application of NTP in foods, with different devices employed for generating the plasma discharges at atmospheric pressure with both direct or indirect food exposure. The schematic representation in Figure 1 shows a direct plasma exposure on target food materials with devices like dielectric barrier discharge (DBD) plasma, corona discharge plasma, gliding arc discharge plasma, and microwave cold plasma. Other plasma discharge devices are dielectric barrier grating discharge plasma, radio frequency plasma, nanosecond pulse plasma, and multijet atmospheric plasma discharges shown in Figure 2 (Chiang et al., 2010; Cullen et al., 2017; Gallagher Jr et al., 2007; Joubert et al., 2013; Kim, Oh, Won, Lee, & Min, 2017; Korachi, Gurol, & Aslan, 2010; Liao et al., 2017b; Moreau et al., 2007; Pankaj et al., 2015; Park et al., 2015). A comprehensive description of

Table 1—Effect of NTP treatment on the functional components of food.

NTP type	Treatment conditions	Bioactive compounds	Food commodity	Matrix	Observation	References
Atmospheric pressure plasma jet	0.20, 40, 80, and 120 s; 35 W; 27.12 MHz;	Flavonoids	Lamb lettuce	Lettuce leaf	<ul style="list-style-type: none"> <li>Reduction in phenolic acids levels.</li> <li>Decrease in caffeic acids.</li> <li>Increase in diosmetin.</li> </ul>	Grzegorzewski et al. (2011b)
Cold atmospheric gas phase plasma	3 and 5 min; 4 W; 25 kHz; argon gas; 3, 5, and 7 cm <sup>3</sup> sample volume.	Hydroxycinnamic acids, flavonols, polyphenols,	Chokeberry juice	Juice	<ul style="list-style-type: none"> <li>Increase in hydroxycinnamic acids.</li> <li>Increase in flavonols</li> <li>loss of anthocyanins.</li> <li>Reduction in extraction time of anthocyanins.</li> <li>Increase in concentration of neochlorogenic acid.</li> </ul>	Kovačević et al. (2016a)
High-voltage atmospheric cold plasma	0, 1, 2, 3, and 4 min; 80 kV; 46% RH.	Phenols, flavonoids, and flavonols	White grape	Juice	<ul style="list-style-type: none"> <li>A decrease in total phenolics.</li> <li>A decline in flavonoids.</li> <li>Increase in total flavonols.</li> </ul>	Pankaj et al. (2017)
Cold Atmospheric pressure plasma	0, 2.5, 5 and 10 min; 3 kHz; 9 kV; Air;	Flavonoid glycosides	Pea	Seed and 15-d old Pea seedlings	<ul style="list-style-type: none"> <li>A reduced concentration of quercetin glycosides.</li> <li>Kaempferol glycosides concentrations were decreased.</li> </ul>	Buřler et al. (2015)
Cold atmospheric gas phase plasma	3, 5, 7 min; 4 W power; 25 kHz; 0.75, 1, 1.25 dm <sup>3</sup> gas flow rate	Anthocyanin	pomegranate	Juice	<ul style="list-style-type: none"> <li>Increase in anthocyanin content.</li> <li>Positive impact on anthocyanin stability.</li> </ul>	Kovačević et al. (2016b)
Radio-frequency (RF)-glow low-pressure oxygen plasma	20-300 s; 75 W, and 150 W; O <sub>2</sub> gas at 0.5 mbar	Phenolic acids, Flavonoids	Lamb's lettuce	Leaf	<ul style="list-style-type: none"> <li>Increase in protocatechuic acid.</li> <li>Increase in luteolin and diosmetin.</li> </ul>	Grzegorzewski et al. (2010a)
Atmospheric RF-plasma jet	60 s; 20 and 40 W; 20-600 kHz	Total phenolics content	Dragon fruit	Dragon fruit slice	<ul style="list-style-type: none"> <li>Reduction in total phenolic contents.</li> </ul>	Matan et al. (2015)
Atmospheric double barrier discharge plasma	Air, 60% RH; 15 kV; 10+10 and 20+20 min.	Total phenolics content, Carotenoids	Kiwifruit	Fresh-cut Kiwifruit	<ul style="list-style-type: none"> <li>No significant change in total phenolic contents.</li> <li>A decrease in total carotenoids.</li> </ul>	Ramazzina et al. (2015)
Cold plasma	N <sub>2</sub> gas; 10, 30, and 50 mL/min flow rate; 5, 10 and 15 min; 80 kHz; 30 kPa vacuum conditions.	TPC and TFC	Cashew apple juice	Juice	<ul style="list-style-type: none"> <li>Increase in TPC and TFC at a higher gas flow rate.</li> <li>Overexposure led to degradation of TPC and TFC.</li> </ul>	Rodríguez et al. (2017)
Cold atmospheric gas phase plasma	Argon gas; 3, 5, and 7 min; 25 kHz; 4 W; 3, 4, and 5 cm <sup>3</sup> sample volume; 0.75, 1, 1.25 dm <sup>3</sup> /min flow rate.	Phenolic compounds	Pomegranate juice	Juice	<ul style="list-style-type: none"> <li>Increase in concentrations of ellagic acid, chlorogenic acid, ferulic acid, catechin and punicalagin 1.</li> <li>Reduction in contents of protocatechuic acid, caffeic acid and punicalagin 2.</li> </ul>	Herceg et al. (2016)
Atmospheric cold plasma	Air; 15, 30, 45, and 60 s; 70 kV; 50 Hz; 22 mm electrode distance;	TPC	Prebiotic orange juice	Juice	<ul style="list-style-type: none"> <li>Reduction in TPC irrespective of direct or indirect exposure.</li> </ul>	Almeida et al. (2015)
Atmospheric cold plasma	30, 60, 90, and 120 s; 650 W; 3000 L/h gas flow rate; 25 kHz.	TPC	Sour cherry nectar, apple, orange, and tomato juices	Juice	<ul style="list-style-type: none"> <li>An overall increase in TPC in all treated juices after 120 s.</li> </ul>	Dasan and Boyacı (2018)
Gas phase plasma	3, 4, and 5 min; Ar gas; 4 W; 2.5 kV; 25 kHz; 2, 3, and 4 mL sample; 0.75, 1, 1.25 L/min gas flow rate.	TPC and TAC	Sour cherry Marasca juice	Juice	<ul style="list-style-type: none"> <li>Higher TPC was recorded at shorter treatment time.</li> <li>Lower TAC observed at longer treatment time.</li> </ul>	Garofulić et al. (2015)
Atmospheric cold plasma	Air as gas; 0, 2, and 5 min; 60 and 80 kV; 50 Hz.	TPC, TFC, and anthocyanin.	Blueberry	Fruit	<ul style="list-style-type: none"> <li>A significant increase in TPC and TFC after 1 min plasma exposure.</li> <li>Significant reduction in anthocyanin with extended treatment time.</li> </ul>	Sarangapani et al. (2017)
Atmospheric cold plasma	Air; 0, 15, 30, 45, 60, 90, and 120 s; 549 W; 47 kHz; 7.5 cm electrode distance; 113.27 L/min flow rate.	Anthocyanin	Blueberries	Fruit	<ul style="list-style-type: none"> <li>A significant decrease in TAC after 90 s.</li> </ul>	Lacombe et al. (2015)

(Continued)

Table 1–Continued.

NTP type	Treatment conditions	Bioactive compounds	Food commodity	Matrix	Observation	References
Microwave-powered cold plasma	N <sub>2</sub> gas; 2, 5, and 10 min; 900 W; 0.25 W m <sup>-2</sup> wave; 20 L/min gas flow rate	TPC	Mandarin flesh and mandarin peel	Fruit	<ul style="list-style-type: none"> <li>No significant increase in TPC within the flesh.</li> <li>A significant increase in TPC in the peel.</li> </ul>	Yeon et al. (2017)
Cold plasma	Ar; 1 L/min; 0, 3, 5, 7, 9, 10, and 11; 15 kV; 12 kHz.	TPC	Walnut	Nut	<ul style="list-style-type: none"> <li>No effect on TPC on fresh and dried walnut.</li> </ul>	Amini and Ghoranneviss (2016)
Microwave-powered cold plasma	He gas; 1 L/min; 10, 14, 25, 36, and 40 min; 400, 474, 650, 826, and 900 W; 0.7 kPa.	Quercetin content	Onion powder	Powder	<ul style="list-style-type: none"> <li>No significant alteration in quercetin content.</li> </ul>	Kim et al. (2017)
Dielectric barrier discharge atmospheric cold plasma	Air; 30, 40, and 50 W; 0, 5, 10, 15, 20, 30, and 40 s; 2 mm electrode distance.	TPC	Apple juice	Juice	<ul style="list-style-type: none"> <li>A decline in TPC with increase and treatment time and power.</li> </ul>	Liao et al. (2018)

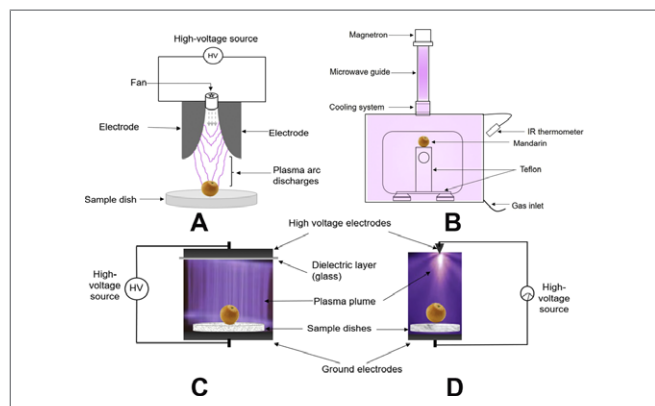


Figure 1—NTP discharge devices during direct exposure on food materials, (A) gliding arc discharge equipment, (B) microwave cold plasma generator (Yeon et al., 2017), (C) DBD plasma generator, and (D) corona discharge generator.

these devices is extensively discussed elsewhere (Lu, Laroussi, & Puech, 2012; Misra et al., 2016; Muhammad et al., 2018; Schlüter & Fröhling, 2014; Scholtz et al., 2015; Yildirim et al., 2008). Given the scale and generally low-value-added nature of food processing, the choice of gas is very important. Examination of the rapidly expanding literature shows a clear trend toward the use of atmospheric air as the operational gas of choice (Pignata et al., 2017), offering a cheap processing aid for food applications (Saragapani, Patange, Bourke, Keener, & Cullen, 2018).

### Mechanism of NTP Interaction with Functional Food Components

Despite the number of research articles on the application of NTP in food, uncertainty exists between the mechanism of interaction of bioactive compounds and plasma RS. Unraveling the mechanisms involved is particularly challenging given the highly dynamic nature of plasma species. This relationship is likely to depend on several control conditions, such as the process gas, plasma source, input power, duration of exposure, and the distance between the discharge and the target. Elucidation of the mechanism is important for future approval of plasma as a food processing aid. The impact of NTPT on the functionality and stability of phenolic compounds is a structure-dependent phenomenon which may be explained by the synergistic effects of the

various active plasma RS. A strong surface oxidation effect was proposed to have led to the addition of new carbonyl and carboxylic groups followed by heightened oxygen formation (Grzegorzewski, Michaela, Rohn, Kroh, & Schlueter, 2011b; Grzegorzewski, Rohn, Kroh, Geyer, & Schlüter, 2010b). Further addition of functional groups such as hydroxyl groups in the aromatic rings of phenolic compounds was documented by Aadil, Zeng, Han, and Sun (2013).

Comparably, the degradation of thermally treated model solutions of flavonoids (rutin and quercetin) is reported to be due to the presence of molecular oxygen (O<sub>2</sub>) and ROS such as O<sub>2</sub><sup>-</sup>, OH•. The final compounds formed were due to the shifting of some of the hydrogen atoms in the B-ring. Although the scavenging potentials of these compounds were reduced, they retained about 20% of their scavenging activity (Buchner et al., 2006; Patras, Brunton, O'Donnell, & Tiwari, 2010). Likewise, Makris and Rossiter (2002) have linked the degradation of phenolic compounds by plasma to that of a heat-induced oxidative cleavage path. However, Grzegorzewski and group hypothesized that the plasma-induced phenolics degradation was neither caused by photodesorption nor thermal desorption processes, it was rather induced by the combined effects of numerous plasma RS (Grzegorzewski et al., 2009; Grzegorzewski, Ehlbeck, Schlüter, Kroh, & Rohn, 2011b). In quercetin, for example (Figure 3A), an initial hydrogen removal from the hydroxyl group in the C-4' position is due to the potent influence of atomic oxygen (O) and OH•. A subsequent slower degradation is due to hydrogen inhibition via substitution with β-O-linked D-glucose (quercetin-4'-O- monoglucoside) or steric interference in an adjacent position to quercetin-3,4'-O-diglucoside at C-3' (Grzegorzewski et al., 2010b).

In a similar study, Makris and Rossiter (2002) proposed a hydroxyl free radical oxidative degradation of flavonols (quercetin and morin) that formed low-molecular-weight phenolic compounds (Makris & Rossiter, 2002). It was claimed that both compounds have similar degradation pathways that depend on B-ring and 3-hydroxyl group substitutions (Grzegorzewski et al., 2009; Makris & Rossiter, 2002). Grzegorzewski and group speculated that flavonoids degraded much faster than phenolic acids during NTP exposure. Their assertion was based on the radical-scavenging potential of polyphenols, which can scavenge the plasma-generated RS. This has allowed phenolic compounds to resist the degradation to a greater extent than flavonoids (Grzegorzewski et al., 2011b).

Another proposed mechanism of degradation for low-molecular-weight organic compounds was ozone-induced



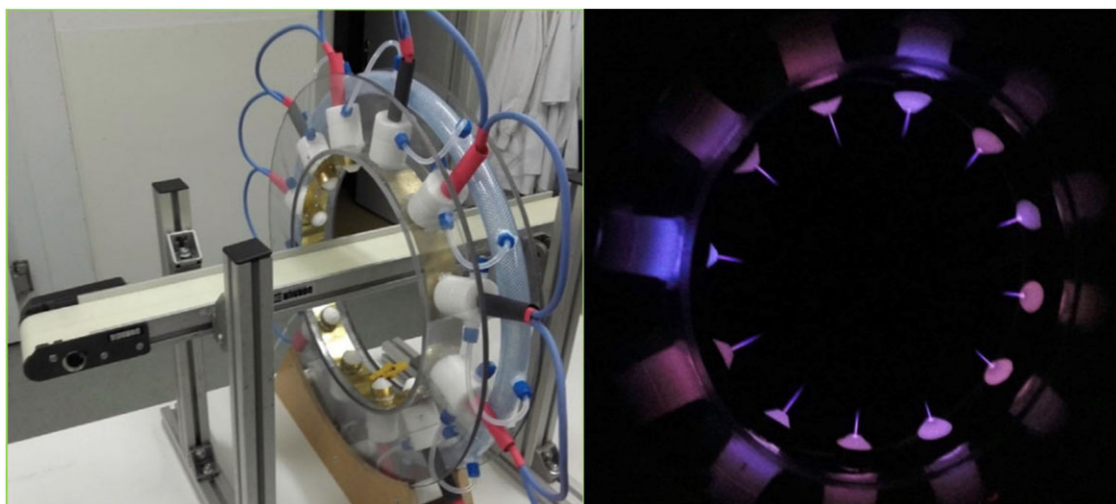


Figure 2—(A) 450-mm-diameter multijet plasma discharge designed for continuous treatment of food materials conveyed using a conveyor belt and a surrounding wall which helps in the retention of plasma RS (Cullen et al., 2017).

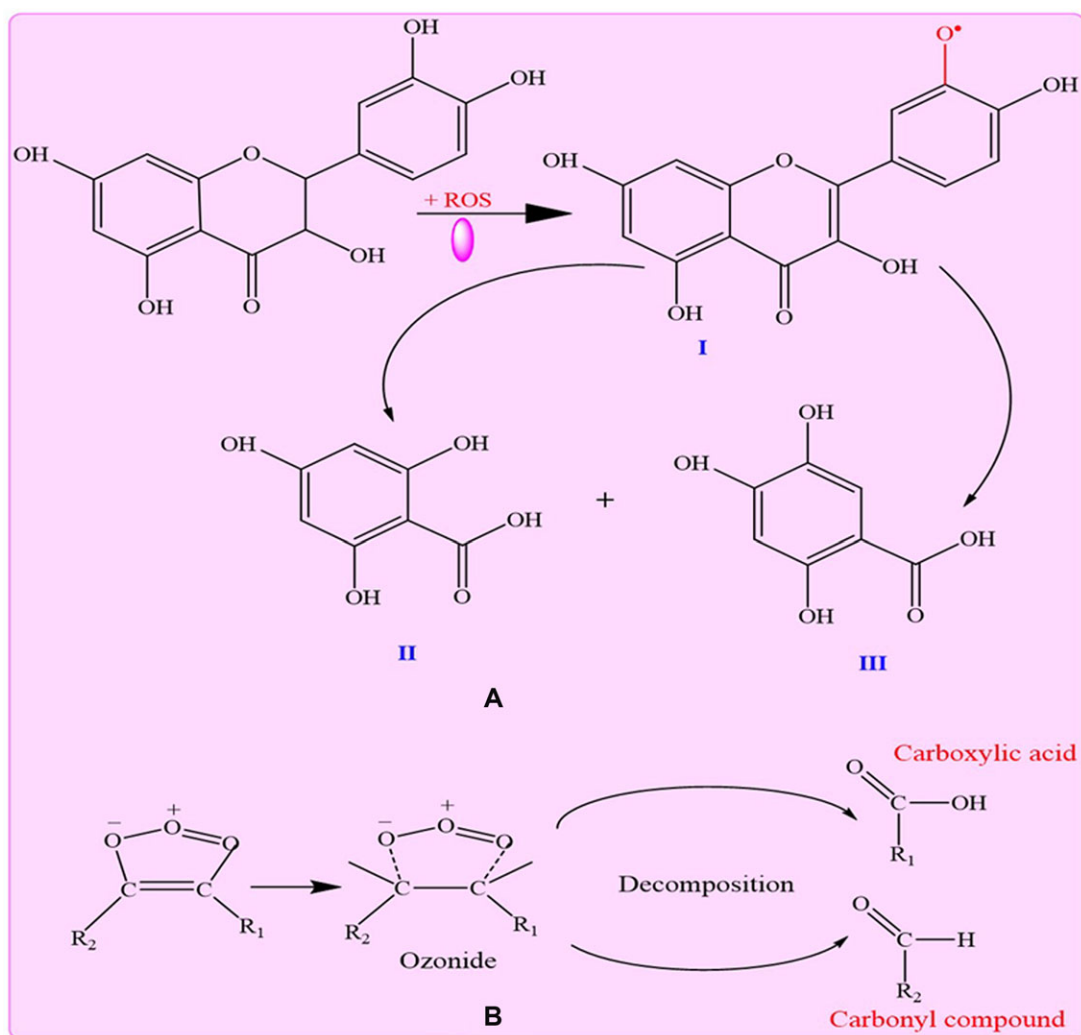


Figure 3—(A) Radical-induced oxidative degradation of quercetin leads to the formation of low-molecular-weight phenolic compounds II and III. The degradation path is similar to heat-induced oxidative cleavage (Grzegorzewski et al., 2010b). (B) Direct reaction of ozone and subsequent decomposition of ozonide to carboxylic acids and carbonyl compounds (Tiwari et al., 2009).

Table 2—Effect of NTP treatment on antioxidant activity, antioxidant contents, and scavenging potential of functional food components.

NTP type	Treatment conditions	Scavenging assay	Food commodity	Matrix	Observations	Reference
High-voltage atmospheric cold plasma	0, 1, 2, 3, and 4 min; 80 kV; 46% RH.	DPPH	White Grape	Juice	<ul style="list-style-type: none"> <li>A decrease in DPPH free radical scavenging activity.</li> <li>Reduction in antioxidant capacity</li> </ul>	Pankaj et al. (2017)
Atmospheric double barrier discharge plasma	Air, 60% RH; 15 kV; 10+10 and 20+20 min.	ABTS, DPPH, and FRAP	Kiwifruit	Fresh-cut Kiwifruit	<ul style="list-style-type: none"> <li>No significant change in antioxidant activity and antioxidant contents in all assays</li> </ul>	Ramazzina et al. (2015)
Atmospheric cold plasma	Air, 60% RH; 15 and 30 min; 70 mm discharge distance; 15 kV.	ABTS and ORAC	Radicchio	Radicchio leaves	<ul style="list-style-type: none"> <li>Slight decrease in antioxidant activity in both assays</li> </ul>	Pasquali et al. (2016)
Cold plasma	N <sub>2</sub> gas; 10, 30, and 50 mL/min flow rate; 5, 10, and 15 min; 80 kHz; 30 kPa vacuum conditions.	FRAP, DPPH, and ABTS	Cashew apple juice	Juice	<ul style="list-style-type: none"> <li>An initial increase in antioxidant activity in the 1st 5 min at a low flow rate.</li> <li>Reduction in antioxidant activity at high flow rates.</li> </ul>	Rodríguez et al. (2017)
Atmospheric cold plasma	Air; 15, 30, 45, and 60 s; 70 kV; 50 Hz; 22 mm electrode distance;	DPPH, and ABTS	Prebiotic orange juice	Juice	<ul style="list-style-type: none"> <li>No significant change in antioxidant capacity using DPPH assay.</li> <li>A significant decrease in antioxidant capacity using ABTS assay.</li> </ul>	Almeida et al. (2015)
Microwave-powered cold plasma	N <sub>2</sub> gas; 2, 5, and 10 min; 900 W; 0.25 W/m <sup>2</sup> wave; 20 L/min gas flow rate	DPPH	Mandarin flesh and mandarin peel	Fruit	<ul style="list-style-type: none"> <li>No effect on scavenging activity of the flesh.</li> <li>A significant increase in activity for the peel.</li> </ul>	Yeon et al. (2017)
Cold plasma	Ar; 1 L/min; 0, 3, 5, 7, 9, 10, and 11; 15 kV; 12 kHz.	FRAP and DPPH	Walnut	Nut	<ul style="list-style-type: none"> <li>No effect was observed in all samples.</li> </ul>	Amini and Ghoranneviss (2016)
Microwave-powered cold plasma	He gas; 1 L/min; 10, 14, 25, 36, and 40 min; 400, 474, 650, 826, and 900 W; 0.7 kPa.	DPPH	Onion powder	Powder	<ul style="list-style-type: none"> <li>Increase in antioxidant activity.</li> </ul>	Kim et al. (2017)
PAW	98% Ar and 2% O <sub>2</sub> ; 5 L/min flow rate; 10 kHz; 10 mm working distance; PAW-5, 10 and 15 min.	UV/Vis spectrometer	Button mushroom	Mushroom	<ul style="list-style-type: none"> <li>Increase in antioxidant.</li> </ul>	Xu et al. (2016)
Cold plasma	400 and 900 W; 10 min; N <sub>2</sub> , He, N <sub>2</sub> +O <sub>2</sub> ;	DPPH and ABTS	Lettuce	Vegetable	<ul style="list-style-type: none"> <li>No significant effect on the antioxidant activities.</li> </ul>	Song et al. (2015)
Microwave-powered cold plasma	N <sub>2</sub> ; 0, 2, 5, 10, and 20 min; 900 W; 667 Pa;	ABTS	Radish sprout	Vegetable	<ul style="list-style-type: none"> <li>Antioxidant activity was not affected.</li> </ul>	Oh et al. (2017)
Dielectric barrier discharge atmospheric cold plasma	Air; 30, 40, and 50 W; 0, 5, 10, 15, 20, 30, and 40 s; 2 mm electrode distance.	DPPH	Apple juice	Juice	<ul style="list-style-type: none"> <li>A slight decrease in antioxidant capacity.</li> </ul>	Liao et al. (2018)

oxidative cleavage of the double bonds of organic compounds, which leads to the formation of unstable ozonide, which subsequently degrades. Such degradation follows either a direct reaction with O<sub>3</sub> or indirect reaction with another ROS such as O<sub>2</sub><sup>•−</sup> or OH<sup>•</sup>. The indirect reaction leads to electrophilic and nucleophilic reactions with aromatic compounds which are replaced with an electron donor (OH<sup>•</sup>) with high electron affinity in the ortho and para positions. The direct reaction is explained by the Criegee mechanism, which involves subjecting ozone molecules to 1–3 dipolar cycloaddition with the double bonds. This results in the formation of ozonides (1,2,4-trioxolanes) from the unsaturated alkenes and then ozone with an aldehyde or ketone oxides, which have finite lifetimes. However, due to the instability of the ozonides, their oxidative degradation yields carbonyl compounds, and carboxylic acids or ketones, as shown in Figure 3(B) (Criegee, 1957; Tiwari, O'Donnell, & Cullen, 2009).

### Influence of NTP Treatment on Bioactive Compounds

Polyphenols are bioactive compounds that are mostly derived from plants. They consist of flavones, flavonols, flavan-3-ols, isoflavones, anthocyanidins, lignans, and so on. When consumed, they are metabolized in the body with synergistic effects of antioxidant, anti-inflammatory, and antimicrobial properties, resulting in a healthy body (Kristbergsson & Ötles, 2016; Scalbert, Johnson, & Saltmarsh, 2005; Siddiq, Ahmed, Lobo, & Ozadali, 2012). NTP processing of F&V results in the alteration of composition and functionality of polyphenols. Table 1 highlights the effect of NTP treatment on various functional food components. For example, anthocyanins are phenolic flavonoids situated in the cell vacuole. NTP disruption of the cell membrane leads to the release of intracellular substances into the extracellular environment. Consequently, an improved mass transfer and faster penetration of solvents into the cell enhance the extraction of polyphenols



Table 3—Effect of NTP treatment on vitamins (Vc).

NTP type	Treatment conditions	Assay type	Food commodity	Matrix	Observations	Reference
Cold plasma	N <sub>2</sub> gas; 10, 30, and 50 mL/min flow rate; 5, 10, and 15 min; 80 kHz; 30 kPa vacuum conditions.	UV/Vis spectrophotometer	Cashew apple juice	Juice	<ul style="list-style-type: none"> <li>• An initial increase in Vc content in the 1st 5 min at a lower flow rate.</li> <li>• Reduction in Vc content at high treatment conditions.</li> </ul>	Rodríguez et al. (2017)
Atmospheric cold plasma	15, 30, 45, and 60 s; 70 kV; 50 Hz; 22 mm electrode distance.	HPLC	Prebiotic Orange juice	Juice	<ul style="list-style-type: none"> <li>• Increase in ascorbic acid content.</li> </ul>	Diva et al. (2017)
Atmospheric cold plasma	Air as gas; 0, 2, and 5 min; 60 and 80 kV; 50 Hz.	HPLC	Blueberry	Whole fruit	<ul style="list-style-type: none"> <li>• A significant increase in ascorbic acid content at 1 min and 80 kV.</li> <li>• Significant decrease after 5 min treatment time and 80 kV.</li> </ul>	Sarangapani et al. (2017)
Microwave-powered cold plasma	N <sub>2</sub> gas; 2, 5, and 10 min; 900 W; 0.25 W/m <sup>2</sup> wave; 20 L/min gas flow rate	HPLC	Mandarin flesh and mandarin peel	Whole fruit	<ul style="list-style-type: none"> <li>• Insignificant change in ascorbic acid concentration.</li> </ul>	Yeon et al. (2017)
PAW	98% Ar and 2% O <sub>2</sub> ; 5 L/min flow rate; 10 kHz; 10 mm working distance; PAW-5, 10, and 15 min.	UV/Vis spectrometer	Button mushroom	Mushroom	<ul style="list-style-type: none"> <li>• Increase in Vc concentration with increase PAW.</li> </ul>	Xu et al. (2016)
Atmospheric cold plasma	Air; flow rate 5 slm; 30 mA; 500 V; 0, 30, 60, 90, 150, and 240 s.	HPLC	Cucumber Carrot Pear	Fruit Slices	<ul style="list-style-type: none"> <li>• 3.6% loss in Vc content in cucumber slices.</li> <li>• 3.2 % loss in carrot slices.</li> <li>• 2.8% loss in pear slices.</li> </ul>	Wang et al. (2012)
Cold plasma	400 and 900 W; 10 min; N <sub>2</sub> , He, N <sub>2</sub> +O <sub>2</sub> ;	HPLC	Lettuce	Vegetable	<ul style="list-style-type: none"> <li>• No significant effect was observed.</li> </ul>	Song et al. (2015)
Microwave-powered cold plasma	N <sub>2</sub> ; 0, 2, 5, 10, and 20 min; 900 W; 667 Pa.	HPLC	Radish sprout	Vegetable	<ul style="list-style-type: none"> <li>• MCP did not decrease the ascorbic acid concentration.</li> </ul>	Oh et al. (2017)
High-voltage atmospheric cold plasma	90 kV; 60 Hz; 30, 60, and 120 s; 4.44 cm electrode gap.	HPLC	Orange juice	Juice	<ul style="list-style-type: none"> <li>• 120 s direct treatment reduce Vc content by 22%.</li> </ul>	(Xu et al., 2017)

(Kobzev et al., 2013; Landbo & Meyer, 2001). Grzegorzewski and coresearchers hypothesized that plasma ROS such as OH• and Ar<sup>+</sup> have caused etching of the upper epidermis of lamb's lettuce which stimulated the release and degradation of flavonoids and other compounds from the central vacuoles of the guard cells (Grzegorzewski et al., 2011b). To study the degradation of NTP-treated chokeberry juice, Kovačević and coresearchers employed high-performance liquid chromatography equipped with UV/Vis-photo diode array detection (HPLC-DAD). Their results showed a 23% loss in anthocyanins due to their low stability in the juice coupled with the oxidative effect of the plasma RS. Also, increases in the concentration of neochlorogenic acid, quercetin-3-rutinoside, and quercetin-3-glucoside were observed. On extraction of the plasma-treated phytochemicals, a reduction in the extraction time of anthocyanins, and a decrease in the percentage volumes of neochlorogenic acid (5%), caffeic acid (2%), and quercetin-3-rutinoside (9%) were recorded (Kovačević et al., 2016a). In another study on cold atmospheric gas plasma, the anthocyanin content of pomegranate juice was increased by about 21–35%, thus affirming NTP's positive impact on anthocyanin stability (Kovačević et al., 2016b).

The contents of protocatechuic, chlorogenic, and caffeic acids in fresh lamb's lettuce were decreased by 16%, 29%, and 35%, respectively, after plasma exposure, while the diosmetin content was increased by 44%, and the pure flavonoids (model solution) showed a strong time-dependent decrease after NTP treatment (Grzegorzewski et al., 2011b). Comparatively, negligible changes in the contents of chlorogenic and caffeic acids after UV-C expo-

sure were reported by the same researchers. Conversely, protocatechuic acid, luteolin, and diosmetin were reported to be increased by 70%, 53%, and 101%, respectively, due to the damaging effects of UV-C on the epidermal and mesophyll cells (Grzegorzewski et al., 2011b). A divergent result of a 2-fold increment in the protocatechuic acid and luteolin contents was observed after a 120-s treatment irrespective of the input power. The diosmetin content was also increased 2.5-fold in a similar manner (Grzegorzewski et al., 2010a).

Pankaj and coresearchers studied the effect of high-voltage cold atmospheric plasma on white grape juice, with an increase in the total flavonols content observed. It was further stated that the total phenolic and flavonoid contents (TPC and TFC) reduced drastically with increased treatment time (Pankaj, Wan, Colonna, & Keener, 2017). In agreement with Pankaj et al. (2017), Herceg and coresearchers reported a 33.03% increase in TPC of plasma-treated pomegranate juice. Additionally, the ellagic acid content was 3 times higher in the plasma-treated juice than the untreated juice. This might be due to the plasma RS bombarding the cell membrane to induce hydrolysis and degradation of ellagitannins leading to increases in the ellagic acid increment (Herceg et al., 2016). The influence of ACP on orange, tomato, and apple juices, and on sour cherry nectar, was also reported. Following 120 s of treatment, the TPC in all the juices were increased by 9.52%, 14.81%, 14.43%, and 14.47%, respectively (Dasan & Boyaci, 2018). From another research on sour cherry Marasca juice exposed to gas phase plasma, a time-dependent effect was observed. The highest concentration of total anthocyanins

(TAC; 223.96 mg/100 g) was recorded after 3 min of treatment. Likewise, a higher TPC of 163.36 mg/100 g was observed at the shortest exposure time of 3 min, as against the samples treated for 5 min (Garofulić et al., 2015). These results point to the impact of NTP on phenolic degradation. Prolonged exposure of both anthocyanins and phenolic acids confirmed the reaction with plasma-induced ROS (Grzegorzewski et al., 2011b). Regardless of the applied voltage, a marked increase in TPC and TFC of blueberries after just 1 min of ACP treatment was reported. The research further showed a slight drop in both TPC and TFC as compared with untreated samples after the treatment was extended to 5 min. Meanwhile, the anthocyanin content significantly dropped over extended periods of plasma treatment at higher input voltage (Sarangapani, O'Toole, Cullen, & Bourke, 2017). The TPC in mandarin peel significantly rose after microwave-powered plasma treatment, while that in the mandarin flesh was not altered (Yeon, Jo, & Min, 2017). An increase in TPC and polyphenolics could be described by the accumulation of phenolic compounds within the epidermal cells which are triggered by plasma RS such as UV that enhances their biosynthesis (Grzegorzewski et al., 2010a; Laroussi & Leipold, 2004). Likewise, Matan and coresearchers suggested that NTP treatment alone caused a slight drop in TPC from  $2.0 \pm 0.2$  mg 100/g to  $1.9 \pm 0.1$  mg 100/g in dragon fruit. However, upon combining the dragon fruit with 5.0% green tea extract, a marked increase in TPC was noted (Matan, Puangjinda, Phothisuwan, & Nisoa, 2015).

Meanwhile, a different result was reported by Almeida and coresearchers after NTP treatment of prebiotic orange juice. The result showed a marked reduction in the TPC from  $2.52 \pm 0.20$  to  $2.37 \pm 0.10$  g/L and  $1.93 \pm 0.12$  g/L for direct and indirect exposure, respectively. In the indirect exposure, the TPC was significantly affected at 60 s of treatment. Likewise, after ozone treatment of the same juice, the TPC was slightly reduced to  $2.33 \pm 0.07$  g/L (Almeida et al., 2015). It is also worth noting that  $O_3$  is generally present in significant amounts where the plasma inducer gas contains some level of oxygen (Mir et al., 2016; Misra et al., 2015; Surowsky et al., 2014). Apple juice treated with atmospheric cold plasma-DBD plasma showed a slight decrease in TPC at an input power of 30 and 40 W. But after increasing the exposure time at 50 W, the reduction in TPC was significant (Liao et al., 2018). Lacombe and co-researchers observed a significant decline in TAC for plasma-treated blueberries after 90 s of exposure (Lacombe et al., 2015), although many factors could have resulted in the change of anthocyanin stability. The processing temperature could accelerate the rate of degradation of anthocyanin via tempering with the enzymatic activity of  $\beta$ -glucosidase and polyphenol oxidase (Patras et al., 2010). In the flavonol glycoside profile of pea seeds, seedlings, and sprouts, a dose-dependent decline in the concentrations of flavonol was observed after NTP treatment. The concentrations of quercetin and kaempferol glycosides were reduced as the treatment time was extended. This might be due to their protective effects against oxidative stresses (Bußler et al., 2015).

Another research group reported non-significant effects of NTP treatment on TPC for some food products. Amini and Ghoranneviss (2016) recorded no effects for fresh and dried argon plasma-treated walnuts after an 11-min exposure. Meanwhile, in onion powder, the content of quercetin was not significantly affected following the microwave plasma treatment. Although onions have a high concentration of quercetin and quercetin glycosides, which degrade upon thermal processing (Aguiló-Aguayo et al., 2013), the plasma-treated quercetin content remained intact even after

storage at 4 °C for 28 d (Kim et al., 2017). This was possibly due to the mild nature of the plasma treatment and the defense mechanism against oxidation. An onion of 10 g could provide about 4 mg of quercetin, which is equivalent to the allowable daily intake of 8 to 10 mg/d of vitamin E for an adult (Bahram-Parvar & Lim, 2018).

This section clearly highlights improvements, declines, and no notable effects of polyphenols after NTP treatment. These divergent results may be due to differences in the food matrices, plasma equipment configuration, and processing parameters, particularly the gas used. From the food processing perspective, the after-effect of NTP treatment on polyphenols warrants a comprehensive optimization of all process condition, in order to fully understand their interactions with target food matrices.

## Antimicrobial Peptides

Antimicrobial peptides (AMPs) are low-molecular-weight biomolecules with a wide range of antimicrobial effects against fungi, bacteria, yeasts, virus, and cancer cells. These biomolecules are found naturally in living organisms as the first line of defense, with a varying number of amino acids (Bahar & Ren, 2013; Bazaka, Jacob, Chrzanowski, & Ostrikov, 2015; Villa & Viñas, 2016; Zhang & Gallo, 2016). However, bacteriocins are a subgroup of AMPs produced by bacteria, which can inhibit or kill closely related or nonrelated bacteria without posing any harm to the bacteria themselves (Yang, Lin, Sung, & Fang, 2014). The majority of the bacteriocins are produced by lactic acid bacteria and are used as starters in food fermentation or as preservatives. These bioactive peptides can also be added as hurdle technologies in packaging systems for shelf life extension. For instance, nisin produced by *Lactococcus lactis* was approved by the US Food and Drug Administration (FDA) to be used in processed cheese in 1988 (Røssland, Langsrud, Granum, & Sørhaug, 2005; Villa & Viñas, 2016). The pathway in which these peptides lead to bacteria death is via inhibiting the protein synthesis and DNA replication pathways thereby subduing the cellular functions (Brogden, 2005). Most AMPs are positively charged with hydrophilic and hydrophobic groups. These enable the peptides to target bacterial cell membranes by binding to the lipid and phospholipid components, which cause decomposition of the lipid bilayer (Izadpanah & Gallo, 2005; Shai, 2002; Lijuan Zhang, Rozek, & Hancock, 2001).

## Advantages and Limitations

Interestingly, bacteriocins do not harm the producing strain due to specific immune proteins. Likewise, AMPs are stable to heat, can extend food preservation duration, and treat malignant cancers and pathogenic diseases. These peptides could potentially replace antibiotics to treat multiple drug-resistant pathogens (Ghrairi, Chafar, & Hani, 2012; Lancaster, Wintermeyer, & Rodnina, 2007; Van Heel, Montalban-Lopez, & Kuipers, 2011; Yang et al., 2014). However, despite the aforementioned benefits, there are some impending issues to their application in general. AMPs are susceptible to proteases such as pepsin and trypsin (Cleveland, Montville, Nes, & Chikindas, 2001), could potentially be toxic to humans (Pacor, Giangaspero, Bacac, Sava, & Tossi, 2002), are costly to produce (Bommarius et al., 2010), are bacterial resistance to some AMPs (Bader et al., 2005), and lack of selectivity against specific strain (Eckert et al., 2006).

Based on these challenges, AMPs could possibly be modified by NTP to improve some of the functionalities. Arndt and coresearchers reported the activation of  $\beta$ -defensin during wounding

after NTP exposure (Arndt et al., 2015). Given the potential of this avenue of research, more NTP food-related research ought to be conducted to determine the possible AMPs enhancements for immobilization on food packaging materials. This could be potential new research area in active food packaging.

### Influence of NTP Processing on the Antioxidant Activity, Antioxidant Contents, and Scavenging Potential of Functional Food Components

The major antioxidant and scavenging compounds in F&V are vitamin C, vitamin E, and phenolic compounds. These bioactive compounds have the capacity to scavenge free radicals responsible for many diseases caused by oxidative stress and thereby minimize their risk (Aadil et al., 2013; Bajpai, Mishra, & Prakash, 2017). The antioxidant components in fruit and vegetable tissues are liable to degrade upon interaction with light, oxygen, or exposure to enzymes, such as polyphenol oxidase, ascorbate oxidase, cytochrome oxidase, and peroxidase, after wounding (Gil, Aguayo, & Kader, 2006). One major obstacle in the antioxidant determination is identifying the assays suitable for a particular application, as the antioxidants can induce numerous reactions, such as hydrogen peroxide or hydroperoxide decompositions, radical-scavenging, repairing biological damage, and quenching of active pro-oxidants. In such situations, the choice of the antioxidant assay should be based on its predefined function being measured (Apak, Özyürek, Güçlü, & Çapanoğlu, 2016; Niki & Noguchi, 2000). The antioxidant assays commonly used include organic radical-scavenging ability (2,2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, ABTS, and 2,2-diphenyl-1-picrylhydrazyl, DPPH), electron transfer ability (Folin-Ciocalteu, FC), and metal-reduction ability (ferric-reducing antioxidant power, FRAP) (Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot, 2017). Over the years, there has been confusion on what is being determined in antioxidant capacity and phenolic contents using the FC method. This assay determines phenolic contents, which are not a measure of the antioxidant capacity of the sample, although they are related. However, this method should be used with caution as it can be influenced by the presence of other antioxidants and type of polyphenol (Apak et al., 2016; Prior, Wu, & Schaich, 2005). Therefore, careful selection of one or more antioxidant assay can provide a broad interpretation of the antioxidant capacity of foods, provided that they were selected based on a predefined objective.

The radical-scavenging potential of functional components in food altered during NTP processing could be of benefit or disadvantageous. Such changes are particularly important for high-value foods with clear functional properties like prebiotic juices and whole F&V. Table 2 presents a summary of literature related to the influence of NTP processing on antioxidant capacity and scavenging potential. The DPPH free radical-scavenging activity of high-voltage atmospheric cold plasma-treated grape juice declined by 10.66% following 4 min of treatment. In the same way, the antioxidant capacity was found to drop drastically in a similar time-dependent manner (Pankaj et al., 2017). Likewise, the effects of NTP and ozone on the antioxidant activity of prebiotic orange juice have also been investigated. DPPH showed no significant changes among the treated and untreated samples irrespective of the mode of exposure. Meanwhile, in the ABTS assay, a pronounced (50% reduction) in the antioxidant activity with direct exposure at 60 s was recorded. It was hypothesized that the ABTS method was more responsive than the DPPH method be-

cause of the reaction that occurred between the ABTS radicals and the antioxidant compounds in the juice. Unlike the NTP-treated juice, ozonated juice lost its antioxidant capacity by 18% when compared with the untreated. Although the dosage, 0.23 mg O<sub>3</sub>/mL, was far beyond the necessary dosage needed for pathogen inactivation (Almeida et al., 2015). The antioxidant activity of NTP-treated cashew apple juice using DPPH and ABTS was also reported. In both assays, the common trend was an increased antioxidant activity after a 5-min treatment at an N<sub>2</sub> flow rate of 10 mL/min. Following an increase in treatment time and N<sub>2</sub> flow rate in the FRAP assay, the antioxidant activity was elevated, while a significant drop in antioxidant activity was observed in the DPPH assay. Therefore, low N<sub>2</sub> plasma exposure at the lesser time led to an increased antioxidant activity, whereas extended treatment times and higher flow rates led to decline in the antioxidant activity. The influence on the antioxidant potential might be due to the higher vitamin C content in the juice (Rodríguez, Gomes, Rodrigues, & Fernandes, 2017). An insignificant reduction in the antioxidant capacity of apple juice was reported following NTP exposure, however, a sharp decline was noticed with increasing the input power to 50 W for 30 s (Liao et al., 2018). During the exposure of radicchio leaves to NTP, an insignificant reduction in the antioxidant activity of the radicchio leaves was observed. The researchers, however, reported difficulty in investigating the plasma effect due to synergistic interactions of ROS, which might follow several reaction pathways (Pasquali et al., 2016).

Ramazzina and coresearcher used ABTS, DPPH, and FRAP assays to observe the effect of DBD plasma on the antioxidant activity and antioxidant contents of kiwifruit. The result showed no alteration in all the assays conducted after the NTP treatment. Generally, plasma-ROS should have caused oxidation of the phenolic compounds responsible for the antioxidant activity, however, due to the counteractive effect of the tissue response mechanisms in the kiwifruit, the ROS-induced oxidation was impeded (Ramazzina et al., 2015). The NTP treatment of fresh walnuts was found to have no effect on the antioxidant activity after 11 min of treatment. The FRAP and DPPH of the fresh walnuts were 233–240 and 226–240  $\mu$ mmol TAE/g, respectively (Amini & Ghoranneviss, 2016). A similar assertion was made for lettuce, in which antioxidant activity was insignificantly altered after exposure to NTP, regardless of assay type, power, treatment time, and type of gas used (Song et al., 2015). Another insignificant effect on the antioxidant capacity was observed in microwave-powered cold plasma-treated radish sprouts after 10 min of exposure at 900 W (Oh, Song, & Min, 2017). Equally, the scavenging activity of plasma-treated mandarin flesh was not altered after exposure; however, that of the peel was significantly increased following the DPPH assay (Yeon et al., 2017). Similarly, the DPPH-scavenging activity of plasma-treated onion powder was increased from 80.71% to 84.94% after treatment at 400 W for 40 min (Kim et al., 2017). An alternative approach for delivering plasma-generated RS to the target is the use of PAW, which had recently been demonstrated to have significant antimicrobial activity (Figure 4). However, there are sparse data on the effects of PAW on the nutritional and functional properties of food products. One study reports the antioxidant capacity of button mushroom was extended with increases in PAW processing time. Among the processing times, the PAW-15 min treatment resulted in the highest antioxidant activity (47.25%) (Xu, Tian, Ma, Liu, & Zhang, 2016). Overall, most of the studies have restricted their research to either reporting an increase or decrease in the antioxidant potential of NTP-treated food. Further work is needed to clarify the

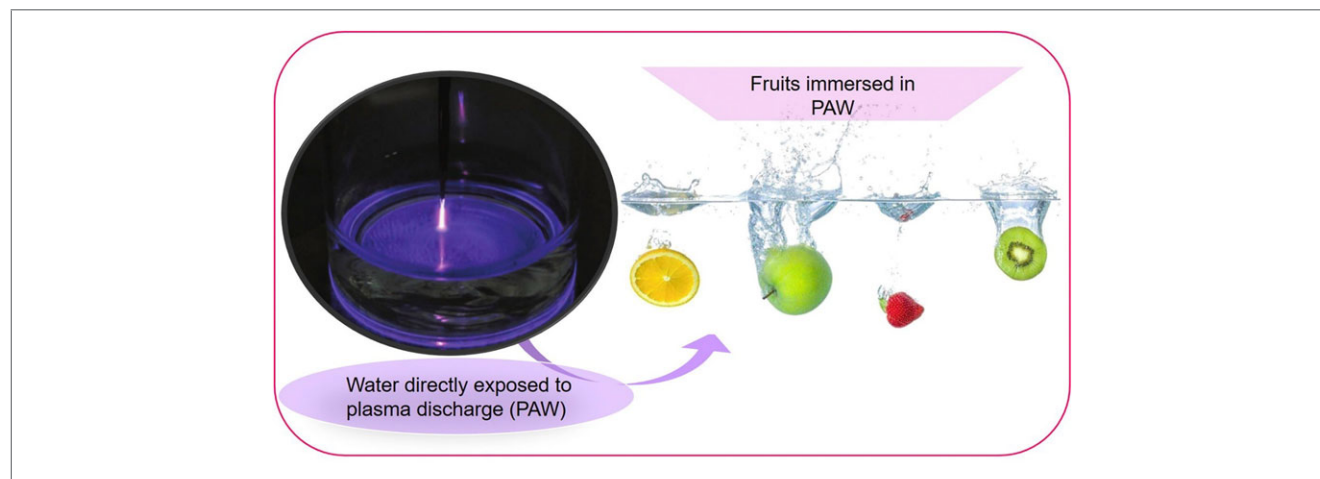


Figure 4—PAW generated from plasma exposure was used to immerse fruits for microbial inactivation.

reaction chemistry between plasma RS and antioxidants in food products.

### Influence of NTP on Vitamins

The importance of F&V as sources of different kinds of antioxidants has been discussed; however, there are also natural sources of vitamins such as biotin, riboflavin (B2), and pyridoxine (B6) (Altemimi et al., 2017; Pankaj, Wan, & Keener, 2018). These vitamins are usually stable. Others such as lycopene, carotenoids, vitamin A, C, and E, and thiamin (B1) are liable to change during processing (Pankaj et al., 2018). Various researchers have reported NTP-induced effects on the concentrations and scavenging potential of vitamin C (Vc) (Aguiló-Aguayo et al., 2013; Bevilacqua et al., 2017; Bravo et al., 2012; Pankaj et al., 2018; Rodríguez et al., 2017). This might be connected to its antioxidant potential for the regulation of ROS and RNS via quenching their induced damage to the surrounding tissues and cells (Amatore, Arbault, Ferreira, Tapsoba, & Verchier, 2008; Moldau, 1998).

Numerous articles report a positive effect of NTP processing on vitamins (Table 3). For instance, the Vc content of cashew juice was increased by 10.4% and 10.8% after 5 and 10 min NTP treatment, respectively. Upon increasing the N<sub>2</sub> flow rate and treatment time, the Vc content declined (Rodríguez et al., 2017). Similarly, the ascorbic acid content in NTP-treated prebiotic orange juice was increased from  $35.1 \pm 0.35$  mg/100 mL to  $41.11 \pm 0.33$  (direct exposure) and  $49.21 \pm 0.88$  mg/100 mL (indirect exposure) after 60 s of treatment (Diva et al., 2017). The increment was attributed to various mechanisms, such as cell distortion, dissociation of smaller-sized particles, or due to chemical reactions induced by the action of ROS. The same group reported a similar increment in the same juice treated with high-pressure processing at 450 MPa for 5 min. Meanwhile, the ascorbic acid content of blueberries (8.91 mg/100 g) increased drastically to 14.01 mg/100 g following 1 min of NTP treatment at 80 kV. On extending the treatment time to 5 min, the ascorbic acid content declined (Sarangapani et al., 2017). The treatment of button mushroom with PAW increased the concentration of Vc. However, the researchers did not give further details, only linking the increment to a postharvest storage of 7 days (Xu et al., 2016).

Yeon and co-researchers reported a distinct result after a whole mandarin was subjected to microwave cold plasma treatment. The ascorbic acid concentration in the flesh recorded an insignificant

change, which ranged between 0.5 and 0.6 mg/mL. Although this was linked to the level of energy applied, the presence of the thick mandarin peel might have shielded the target from the generated ROS (Yeon et al., 2017). Using similar plasma equipment, no reductions in the concentration of ascorbic acid was noticed after 10 min 900 W NTP treatment. Additionally, no accelerated degradation was observed during its storage at 4 °C and 10 °C (Oh et al., 2017). Irrespective of the NTP processing parameters (power, time, plasma gas), NTP-treated lettuce showed no significant effects on the concentration of ascorbic acid even after 12 days of storage (Song et al., 2015).

In contrast to the above results, a loss of 3.6%, 3.2%, and 2.8% for cucumber, carrot, and pear slices was recorded, respectively, after NTP treatment (Wang et al., 2012). Another loss of Vc concentration was found for high-voltage atmospheric cold plasma-treated orange juice. The loss of concentration was a function of treatment time (Xu, Garner, Tao, & Keener, 2017).

Looking at the aforementioned findings, it is evident that NTP had more positive than negative impacts on Vc. The critical factors found for ascorbic acid degradation are the food matrix, process gas, higher input power, and extended exposure times. Further studies on the influence of NTP on other vitamins is recommended.

### Effect of NTP Species and Their Toxicity

ROS and RNS in NTP are the most important species generated for food applications. However, it is poorly understood which species could adversely cause health-related effects upon interaction with food matrices. Liao et al. (2018) reported increased concentrations of O<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, and nitrate with treatment time and power. The accumulation of nitrates and nitrites is a concern due to induced changes in cell viability (Tresp, Hammer, Weltmann, & Reuter, 2013). Furthermore, ROS and RNS detected in PAW treated with helium gas plasma have resulted in significant effects on the rate of apoptosis (Chen, Lin, Cheng, Gjika, & Keidar, 2016), while H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>•−</sup> produced have led to generation of OH• in cells via the Haber-Weiss reaction, which resulted in apoptosis and cell death (Xu et al., 2015).

Similarly, plasma RS has induced chemical changes in food constituents, such as the modification of amino acid in proteins, oxidation of higher-molecular-weight compounds to organic acids, and lipids peroxidation, which could result in toxic metabolites



like short-chain aldehydes (Muhammad et al., 2018). The only toxicity plasma research on edible film coatings conducted on rats reported very low toxicity in the edible films, which suggested that the plasma-treated films had no harmful byproducts (Han, Suh, Hong, Kim, & Min, 2016).

Apart from the aforementioned, the potential rise in concentrations of nitrogen compounds in other food products, such as apple juice, needs in-depth analysis from researchers. Their concentration may exceed WHO standards such as 50 mg/L nitrate and 3 mg/L nitrite for drinking water, or the acceptable daily intake (ADI) of 222 mg/day for a 60-kg adult (FAO/WHO, 2012). Therefore, more scientific approaches using both animals and human subjects are required to elucidate on the interactions with plasma RS.

### Disadvantages and Limitations of NTPT

In spite of the immense contribution of NTP in various studies, its intricate RS chemistry is challenging in terms of regulatory approval and process validation. The abundant RS generated is already a complicated phenomenon, and their interactions with food materials become even more complex to understand because of the multicomponent nature of the food (starches, proteins, lipids, minerals, vitamins, and water). The reaction chemistry could be better predicted when these food components are studied in isolation (Muhammad et al., 2018). Moreover, the difficulty in the precise control of plasma reaction chemistry is worth mentioning due to the diverse moisture contents of foods (Coutinho et al., 2018). NTP treatment has caused increased lipid oxidation in high-fat foods such as walnut, peanuts, and milk, cheese, and oil after extended processing times. This was due to the oxidizing effect of radicals such as  $\text{OH}\cdot$  which might have oxidized the molecules of the lipids. Other detrimental effects were declined pH, fruit firmness, and color, whereas increased acidity and formation of off-flavors were equally mentioned (Coutinho et al., 2018; Kim et al., 2015; Muhammad et al., 2018; Thirumdas et al., 2014). These are major concerns that need an exhaustive sensory evaluation for novel food processes.

Many studies have employed a variety of gases, such as argon, helium, or their combination with oxygen, as plasma process gas (Khani, Shokri, & Khajeh, 2017; Kim et al., 2011; Rød, Hansen, Leipold, & Knöchel, 2012). Irrespective of the gases used, both ROS and RNS will still be generated, even when the process gases do not contain either of  $\text{O}_2$  or  $\text{N}_2$  (Brandenburg et al., 2007). Economic analyses are also scarce; however, the technology could be affordable when atmospheric air is used as process gas instead of the expensive noble gases. Cullen et al. (2017) highlighted the likely approach to choose a cheaper alternative (air) for industrial scale-up looking at the large-scale volume encountered in food processing. The researchers stated that the limiting factor would be the dielectric strength of air ( $3 \times 10^6 \text{ V/m}$ ), which requires high voltages to break down at atmospheric conditions (Cullen et al., 2017). Apart from the cost of the equipment design, all recurrent costs, including power and inducer gas, will probably help in estimating the operational cost. The rise in wattage consumed from the laboratory to industrial scale will be in accordance with size and capacity of the plasma equipment, ranging from watts to several thousand kilowatts. This should be compared to existing conventional and nonthermal technologies at the industrial level. For plasma systems, Niemira (2012) estimated the cost of power consumption in kWh as \$0.05. This implied that for every 1000 h of operation, \$4500 will be the approximate electricity cost.

### Conclusion and Recommendation

Despite NTPT being at a nascent stage, it is rapidly gaining interest from researchers and industry alike. There have been numerous research studies focused on microbial inactivation, while less attention has been given to the effects on food components. This review highlights the complexity of plasma RS interactions with various bioactive compounds, antioxidants, and vitamins. Moreover, this article has explained the plasma chemistry as a driver of NTP enhancement of bioactive compounds and their antioxidant potentials. Other applications include improvements in polyphenol extraction and reductions in the required extraction times. Reaction chemistry is critical in NTP modification of functional food components, which are influenced by process conditions such as voltage, process gas, and treatment time. Oxidative degradation and double bond cleavage of polyphenols induced by ROS, such as  $\text{OH}\cdot$ ,  $\text{O}_3$ , and  $\text{O}_2^-$ , are the likely mechanisms that lead to the formation of compounds with carbonyl and carboxylic groups. There is a need for further elucidation of the interaction with polyphenols and vitamins, especially vitamins that have quenching effects against ROS and RNS-induced changes. Furthermore, a potential NTP interaction with AMPs for possible enhancement could be an interesting research topic that needs attention.

In addition, the establishment of safe NTP dosages (concentrations, treatment times, input power) at which toxic effects can occur on target food matrices is important. This might be difficult due to different plasma equipment configurations and the diverse moisture levels of food products. However, this can be achieved through process validation, optimization, and control to reduce the negative impacts on high-value food products such as (F&V), milk, meat, spices, and beverages. More *in vivo* studies to ascertain the toxicity of plasma-treated food materials are highly recommended as their safety is essential for regulatory approval for industrialization of the NTP technology.

### Nomenclature

ABTS	2,2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid
ACP	Atmospheric cold plasma
AMPs	Antimicrobial peptides
Ar	Argon
DBD	Dielectric barrier discharge plasma
DPPH	2,2-diphenyl-1-picrylhydrazyl
FC	Folin–Ciocalteu
FDA	Food and Drugs Administration
F&V	Fruits and vegetables
FRAP	ferric-reducing antioxidant power
He	Helium
N	Atomic nitrogen
$\text{N}_2$	Excited nitrogen
Ne	Neon
$\text{NO}\cdot$	Nitric oxide
NTP	Nonthermal plasma
NTPT	Nonthermal plasma technology
O	Atomic oxygen species
$\text{O}_2^-$	Superoxide anion
$\text{O}_2$	Molecular oxygen
$^1\text{O}_2$	Singlet oxygen
$\text{OH}\cdot$	Hydroxyl radical
$\text{O}_3$	Ozone
PAW	Plasma activated water
RF	Radio frequency
RNS	Reactive nitrogen species
RS	Plasma reactive species



ROS	Reactive oxygen species
TAC	Total anthocyanins
TPC	Total phenolic content
TFC	Total flavonoid content
UV-C	Ultraviolet irradiation
Vc	Vitamin C

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## Conflict of Interest

P. J. Cullen is CEO of a Plasma Technology Comp.; PlasmaLeap Technologies.

## Author Contributions

Aliyu Idris Muhammad and Xinyu Liao drafted the manuscript. Patrick J. Cullen, Tian Ding, and Donghong Liu critically revised the article. Qisen Xiang, Shiguo Chen, and Xingqian Ye conceptualized the idea and drafted the outline.

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