

Review

Effects of nonthermal plasma on food safety and food quality attributes: a review

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Abstract

The use of nonthermal plasma (NTP) is a promising technology that has high efficiency, safe for the environment, and free from toxic residues. Therefore, NTP has been applied in the food industry to reduce the activity of microorganisms on foods. Even after NTP treatment, the foods exhibit satisfactory high quality in terms of physical (colour and texture) and chemical (pH, titration acidity, nutrients, and enzymes) characteristics. In the present review, the effects and mechanisms of microbial inactivation conducted using NTP on foods are reviewed. In addition, the effects on food quality attributes after plasma treatment are also discussed. Finally, the conclusions of NTP pertaining to food safety, food quality attributes, and some of the related challenges are proposed. The present review provides deeper understanding pertaining to the viability of plasma technology in food processing applications.

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Introduction

Due to worldwide awareness on food safety, controlling food spoilage and avoiding food poisoning caused by microorganisms have become trending concerns. Food spoilage and foodborne diseases caused by microbial contamination might result in massive food wastage and threat to human health, respectively (Schnabel *et al.*, 2012). According to a survey, tons of vegetables and fruits were wasted in Germany, especially fresh fruits (30%) (Schnabel *et al.*, 2012). The consumption of fresh but contaminated agricultural produce has been widely reported to be the cause of foodborne diseases in humans (Butscher *et al.*, 2016a). In 2015, FoodNet reported 4531 hospitalisations and 77 deaths, including 15% of Americans, due to nine types of food pathogens at ten locations (Pignata *et al.*, 2017). Callejon *et al.* (2015) reported 3000 patients with diarrhoea, more than 800 patients with haemolytic-uremic syndrome, and 53 deaths due to the consumption of contaminated germinating fenugreek seeds in the European Union in 2011. Therefore, the inactivation of microorganisms is critical to enhance food safety. To minimise health risks resulting from consuming contaminated foods and to ensure food safety, alternative decontamination technologies are required.

Conventional thermal treatments are not suitable for food preservation due to changes caused to food nutrients, which consequently influence

consumers' acceptance. Thus, some nonthermal technologies, such as the use of ultrasound, high-voltage pulses, and ozone have been developed to prevent the undesired effects of thermal technologies (Phan *et al.*, 2017). However, these techniques do not meet the food quality requirements of consumers. In particular, the use of ozone requires high-cost detection equipment, and the sterilisation effect is unsatisfactory (Schnabel *et al.*, 2012). Therefore, considerable effort is required to develop innovative technologies or approaches to ensure food safety and quality.

Nonthermal plasma (NTP) is considered a promising technology for food preservation due to its specific advantages such as shelf-life extension, improved quality retention, low energy consumption, moderate operational conditions, efficient decontamination ability, and environmental sustainability (Phan *et al.*, 2017; Cullen *et al.*, 2018). Plasma is defined as ionised gas that includes electrons, neutrons, ions, and radicals with strong oxidative effects (Tu *et al.*, 2011), that can decontaminate microbial species to ensure food safety and quality (Dirks *et al.*, 2012; Rød *et al.*, 2012). Recently, studies published pertaining to the application of plasma in the food industry have increasingly focused on microbial inactivation (Misra *et al.*, 2011; Bourke *et al.*, 2017) and food quality retention (Niemira and Sites, 2008). This review thus summarises the current studies pertaining to microbial inactivation in food by plasma treatment. The effects and mechanisms of microbial inactivation in food are

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critically discussed. Moreover, the effects of plasma exposure on food quality attributes are also analysed. Finally, the outlooks of NTP on food safety and food quality attributes with some of the related challenges and limitations are also proposed. The content of this review is further illustrated in Figure 1.

Microbial inactivation by NTP

Effect of NTP on microbial inactivation

Microbial pathogens, foodborne viruses, bacterial toxins, and mycotoxins are caused by microorganisms, which are considered critical causes of food safety issues (Van Boesrael *et al.*, 2013). Many publications have provided exhaustive reports pertaining to the use of NTP on solid foods like fruits, vegetables, meats, and grains for the inactivation of microorganisms. In addition to the generation of plasma in the gas phase, it can also be formed in the liquid phase to treat liquid foods. Many studies have indicated that NTP is highly efficient in the sterilisation of liquid foods such as milk and juice. All these works are shown in Tables 1 and 2, and discussed accordingly.

Microbial inactivation on solid food

The effects of NTP on microbial inactivation depend on microbial exposure patterns (direct and indirect), food surface characteristics, type of microorganisms involved, and operation parameters (voltage, frequency, power, treatment time, post-storage time, relative humidity, and carrier gas composition).

Exposure patterns

Studies have indicated that microbial

exposure patterns serve vital roles in microbial inactivation. For example, Hertwig *et al.* (2015) studied the inactivation performance of direct and indirect plasma treatment on *Bacillus subtilis* spores, *B. atrophaeus* spores, and *Salmonella enterica* inoculated on black pepper. They found that indirect plasma method exhibited higher inactivation performance, which was probably due to different mechanisms available for various plasma systems and matrix surfaces. Similarly, Ziuzina *et al.* (2014b) applied direct and indirect DBD argon plasma to inactivate *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes* present on the surface of cherry tomatoes. It could be summarised that low-density ozone transferred from oxidative free radicals might be the major active species on a complex surface without the use of direct plasma flumes, thus yielding lower inactivation performance. Moreover, the use of indirect plasma facilitates post-treatment retention of active species, promotes the diffusion of those species, and achieves better sterilisation (Ziuzina *et al.*, 2013; 2014a). Schnabel *et al.* (2012) compared the effect of direct DBD plasma with that of indirect microwave plasma involving air on contaminated *Brassica napus* seeds. The results revealed a significant decrease in *B. atrophaeus* endospores. Moreover, after 15-min indirect plasma treatment, the population of *B. atrophaeus* was below detectable levels. The aforementioned results revealed that indirect plasma treatment showed better inactivation performance than did direct plasma treatment due to the post-treatment retention of active species. All the aforementioned studies are listed in Table 1.

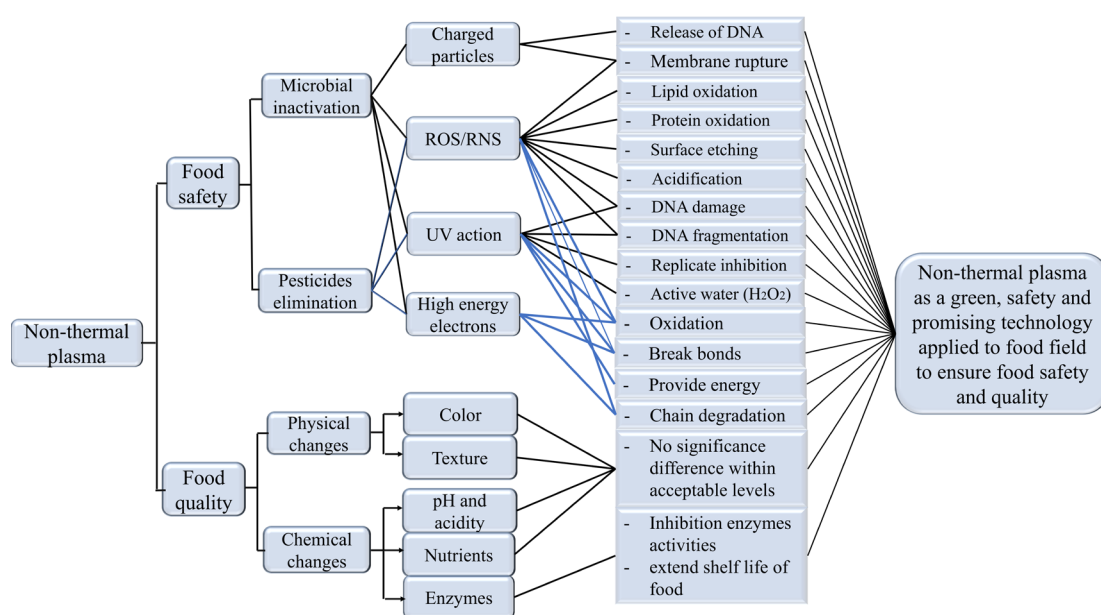


Figure 1. The removal mechanisms of microorganisms on foods by using nonthermal plasma and their corresponding effects on food quality attributes.

Table 1. Overview of the key results pertaining to the plasma inactivation of microorganisms for solid foods by nonthermal plasma.

Micro organism	Plasma parameter	Exposure type	Surface	Result/Max. reduction	Reference
<i>Escherichia coli</i>	DBD; Air; 20 mm;	Direct	Wheat	4.84 log reduction	Thomas-Popo et al. (2019)
<i>Salmonella enterica</i>	44 KV; 60 Hz; 20 min			4.32 log reduction	
<i>Listeria monocytogenes</i>	Plasma jet; air; 7.5 cm; 40 s	Direct	Apples	4.6 log reduction	Ukuku et al. (2019)
<i>Aspergillus sp.</i>	Surface DBD; 6.2 kV AC; 30 kHz; air; 0.3 L/min	Direct	Black pepper	3 log reduction after 4 min	Tanino et al. (2019)
<i>Salmonella Epidermidis</i>	Non-pulsed glow discharge; 2.7 kV;	Direct	Plastic cups	4 log reduction within 5 s	Kordová et al. (2018)
<i>Cladosporium sphaerospermum</i>	500 µA; 10% hydrogen peroxide in			3 log reduction within 30 s	
<i>Aspergillus niger</i>	air; 2.0 L/min			3 log reduction within 30 s	
<i>Total aerobic bacteria</i>				2.2 log reduction after 3 min	
<i>Escherichia coli</i>	Corona discharge plasma jet; dry air;	Direct	Radish seeds	2.0 log reduction after 3 min	Puligundla et al. (2017)
<i>Bacillus cereus</i>	2.5 m/s; 5 mm; 20 kV DC; 58 kHz			1.2 log reduction after 3 min	
<i>Salmonella spp.</i>				1.7 log reduction after 3 min	
				1.0 log reduction	
<i>Escherichia coli</i>			Tomato	4.9 log reduction	Jiang et al. (2017)
			Cantaloupe rind	1.5 log reduction	
			Spinach leaves	1.3 log reduction	
			Tomato	1.3 log reduction	
<i>Salmonella Typhimurium</i>	17 kV Corona Discharge; H ₂ O ₂ (7.8%); 9.7 ml/min air 15 psi pressure; 45 s treatment; 30 min dwell time	Indirect	Cantaloupe rind	4.2 log reduction	
			Spinach leaves	1.3 log reduction	
			Tomato	3.0 log reduction	
<i>Listeria innocua</i>			Cantaloupe rind	4.0 log reduction	

<i>Aspergillus flavus</i>	Fluidized bed plasma system with diameter 49 mm; Air/Nitrogen; 5 min; 25 kHz; 655 W; 3000 L/h	Direct	Maize	5.48 log reduction by air 4.62 log reduction by nitrogen 5.20 log reduction by air 4.68 log reduction by nitrogen	Dasan et al. (2016)
<i>Aspergillus parasiticus</i>					
<i>Bacillus cereus</i>	DBD; Air; 20 min; 250 W; 15 kHz	Direct	Brown rice	1.30 log CFU/g reduction 1.29 log CFU/g reduction	Lee et al. (2016)
<i>Bacillus subtilis</i>					
<i>Escherichia coli</i>	DBD; Air; 80 kV; post-treatment storage time 24 h; 5 min	Direct	Lettuce piece	3.3 log reduction 2.4 log reduction 2.3 log reduction	Ziuzina et al. (2015)
<i>Salmonella</i>					
<i>Listeria monocytogenes</i>					
<i>Bacillus subtilis</i> spores	Microwave-driven remote plasma; Argon; 30 min; 2.45 GHz; 1.2 kW	Indirect		2.4 log reduction 2.8 log reduction 4.1 log reduction	
<i>Bacillus atrophaeus</i> spores					
<i>Salmonella enterica</i>			Black pepper	0.8 log reduction 1.3 log reduction 2.7 log reduction	Hertwig et al. (2015)
<i>Bacillus subtilis</i> spores	Radio frequency plasma jet; Argon; 15 min; 30 W	Direct			
<i>Bacillus atrophaeus</i> spores					
<i>Salmonella enterica</i>					
<i>Escherichia coli</i>	DBD plasma; 70 kV; 70% N ₂ +30% CO ₂ (gas 1); 90% N ₂ +10% O ₂ (gas 2); air (gas 3); 70% O ₂ +30% CO ₂ (gas 4); 300 s; 24 h post-treatment storage	Direct	Plates	6.95, 2.31, and 4.23 log reduction using gases 1, 2, and 3; undetectable using gas 4 6.60, 4.72, and 3.54 log reduction using gases 1, 2, and 3; undetectable using gas 4; 6.10 log reduction using gases 1; undetectable using gases 2, 3, and 4	Han et al. (2016)
<i>Staphylococcus aureus</i>					
<i>Listeria monocytogenes</i>					
			Glass beads Glass helices Molecule sieve <i>Brassica napus</i> seeds	2.4 log reduction 2.1 log reduction 0.5 log reduction 0.7 log reduction	Schnabel et al. (2012)
<i>Bacillus atrophaeus</i> endospores	DBD plasma (direct); 8.7 kV; 5.7 kHz; argon; 10 min	Direct			

<i>Escherichia coli</i>	Microwave plasma (indirect); 2.45 GHz; 1.2 kW; air; 5 min	Glass beads	5.2 log reduction	Ziuzina et al. (2014b)
		Glass helices	3.4 log reduction	
		Molecule sieve	0.5 log reduction	
		<i>Brassica napus</i> seeds	2.4 log reduction	
		Cherry tomato	3.1 log reduction	
<i>Salmonella</i>	DBD plasma; Air; 120 kV; 50 Hz	Strawberry	3.5 log reduction	Ziuzina et al. (2014b)
		Cherry tomato	6.3 log reduction	
		Strawberry	3.8 log reduction	
<i>Listeria monocytogenes</i>		Cherry tomato	6.7 log reduction	
		Strawberry	4.2 log reduction	
		Lettuce	2.72 log reduction after 15 min	
<i>Salmonella</i>	Plasma jet; nitrogen; 1 kHz; 1 W; 12 standard litres per minute	Strawberry	1.76 log reduction after 15 min	Fernández et al. (2013)
		Potato	0.94 log reduction after 15 min	
		Membrane filters	2.7 log reduction after 5 min	
<i>Escherichia coli</i>	Plasma jet; Argon; 3.95-6.90/12.83 kV; 60 Hz; 10 min	Lettuce	0.5 log reduction at 6.90 kV	Bermudez-Aguirre et al. (2013)
		Tomato	1.7 log reduction at 12.83 kV	
		Carrot	1.7 log reduction at voltage 12.83 kV	
<i>Geobacillus stearothermophilus</i>	DBD; Argon; pulse frequency 5 - 15 kHz; pulse voltage 6 - 10 kV	Wheat grain	Less than 0.5 log reduction	Butscher et al. (2016b)
		Flat PP	0.8 log reduction after 5 min;	
		Granules PP	2.0 log reduction after 1 min	
			2.7 log reduction after 1 min;	
			5 log reduction after 5 min	
<i>Salmonella</i> Enteritidis	Resistive barrier discharge; 10 - 90 min; 35 and 65% relative humidity;		2.5 log reduction (90 min, 35% RH)	Ragni et al. (2010)
<i>Salmonella</i> Typhimurium	15 kV; air	Eggshells	3.5 log reduction (90 min, 65% RH)	
<i>Salmonella</i> Enteritidis			4.5 log reduction (90 min, 65% RH)	

Food surface properties

Schnabel *et al.* (2012) investigated the inactivation effects of plasma treatment on *B. atrophaeus* on glass beads, glass helices, molecular sieve, and *B. napus* seeds. Their findings indicated that the inactivation of *B. atrophaeus* on glass beads was quantitatively higher than that on other contaminated surfaces. Butscher *et al.* (2016b) reported a faster inactivation rate of *Geobacillus stearothermophilus* on smoother wheat grain than polypropylene (PP) samples. Moreover, Ziuzina *et al.* (2014b) found higher inactivation performance on the smooth surfaces of tomatoes than on the rough surfaces of strawberries by DBD argon plasma. Determining protection barriers that can be used for more complex surfaces which can cease the direct reaction of plasma flumes and radicals is crucial. Consequently, secondary active species, such as ozone and nitrogen oxides, are the major mechanisms that influence microbial inactivation in foods. Critzer *et al.* (2007) studied the inactivation curves of various pathogens on agar plates and found that the surface structures of cantaloupe and lettuce leaves hindered the inactivation of various pathogens. According to these studies, comparably complex topographical features of food surfaces can block microbial inactivation of direct plasma and limit the inactivation of secondary active species (Bermudez-Aguirre *et al.*, 2013). Therefore, the topographical features of solid foods have a vital influence on the efficacy of NTP on microbial inactivation. The detailed data are listed in Table 1.

Microbial characteristics

The characteristics of target microorganisms are another critical factor for achieving efficient decontamination by NTP technology. Ziuzina *et al.* (2014b) revealed that *Salmonella* and *E. coli* (Gram-negative) were more sensitive to plasma treatment when compared with *L. monocytogenes* (Gram-positive), because Gram-positive microorganisms have thicker cell walls. Frohling *et al.* (2012) and Ermolaeva *et al.* (2011) drew the same conclusion. However, Fan *et al.* (2012) believed that Gram-positive *Listeria* was more sensitive to NTP than Gram-negative *E. coli* on the surface of tomatoes. Other studies have presented that Gram-positive and Gram-negative microorganisms have similar susceptibility to inactivation by NTP (Kostov *et al.*, 2010; Klampfl *et al.*, 2012). The target microbial characteristics significantly influence microbial inactivation. However, the use of different plasma systems, inactivation processes, matrix surfaces, and microbial types might cause complex

interactions while determining inactivation performances (Ziuzina *et al.*, 2014b). The results of relevant studies are listed in Table 1.

Operational parameters

Operational conditions such as carrier gas composition, relative humidity, input energy, and treatment time can also influence the inactivation efficacy. Hury *et al.* (1998) investigated the inactivation efficiency of *Bacillus* spp. spores by using plasma with different types of carrier gases. The findings revealed that pure oxygen plasma exhibited stronger inactivation effects than pure argon plasma. Moreover, Han *et al.* (2016) applied plasma with different gas mixtures to inactivate *E. coli*, *L. monocytogenes*, and *Staphylococcus aureus*. Their results revealed that the inactivation rate for all target microorganisms increased with an increase in the plasma treatment time and oxygen content of carrier gases. The production of more reactive oxygen species (ROS) enhanced the microbial inactivation rate (Cheng *et al.*, 2014; Lu *et al.*, 2014). Furthermore, Ragni *et al.* (2010) applied air plasma to inactivate *Salmonella* Enteritidis from eggshells at different humidity conditions. After treatment for 90 min, more reduction for the population of *S. Enteritidis* was observed under higher humidity conditions, which was attributed to the formation of OH radicals. Moreover, Bermudez-Aguirre *et al.* (2013) used argon plasma with a special reactor to inactivate *E. coli* from food surfaces. The microbial inactivation was obtained as functions of plasma treatment time, input energy, and initial microbial concentration. The increase in input energy could generate more active species, and enhance microbial inactivation. Some representative findings for microbial inactivation by NTP are listed in Table 1.

These studies have indicated that exposure patterns, food surface characteristics, microbial types, and operation conditions can influence microbial inactivation efficiency on solid food surfaces. A conclusion can be drawn based on these studies that NTP has the potential of cleaning a food surface. For example, when rough surface features pose a significant challenge to microbial inactivation, high inactivation efficiency can be achieved using the in-package design to retain the active species and optimise parameters (Bourke *et al.*, 2017).

Microbial inactivation in liquid foods

An overview of the key results on plasma inactivation of microorganisms in liquid foods is summarised in Table 2. Some key factors such as operation parameters (voltage, power, treatment

time, and post-storage time), liquid environment, and gas compositions are discussed due to their roles in microbial inactivation.

Operation parameters

Plasma parameters serve a vital role in inactivating microorganisms. Surowsky *et al.* (2015) applied pulsed plasma to apple juice, and achieved a reduction of approximately 5 log for *E. coli*. In another study, Gurol *et al.* (2012) used a corona discharge plasma with an AC power supply to treat whole (3% fat), semi-skimmed (1.5% fat), and skimmed milk (0.1% fat). *E. coli* densities in the three types of milk were immediately analysed. The results revealed a significant decrease in densities with an increase in treatment time for all three kinds of milk samples. Similarly, Kim *et al.* (2015) and Van Gils *et al.* (2013) have reported a decrease in the populations of microorganisms in a liquid solution as a function of plasma treatment time. Lin *et al.* (2006) employed a DBD plasma reactor to inactivate *E. coli* in water, apple cider, and orange juice, and achieved a reduction of 5 log CFU/mL in the amount of *E. coli* in the three liquids at 30 kV and a flow rate of 150 mL/min. These results indicate that microbial inactivation depends on the input energy, plasma system, and exposure time. Moreover, the magnitude of microbial inactivation not only relies on the aforementioned parameters but also on post-storage time. For instance, Surowsky *et al.* (2014) found the permeabilisation percentage of *Citrobacter freundii* had an insignificant increment with more direct argon plasma treatment time. However, it was observed that the permeabilisation ratio of microorganisms grew rapidly after one-day storage. This phenomenon revealed that after plasma treatment, some reactive species remained in the apple juice and continued the sterilisation process during the storage period. This thus caused an increase in membrane permeabilisation, and highlighted the requirement of storage. These findings indicate that plasma parameters are considered key factors for the elimination of microorganisms.

Gas compositions

Gas composition is another crucial parameter for microbial inactivation in a liquid environment when the NTP system is used. This parameter has been widely discussed. For instance, Ma *et al.* (2002) discovered that the orange juice and milk with gas bubbles exhibited much more effective microbial inactivation than liquids without bubbles. Moreover, oxygen bubbles achieved better reduction

of microorganisms in the reaction tank than air. This phenomenon was attributed to the higher ionisation energy requirement for air than that for oxygen. Consequently, under the same parameters, the concentration of reactive species was lower in air than in oxygen. Similarly, Surowsky *et al.* (2014) also observed that the antimicrobial behaviour of NTP for *C. freundii* was enhanced by adding oxygen in the carrier gas of apple juice. By adding 0.025, 0.05, 0.075, and 0.1% of O₂ to the process gas at an exposure time of 8 min, the inactivation of *C. freundii* was achieved to be approximately 1.5, 2.1, 3.6, and 4.4 log reduction, respectively. The aforementioned results highlight the effect of gas composition, especially oxygen content, on microbial sterilisation of plasma in liquid.

Microbial response in different liquid media

The liquid-environment-based effect of NTP technology was examined to obtain a better understanding of microbial inactivation. Oehmigen *et al.* (2010) reported a 2.5 log reduction in spores present in physiological saline after plasma treatment for 30 min. However, no such spore inactivation was found in PBS-based water. A possible reason for this behaviour could be that plasma treatment caused a higher amount of acidification in physiological saline than in water. By contrast, Van Gils *et al.* (2013) claimed less inactivation of *Pseudomonas aeruginosa* in saline solution than in water by NTP. The results showed that the saline solution required longer plasma treatment time than water to achieve a similar bacterial inactivation. Moreover, milk was another widely used liquid for microbial inactivation studies under different components. Martin *et al.* (1997) reported that lower microbial inactivation was obtained in milk than in buffer solutions due to the complex composition of milk that inhibited microbial inactivation. Similarly, Grahl and Markl (1996) found that milk fat provides protection for microorganisms against plasma reactive species. However, Gurol *et al.* (2012) reported that the inactivation of *E. coli* in milk by NTP system was not affected by the fat content of milk. El-Hag *et al.* (2008) used NTP to treat *S. aureus* and *L. monocytogenes* inoculated in whole and skimmed milk, and found results similar to the aforementioned results. Differences between the results of the two studies could likely be due to the use of different systems and operation parameters (Gurol *et al.*, 2012). These findings demonstrated that the composition of a liquid influence the efficiency of microbial inactivation.

As aforementioned, these studies indicated

Table 2. Overview of the key results pertaining to the plasma inactivation of microorganisms for liquid food by nonthermal plasma.

Medium	Plasma parameter	Result/Max. reduction	Reference
Milk (whole, semi-skimmed, and skimmed)	Corona discharge; Air; 9 kV; 3, 6, 9, 12, 15, and 20 min	54% reduction in <i>E. coli</i> in three types of milk after 3 min; 4.15, 4.38, and 4.44 log CFU/mL of <i>E. coli</i> reduction in whole, semi-skimmed and skimmed milk after 20 min; 3.47, 3.6, 3.88, and 3.94 log CFU/mL of <i>E. coli</i> reduction in whole milk after 6, 9, 12, and 15 min	Guroi <i>et al.</i> (2012)
	Plasma jet; Argon and 0.025 - 0.1% oxygen; 0 - 480 s; 5 slm; 65 V	1.5, 2.1, 3.6, and 4.4 log cycles of <i>C. freundii</i> were obtained after 8 min treatment with 0.025%, 0.05%, 0.075%, and 0.1% O ₂ ; 9.7, 16.6, and 53.4% of permeabilised cells were obtained after 8 min treatment with 0, 3, and 24 h storage time	Surowsky <i>et al.</i> (2014)
	DBD plasma; Air; 30 - 50 W	3.98 - 4.34 log reduction in <i>E. coli</i> after less than 40 s treatment 3.85, 4.03, and 3.75 log CFU/mL of <i>E. coli</i> , <i>L. monocytogenes</i> , and <i>S. Typhimurium</i> reduction after 10 time; 4.76, 5.17, and 4.74 log CFU/mL of <i>E. coli</i> , <i>L. monocytogenes</i> , and <i>S. Typhimurium</i> reduction after 5 time	Liao <i>et al.</i> (2018)
Milk	DBD; Air; 250 W; 15 kHz; 5 and 10 min	1.6, 4.1, 6.1, 5.8 m and 6.5 log reduction of <i>P. aeruginosa</i> in saline solution after treatment time for 20, 40, 60, 80, 100 and 120s ; 0.2, 0.5, 2.3, 3.8, and 6.2 reduction of <i>P. aeruginosa</i> in water after treatment time for 20, 40, 60, 80, 100 and 120s	Kim <i>et al.</i> (2015)
Saline solution	Remote radio-frequency plasma		Van Gils <i>et al.</i> (2013)
Water	jet; argon; 1.5 slm; 1.4 W		
Saline solution	DBD plasma; 10 kV; 20 kHz; 30 min	2.5 log reduction of spores in physiological saline; no spore inactivation in PBS-based water	Oehmigen <i>et al.</i> (2010)
Water			
Tangerine juice	Direct-in-liquid discharge; Air; 3 min; 17 - 30 kV; 40 Hz; 25°C	4.8 and 7 log ₁₀ CFU/mL of <i>E. coli</i> reduction after 2- and 3-min treatment at 30 kV, 40 Hz and 25°C;	Yannam <i>et al.</i> (2018)
Apple juice	Needle-plate pulse plasma; Air; 9 kV; 1000 Hz;	0.54, 0.8, 1.1, 1.5, 2.1, 2.3, 6.2, and > 7 log reduction in <i>E. coli</i> with increasing pulse numbers (100, 300, 500, 1000, 2000, 2500, 3000, and 4000)	Montenegro <i>et al.</i> (2002)
Orange juice	DBD; Air; 60 kHz; 20 kV; 1.14 W/cm ²	More than 5 log of <i>S. aureus</i> , <i>E. coli</i> , and <i>C. albicans</i> after treatment for 12, 8 and 25 s	Shi <i>et al.</i> (2011)

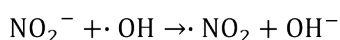
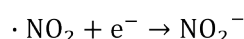
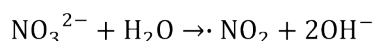
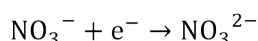
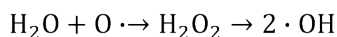
However, Vleugels *et al.* (2005) reported that the UV photon radiation has a minor role in microbial inactivation. These findings demonstrated that the antimicrobial behaviour of UV light is based on the intensity of UV radiation.

In summary, various reactive species are involved in microbial inactivation, thus leading to complex interaction mechanisms. The specific interaction mechanisms between various reactive species and microbial components are currently under research (Misra *et al.*, 2011). After obtaining this information, we can further understand the interaction mechanisms to achieve target control of microbial inactivation.

Mechanisms of microbial elimination in liquid foods

Many studies have shown that plasma-induced chemical reactions were consistent with microbial inactivation mechanisms in liquids (Surowsky *et al.*, 2015). According to different reaction behaviours, the mechanisms of microbial inactivation can be divided into three sections: (1) acid-base environment, (2) ROS, RNS, and free radicals, and (3) UV light radiation (Lukes *et al.*, 2012). These mechanisms are further elaborated in this section, as shown in Figure 2b.

Plasma applied to inactivate microorganisms in liquid food creates an acid-base environment, which is mainly due to excited nitrogen and oxygen species. These species react with each other, and generate secondary species, such as nitrous acid (NO_2^-) and nitric acid (NO_3^-) (Surowsky *et al.*, 2015). Moreover, plasma-treated liquids produce a higher concentration of H_2O_2 that leads to the generation of acidic H_3O^+ and a decrease in the pH value (Oehmigen *et al.*, 2010). All the reactions in liquid phase could be summarised as follows (Zhang *et al.*, 2012).



Moreover, Oehmigen *et al.* (2010) has proved that the acid-base environment alone did not produce comparable microbial inactivation, but the microbial inactivation performance of NTP was

significantly enhanced as the pH decreased.

The key antimicrobial active plasma components are ROS, RNS, and free radicals. The major ROS formed in liquids is $\text{OH} \cdot$, and stable molecular compounds like H_2O_2 are generated by recombination reactions (Surowsky *et al.*, 2015). When compared with $\text{OH} \cdot$, H_2O_2 is highly soluble in water, has a very long life, and increases the overall oxidising power of NTP. Especially, the presence of ROS stimulates the inactivation efficacy of RNS, which highlighted the importance of ROS in microbial inactivation (Sureshkumar *et al.* 2010; Surowsky *et al.*, 2014). Besides, ROS, especially $\text{OH} \cdot$, would react with C=C double bond of mycotoxin molecules in acidic environment. As a result of which hydroxyls are added to the double bond for the detoxification (Zhang *et al.*, 2012).

UV radiation is another key factor for inactivating microorganisms and the UV radiations could damage DNA strands as mentioned before. Among them, UV-C radiation has the highest lethal influence on microorganisms (Surowsky *et al.*, 2014). Moreover, studies presented that the UV light below 280 nm, such as UV-A and UV-B, is completely absorbed by liquid (Misra *et al.*, 2015). As is known from photochemistry, H_2O can be effectively dissociated using a significant value of UV flux with the production of numerous $\text{OH} \cdot$ (Buřle *et al.*, 2017). This method oxidises unsaturated fatty acids of the lipid bilayer, cuts peptide bonds, and oxidises amino acid side chains (Laroussi and Leipold, 2004). In general, UV has a positive role in microbial inactivation.

These findings showed that interactions between plasma and liquid molecules trigger various chemical reactions in the liquid phase. These chemical reactions in the liquid phase are more complex than those in the gas phase. Therefore, a systematic study on elimination mechanisms in the liquid environment has been widely studied currently. Further investigation on the microbial inactivation of NTP in liquid foods and determination of the plasma interactions with bacteria cell systems are necessary.

Effect of plasma on food quality

The effect of NTP on food components and properties is an indicator for determining whether NTP is a qualified potential technology to ensure food quality. A comprehensive analysis was conducted for determining the effects of NTP on the physical (colour and texture) and chemical (pH, acidity, nutrient, and enzymes) changes of food, as summarised in Table 3.

Table 3. Overview of the representative results pertaining to the effects of nonthermal plasma on food quality (colour, nutrients, and surface properties).

Sample	Plasma Parameter	Property and Results	Reference
Cashew apple juice	Benchtop plasma system; 10, 30 and 50 ml/min Nitrogen; 80 kHz; 10 min	Approx. 10% of vitamin C at 10 mL/min gas flow; Approx. 5% of vitamin C at 50 mL/min gas flow; Slight decrease in flavonoid content; Slight increase in polyphenol content; General degradation of sugar caused by the plasma Distinct perceivable colour;	Rodriguez <i>et al.</i> (2017)
Cherry tomato	DBD; Air; 30 kV, 50 Hz; 20 ± 2°C; 60 ± 5% RH; 30 - 180 s	Less firmness for the intact tissue structure; No significant change in pH	Misra <i>et al.</i> (2014)
Brown rice	DBD; Air; 25 W, 15 kHz; 5 - 20 min	Slight decrease in the yellowness and redness; Wide and shallow surface destruction at 1 kV; Narrow and deep surface destruction at 3 kV; 20 - 25% higher water absorption after 5 h soaking; Significant increase in the activity of α -amylase	Lee <i>et al.</i> (2016)
Blueberry	Plasma jet; Air; 549 W; 47 kHz; 0 - 120 s	Significant impactation of firmness	Lacombe <i>et al.</i> (2015)
Lettuce; Carrot and Tomato	Plasma jet; Argon; 60 Hz; 3.95 - 12.83 kHz; 30 s - 10 min	No significant difference on the colour of lettuce and carrot; Possible increase in redness on the colour of tomato	Bermudez-Aguirre <i>et al.</i> (2013)

Lettuce and Cabbage	Cold oxygen plasma (COP) light; 2.54 mW/cm ² energy; 10 min (5 + 5 min for each side)	No significant difference on the colour of lettuce and cabbage; No significant difference on the surface fitness of lettuce; The fitness of lettuce increments by 10% Discoloration (dark, brownish colour); Less elasticity and rougher surface; Overall dry appearance; Chlorogenic acid reduction by approx. 29%; Caffeic acid reduction by approx. 35%; Protocatechuic acid reduction by approx. 16%; No significant difference in Luteolin; Diosmetin increase by approx. 44%; Decreased lightness and longer maintenance time for colour; No significant difference in firmness, Electrolyte leakage and Soluble solid content (SSC); Ascorbic reduction by approx. 7% after 4-day storage; Chlorophyll reduction by approx. 15% Significant visual quality impact after 1-day, 4°C storage; Significant decrease of sensory evaluation after 3-day, 4°C storage Insignificant change in visual colour; Insignificant change in pH and TA; Insignificant change in SSC; Insignificant change of TPCs and antioxidant in mandarin flesh; Faster decrease TPCs and antioxidant in mandarin peel	Srey et al. (2014)
Lamb's lettuce (<i>Valerianella locusta</i>)	APPJ; Argon; 35 W; 23.12 kHz; 40 s		Grzegorzewski et al. (2011)
Kiwifruit	DBD; Air; 15 kV; 22°C; 60% RH; 40 min (20 + 20 min for each side)		Ramazina et al. (2015)
Radicchio	DBD; Air; 15 kV; 22°C; 60% RH; 12.5 kHz; 30 min		Pasquali et al. (2016)
Mandarin	Microwave; 1 L/min Nitrogen; 0.7 kPa; 400, 650, and 900 W; 2 - 10 min		Won et al. (2017)
Pomegranate juice	Plasma jet; 0.75 - 1.25 L/min argon; 2.5 kV; 3 mA; 4 W; 2.5 kHz; 3 - 7 min	Increment of TPCs by 48.99%	Herceg et al. (2016)

Pomegranate juice	Plasma jet; 0.75 - 1.25 L/min argon; 2.5 kV; 3 mA; 4 W; 2.5 kHz; 3 - 7 min	Less colour change with increased gas flow; Positive effect on the stability anthocyanins Slight increase in lightness; Significant pH decrease (4.43 to 4.0); Sugar and oligosaccharide degradation; Slight decrease of TPCs during direct plasma treatment Significant decrease of TPCs during indirect plasma treatment No significant change in lipid; Significant increase in total alcohol, especially 1-octanol	Kovacevic <i>et al.</i> (2016)
Orange juice	DBD-ACP; Air; 70 kV; 50 Hz; 20 s direct plasma/ 40 s indirect plasma		Almeida <i>et al.</i> (2015)
Raw milk	Direct-in-liquid discharge; Air; 9 kV; 90 mA; < 35°C; 3 - 20 min		Korachi <i>et al.</i> (2015)
Raw milk	Direct-in-liquid discharge; Air; 9 kV; 90 mA; < 35°C; 3 - 20 min	No significant change in colour; No significant change in pH	Gurol <i>et al.</i> (2012)
Milk	DBD; Air; 25 W; 15 kHz; 10 min	Increase in lightness and redness; decrease in yellowness; No significant change in fatty acid and lipid	Kim <i>et al.</i> (2015)
Milk	Direct-in-liquid discharge; 30.7 L/min argon; 13.56 MHz; 15 min	No significant change in the content of α -casein and whey protein	Tammineedi <i>et al.</i> (2013)
Melon	DBD; Air; 15 kV; 22°C; 60% RH; 12.5 kHz; 60 s	18% activity reduction of Peroxidase (POD); 6% activity reduction of pectin methylesterase Slightly noticeable colour change in hue; Citric acid increase along the treatment time; Significant pH decrease (4.43 to 3.90); Slight increase in vitamin C (35.11 to 41.11 mg/100 ml); 50% loss of antioxidant activity	Tappi <i>et al.</i> (2016)
Orange juice	DBD-ACP; Air; 70 kV; 50 Hz; 15 - 60 s		Almeida <i>et al.</i> (2017)

Sour cherry Marasca juice	Plasma jet; 0.75 - 1.25 L/min argon; 2.5 kV; 3 mA; 4 W; 2.5 kHz; 3 - 5 min	Total phenolic acids increase in short time exposure and degradation in longer exposure;	Garofulic <i>et al.</i> (2015)
		Total anthocyanins increase in short time exposure and degradation in longer exposure	
Apple and potato	Microwave generator; 2.5 GHz; 20 L/min air; 1.2 kW; 22°C; 1 - 10 min	52% PPO activation remaining on warm-dried apple, further decrease for more treatment time;	Bušle <i>et al.</i> (2017)
		Activation of PPO and POD remaining continuously around or below 10% on freeze-dried apple;	
		Dose-dependent inactivation of PPO and POD on potato (warm/freeze-dried)	
Fresh cut apple	DBD; 0.8 m/s air flow; 15 kV; 150 W; 12.7 kHz; 10 - 30 min	Trendily increase in firmness;	Tappi <i>et al.</i> (2014)
		No significant difference in titratable acidity (TA);	
		Limited increments of Soluble solid content (SSC);	
		Roughly linear decrease in the activity of polyphenoloxidase (PPO); 12, 32, and 58% activity decreased after 10, 20, and 30 min treatment	

Physical quality

Colour

Colour is a visible attribute for any food due to its direct influence on the perception of consumers. Parameters such as lightness (L^*), redness (a^*), and yellowness (b^*) are acknowledged by International Photometric Commission (CIE) as indicators for evaluating the human visible colour distinctions. Note that a^* and b^* indicate the chroma aspect, whereas L^* reflects the brightness aspect. In general, fruits and vegetables with higher chroma and brightness values are more attractive to customers. Thus, L^* , a^* , and b^* are commonly measured to evaluate colour changes in food before and after plasma treatment. Many studies have reported colour changes in various treated foods. The results of some studies are consistent with the review that NTP treatment can slightly contribute to visible change in the colour of foods. For instance, Ramazzina *et al.* (2015) measured the surface colour change of kiwifruits, and found a decrease in L^* after DBD air plasma treatment. Yong *et al.* (2017) investigated the processing of packaged beef jerky by a flexible thin-layer plasma to evaluate the effects on the colour. The results revealed that L^* value decreased, while a^* value and the total colour difference (ΔE) value increased in the treated samples. Liao *et al.* (2018) treated apple juice with atmospheric cold plasma (ATP) and observed its colour changes. The results showed that the L^* value of ATP treated apple juice declined with the increase in treatment time, while the increase of a^* and b^* value was observed. But Xiang *et al.* (2018) found that L^* value of apple juice did not significantly change after DBD plasma treatment, but average a^* and b^* values of DBD plasma treated samples were significantly lower than those of untreated samples. Anyway, DBD plasma caused adverse effects on certain colour parameters of apple juice. However, some studies have obtained a contradicting conclusion than the aforementioned conclusions. Bermudez-Aguirre *et al.* (2013) found no significant change in the surface colour of lettuce leaves and carrots after exposing the vegetables to an argon plasma jet for 10 min. This result is in accordance with the result obtained by Gurol *et al.* (2012), in which little effect of the corona plasma on the colour of three types of milk samples with different fat contents was discovered. Muhammad *et al.* (2019) treated tiger nut milk with ATP, and the results showed that L^* , a^* , and b^* values did not significantly change. In fact, the colour change was difficult to be detected. In addition, Hou *et al.* (2019) researched the effect of NTP treatment on the quality of blueberry juice. When compared with the heat

treatment technique, the colour of blueberry juice treated by NTP was more similar to that of untreated. No significant change was found after NTP treatment, thus indicating that NTP is a high-efficiency and low-damage technology for food production.

Texture

Many of the reported studies have examined changes in the texture of food products including vegetables, fruits, and grains, after NTP treatment. For example, Grzegorzewski *et al.* (2011) applied an argon plasma jet to lamb's lettuce. The frequent impact of energetic ions and radicals resulted in a less elastic and rougher surface condition, and an overall dry appearance. Similarly, a study investigated that in-package NTP-treated cherry tomatoes did not exhibit any negative effects in terms of their weight, pH level, and firmness (Misra *et al.*, 2014). Moreover, an increase in the firmness of freshly cut apple was revealed by Tappi *et al.* (2014) by utilising DBD air plasma. Such a phenomenon is possibly due to ozone molecules and high oxygen atmosphere created by NTP discharge that caused a strong reduction in the ripening rate (Runguang, 2011). Lee *et al.* (2016) and Chen *et al.* (2016) conducted NTP treatment on grains. Lee *et al.* (2016) applied DBD air plasma to brown rice at different operating voltages. A narrow and deep surface etching at 3 kV seemed to be more destructive, and led to higher water absorption when compared with a wide and shallow etching at 1 kV. In another study conducted by Chen *et al.* (2016), the water absorption of brown rice was reported to be 30.2% higher because of NTP etching on the rice surface. The higher water absorption of rice grains enabled easy attachment of water to rice kernels. This easy attachment consequently reduced the cooking time and germination time (Mohapatra and Bal, 2006, Chen *et al.*, 2012). Such a treatment is beneficial for rice products.

Irrespective of the aforementioned studies, we believe that there are a few negative effects, to a certain extent, on food texture after plasma treatment. Texture evaluation was conducted, and the texture was found to be within the acceptable state. This finding indicated the feasibility of plasma application in the food industry.

Chemical quality

pH and titratable acidity

The pH value and titratable acidity (TA) are directly linked with the taste and flavour of food products, especially fruits and vegetables. A low pH value and high acidity value indicate a large amount

of H⁺ and –COOH in the food, and might contribute to sour and sharp tastes, thus influencing the food quality. Different foods have different pH levels in the acceptable range. For example, fruit juice with a pH value higher than 2.8 was considered to be within the quality standards (Almeida et al., 2015; 2017), whereas the pH value of qualified milk products was approximately 6.2 (Liu et al., 2005). Plasma treatment might contribute to acidification in a liquid environment (Oehmigen et al., 2010). Thus, the studies of plasma effect on pH and TA of food are crucial. According to studies conducted by Misra et al. (2014), Tappi et al. (2014), and Won et al. (2017), no significant variations in TA were reported on the samples of cherry tomatoes, apples, and mandarins after air plasma treatment, respectively. Almeida et al. (2015; 2017) noticed a significant decrease in the pH of orange juice from the initial value of 4.43 to approximately 4.00 after conducting DBD-atmospheric cold plasma treatment twice, which is within the quality limitation of citric fruit juice. A possible explanation for acidification could be the fact that nitric acid was generated by nitric oxide and reactive nitrogen species in air plasma discharge (Oehmigen et al., 2010). In conclusion, NTP treatment may lead to lower pH values and higher TA values, but have no quality influence on the food products. This finding proves that NTP is a reliable technique in terms of food safety.

Nutrients

Different nutrients can be used to evaluate different characteristics of food products. For example, carbohydrates serve a crucial role in assessing food quality and storage. Vitamin C and anthocyanins are essential nutritional properties of fruits, vegetables, and their by-products. Lipid oxidation is a major concern for most high-fat products. According to a study conducted by Ramazzina et al. (2015), Tappi et al. (2014), and Won et al. (2017), the soluble solid content (SSC) including carbohydrates, vitamins, acids, and minerals in apple, kiwifruit, and mandarin samples, respectively, presented no significant difference after NTP treatment. In another study, Almeida et al. (2017) made an inspiring discovery that plasma could promote vitamin C content in orange juice as the treatment time was increased. Kovacevic et al. (2016) reported a positive effect on the stability of anthocyanins in pomegranate juice by using an argon plasma jet. Moreover, NTP experiments on milk samples conducted by Kim et al. (2015) and Korachi et al. (2015) have separately reached the same conclusion that no lipid oxidation occurs due to the

NTP treatment. Similarly, no significant change in the content of α -casein and whey protein was observed in milk samples treated by direct-in-liquid argon plasma by Tammineedi et al. (2013). Kim et al. (2014) found no significant influence on capsanthin values in red pepper after NTP treatment. Moreover, the treatment of fresh chicken by in-package plasma exhibited no negative effects on the appearance of chicken, and even extended the shelf life up to 14 days (Wang et al., 2016).

Enzyme activity

Similar to other chemical problems, enzyme activity is also an indicator of food product quality. The activity influence of NTP treatment represents the reliability of NTP in the food industry. The activities of several common enzymes, such as polyphenoloxidase (PPO), peroxidase (POD), and α -amylase after NTP treatment are analysed in this section. For instance, Tappi et al. (2014) found the activities of PPO in freshly cut apples to show a significant and substantial linear decrease with an increase in DBD NTP treatment time by causing the damage to the structure of the enzyme amino acid. Moreover, Tappi et al. (2016) observed the activities of POD and PME significantly reduced by 83 and 93%, respectively, after plasma treatment. Muhammad et al. (2019) found that the POD activity of the tiger nut milk was significantly reduced by DBD plasma treatment. Bußle et al. (2017) also reported that when freshly cut apples and potatoes were exposed to NTP for 10 min, the activities of PPO and POD significantly reduced from 38 to 23% and from 35 to 11%, respectively. This result was observed due to the less stable structure of POD after NTP treatment than that of PPO, and is consistent with that of the study conducted by Pankaj et al. (2013). Takai et al. (2012) found that the reactive species produced by the plasma jet induced a change in the secondary structure of enzymes, and the chemical modifications of the side chains of amino acids caused the inactivation of enzymes. These studies provided evidence that plasma treatment can be effective for degrading the activity of enzymes, such as PPO, POD, and PME, thus preventing food from blackening, and in turn prolonging their shelf life. In addition, the activities of α -amylase, phytase, and some antioxidase including superoxide dismutase (SOD) and catalase (CAT) can be increased by applying cold plasma (Han et al., 2019). Farasat et al. (2018) reported that the exposure of phytase solution with atmospheric pressure cold plasma (ACP) resulted in the enzyme activity showing a sharp rise (125% increase) for the next

four hours.

As aforementioned, the studies provided evidence for the applicability of plasma technology. Plasma technology not only achieves food decontamination and ensures food safety, but also has a slight influence on food quality and extends the shelf life of food. This suggests that plasma technology has a wide range of potential applications in the food industry.

Conclusion

The present review has summarised the applications of NTP for microbial inactivation to ensure food safety, and the related mechanisms. Researchers worldwide have reported that NTP treatment has been effective for eliminating microorganisms present on various food products. Microbial inactivation has met customer requirements and quality standards. However, the inactivation mechanisms involving NTP for solid and liquid foods have revealed significant differences. The mechanisms for solid foods generally include a direct reaction between the plasma-generated active species and bacterial cells. Consequently, damage will be inflicted on the membrane, protein, and DNA strands of bacterial cells, and cells will finally be inactivated. In contrast, the inactivation mechanism for liquid foods is based on the diffusion of active species generated from plasma discharge and water molecules, such as ROS and RNS. Moreover, the acidification of a liquid environment by conducting plasma treatment might promote microbial inactivation. Furthermore, the quality of treated food samples in terms of physical (colour and texture) and chemical (pH, nutrients, and enzymes) properties have been evaluated in different studies. Insignificant differences that are within the acceptable range of qualification between the properties of foods before and after NTP treatment has made the use of NTP as a reliable technology in the food industry, and a guarantee to ensure food quality.

NTP is considered as an environmentally safe and ecological technology that does not include chemical residues. However, this technology contains some challenges as follows: (1) the mechanisms of microbial inactivation in foods require further validation, (2) the control of process parameters to achieve specific degradation effects requires further investigation, (3) studies on the toxicological properties of food after plasma treatment are insufficient, and (4) the transition from laboratory to industrialisation is difficult. The

industrialisation of plasma in the food industry still requires considerable research.

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