Atmospheric Cold Plasma in the Food Industry

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Contributor: Darin Khumsupan , Shin-Ping Lin , Chang-Wei Hsieh , Shella Permatasari Santoso , Yu-Jou Chou , Kuan-Chen Hsieh , Hui-Wen Lin , Yuwen Ting , Kuan-Chen Cheng

The cost-effectiveness and high efficiency of atmospheric cold plasma (ACP) incentivise researchers to explore its potentials within the food industry. The destructive nature of this nonthermal technology can be utilised to inactivate foodborne pathogens, enzymatic ripening, food allergens, and pesticides.

atmospheric cold plasma food modification active packaging

1. Introduction

Incidents of foodborne outbreaks and subsequent recalls of food products are frequently caused by ineffective disinfection methods. In addition to concerns of microorganisms' resistance to standard food processing practices, producers also need to consider other marketing factors, such as chemical-free products, minimal processing, and safety, to satisfy consumers' demands [1][2][3]. Conventionally, thermal processes which involve the incorporation of heat, including pasteurisation and sterilisation, have been extensively exploited. However, the techniques have several drawbacks: long processing time, loss of sensory properties, and degradation of thermally sensitive nutrients. Scientists have looked into other approaches to overcome these problems.

Plasma is commonly referred to as the fourth state of matter based on the levels of energy, after solid, liquid, and gas. It comprises electrons, ions, neutral species, photons, and metastable and other excited gaseous atoms. Both man-made and naturally occurring plasmas can have a wide range of temperatures and densities. The ability to regulate their behaviour is of great interest to the scientific community [4][5][6]. The first application of plasma was conducted by Werner von Siemens in 1857 when he created a dielectric barrier discharge ozoniser to treat water. Then, the term plasma was later coined in 1928 by Irving Langmuir [7][8]. Plasma can be classified into two types: thermal and nonthermal (low temperature) [3]. Non-equilibrium or "cold" plasma, which are both types of nonthermal plasma, are states that are not in local thermodynamic equilibrium (usually less than 60 °C) [9].

As a result, cold plasma at atmospheric pressure has attracted substantial attention within the food industry in the past decade since the expensive vacuum pump can be omitted while the generated cold plasma still maintains similar properties. In food technology, a diverse application of atmospheric cold plasma (ACP) can be observed since it offers numerous benefits, as summarised in **Figure 1**. ACP is considered as an economical processing technology as it does not require heat, pressure, water, or additional chemical solvents [10]. Moreover, it utilises less energy than conventional methods due to shorter treatment time [3]. However, ACP still encounters some constraints that limit its full potential within the food processing industry.

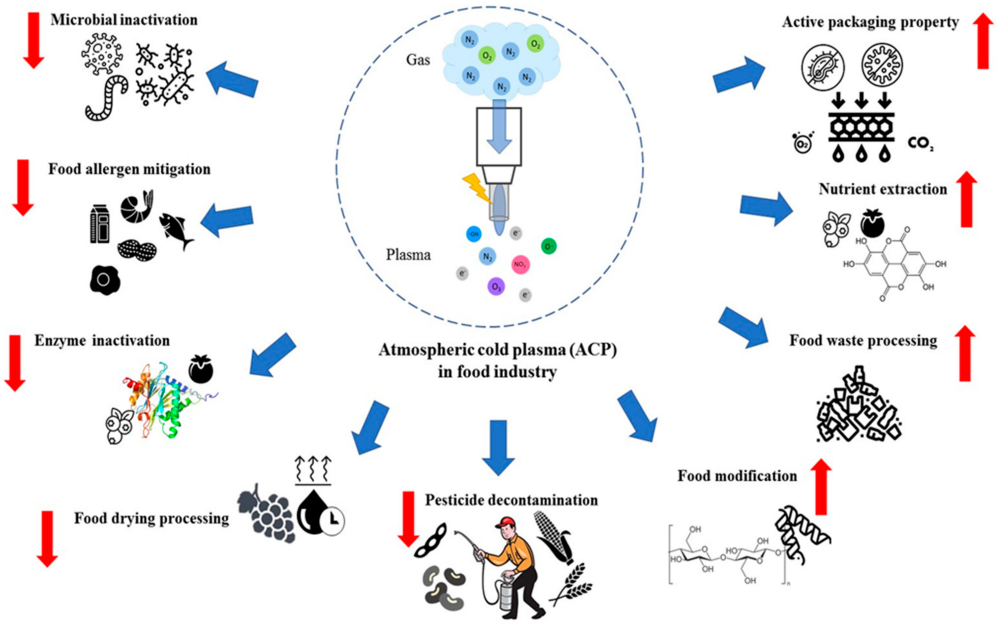

Figure 1. Applications of atmospheric cold plasma (ACP): Depending on the set parameters, ACP can be utilised to inactivate pathogens and compounds, or modify food products.

2. Atmospheric Cold Plasma (ACP) in Food Technology

2.1. Microbial Inactivation

Most studies focus on bacteria such as *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* species, which are some of the most common food pathogens. *Escherichia coli* is a Gram-negative bacterium commonly found in the gut of humans and animals. While most strains of *E. coli* are benign, some can be pathogenic and trigger severe diarrhoea. Each year, more than 1.7 billion people suffer from severe diarrhoea; in addition, approximately 760,000 children under 5 years old die from diarrheal diseases every year, making it the second leading cause of death amongst children under 5 years old [11]. Similarly, another rod-shaped, Gram-negative bacterium that has always been a major public health concern is *Salmonella*. *Salmonella* accounts for 155,000 deaths annually and it is one of the main causes for gastroenteritis [12]. Both *E. coli* and *Salmonella* can live in a variety of food products including meat, dairy, and vegetables [11][12].

Moreover, *Listeria monocytogenes* is another bacterial pathogen which results in 19% of the deaths of Americans each year in cases related to food contamination [13]. The growing popularity of ready-to-eat products as well as the refrigeration system in industrial-scale food processes make the bacteria such a prevalent pathogen as they can develop resistance to environmental stress and multiply in cold temperatures [14]. The research of ACP on tender coconut water concluded that ACP can be utilised as a nonthermal process to inactivate Gram-negative bacteria *E. coli* and Gram-positive bacteria *L. monocytogenes*, lengthening the shelf life of tender coconut water by up to 48 days [15]. The experiment was carried out using dry and modified air M65 (65% O_2, 30% CO_2, and 5% N_2) with the treatment time at 120 s at 90 kV. According to the results from optical emission spectroscopy, RONS were responsible for bacterial cell leakage or induced other morphological changes in bacteria [15].

Overall, the mechanism of ACP to inactivate bacteria was examined and it was concluded that in Gram-negative bacteria, RONS generated by ACP damage the lipoproteins and peptidoglycans of the cell envelope which lead to cell leakage and disruption. Meanwhile, ACP does not promote cell leakage in Gram-positive bacteria but instead impair essential cellular components such as DNA [16].

ACP has also been extended to the inactivation of other microbes, including viruses, yeasts, and fungi. Although viral contamination in food is less frequent than that of bacteria, its effect on public health is as severe. Human norovirus is one of the viral pathogens which can also cause gastroenteritis. As the virus usually exists in water, it can pose danger to any food products that carry water, such as fruits, vegetables, and shellfish. Currently, human norovirus cell culture systems cannot be cultivated in a laboratory setting and the researchers have to rely on murine norovirus and Tulane virus as surrogates [17]. A viral inactivation experiment was performed on the Tulane virus and murine norovirus on blueberries using a plasma jet under the following conditions: 4 cubic feet/min (cfm), 0–60 s treatment time, and 7.5 cm treatment distance. The study observed a notable reduction of Tulane virus (1.5 PFU/g) compared to the control after 45 s of treatment time, which demonstrated the potential of ACP on blueberry processing [18]. In viruses, RONS are the main contributors of viral inactivation as they penetrate through capsid by diffusion and cause damage to the RNA [19].

The research on other pathogens was investigated. The effect of ACP on a yeast strain *Saccharomyces cerevisiae* was studied and it was reported that this medium can also influence the sterilisation efficacy in addition to plasma conditions (oxygen gas level, electrical power, and treatment time). According to the results, ACP showed the highest inactivation efficiency when *S. cerevisiae* is in water and saline solution. This outcome elucidated that ACP may be the most suitable food processing choice when the media are water or other biological fluids containing NaCl. Contrastingly, the plasma treatment of *S. cerevisiae* in YPD media exhibited the least efficiency, which asserts that the ability of ACP to inactivate microorganisms is reduced in nutrient-rich solutions [20]. Moreover, the study also concluded that OH radicals played the most vital role in yeast cell inactivation. The experiment assessed the level of oxygen reactive species and reported that the water media contained the highest level of OH radicals, whereas YPD had the lowest. One possible explanation is that oxygen radicals were quenched when ACP were treated in YPD solution, which resulted in less lipid peroxidation and subsequently less cell damage [20].

Aflatoxins, produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, are mutagenic and carcinogenic compounds discovered in 1960 during an outbreak called “Turkey ‘X’ Disease” in the UK [21]. B1 and B2 aflatoxins are produced by *A. flavus*, whereas B1, B2, G1 and G2 aflatoxins are all produced by *A. parasiticus* [22]. The most toxic aflatoxin is B1 as it is linked to liver cancer. All aflatoxins can commonly be found in poorly dried foods such as cereals, spices, and nuts, where the fungi can proliferate. Since aflatoxins are extremely tolerant to both heat and freezing temperatures, they can exist in food indefinitely [21]. Devi and team [22] published the results on the effect of ACP on fungal growth in groundnuts and elimination of aflatoxins. After the inoculated groundnuts were plasma-treated with 60 W for 15 min, they observed the reduction in *A. parasiticus* by 97.9% and that of *A. flavus* by 99.3%. The results from electron microscopy showed that RONS generated electroporation and etching of fungal spore membranes. This study indicated the potential application of ACP to eliminate pathogenic fungi and

their toxins. Despite these diverse scientific studies, it should be noted that the efficacy of ACP depends on the microbial species, density of pathogens, plasma system, and treatment conditions [16].

2.2. Active Food Packaging

Packaging can improve food quality and safety by protecting the product from undesirable external conditions such as moisture, microorganisms, dust, and extraneous materials. However, traditional packaging is limited in extending the shelf life of food; hence a functional packaging, also known as active packaging, is explored. According to the statement issued by Commission Regulation (EC) No. 450/2009, the active packaging aims to "deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food" [23].

Wong et al. [24] investigated the effectiveness of ACP-treated PE film coated with chitosan and gallic acid film for tilapia fillet preservation. The addition of 1% chitosan and gallic acid extended freshness of the fillet for 14 days as it can hinder bacteria accumulation by 1.52 log CFU/g compared to control, and delayed volatile basic nitrogen and thiobarbituric acid by 89.9% and 33.3%, respectively [24]. At the molecular level, the bombardment of reactive species, particularly OH radicals, oxidises covalent bonds on the film surface to form carboxyl and other oxygen-containing functional groups [25]. This process also enhances the roughness of the film surface which can facilitate the incorporation of other antioxidant or antimicrobial compounds [26][27]. Thus, it can be perceived that ACP has the potential to be integrated into many steps within the food packaging process, from the production of active packaging to post-contamination prevention. The ability to control the production of OH radicals and other reactive species would certainly be beneficial to the progress in this technology.

2.3. Food Allergen Mitigation

Food allergy is *a critical* food safety issue, and its prevalence is growing continuously between 2 and 10% [28]. Many studies investigate the influence of ACP on food allergens. The eight major allergens, such as milk, eggs, fish, shellfish, tree nuts, peanuts, wheat, and soybean, account for 90% of food allergies and serious allergic reactions in the world [29]. While thermal processing is commonly used to reduce allergenicity in food, most allergenic proteins are thermally stable [30].

ACP has become an alternative approach to deactivating allergens via structural changes in proteins. Ng et al. [31] treated milk allergens casein, β-lactoglobulin, and α-lactalbumin through spark discharge (SD) and glow discharge (GD) ACP. After SD-ACP and GD-ACP treatment for 30 min, the antigenicity of casein was decreased by 49.9 and 91.1%, whereas that of α-lactalbumin was reduced by 49.5 and 45.5% compared to control, respectively. In milk, RONS from ACP decrease the antigenicity by denaturing α-lactalbumin and β-lactoglobulin proteins. This is achieved by the destruction of hydrogen bonds, which stimulates protein aggregation through disulphide bonding [32]. Essentially, ACP induces intermolecular cross-linkage from the cysteine residues of milk allergens, which affects primary and secondary structures and hence their binding capacity to antibodies [33].

A similar mechanism also occurs in the egg allergen. In more than 35% of patients who are allergic to eggs, lysozyme is the main cause of their allergic reactions [34]. Lysozyme has been used extensively as a food additive as it can hydrolyse the cell wall of Gram-positive bacteria and induce cell disruption, increasing products' shelf life. Consequently, ingredient labels must add the information about any egg-derived additives including lysozyme for safety purposes. Using a low-frequency plasma jet on a 0.3 mL lysozyme sample containing 0.1 mg/mL in 10 mM phosphate buffer (pH 7.4), a denaturation of lysozyme could be detected [35]. It was proposed that the denaturation mechanism of lysozyme by ACP was attributed to the RONS, which induce chemical changes in certain amino acids such as cysteine, phenylalanine, tyrosine, and tryptophan. After the exposure to ACP, the allergenic proteins are reported to have lost their secondary structures, such as α-helices and β-sheets, which result in the destruction of enzymes' binding sites [35]. This study highlights the effect of ACP on lysozyme's activity, which is caused by structural changes of the protein. Nevertheless, it is also possible for new proteins to be formed after the interaction with active species [16].

Peanut allergy is the most prevalent food allergy in many Western countries. The effects of ACP (60 min of treatment time) on major peanut allergens Ara h 1 and Ara h 2 were observed using whole peanut and defatted peanut flour, which showed that the antigenicity was reduced by 65% for Ara h 1 and 66% for Ara h 2. The decrease in antigenicity may be due to the ability of plasma reactive species to change secondary structures of the allergens, which also reduces peanut protein solubility [36]. Liu et al. [37] indicated that the conformational alteration is caused by an oxidation of peptide bond amino groups, such as Trp, Tyr, and Phe amino acid residues. Furthermore, the cleavage of these polypeptide chains can partially diminish linear epitopes. It is intriguing to note that moderate ACP treatment improves the functionality of soy protein, such as solubility, emulsification, and foaming properties, while overexposure may result in denaturation of the soy protein [37][38].

2.4. Enzyme Inactivation

Studies of endogenous food enzyme inactivation have been investigated using DBD and a plasma jet to increase shelf life. One such food is wheatgerm. After treating wheatgerm at 24 kV for 25 min, the results showed that lipase decreased by 25.03% while lipoxygenase dropped by 49.98%. However, the extension of treatment time beyond 25 min did not drastically improve the inactivation efficiency. It is also interesting to note that the inactivation effect was not permanent, as the both lipase and lipoxygenase enzymes recovered some of their activities during the storage period. However, the result showed that ACP did not affect the phenolic content of wheatgerm. This suggests that ACP can be a great boon when it comes to inactivation of endogenous enzymes in food processing [39].

Other endogenous enzyme inactivations are studied, including pectin methylesterase (PME), polyphenol oxidase (PPO), and peroxidase (POD). All of them are normally found in fruits and vegetables as they are responsible for ripening and softening [40][41]. The presence of these enzymes in food commodities can shorten shelf life, which depreciates their market values. Typically, pasteurisation is implemented in the food process to inactivate these enzymes as well as other microorganisms [41].

Tappi and team [42] used fresh-cut melon to observe the effect of ACP on POD and PME. According to the result, POD and PME were reduced by 17% and 7%, respectively, after 15 min of plasma exposure. After the treatment, the samples could be stored up to 4 days at 10 °C compared to 2.5–3 days of untreated samples [42]. The study highlights ACP's ability to inactivate undesirable enzymes and microorganisms in food processing. Further developments of the method may be highly valuable to the food industry.

2.5. Food Drying Pre-Treatment

In order to lengthen food shelf life and reduce transportation costs, dehydration is the process that is commonly used. However, conventional drying techniques may cause degradation to heat-sensitive compounds which results in quality deterioration, such as loss of texture, nutrients, pigment, and aroma. For this reason, there has been an increasing interest in the use of nonthermal processing which aims to enhance the drying process.

The drying processing is a major operation, especially in agro products. Conventionally, a pre-treatment method, such as dipping in an alkaline solution, is employed to hasten the drying step and extend the shelf life, but concerns regarding chemical wastewater and toxicity from chemical residues are raised. Using a plasma jet, Huang and colleagues [43] pre-treated the grape surface three times at a power of 500 W and a frequency of 25 kHz to observe the drying rate of plasma-treated grapes. The results showed that the rate of moisture loss increased as the distance between the plasma nozzle and grape decreased. The experiment reported that no changes in appearance, colour, and antioxidant content of the sample were detected after the ACP treatment [43]. Moreover, they also detected an increase in the total phenolic content (TPC) from approximately 30 mg to 60 mg per 100 g of raisin, in addition to the change in antioxidant capacity from 4.5% to 10%. This is due to the efficiency of moisture diffusivity which reduces the drying time and energy consumption by up to 26.27 and 26.30%, respectively [43]. Similarly, in wolfberry, 45 s of ACP treatment could also shorten the drying time by 50% and increase the rehydration ratio by 7–16%. As the detection of phytochemical contents increased after the ACP treatment, the authors speculated that ultrastructure alteration results in the release of compounds that were trapped within cells, thereby raising the phytochemical contents [44].

Corn is one of the major grains in the world. Fresh corn kernels are easily spoiled due to their high moisture content, which means that drying technology is a crucial operation in corn post-harvest handling to extend the storage period [45]. ACP pre-treatment can be implemented to improve the drying efficiency of corn kernels. Setting the parameters at 500 W for 30 s, the drying time was reduced by 21.52%, and the drying rate was increased by 8.15%. The activation energy of drying kinetics from ACP was 47.79 kJ mol^{-1}, compared to 54.82 kJ mol^{-1} of the control group. Furthermore, atomic force microscopy displayed the surface topography of plasma-treated corn kernel having shrunk or damaged granules [45][46]. This result may help explain the high effectiveness of ACP in the drying pre-treatment process.

2.6. Pesticide Decontamination

One of the most common pesticides in the world is an organophosphate chemical called chlorpyrifos. Having been introduced in 1965, chlorpyrifos is widely used in the cultivation of various plants, such as fruits, vegetables, nuts, and grains [47]. Consequently, many food crops are found to contain high concentrations of this pesticide. The publication of chlorpyrifos and carbaryl degradation on the corn surface using ACP was observed. Based on the optimisation study, the treatment time of 60 s, air flow rate of 1000 mL/min, power of 20 W, and frequency of 1200 Hz resulted in 86.2% and 66.6% degradation efficiency of chlorpyrifos and carbaryl, respectively. Moreover, the treatment did not significantly affect the nutritional quality of corn, showcasing that ACP can be a promising food processing treatment for pesticide degradation [48].

Another study of chlorpyrifos was conducted along with a pesticide called diazinon on apples and cucumbers. The pesticide-dipped fruits were used to test the efficiency of ACP on the toxic degradation in which the humidity, firmness, colour, and sugar percentage of the fruits were determined. According to the results, ACP had relatively minimal effects on those parameters when it was set at 10 min exposure and 13 kV; however, the changes occurred when the treatment time increased [49]. Another interesting point should be mentioned, since the study illustrated varying detoxification efficiency between halogenated pesticides such as chlorpyrifos and the non-halogenated ones such as diazinon. Due to the chemical composition, polarity, or penetration of plant tissue, ACP was able to remove dianizon more effectively than chlorpyrifos [49]. According to the study, ACP may be highly applicable in food processing as a pesticide decontamination method.

2.7. Food Modification

Corn starch is one of the food materials that can be altered to improve its poor physical and functional properties, enhancing its solubility or viscosity. A study reported physicochemical changes in corn starch after being treated with ACP for 30 min at 400–800 W. After washing with distilled water, the peak viscosity, final viscosity, and setback of starch samples were reduced by 87.1%, 92%, and 93.3%, respectively. The results highlight that ACP causes etching on the starch grains, which contributes to solubility and clarity [50].

Wheat is one of the most common staple crops in the world as it is used as an ingredient in bread, pasta, and other bakery products. Consequently, numerous chemicals and enzymes are used on wheat as oxidising or bleaching agents. To avoid potential toxicity from these additives, the effect on wheat flour of nonthermal technology, such as high pressure processing and ACP, has been widely studied. Since ACP can generate strong oxidising agents, such as RONS, the technique can replace the conventional oxidising agent without leaving any toxic residues during wheat processing. According to the report on ACP on wheat flour, treatment times ranging between 5 and 30 min at 80 kV induce depolymerisation of starch and reduces its crystallinity, which essentially increases the hydration and viscosity of wheat flour [51].

Similar results could also be observed in xanthan gum. While various chemical and enzymatic techniques to improve its functionality exist, the treatments can be costly or involve tedious procedures. Using ACP treatment (60 W for 20 min), Bulbul et al. [52] found an increase in the porosity and compressibility index of xanthan gum. Moreover, another study demonstrated similar results after exposing xanthan gum to ACP for 20 min at 3.5 kV. The

samples showed lower shear viscosity and increasing emulsifying capacity without any effect on their whiteness [53]. The research investigation concluded that ACP can be a practical processing technique for xanthan gum to expand its functional characteristics.

Under the scope of protein modification, bovine serum albumin (BSA) was treated with ACP, which caused protein unfolding and changes in the secondary structure. This finding suggests that ACP promotes the structural alteration, aggregation, peptide cleavage, and side-group modification of proteins [54]. Based on the characterisation study, ACP promotes structural conformation and unfolding of the polypeptide chain, which leads to more hydrogen bonding [55]. To summarise, while many researchers study the ability of ACP to alter polysaccharide and protein structures [56][57][58][59], few publications actually evaluate its exact mechanism, particularly on the physicochemical reactions between proteins and active species. Thus, more research on the topic is vital to gain better insights on its efficacy.

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