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The Study of cold plasma on extending of shelf life of Oyster mushroom

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ABSTRACT: In the past, cold plasma is used for sterilization of sensitive materials and now it is extended to food industries as a novel technology. For years cold plasma processing has been viewed as useful for microbial inactivation while maintaining quality of fresh produce. However, this process is not effective for in vitro model food systems for inactivation of microbes or enzymes which are present in intact tissues, as it is a surface phenomenon. Cold plasma technology is also used to inactivate endogenous enzymes which are responsible for browning reactions particularly polyphenoloxidase and peroxidases. Several research investigations showed a reduced growth of microorganism via different mode of actions by etching phenomenon, cell disruption by electrophoration etc. Plasma technology is considered as modern non conventional technique which is used for the preparation of modified Oyster mushroom, altering its physical and chemical properties. Overall application of cold plasma for microbial destruction on different food substrates Oyster mushroom. was discussed. Besides this, it is also used to alter the germination rate of seeds. It is an eco-friendly process which is used in the preservation of food and other potential applications as an alternative to common techniques.

Keywords: *Cold Plasma; Microbial Inactivation; Reactive Species; Surface Modifications*

INTRODUCTION

Matter on earth exists mostly in three distinct phases (gas, liquid and solid) but when universe is considered as fourth state of matter which abundantly exists. So, Plasma is hence referred to as the fourth state of matter, next to solids, liquids and gases. The term 'Plasma' was first employed by Irving Langmuir in 1928 to define this fourth state of matter which is partially or wholly ionized state of gas and discovered plasma oscillations in ionized gas [1]. The change of phase from solid to liquid and further to gas occurs as we increase the energy input likewise increasing the energy input beyond a certain level in gas state causes ionization of molecules which yields the plasma state [2]. d Agostino et al. [3] reported that plasma can be obtained either in low temperature, non-equilibrium glow discharge or high temperature, equilibrium thermal plasma. Based on the properties of plasma, it is used in various fields like textile, electronics, life sciences, packaging etc. [4]. The application of plasma technology as a surface

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cleaning tool has been commercially adopted for the removal of disinfection chemicals applied to medical devices manufactured from heat sensitive plastics [5]. In the biomedical sector plasma technology is used for cold sterilization of instruments and prostheses as well as many thermo labile materials used in the biomedical technology sector for its particular advantages, including its moderate or negligible impact on substrate materials and use on nontoxic compounds [6]. Conventionally, sterilization methods such as heat, chemical solutions are used for the surface disinfection of fruits, seeds, and spices etc., which are often time-consuming and damaging or have toxic residues [7]. Van de Veen et al. [8] reported that the effect of cold plasma on bacterial spores is more than the conventional techniques like heat, chemicals and UV treatment. The objective of this review are first, to present recent knowledge on effect of cold plasma on microbial inactivation and structural modifications of packaging materials as many reviews has been published on these topics. Secondly, the effect of cold plasma on endogenous enzymes, seed germination, Oyster mushroom modifications and limitations for its potential application in food sector as novel technology. One of the important challenges associated with cold plasma technology is ensuring high microbial inactivation while maintaining sensory qualities that ensure there fresh appearance. Plasma Chemistry: Process & Categories In plasma processing ionization is always considered as first important element followed by other factors like reaction rate, rate constants, the mean free path, the electron energy distribution [9]. Plasma chemical process can be divided into two categories based on reactions i) homogeneous gas-phase reaction (for example generation of N3 from N2) and ii) heterogeneous reaction where plasma comes in contact with the solid or liquid medium. The heterogeneous reaction is further classified into three sub categories. In the first sub category, material is removed from the surface termed as etching or ablation; in the second sub category, material is added on the solid surface in the form of thin film observed during plasma polymerization by a process called plasma enhanced chemical vapour deposition and in third sub category there is no material added or removed but the substrate surface is modified physically and chemically during exposure to plasma [3]. Different types of possible mechanisms that the gas used for plasma generation may act on the substrate during the plasma processing are shown in (Fig. 1). Plasma can be produced by subjecting a gas to an electric field (between two electrodes), either of constant (directcurrent field) or alternating amplitude (usually high frequency field). Plasma state can be attained by the application of energy in several forms including; thermal, electric or magnetic fields and radio or microwave frequencies, which increase the kinetic energy of the electrons resulting in increased number of collisions in the gas forming plasma products like electrons, ions, radicals and radiation of varying wavelengths including that in the UV ranges. The various approaches used for plasma generation includes the corona discharge, dielectric barrier discharges (DBD), radio frequency plasma (RFP) and the gliding arc discharge [10]. Cold plasmas, including lowpressure DC and RF discharges (silent discharges), discharges from fluorescent (e.g., neon) illuminating tubes, DBDs may be found both at low pressure or atmospheric pressure [11]. The dielectric barrier discharges (DBDs), historically also called ‘silent discharges’. They also operate at approximately atmospheric pressure (typically 0.1–1 atm). An A.C. voltage with amplitude of 1–100 kV and a frequency of a few Hz to MHz is applied to the discharge, and a dielectric layer (made of glass, quartz, ceramic material or polymers) is again placed between the electrodes. When a potential difference is applied between cathode and anode, a continuous current will flow through the discharge; giving rise to direct current (D.C) glow discharge. Capacitively coupled (CC) radio-frequency (RF) discharges are produced when alternating voltage is applied between the two electrodes, so that each electrode will act alternately as the cathode and anode. The frequencies generally used for these alternating voltages are typically in the radiofrequency (RF) range (1 kHz–103 MHz; with a most common value of 13.56 MHz). Non thermal gas discharges at atmospheric pressure are of interest for the food industries as they don’t subject the food system to extreme conditions. Evolution of Plasma’s Research or Application Going back to history in 1960’s for the first time sterilization property of plasma was introduced, and a patent was filed in the year 1968 [12]. It was reported that the destruction

of 106 spores in inner surface of vials occurred in less than second using argon plasma by pulsed RF field atmospheric plasma. A series of patents were even filed by Ashman and Menashi [13], Boucher [14], and Bithell [15] reported that electrical discharge in particular gases can lead to complete sterilization.

Boucher [14] in his patent explained the role of UV radiations on microbial inactivation along with plasma. It was reported that UV photon can penetrate to the depth of only one micrometer, where as plasma penetrated to the depth of 10 micrometer was observed which is helpful for the destruction of sporulated bacteria. Jacobs and Lin [16] used H2O2 as sterilizing agent and applied plasma to remove the residues of chemical on the sterilized products. The first work with plasma was made from oxygen which was proposed in 1989 and its lethal activity was defined as it interferes with the biological matter. Nelson and Berger [17] reported that O2 plasma showed efficient biocidal action on B. subtilis and Clostridium sporogenes as these two were considered as the most resistant bacteria. Plasmas generated at 200 W were sufficient to reduce the population of B. subtilis more than 3.5 log10 in 5 min [18]. From then the utilization of plasma for sterilization was commercialized. Plasma treatment can effectively inactivate a wide range of microorganisms including spores [19–21] and viruses [22]. Feichtinger et al. [19] reported that cold plasma technology preferred as alternative source for surface sterilization and disinfection process which can act on both vegetative cells and spores with shorter periods of time. The chemical composition of plasma contains free radicals, highly reactive species and radiations are often generated in varying range from UV to visible. It is believed that the role of different constituent depends on the gas and operating pressure. The destruction of microbial DNA by UV irradiation, volatilization of compounds from spore, so-called "etching" of the spore surface by adsorption is because of reactive species like free radicals [23]. Cold plasma can be successfully employed for microbial destruction on fresh products to increase shelf life. In recent investigation of Misra et al. [24] reported that decrease in total mesophilic count was 12–85 %, yeast and mould count by 44–95 % in cold plasma treated strawberries. Raw milk was treated for destruction of E. coli using low temperature plasma by Gurol et al. [25]. They observed 54 % reduction of E.coli after the treatment of 3 min. Application of air plasma and SF6 on nuts (pea nuts, hazel nuts and pistachio) found that 50 % reduction in total aflatoxins using air plasma and 20 % reduction using SF6 [26]. Fernandez et al. [27] reported that treating with plasma for 15 min can achieve 2.72, 1.76 and 0.94 log reductions of S. Typhimurium on lettuce, strawberry and potato respectively. The efficiency of microbial inactivation depends upon the surface of treating produce, plasma device, gas composition, and mode of exposure. Produces like potatoes, strawberries took more time for complete destruction of microbes due to grooves and uneven surfaces [27, 28]. Vannini et al. [29] applied the gas plasma for the decontamination of Salmonella Enteritidis and Listeria monocytogenes from table eggs. The maximum reduction was observed to be 4 and 5 log reduction of Salmonella Enteritidis and Listeria monocytogenes respectively. The efficiency of microbial reduction was improved with increase in humidity of air. Experimental results of several authors suggested that the efficacy of cold plasma on particular microorganism depends upon the treated surface. For example, the destruction of Listeria monocytogenes was high in sliced cheese when compared to sliced ham [30]. It is also reported that there is more than 8 log reduction after exposure to 150 W for 120 s in sliced cheese and in sliced ham after exposure to 120 s there is 0.25 to 1.73 cfu/g reduction in microbial growth. Citrobacter freundii loads in apple juice were reduced by about 5 log cycles after a plasma exposure of 480 s using argon and 0.1 % oxygen plus a subsequent storage time of 24 h reported by Surowsky et al. [31]. Recent findings in the area of cold plasma for the inactivation of microorganisms on food surfaces are shown in Table 1. Functionality of Plasma Effect of Plasma on Microbial Cells The effect of plasma treatment on microbial cells is mainly due to the plasma ions and cell interactions. The reactive species in plasma have been widely associated with the direct oxidative effects on the outer surface of microbial cells. The effect of plasma is highly dependent on the presence of water, highest effect was observed in moist organism compared to lowest in dry organism [45]. The potential application of plasma in

inactivation is based on the fact that plasma reactive species damage the deoxyribonucleic acid (DNA) in the chromosomes. Wiseman and Halliwell reported that the results of radiobiology proved that the mechanism of plasma on a cell is through formation of ROS directly in the vicinity of a DNA molecule inside a cell nucleus. The ROS of interest in plasma processing are hydroxyl radicals, hydrogen peroxide, and the superoxide anion [46]. The application of plasma results in formation of malondialdehyde (MDA) in microbial cells, which participates in the formation of DNA adducts resulting in damage to cells [45]. In particular, reactive species interacts with water, leading to the formation of OH* ions [47] which are most reactive and harmful to the cells (Fig. 2). It is worth mentioning that the OH* radical is most important; these radicals formed in the hydration layer around the DNA molecule are responsible for 90 % of DNA damage. Hydroxyl radicals can then react with nearby organics leading to chain oxidation and thus destruction of DNA molecules as well as cellular membranes and other cell components [45]. Although it is likely that several active species are reacting with cells, it is well documented that reactive oxygen species such as oxygen radicals can produce profound effects on cells by reacting with various macromolecules. The microorganisms are more susceptible to singlet state oxygen leading to deformation of cells [48]. The lipid bi-layer of microbial cell is more susceptible to atomic oxygen as the reactivity of atomic oxygen is much higher than the molecular oxygen, which can degrade lipids, proteins and DNA of cells. The damage of the double

Table 1. Recent finding of microbial inactivation using cold plasma

Microorganism	Substrate	Plasma source	Exposure time	Log reduction	Reference
S.enteritidis(01)	Table Egg	RBD prototype	90 min	4-5	[32]
L.monocytogenes					
E.coli, C.jejuni	Chicken skin	Pulsed gas plasma	24 s	Up to 8	[33]
Listeria innocua	Chicken meat	discharge	4 min	>3.5	[33]
E.coli, S.typhimurium	Bacon	He-O2 plasma	90 s	2-3	[34]
L.monocytogenes		APP			
L.monocytogenes	Sliced ham		120 s	Up to 1.73	
	Sliced cheese	APP	120 s	>8	[35]
S.typhimurium	Lettuce	APP	15 min	Up to 2.72	
	Strawberry		15 min	1.76	[27]
E.coli, S.Stanley	Red apples	CAP	3 min	Up to 3.7	[36]
		Gliding are			
Yeast/mouls	Strawberries	CP	5 min	Up to 3	[24]
E.coli	Apple juice	DBD	-	7	[37]
E.coli	Almonds	Needle/plate system	30 s	1.8-5	[38]
S.aureus, E.coli, C.albicans	Orange juice	Dielectric discharge	25 s	>5	[39]
A.parasiticus hazelnuts, peanuts, pistachio nuts		DBD	20 min	5	[26]
G. liquefaciens, P.agglomerans, S.cerevisiae	Mango & melon skin	Low pressure plasma	25 s	1-3	[40]
E. coli	Mango & melon skin	APJ	5 s	Up to 3	[40]
A. parastiticus, penicillium	Grains and cereals	APJ	15 min	3	[41]
L. sakei	Cold-smoked salmon	SF6 CP	-	1-5	[42]
S.typhimurium	Tomatoes	DBD	300 s	3.8	[28]
A.hydrophila	Lettuce	DBD	5 min	5	[43]
A.flavus	Pepper powder	COP	20 min	2.5	[44]
		Microwave-CPT			

DBD-Dielctric barrier discharge, APJ-Atmospheric plasma jet, CP-cold plasma, COP-Cold oxygen plasma, APP-Atmospheric pressure plasma

bonds in lipid bi-layer cause impaired transportation of molecules in and out of cell. The bombardment of reactive oxygen species (ROS) on the surface of bacterial cell also disrupts the membrane lipids [49–51].

During application of plasma, microorganisms are exposed to an intense bombardment by the radicals most likely provoking surface lesions that the living cell cannot repair sufficiently fast this process is termed "etching". Plasma etching is based on the interaction of relative energetic ions and activated species with the molecules of the substrate. The accumulation of charges imparts an electrostatic force at the outer.

Action of Plasma on Endogenous Enzymes Oyster mushroom

The plasma can be applied not only to microorganisms, but also to simpler biological compounds, like enzymes [45]. Fruits and vegetables mostly spoil due to the enzymatic browning which is considered as secondary loss during post harvest handling and during storage. The endogenous enzymes particularly polyphenoloxidase and peroxidase are the major causes for enzymatic browning as they oxidize phenols at the expense of H2O2 leading to off flavours [57]. Different methods used to prevent the enzymatic browning are heating, blanching, commercial sterilization [58, 59]. Enzymes are inactivated through oxidation reactions mediated by free radicals and atomic oxygen [60]. The other non conventional techniques used in inactivation of endogenous enzyme are the pulsed electric field [61, 62], irradiation [63], high pressure processing [64]. Dobrynin and his colleagues observed there is decrease in enzymatic activity of trypsin (zero at 4 Jcm−2) after the application of plasma [45]. They reported that the plasma was able to change the 3D structure of proteins in trypsin enzymes due to cleavage of peptides bonds. In our investigation we found that the activity of polyphenoloxidase in cold plasma treated guava (Psidium guajava) pulp and whole fruit was reduced by 70 % and 10 % in 300 s at 2 kv respectively. In recent investigations of Pankaj et al. studied the kinetics of inactivation of tomato peroxidase enzymes fitting in different kinetic modeling like first-order, Weibull and logistic models [65]. They observed that there is a decrease in the enzymes activity at different voltages using atmospheric air dielectric barrier discharge plasma. The hypothesis of Meiqiang et al. [66] showed there is increase in the activity of lipase and dehydrogenase enzymes in the hypocotyls of tomato root cells. They concluded that there is no detrimental effect of magnetized plasma on the tomato growth and yield but treatments should be optimized. The relative lipase activity was increased from 1 to 1.4 with the increase in treatment time from 0 to 50 s using helium RF atmospheric pressure discharge [67]. They also reported that there is increase in activity of lipase with increase in plasma treatment time due to changes in the molecular structure of lipase protein which were confirmed by circular dichroism [CD] and fluorescence spectrum. The changes in activity of antioxidant enzymes was studied in the body tissues of plasma treated Indian meal moth larvae by Aziz et al. [48] It was reported that the activity of catalyse, lipid peroxide and glutathione S-transferase enzymes was observed significant increase and no change in glutathione peroxidase activity.

Effect on Phenolic and Antioxidant Oyster mushroom

Antioxidants are considered as first line of defence against free radicals. It is therefore of particular interest to elucidate and understand the basic interactions of plasma species with bioactive compounds, in order to avoid nutritional degradation or any other undesired effects in future applications. Antioxidants protect cells against the damaging effects of ROS, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl ions and peroxynitrite etc. [78]. There are only a few literatures available on effect of plasma treatment on the phenolic compounds. Harborne & Williams [79] reported that several plant species are tolerant to UV radiations accumulating flavonoid metabolites in epidermal cells. During plasma generation the UV radiations formed may be responsible for the formation of phenolic compounds which are extracted from the cells of upper epidermis of leaves Recent investigations of Grzegorzewski et al. [80] using low-pressure oxygen plasma, that a time and structure dependent degradation flavonoids can be observed, due to plasmaimmanent reactive species such as ozone or hydroxyl radicals. To evaluate possible protective

effects of the plant's matrix against plasma-induced degradation of secondary plant metabolites Grzegorzewski et al. [81] worked on flavonols and phenolic acid of lamb's lettuce leaves which were exposed to the cold oxygen plasma. It was reported that there is increase in phenolic contents particularly protocatechuic acid and luteolin was observed doubled upon plasma treatment. The diosmetin content was found to be increased more than others, whereas there is no change in chlorogenic acid content. The use of cold plasma technique over the conventional sources like pasteurization for the treatment of sour cherry marasca juice showed a higher percentage of phenolic acid and anthocyanin content reported by Garofulic et al.

Effect on Packing Oyster mushroom

Food packaging materials are responsible for protection of food materials from the outside environment during handling, transportation and distribution. Cold plasma is used in decontamination of packing material externally where chance of shadow effect is negligible as plasma flows all round the surface [89]. Plasma processing is well known to change or make surface modifications in the case of packing materials. It serves purposes for surface treatment such as cleaning, coating, printing, painting, and adhesive bonding [90]. Lowtemperature gas plasma sterilization allows fast and safe sterilization of packaging materials such as plastic bottles, lids and films without adversely affecting the properties of the material or leaving any residues. Cold plasma can be used for sterilization of heat sensitive packing materials like polythene ethylene and polycarbonate as the temperature is low. Surfaces of polymers particularly for edible packaging films nature of surface should be more hydrophobic with lower surface energies [91]. Using plasma as the transport mechanism and the catalyst, one material can be deposited (in a very thin layer) onto the surface of another material; thereby transferring some of its qualities. Hedenqvist and Johansson studied the oxygen transport properties of the SiOx coating on polyethylene terephthalate [PET], low and high-density polyethylene [LDPE, HDPE] and polypropylene [PP] films, obtained using cold plasma technology and compared experimental data with computer model and found diffusivity was less than normal material [92]. Plasma deposition of heat-sensitive materials such as vitamins, antioxidants and antimicrobials into the packaging material may be sought as potential alternatives in the emerging field of antimicrobial and active packaging. Applications of nanotechnology in packaging as become wide spread to improve the barrier properties of packing material and this can be achieved by cold plasma processing. Pankaj et al. [93] studied the surface topography of zein film using atomic forc microscopy [AFM]. The roughness of plasma treated zein film increased with a root mean square of 100 nm from 20 nm in untreated films this is due to surface etching occurred during plasma treatment.

CONCLUSIONS

Cold plasma is a unique technology which is responsible for microbial destruction and surface modification of substrate as conventional preservatives techniques as some detrimental effects on nutritional quality. Plasma sterilization provides high efficacy, preservation and does not introduce toxicity to the medium. The most important is to select (choosing) a particular gases which already possess germicidal properties so that the efficiency of plasma sterilization can be increased. The cold plasma techniques are preservation treatments that are effective at ambient temperatures, thereby minimum thermal effects on nutritional and sensory quality parameters of food with no chemical residues. Plasma can be used for Oyster mushroom modification as additive and as filler component in packing materials. Although cold plasma technology is not yet used commercially on a large scale, the equipment should be readily scalable. However, research efforts must be taken to evaluate the expenditure for the treatment for large quantities of food commodities at industry level and also the quality, safety, wholesomeness of food commodities.Conclusion can be drawn that in future we hope plasma processing becomes common processing at food industries.

FUTURE SCOPE

Cold plasma is used efficiently for sterilization and

modifications of packaging polymers purpose but there is a huge application in food processing. d' Agostino et al. [3] reported that there are 14 research areas where application of plasma technology can be increased which are determined by international research scientists. The amount of energy consumptions and stability depends up on the type of discharges used for treatment. Based on this parameters for the application of plasma should be optimized for maximum efficiency at low cost of operation. Many scientists successfully applied plasma on foods (solids and liquids) for the microbial inactivation but they did not explain its effects on the nutritional qualities and toxicology of treated foods. There is a necessary that application of plasma on foods should be recognized as GRAS after intense study and research (in vitro and in vivo) in this field. Future studies should be done on applications of plasma on food surfaces to change its physical and chemical properties with cost effective.

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