



Cold plasma technology – An overview of basics and principle

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ABSTRACT

Thermal processing can produce non-enzymatic browning, protein denaturation, flavor alterations, and vitamin loss in food products. A cold plasma treatment, which is non-thermal, is the greatest option for preserving food products, keeping bioactive ingredients, and prolonging shelf life. It is used for brief treatment durations at moderate temperatures. The review's goal is to discuss cold plasma procedures, parameters, and processes for microbial and enzyme inactivation. It also discusses the numerous uses in the dairy business as well as their impact on quality factors. The cold plasma technique shows an excellent performance in the elimination of spoilage microorganisms and maintaining the quality characteristics of food products.

Introduction

Milk is a nutritious liquid food that contains fatty acids, good quality protein, lactose, and micronutrients such as minerals and vitamins. Various health benefits are associated with the consumption of milk and milk products. Milk in diet can fulfill the deficiency of essential nutrients like calcium, vitamin D, vitamin K, phosphorous, and magnesium. It helps to maintain your body healthy and strong. Although the nutritious quality of milk makes it more susceptible to microbial attack, that is why it is needed to preserve the milk. Consumption of raw milk is having no risk at the nutritional level but it could have a detrimental effect on health by consuming potential pathogens with it. Pathogen like *Campylobacter* spp., *Salmonella* spp., *E.coli* O157:H7, *Y.enterocolitica*, *L. monocytogenes*, and *S. aureus* are mostly the reason behind the health hazard caused by milk (De Buyser *et al.*, 2001; Gillespie *et al.*, 2003; Lee and Middleton, 2003; Oliver *et al.*, 2009). The risk of consuming pathogen with milk is reduced or even eliminated with the help of thermal treatment. Pasteurization, thermization, sterilization, and Ultra high treatment (UHT) are thermal methods that have different time-temperature combination by

which different level of bactericidal effect is achieved. UHT and Sterilization are known to kill almost all vegetative and sporulating pathogenic microorganisms including spores of *B. cereus* (Lindstrom *et al.*, 2010). At the same time, any heat processing has the potential to alter the nutritional and sensory properties of food. UHT has been reviewed to show the degrading effect on milk nutrition. Protein, vitamin, and to some extent lactose are lost in UHT processing. Also, ultra heat-treated milk is not suitable for the production of several products like cheese and yogurt. To preserve the nutritional loss made by heat method, non-thermal preservation techniques for decontamination of milk came into frame. One of such emerging non-thermal preservation techniques is cold plasma technology. A plasma state is attained when the gas molecule reaches a certain energy level and gets ionized. The energy for ionizing the gas molecule could be from any source. It could be electrical, thermal, microwave which ultimately causes elevating the kinetic energy of electrons within gas molecules. The increase in kinetic energy causes a collision which results in the formation of ions, radiation of

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different wavelengths and radicals, there are various methods for the generation of plasma such as dielectric barrier discharge (DBD), corona discharge, gliding arc discharge, and radiofrequency plasma(RFP) (Conrads and Schmidt, 2000). Plasma can be classified based on temperature as thermal plasma and non-thermal plasma. In thermal plasma temperature of an electron is almost equal to heavy particle temperature which is approx. 10000 K (Tendero *et al.*, 2006). The non-thermal plasma in the context of temperature, the density of ions, and energy level is said as a non-equilibrium plasma or cold plasma. Nowadays this technique is seeking more attention as it can show a decontamination effect on relatively low temperature and at atmospheric pressure (Roth *et al.*, 2007). Cold plasma is produced at 30-60 °C under low or atmospheric pressure (Thirumdas *et al.*, 2015) Its low-temperature operation makes it applicable in the food industry for heat-sensitive food (Tolouie *et al.*, 2018). In the medical industry, it has been utilized for surface disinfection of devices that are heat sensitive. In packaging industry also uses this technique for sterilization of package surfaces (Pankaj *et al.*, 2014). Also, it has been reported that bacterial spores can be eliminated with better efficiency with plasma technology than the thermal process which is generally used in food industries. In the dairy industry, milk and its products require sterilization to make the product microbiologically safe, to enhance the shelf life by elimination of deteriorative micro-organism but these methods consist of severe thermal operation which interferes with the nutritional quality of the end product. This review is aimed to collect and share knowledge regarding the work done with cold plasma technology on food products.

Cold plasma generation

There are various methods of achieving cold plasma. This requires energy to generate and maintain a plasma state, it could be thermal light or electrical energy. Generally, electrical energy is utilized for plasma generation. Various methods for the generation of cold plasma are discussed below.

Dielectric barrier discharge method.

It utilizes a dielectric barrier for plasma generation. DBD constitutes two metal electrodes, between these two, a high potential difference is maintained

(Table 1). The frequency for the operation ranges between 0.05 to 500 kHz for different pressure conditions (Zhang *et al.*, 2017). In DBD arrangement either one or both, the electrode is covered with a dielectric material. The dielectric material collects the charge into it and prevents it from igniting, which cause retardation in potential difference. In these dielectric barrier discharge setup, charge is self pulsed which does not allow the current to discharge to such level that causes arcing. The plasma produced by DBD method contains large number of filament but it is found in very random manner hence the plasma produced is non uniform. The productivity of plasma by DBD method depends upon factor such as, gas employed, gap between electrodes and level of voltage maintained for operation (Ehlbeck *et al.*, 2010). Advantages with DBD method is that, plasma can be produced with wide variety of gas, flow of gas could be low or no flow is required, and also different shape of electrode could be used. It is good source for large surface application (Phan *et al.*, 2017). Its disadvantage involve voltage requirement of at least 10 kV for the operation

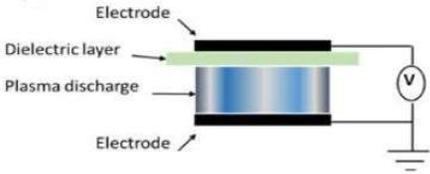
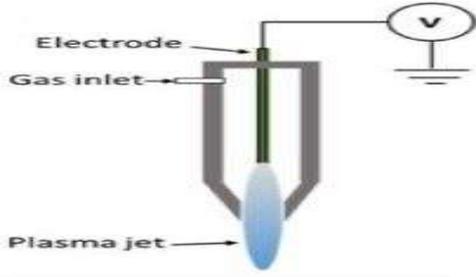
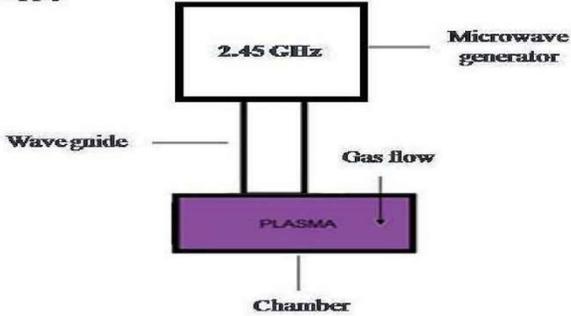
Atmospheric plasma jet

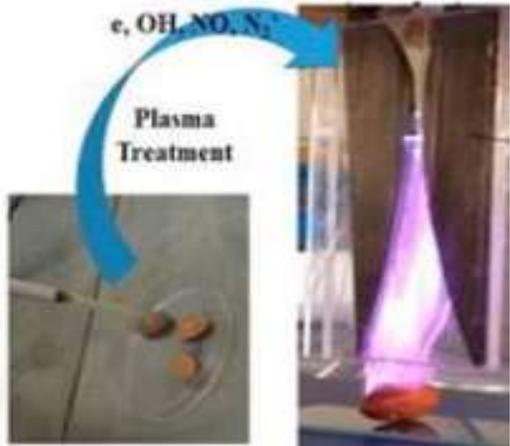
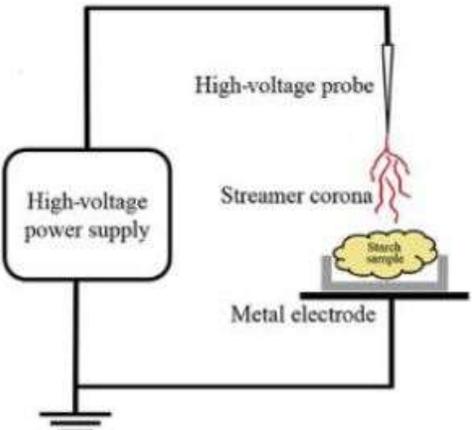
In plasma jet there is two electrodes arranged in concentric manner. The gas is passed between these two electrodes. The electrode at outer side is grounded and inner electrode is applied with high frequency (at radio frequency range commonly at 13.56MHz) and high voltage (100-250 V), these cause the ionization of passing gas. The field produced by radio frequency excite the free electron which produces plasma products (free radicals, excited atoms and molecule) by inelastic collision (Misra *et al.*, 2016). At few millimetres subjected sample is placed the gas plasma impact it through a nozzle. In this process noble gas like helium and argon are used. Flow of gas for so needed to maintain at very high rate (> 10 slm), which make it very expensive process. Its advantage lies in its potential to penetrate in narrow spaces and its direct applicability (Weltmann *et al.*, 2008).

Gliding arc discharge method

Gliding arc discharge method involves plasma generation within a large reactor which constitutes two or more metallic electrode kept at high potential difference of 9kV (Table 1). Gas is forced

Table 1: Different methods of cold plasma generation.

Method	Configuration	Parameter	Application	Used for	Reference
<p>Dielectric barrier discharge</p>  <p>(Pankaj <i>et al.</i>, 2017)</p>	<p>Two electrode kept at high potential difference. Dielectric material is placed between these two electrodes</p>	<p>Frequency 0.05-500 KHz power consumption 10 - 100 W Gas pressure 10^4-10^6 Pa Gap distance 0.1 mm-several centimetre</p>	<p>Orange juice Cold-smoked salmon Tomatoes Strawberries Sliced Cheese Cheddar cheese</p>	<p>Ideal for large surfaces application</p>	<p>(Shimizu <i>et al.</i>, 2018) (Segat <i>et al.</i>, 2016) (Pankaj <i>et al.</i>, 2017) (Zhang <i>et al.</i>, 2017) (Saragapani <i>et al.</i>, 2017)</p>
 <p>Plasma jet (Pankaj <i>et al.</i>, 2017)</p>	<p>Two coaxial electrode central electrode is excited at radio frequency Outer electrode grounded</p>	<p>Radio frequency 13.56 MHz Gas flow rate >10 slm*</p>	<p>Mango melon skin</p>	<p>Applicable where penetration and direct application is require.</p>	<p>(Pankaj <i>et al.</i>, 2017) (Weltmann <i>et al.</i>, 2008)</p>
<p>Microwave discharge plasma supply</p> 	<p>Electrode free Microwave driven (Microwave generally produced by magnetron)</p>	<p>Frequency range-300MHz – 10 GHz (generally produced at 2.45 Ghz)</p>	<p>lamb's lettuce carrot apple strawberry</p>	<p>Suitable for surface modification</p>	<p>(Tolouie <i>et al.</i>, 2017) (Schnabel <i>et al.</i>, 2015)</p>

<p>Gliding arc discharge</p>  <p>(Khalili <i>et al.</i> 2018)</p>	<p>Knife edged two electrode made up of steel . Curved at the bottom</p>	<p>Thickness of electrode - 2 mm, length - 80 mm, width - 20 mm gap distance - 6 mm. gas flow rate - 4 lit/min frequency - 20 kHz voltage 8 -14 kV sample distance – 1.6 cm</p>	<p>Almonds Red apples</p>	<p>Irrespective of shape and size of product.</p>	<p>(Khalili <i>et al.</i>, 2018) (Niemira and Sites, 2008)</p>
<p>Corona discharge</p>  <p>(Wu <i>et al.</i>, 2018)</p>	<p>Two electrode - pin to plate electrode Corona like discharge</p>	<p>Pressure- Atmospheric pressure Frequency</p>	<p>Raw Milk and UHT milk</p>	<p>It is applicable for small space with non-uniform distribution . Best for food application.</p>	<p>(Wu <i>et al.</i>, 2018) (Gurol <i>et al.</i>, 2012)</p>

into the gap between the electrodes. Plume like plasma is created by broken and blown arc which is formed at small gap between the electrodes and the process continues by immediate formation of fresh arc for every cycle (Moreau *et al.*, 2008). Gliding arc plasma offers plasma action for products irrespective of their shape and size, as it uses knife shape electrode which is curved at the bottom. The gap between the electrodes is adjustable. Greater extent of thermal non equilibrium is assured by gliding arc plasma generation method. This plasma generation method offers high electron density and high electron temperature its outcome. The gliding arc plasma generation can be utilized for surface treatment of bulky materials. It also permits rapid processing. All these make gliding arc method potentially available selective chemical processes with good efficiency. Outcome of this process is extremely dependent on temperature of both, electrode and discharge.

Microwave discharge plasma

Plasma can be generated without using electrode through microwave. Microwave-driven discharge uses electromagnetic wave produced by magnetron generally at 2.45 GHz Frequency (Tolouie *et al.*, 2017). The electron present in gas gets energized by absorbing the microwave. Kinetic energy of electrons increased which leads to inelastic collision and ionization of gases ultimately (Schlüter and Fröhling, 2014). The plasma through microwave can be obtained at frequency of 300 MHz–10 GHz. Microwave plasma is one of the most suitable plasma to be used for surface modification as its ions having low energy (Kusano, 2009).

Corona discharge

Corona discharge is method that consist two sets of non - uniform electrodes that are fitted with a high-voltage gas ionisation system (Li *et al.*, 2004). Corona is limited emission which can be ignited on application of strong electric field at atmospheric pressure. Mainly the discharge of these setup is around the edges (sharp or pointed) of electrode, where electric field is high enough to excite the electron to level of ionization energy of gas (Phan *et al.*, 2017). Advantage of corona discharge is, it does not require complicated equipments can be produced with simple device also it is not

expensive operation. But the disadvantage with it is applicable for small space with non-uniform distribution.

Parameters of cold plasma technology

The effectiveness of cold plasma process depends upon several factors, of which can be divided into equipment, process products parameter. All of these influence final result of the process. The parameters within the equipment such as plasma generation method, electrode characteristics, distance between electrode effects the final outcome of the process. During plasma generation certain parameter are needed to be controlled or maintained are called process parameter like mode of plasma application, gas composition, flow rate of gas, electrical power , frequency, voltage, relative humidity, mode of exposure of plasma, and time duration for which it is exposed. The product parameter involves composition of raw material, physical state of food, initial microbial concentration, shape, and structure.

Source of plasma

There are many generation sources for plasma-like dielectric barrier discharge (DBD) microwave discharge, radio frequency plasma, etc. Plasma generated through different methods has different characteristics. DBD is used for large surface applications (Phan *et al.*, 2017), the atmospheric pressure plasma jet is used where penetration is required. In addition, the electrode material used and the distance between them result in changes in the plasma generated. Lesser the distance greater will be the efficiency of the treatment (Moreau *et al.*, 2008).

Direct or indirect mode of application

The effectiveness of the operation depends upon mode of application of plasma. There are two ways of exposing plasma direct and indirect. Direct plasma application depicts more short-lived reactive species in its component which is having a life span of millisecond and might be involved in damaging interaction with cells (Misra and Jo, 2017). The indirect mode means plasma is generated in a separated chamber also known as remote or afterglow plasma. Indirect plasma involves more of longer living reactive species such as nitrogen and oxygen reactive species (Surowsky *et al.*, 2015). The amount of heat transferred and charged particles received in the sample is

comparatively less concerning the direct method (Misra *et al.*, 2011). The direct exposure of plasma is more efficient than indirect exposure also the indirect plasma generator is difficult to build and operate (Niemira, 2012).

Gas and their flow rate

The selection of gas for the operation is important as its composition is correlated with the generation of active species. Initially, Nobel gases were used for the plasma treatment because of their high thermal conductivity and ultraviolet ray emission, also it require low power for operation at atmospheric pressure. But nobel gas is expensive which opens the path for utilizing air and gaseous mixture to be used in the plasma generation process. The more the presence of oxygen and nitrogen in the operating gas mix the greater will be the amount of reactive oxygen and nitrogen species in the discharge, which ultimately affects decontamination efficiency (Misra and Jo, 2017). Gas flow rate on the other hand assures the delivery of reactive species into the target sample within their life span. Rapid flow rate enhances the rate of carrying short-lived reactive species into sample, assure enough mass transfer and collision rate for decontamination (Zhang *et al.*, 2017).

Power input & treatment time

The non thermal plasma is generally generated by utilizing electrical energy for increasing the energy of gas to reach the plasma state. The controlled input is required in the process for efficient output. Parameter such as frequency, voltage, power are positively correlated with the microbial reduction rate but with the use of high electrical power other food quality parameter might get disturbed, hence controlled input is required (Liao *et al.*, 2017). Another parameter that is needed to consider is treatment time. The time for which plasma product and sample remain in contact have direct influence on the microbial reduction rate (Nishime *et al.*, 2017).

Relative humidity

In decontamination process through plasma, relative humidity play essential role. Studies show that presence of water vapour enhances the generation of reactive species in the plasma product but excess of it may lead to dilution effect in the product. The generation of peroxy group and OH group increased due to the added water vapour decomposition which thereby boosts the

antimicrobial effect of the process (Guo *et al.*, 2015; Liao *et al.*, 2017).

Parameters related to food

The parameter other than process parameter which influences the effectiveness of non thermal plasma process is of food or sample itself. The chemical composition of food such as in food with high fat composition, reactive oxygen species will lead to oxidation (Saragapani *et al.*, 2017). The activity of plasma depends also upon whether food is in solid or liquid medium. In solid food, its activity is restricted to surface only but in liquid sample penetration into the sample is possible (Surowsky *et al.*, 2015). The penetrability and success of plasma action depend also upon physicochemical characteristics, moisture content and porosity of solid food whereas in liquid food volume of it coming into contact with plasma is important instead of penetration (Surowsky *et al.*, 2016). Other than these, the initial concentration and the type of microorganism into the sample also influence the process. Micro-organism which is in vegetative form and in exponential phase is more susceptible for destruction than those in stationary phase and sporulated form. Efficiency of microbe inactivation of plasma process is reduced with higher initial concentration of micro-organism. Inactivation of gram negative bacteria are more efficient than gram positive bacteria, hence the type of bacterial culture present in sample also affect the process. (Liao *et al.*, 2017). Difficulties caused by the irregular shape and structure of food for the treatment, as it provide site for growth or hide the micro-organism where the plasma is not able to reach (Misra and Jo, 2017).

Working mechanism of plasma for microbial inactivation

Relatively recent non-thermal technology cold plasma, which effectively inactivates microorganisms such as bacteria, their spores, biofilms and fungi. The inactivation of microorganism is done by plasma through activating three fundamental pathways. Those three possible-pathway are etching of surface of cell by the action of reactive species developed during the generation of plasma, degradation of genetic content and compound volatilization, inherent desorption by UV photon (Laroussi, 2005). The operating pressure, level of plasma discharge, plasma sources configuration are the decisive factor

for maximum possible contribution of UV radiation in cell death process (Misra and Jo, 2017). Chemical bond inside the micro-organism is broken and volatile compound such as CO and CH_x, due to irradiation by UV also it may cause desorption within the cell (Schlüter and Fröhling, 2014). Etching of cell surface refers to inability of healing an injury not sufficiently fast which leads to cell death (Fig. 1). The injury caused due to cell surface interaction with energetic radical ion and reactive species. The activities of plasma as microbial destruction tool involve the interaction between plasma products and cell. Application of plasma activate many agents and chemical products such as free radicals (OH & NO), reactive oxygen species (ROS), Reactive nitrogen species (RNS,) radiation of high energy, UV radiations, fluctuating electric field and charged particle all these act synergistically and make it impossible for pathogen to survive against such condition (Ehlbeck *et al.*, 2010). RNS and ROS of plasma effect directly on the outer membrane of cells. Availability of water effect plasma action the most, as it has been found on comparing with plasma action on moist cell and dry cell, maximum effect were detected in moist cell (Dobrynin *et al.*, 2009). It has been reported by Wiseman and Halliwell that on application of plasma, reactive oxygen species is formed directly in the region of DNA inside cell nucleus (Wiseman and Halliwell, 1996). The reactive species of plasma damage the deoxyribonucleic acid (DNA) by forming malondialdehyde (MDA) in microbial cell which is involved in DNA adduct formation (Dobrynin *et al.*, 2009). ROS leads to oxidation of amino and nucleic acid and also converts membrane lipid to unsaturated fatty acid peroxide as it found at surface of cell membrane it is most susceptible for reaction. (Liao *et al.*, 2017). Due to this reaction leakage of macromolecule associated with this lipid is occurred (Schlüter and Fröhling, 2014). On application of plasma, micro-organism experiences intense bombardment. OH and NO (Plasma radicals) get absorbed at surface of micro-organism and form compound like CO₂ and H₂O, which are volatile. These compounds induce surface defect, which cannot be healed by cell, results in death of cell. Also the membrane of cell is revealed to extreme electric field which creates

electrostatic tension and that can induce rupture (Misra and Jo, 2017). The formation of pores helps in liberation of inner fluid and inhibit micro-organism healing activity, simultaneously the active species get their way into cell which can cause cell damage by destructing DNA, protein and other component inside the cell (Phan *et al.*, 2017). Higher membrane permeability resulted due to formation of pores, which directly affect the pH regulation of the cell, with acidification induced by humid plasma air. But this parameter is inessential factor for microbial inactivation as many microbes have buffer capacity due to cytoplasmic protein and very dense cytoplasm which maintain pH within cell (Moreau *et al.*, 2008). In any food there are two type micro-organisms present for which all these preservation techniques are required. One which deteriorates the end food product quality by various microbial actions and the other is which affect the health of one who consumes it. They are commonly known as spoilage micro-organism and pathogen respectively. In milk and milk product spoilage organism are generally psychotropic bacteria such as bacterias of *Bacilli*, *Micrococci*, *Staphylococci species* and pathogen such as *Listeria monocytogenus*, *Bacillus subtilis*, *E.Coli* O157:H7, *Salmonella spp.* etc are checked for quality assurance. Preservation technique for milk and milk product are designed targeting these micro-organisms. Different research work has been done to check the efficiency of cold plasma treatment in reduction of microbial count of these micro-organisms. Table 2 show the list of different microorganism (which is also generally found in milk) inoculated on various substrate for study of capability of non thermal plasma in decontamination of specific microbes inactivated through cold plasma treatment. Cold plasma technique has been successfully studied for log reduction of microbes in the different substrate.

Working mechanism of plasma for enzyme inactivation

Defects in foods are not only caused by microorganism, the enzyme activities also lead to production of some off flavour, browning, vitamin loss etc. In order to make food preserved not only elimination of spoilage micro-organism is taken into consideration but also the enzyme residual activities should be considered (Mastwijk and Groot, 2010). Enzyme in milk and milk products

Table 2: Different microorganism inoculated on various substrates

Micro-organism	substrate	Plasma treatment	Log reduction /time	Reference
<i>Bacillus cereus</i>	-	DBD (3.5 W)	1.0 log	Bayrer <i>et al.</i> , 2020
<i>B. coagulans</i>	-	DBD (3.5 W)	3.3 log	
<i>Listeria monocytogenes.</i>	Sliced Ham	APP	1.73log /120 s up	Lee <i>et al.</i> , 2011
	Sliced cheese	APP	More than 8 log / 120 s	
<i>Salmonella spp. Typhimurium KCTC 1925</i>	Bacon	APP	1.7 log / 90 s	Kim <i>et al.</i> , 2011
<i>Campylobacter jejuni, E.coli</i>	Chicken skin	Pulsed gas plasma discharge	8 log /24 s up	Noriega <i>et al.</i> , 2011
<i>S. aureus, E.coli, C. Albicans</i>	Orange juice	DBD	5 log / 25s	Shi <i>et al.</i> , 2011
<i>E. coli O157:H7</i>	Apple surface	NTAP	1.79log/ 15 min	Calvo <i>et al.</i> , 2020

*APP (atmospheric pressure plasma); CAPJ (cold atmospheric plasma jet); DBD (Dielectric barrier discharge); NTAP (Non thermal atmospheric plasma)

degrade lipids, carbohydrates, and protein by their various actions which result in changes in texture, pH, flavour, colour etc. Some of those activities are desired and some are not desired in the milk and milk products. Enzymes such as lipase which hydrolyze fat and give rancid defect, some proteases that produce bitter flavour by degrading milk protein etc, are not acceptable and suitable for consumption as well as manufacturing of other milk products. Inactivation of these enzymes can be done with non thermal plasma. The mechanism for inactivation depend upon, interaction between reactive species and enzyme component, amount of reactive species produced, power of discharge, structure of enzyme etc. The inactivation of enzyme is mainly due to damage of specific bond or alteration of chemical structure due to action of reactive species. Which if left undamaged or unaltered, will produce secondary structure for catalysis of the chemical reaction (Misra *et al.*, 2016). Enzyme in milk are produced either by bacteria or it find its way into milk from blood of bovine. They perform specific function in the milk most of them are heat sensitive and get inactivated with certain temperature. Various research works has been successfully done for inactivating enzymes with atmospheric plasma application. In a study researchers have been deteriorated the immobilized lysozyme enzyme with plasma produced by mixture of oxygen and nitrogen gas. They hypothesized the destruction is due to the damage of the active site of enzymes by O₂ and N₂ plasma reactive species (Bernard *et al.*, 2006).

Alkaline phosphatase is one of the enzymes generally found in milk, which is used as parameter to check efficiency of pasteurization (Rankine *et al.*, 2010). In a research work alkaline phosphatase is subjected to DBD plasma treatment of 5 sec to 5 min in a discrete voltage of 40- 60 KV. It resulted in significant decrease in activity of alkaline phosphatase (Segat *et al.*, 2016). Different enzymes were studied for inactivation through plasma treatment. Table 3 enlists various enzymes that are also found in milk and treatment provided for their inactivation.

Application in dairy and food industry

Recently the effect of dielectric barrier discharge plasma on different MAP packaged ready to eat Ham was studied where, *L. monocytogenes* has been reduced to >2 log with, 20% O₂ + 40% CO₂ + 40% N₂, MAP composition. Initial cell population on the surface of ham was 8 log CFU/cm². The reduction in microbial count was irrespective of formulation ham (Yadav *et al.*, 2020). In other study on the Korean rice inoculated with, *E. coli*, *S. typhimurium*, *Listeria monocytogenes*, and *Penicillium chrysogenum*, the rice was treated with plasma activated water the cell population reduced by *E. coli* 2.01–2.03 log CFU/g, *S. Typhimurium* 2.08–2.12 log CFU/g, *Listeria monocytogenes* 1.98–2.17 log CFU/g *Penicillium chrysogenum* ~2 log CFU/g (Han *et al.*, 2020). Whereas in the barley grain cheddar cheese DBD based atmospheric cold plasma effects germination parameter, the best result found with the 6 min treatment time (Feizollahi *et al.*, 2020).

Table 3: Various enzymes inactivated using cold plasma technology

Enzyme	Plasma generation method	Surface	Plasma treatment	Gas used	Result	Reference
Lysozyme	Microwave plasma	Enzyme immobilised on polystyrene 96 well plate	(Power: 300 W ,Frequency: 915) Time : 0 - >800 sec	Nitrogen + oxygen	destruction and desorption of Significant protein	Bernard, <i>et al.</i> , (2006)
Lysozyme	Low pressure inductively coupled plasma	Si wafers, glass slides, gold and polystyrene plates	Pressure: 10 Pa; RF power: 200 W	Ar, Ar+N ₂ , Ar+O ₂ mixtures	Ar+O ₂ was most effective in etching the enzyme deposits	Kylian <i>et al.</i> , (2008)
Lipase (from <i>Candida rugosa</i>)	Radio-Frequency (RF) atmospheric pressure glow discharge (APGD)	Stainless steel	Power: 180 W RF Flow rate: 10 L/min; Temperature: <57 °C; Operation time: 0-50 s	Helium	Activity of the lipase increased significantly after 1 min; Enhanced activity attributed to change in secondary and tertiary structures of protein	Li <i>et al.</i> , (2011)
α -chymotrypsin	Cold plasma jet	Aqueous solution (Buffer)	Frequency: 60 Hz; Operation time: 5 min	Air	Secondary structure changes; β -strands decrease	Attri <i>et al.</i> , (2012)
Alkaline phosphatase	Dielectric Barrier Discharge	Buffered solution	Voltage: 40-60 kV; Frequency: 50 Hz; Operation time: 0-5 min;	Air	Voltage and time dependent inactivation; Inactivation follows a sigmoidal logistic function	Segat <i>et al.</i> , (2016)

Various research works has been performed to check applicability of non thermal plasma process in dairy industry as a mean of preservation technique. The inactivation ability of the cold plasma technique is checked by inoculating strains of micro-organism that are generally found in milk and milk products and is evaluated for inactivation through various plasma methods. Gurol *et al.*, (2012) in their experiment inoculated *Escherichia*

coli ATCC 25922 in milk with different fat content with an objective to check capability of low temperature plasma in killing *E. Coli*. Corona discharge plasma for this experiment. The system consist of two tungsten electrode, one is rotating above with the help of dc motor, the other one is dipped into milk, plasma is generated between the milk and upper electrode tip. Power of 9 kV AC is supplied in the system. The temperature

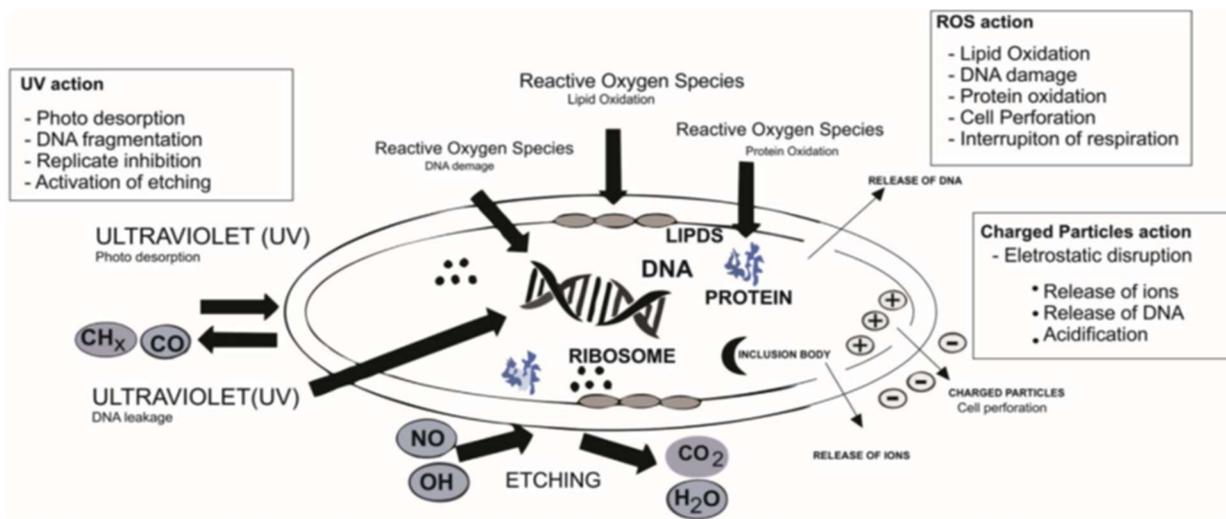


Figure 1: Overview of cold plasma mechanisms involved in microbial inactivation. Adapted from Schlüter and Fröhling, 2014.

during the process was maintained below 35°C. The time dependent effect of plasma process is checked at time interval of 0, 3, 6, 9, 12, 15, and 20 min. They observed that there is significant reduction of 54 percent in *E.coli* population in just after 3 min. There is no remarkable difference in the result due to distinct fat content of the sample. Initial count was 7.78 log CFU/mL which has been reduced to 3.63 log CFU/mL after 20 min of treatment. After one week no viable cell is detected and it last as such for 6 week storage period. Also other quality parameter is evaluated such as pH and color properties of the sample are not significantly altered by the treatment. In another experiment, kim *et al.* (2015) in addition *E.coli* two other pathogen namely *L.monocytogenes* and *Salmonella typhimurium* is inoculated into the milk. To eliminate microbes dielectric barrier discharge (DBD) method was chosen. The setup for encapsulated DBD plasma generation involves a parallel-piped rectangular container of plastic inside which sample was kept. 250W power is provided to the system and voltage at 15 kHz bipolar waveform is given to one electrode and the other one was grounded. Treatment of plasma is given for 5 and 10 min. Initially the aerobic bacterial count was 0.98 log CFU/mL after treatment no viable cell were observed on both 5 and 10 min treated sample. The load of 6.28, 6.43, and 6.21 log CFU/mL was calculated of *E. coli*, *L. monocytogenes*, and *S. Typhimurium* on milk after treatment of DBD plasma, microbial count of

pathogens was reduced to 2.43, 2.40, and 2.46 respectively. Researcher also observed that the milk pH decreased and L*(lightness) and b*(yellow/blue coordinate) values of hunter color for milk increased were as a*(red/green coordinate) value decreased after 10 min of encapsulated DBD plasma treatment.

The evaluation of non thermal plasma technology for decontamination effect is not just limited to milk but researcher performed experiment to evaluate the effect of plasma into solid milk product such as cheese. In a research study by Lee *et al.*, (2012), UV sterilized cheese slices were inoculated with *Escherichia coli* and *Staphylococcus aureus* strain. DBD plasma for treatment is generated at 3.5 kVpp and a bipolar low-frequency voltage is maintained at 50 kHz. Helium and a mixture of helium and oxygen gas are used to improve the inactivation effect. Plasma treatment was given to the sample for 1, 5, 10, and 15 min. Other quality parameters and sensory characteristics were evaluated. Significant log reduction in the microbial count of *E.coli* ranging from 0.09 to 1.47 has been observed with the helium and 0.05-1.98 log with the Helium oxygen mixture gas. *S. Aureus* reduced logarithmically 0.05- 0.45 log and 0.08- 0.91 log with helium and mixture of helium-oxygen gas respectively. Whereas a considerable increase in b* value and decline in L* value were observed in the study. The researcher also discovered that cheese slices got damaged after 10 and 15 min of the

treatment and there is a remarkable reduction in the sensory attribute of the cheese slices which includes flavor, odor, and acceptability. Experiment outcomes show that with the addition of oxygen there is an increase in pathogen inactivation ability although the effect was limited.

Cheese slice were also evaluated for inactivation of pathogen through encapsulated dielectric barrier discharge. Slices were inoculated with *E coli*, *Salmonella typhimurium*, and *Listeria monocytogenes*. Encapsulated DBD plasma is generated in plastic rectangular container though electrical power of 250 W and voltage at frequency 15 kHz. No viable cell was detected after for 90 s, 60 s, and 10 min respectively for pathogen. After treatment of 10 min no visible damage is evident on the cheese slice. Logarithmic reduction in microbial count of *E coli*, *Salmonella typhimurium*, and *Listeria monocytogenes* 2.88, 3.11, and 2.26 were observed respectively after 15 min of plasma treatment. The results show that pathogens were successfully reduced and inactivated by the DBD plasma system in cheese slices (Yong *et al.*, 2015). In another study cheddar cheese slice was inoculated with pathogen strain *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* with DBD plasma (flexible thin layer) with input 100 W power, the voltage at 15 kHz frequency for 0, 2.5, 5 and 10 min of duration. After 10 min of treatment notable microbial count reduction of 3.2, 2.1, and 5.8 logs CFU/g has been observed for pathogen strain respectively (Yong *et al.*, 2015).

Recently the effect of cold plasma has been analyzed on tofu by treating it with plasma-activated water, the polyphenol retained was 80 % of the initial content also, the gumminess reduced up to 32% and immediate softening has occurred in the tofu (Frías *et al.*, 2020).

Cold plasma application on packaging material

Packaging material protects the food from physical damage, contamination also restricts the movement of moisture, gas from both sides (Zhang *et al.*, 2020). Packaging material remains in direct contact with the food. That is why the pre-packing sterilization of packaging materials is needed (Ganesan *et al.*, 2021; Peng *et al.*, 2019). Cold plasma processes involve surface treatment hence

used in sanitization of packaging material surface (Banu *et al.*, 2012). Plastic bottles, films and lids can be sterilized using non thermal plasma as it offers rapid and safe processing without unfavourably altering the characteristics of material and without any residue leaving behind. In recent years, cold plasma has been employed to improve the functionality and interfacial properties of biopolymers (Bahrami *et al.*, 2020).

There are two types of cold plasma treatment for sterilising food packaging materials: direct treatment and indirect treatment. Materials of interest are placed into the plasma discharge region and sterilised by exposure to active species created within the plasma region, as well as high-energy photons such as UV radiation, during direct treatment. Indirect treatment, on the other hand, places the materials to be sanitised outside of the plasma discharge region, allowing some of the active species departing the plasma region to coat the material's surface, similar to surface modification treatments (Peng *et al.*, 2019). The effect of cold plasma is lethal to bacterial cells, resulting in the death of microorganisms (Corradini, 2020); nevertheless, the ionised gas created by plasma is not lethal to fresh produce cells or tissues. This plasma characteristic inhibits mutant cells from forming following treatment, providing some useful information on the effects of plasma treatment on biological materials (Gavahian and Khaneghah, 2019). A plasma's ability to transport energy is determined by its chemical composition and temperature (Hosseini *et al.*, 2020). So, plasma has also been utilised in the packaging industry to modify polymer structure in order to acquire desired qualities in packaging materials (Pankaj *et al.*, 2014). Both Gram-positive and Gram-negative bacteria are inactivated differently by cold atmospheric plasma (CAP), according to studies. The major goal of using CAP is to protect minimally processed goods (such as fruits and vegetables) from microbial and chemical contamination (Sarangapani *et al.*, 2017; Ganesan *et al.*, 2021). At limited exposure times, CAP treatment lowers the total bacterial count in fresh fruits (Rana *et al.*, 2020) and meat products (Kulawik *et al.*, 2018). Cold plasma decontamination, preservation, and sterilisation of

food products is an innovative and extremely dependable technology without affecting the food or the package's properties (Peng *et al.*, 2019). Because it is a dry, ideal for in-line processing, easy to produce and control, chemical-free, and waste-free method, it can be considered a viable technique for improving the attributes of edible films and packaging material (Bahrami *et al.*, 2020).

The non thermal plasma as name suggest operate at low temperatures, that make it acceptable for treatment of heat sensitive packaging materials such as polythene, polycarbonate etc. Surface of food packaging polymer should be more hydrophobic and surface energies should be low (Vesel and Mozetic, 2012). Deilmann *et al.*, (2008) sterilized polyethylene terephthalate bottles by projecting microwave plasma gas mixture into the bottle. For so they had developed a plasma reactor setup which offers three dimensional movement of plasma gas into bottle. Mixture of hydrogen, nitrogen and oxygen had been used in the process. Microbial reduction of 105 and 104 CFU for *Bacillus atrophaeus* and *Aspergillus niger* respectively, has been resulted from microwave plasma treatment of fewer than 5 seconds (Deilmann *et al.*, 2008). Inactivation of *E. coli* K12, *P. Aeruginosa*, *S. Aureus* from the surface of polypropylene by glow discharge plasma has been reported by Gadri *et al.*, (2000). Also, sterilization of packaging material such as polystyrene (PS) films, PET, multi-layer packages like PET/polyvinylidene chloride (PVDC)/polyethylene (PE) through plasma has been reported by Muranyi *et al.*, (2010). Various studies have been done to study the changes caused by the treatment of

plasma on packaging materials such as polypropylene, low-density polyethylene, High-density polyethylene terephthalate. Studies revealed that plasma treatment changes in properties such as an increase in weight, roughness, crystallinity, and decrease in contact angle, wettability, aging effect for different packaging materials (Thirumdas *et al.*, 2015).

Conclusion

The non-thermal plasma has proven its potential in the decontamination or microbial load reduction for various food materials including milk. Results of various researches revealed that cold plasma technology can deal with pathogens and spoilage bacteria. Not only bacteria it is possible to inactivate the residual activities of enzymes in foods utilizing this technology. This review paper converses various research work and studies done on milk and milk products revealing the fact that with Nonthermal plasma or cold plasma process decontamination is done, in less amount of time without reaching high temperature. But the application of this technology is not only restricted to product sanitization or decontamination. Although there are certain limitations with this process and the need for a lot of research work in standardizing the process for the desired outcome without disturbing the quality parameter of food.

Conflict of interest

The authors declare that they have no conflict of interest.

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