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A review on cold plasma – A novel non-thermal preservation method of fish and fishery products

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Abstract
Utilization of plasma technology, a useful non-thermal technique, is encouraged in the food industry because of its effectiveness in preserving the natural aroma and flavor and antimicrobial activity. The cold plasma (CP) technique is used for food processing for enhancing antimicrobial activity, structural modification, decontamination of surfaces, and disinfection of food-processing instruments. Currently, a combination of CP with other promising approaches, such as nanotechnology applications, including nanofiber, nanoemulsion, nanoparticles, and nanoencapsulation, and emerging nonthermal technologies, including pulsed electric field (PEF), pulsed light (PL), and ultrasound, is gaining increased attention. In addition to its many advantages, CP is a low-cost method that can be an alternative to heat-based techniques used for the processing of food products. Therefore, application of CP technology in the seafood sector has been described in this review.

Keywords: Cold plasma, non-thermal, preservation, seafood

Introduction
The fishing industry is one of the established food sectors which supplies an ample amount of food to the growing population [7]. Fish continues to be one of the most traded food commodities worldwide [2]. In the food production sector, 'shelf life' is one of the most essential quality parameters. The high perishability of fish products is mainly due to their peculiar composition and structure, even if storage time and temperature are crucial factors for the final quality of the product. The major cause of fish perishability is attributable to the high content in non-protein nitrogen compounds and to the low acidity of the flesh, which are conditions favourable to the growth of microorganism-producing metabolites that affect the organoleptic properties of the products. Nevertheless, the rate of spoilage is also due to the kind of fish species, the sanitary conditions on board and the amount of food in the guts [9]. Seafood is high in nutritional value, containing high-quality proteins, omega-3 fatty acids, essential micronutrients, minerals and vitamins [24]. Pathogens such as *Listeria monocytogenes*, *Escherichia coli* O157: H7, *Campylobacter jejuni* and *Salmonella spp.* can easily thrive on seafood, causing severe foodborne illness in consumers. Due to increase in health disorders across globe there is demand for safe and nutritious food ensuring higher food safety [41]. Traditionally, the methods used to extend the shelf-life of fish products include fermentation, smoking, salting and marinating, or other treatments such as chilling, refrigeration, freezing, drying, boiling, steaming, etc. However, all these techniques are associated with undesirable changes, from a reduced nutritional value to worsened sensory attributes, which fight against the increasing demand of consumers for minimally processed foods with high quality. Thus, in recent years some alternative methods have been proposed as innovative processing technologies able to guarantee an extension of shelf-life while minimally affecting their organoleptic properties. The use of high heat treatment can result in undesirable effects such as change in color, texture, loss of nutrients etc., motivating researchers to explore more non-thermal alternatives for food processing. Conventional techniques like drying, cooling, pasteurization, are used to enhance shelf-life of foods but employing non-thermal techniques has gained much importance in recent times and Plasma technique is one such nonthermal techniques used for food processing [19]. Cold plasma treatment is a versatile and nascent technology that has found many applications in the food processing sector. This non-thermal processing and preservation technique came into the limelight due to its protean nature [26]. The term 'plasma', originally coined by Nobel prize winner Irving Langmuir, designates in physical sciences a gas where a fraction of the particles is ionised that is, stripped of one electron and converted into an electron–ion couple. The plasma state, which is considered as the fourth state of matter, is thus a mixture of electrons, ions and neutral particles [34]. The term plasma is the Greek word (meaning "moldable substances) was first described by the chemist Irving Langmuir in 1920's [35]. Plasma is a very hot ionised gas which is made up of equal numbers of positively charged particles (protons) and negatively charged particles (electrons). When some external energy is applied to the atoms the high energy causes the electrons to strip away from the atomic nuclei and produces various reactive plasma products such as electrons, ions, neutrons, protons and reactive oxygen, atomic oxygen (O), ozone (O_3), hydroxyl radicals (OH^+) and nitrogen species (N_2, NO, NO_2, nitric oxide radical NO^+). The external energy sources can be electrical energy, magnetic current, radiofrequency waves, intense ultraviolet or laser light [20]. Cold plasma technology is emerging, green processes have evolved in recent times in food industries, and its application on seafood industries has been encouraging. The conversion of solid to liquid and liquid to gas occurs with an increase in the energy of molecules in a system. This causes a change in intermolecular arrangement. The further increment in the energy of gases results in the disappearance of all the interactions, which releases positive and negative ions,

causing some of the molecules and atoms in gases to become ionized, which gives rise to plasma [38]. Cold plasma technology is extensively used to preserve food by arresting the activity of microorganisms and increasing the shelf life of food [23] and also for safeguarding of naturally occurring health beneficial bioactive components in food [1]. This non-thermal technology has shown potential application in preserving packaged food product against pathogenic microbes like *Escherichia coli*, *Salmonella* [36, 43]. Fish processing industries are attracted to this technology because it is environmentally friendly, economical, and has broader food safety and processing applications. Cold plasma (CP) is a recent technical intervention for maintaining food quality and safety. Plasma has a neutral ionised gaseous form consisting of ions, free electrons, gas atoms and molecules, as well as UV photons depending on the process parameters and the gas used [8]. Oxygen seems to be more effective than the other gases due to its ability to cause greater oxidation of nucleic acids and amino acids [29]. Based on this, the review focuses on the effects of cold plasma technology on fish and fishery products as well as microbiological decontamination. The present review aims to describe the primary mechanisms of cold plasma methods applied to preserve quality and safety of fish products. In this paper, we have reviewed the important aspects of cold plasma technology for seafood or fish and fishery products.

Principle, Mechanism and Classification of cold plasma

Plasma sterilisation effect was first documented and patented in the year 1968 by Menashi. When the food surface contaminants are exposed to reactive species produced by plasma there will be an accumulation of electrostatic forces at place where the high energy flows. The energy flow further induces radical bombardment action and hence cell lysis occurs. The impact of radical bombardment causes the lesions on the surface makes the microbial cell impotent to repair quickly which results in cell destruction. This phenomenon is termed as "plasma etching". Plasma etching causes DNA and chemical bonds denaturation, thus produces an antimicrobial effect on the cell [18]. The efficiency of plasma treatment against contamination is based on the several factors such as Power level to generate the plasma, Gaseous mixture and Intensity of gases species, Length of exposure, Flow rate, Pressure and design of the system, Milleu factors - Relative humidity, pH and nature of sample [5, 6]. Plasma consists of activated molecules, free radicals, positive ions, negative ions, and neutral atoms. Cold plasma utilizes atmospheric temperature to generate plasma. Many gases (H_2, O_2, He, N_2, Ar, Ne, CF_4, CH_4, NH_3) can be converted into plasma by subjecting them to electric fields such as alternating current (higher frequency), direct current, thermal, microwave, and radio frequency, magnetic field. It produces plasma particles such as ions, free radicals, and electrons that collide with greater kinetic energies [38]. Plasma changes are influenced by factors such as supply gas proportion, surrounding phase, power, humidity as well as voltage levels [19]. In the context of microbial inactivation, when contamination of the food surface is subjected to plasma-created reactive species, electrostatic forces accumulate at the point where the energy is at its highest. The flow of energy results in far more radical bombardment activity, which results in cell lysis. The effects of heavy bombardment produce surface injuries, leaving the pathogenic bacteria cell incapable of swiftly repairing itself, culminating in cell death. Plasma etching denatures DNA as well as chemical bonds, providing the cell with an antimicrobial effect.

Based on the method of generation, pressure and the relative temperature, plasma can be classified in to two different groups they are 1) Non-thermal plasma 2) Thermal plasma.

Thermal plasma - Thermal plasma contains contain gas species and electrons having same temperature of around 10000°K under high pressure are thermodynamically equilibrium in nature. Hence, Thermal plasma has found its use in effectively treating the hazardous metal wastes [18, 22].

Non-thermal plasma - Non-thermal plasma is also called as cold plasma or nonequilibrium plasma is partially ionised gas which are produced under atmospheric/vacuum temperature of about 30- 60°C. Cold plasma contains various gaseous species possess same energy of above moderate room temperature but the electron poses higher temperature of 20,000K with higher energy [20]. Non-thermal plasma can be subdivided into two categories *viz* Quasi-equilibrium plasma (50–100 ◦C), where the reactive species are in local thermodynamic equilibrium and Non-equilibrium plasma (60 *C) or CP where the heavier species present have a lower temperature than the electrons. Cold plasma could be possible method for food applications to inactivate microorganisms to keep the food safe throughout its shelf life. Fast growing demand for fresh produce poses the food industries to supply minimally processed food in a safe manner to the consumer. Cold plasma technique can be a promising technique for preserving food by destroying microorganisms without affecting its quality [20].

Methods of generation of cold plasma

The energy distribution among the component particles in the cold plasma treatment is distinctively non-uniform (a non-equilibrium), where the electrons are more susceptible to transferring heat through a collision with heavier particles, and so determines the particular matrix's electron component. Furthermore, the medium's temperature (35 ºC) does not change, which is a distinctive aspect appropriate for thermal-sensitive products. Several non-thermal plasma discharge techniques are used depending on the mechanism and desired target response.

A. Dielectric barrier discharge

This approach employs a dielectric substance wrapped around two flat metal electrodes which block electric currents and keeps sparks from forming. In a closed target chamber, any inert gas combination or neutral gas travels involving two electrodes and is ionized to produce plasma products. A high-voltage circuit is connected to one electrode, while the ground is connected to the other. It is a non-equilibrium alternating or direct current discharge that typically works across a wide variety of gas pressures (often around 10^4 to 10^6 Pa), about 0.05–500 kHz, and its operational energy requirements range from 10 to 100 W. Various parameters impact dielectric barrier discharge's efficiency, including the operating voltage, the gas used, and the spacing between the electrodes. For large surfaces, dielectric barrier discharge is the best plasma source.

B. Corona discharge plasma

A changing electric field strength at atmospheric pressure creates plasma in this process. Corona discharge can be seen on sharp edges and along thin wires. When gases surpass their breakdown strength in a very non-uniform electric field, they generate weakly ionized plasma with some luminance. The corona system may be created in a simple device and does not need a complicated apparatus, saving money on setup and maintenance costs. Its primary drawback is that it only affects a small region and does so unevenly. However, corona discharges are still regarded as the best choice for food sterilization applications.

C. Plasma jet discharge

There are two parallel electrodes in plasma jet systems through which the carrier gas flows at various speeds. Radio frequencies are commonly used to produce plasma jets or flames at atmospheric pressures. The excited species exit by a tiny valve or a hole approaching the electrode's terminal with the assistance of a carrier gas. Noble gases like helium or argon at an increased flow rate (>10 s/m) are frequently employed in the process. Plasma jets have the benefit of being directly applicable and may be employed between small areas. Although it is suitable for some biological applications, food processing cannot utilize this due to the high expense associated with the gas flow.

D. Microwave plasma discharge

Instead of employing electrodes with varying potentials, A high frequency electromagnetic field drives microwave plasma discharges generated by a magnetron (usually takes place at 2.45 GHz). Microwave radiation oscillates electrons, which form ions by interacting with gaseous atoms and molecules, resulting in an elevated ionization level in microwave plasma. The electrode-less setup of Microwave-driven discharges is their main benefit since it is user friendly. The drawback, though, is the space constraint. Large regions must be directly decontaminated using a variety of discharges. This approach is commonly used in high-temperature processing.

Application of cold plasma in fisheries

Fish are considered an excellent source of polyunsaturated fatty acid, namely omega -3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid, which has a beneficial effect on human health. An 80 kV DBD atmospheric plasma treatment to in package mackerel fish for 5 min does not affect the oxidation of lipids in fish measured by an increase in malondialdehyde content measured on the same day, but the TBARS value increased for both control and cold plasma-treated sample during storage. The generation of reactive species during cold plasma treatment and their interaction with food lipids causing oxidation of lipids in the food system is not an instant reaction. Hence, the low TBRAS value recorded after cold plasma treatment should not be used as an indication to conclude that cold plasma does not affect the oxidation of food lipids. 5 min of exposure of barramundi fish pieces to high intensity cold atmospheric plasma resulted in a decrease in a load of diseases causing and spoilage microbes without any bad effect on the lipids in fish pieces. Because of the addition of food additives like chitooligosaccharides [40] to fish pieces which are good antioxidants and anti-microbial agents. PV and TBARS value increase with storage time for cold plasma-treated samples, but the lowest PV and TBARS values were observed for samples with chitooligosaccharides subjected to cold plasma [37]. Chitooligosaccaride contains NH_2 and OH groups that can arrest the reactive species / free radicals produced during cold plasma treatment, thereby preventing oxidative damage to lipids in fish. They serve as hydrogen donors and block the lipid oxidation process [16]. Hence the ill effect of cold plasma can be minimized or avoided by the addition of such food additives to food before exposure to cold plasma treatment. Olatunde *et al.* (2019) reported that the cold plasma treatment with a high voltage of 260 V and 50 Hz of Asian sea bass reduced a load of pathogenic microorganisms, including *E. Coli, Listeria monocytogenes, Staphylococcus aureusbut* with an increase in the exposure time beyond 5 min resulted in oxidative damage to lipids. The carrier gas composition with a higher percentage of oxygen showed a negative effect on the quality of lipid compared with the carrier gas with a low percentage of oxygen. The gas composition of (90 Argon: 10 Oxygen) low oxygen formed 2900 ppm ozone with 5 min treatment time, and gas composition with more oxygen (80 Argon: 20 Oxygen) resulted in the formation of 4300 ppm of ozone. The TBARS value increased beyond 5 min treatment time because of the presence of a higher percentage of unsaturation in lipids of Asian sea bass. The study revealed that the higher percentage of oxygen in carrier gas accelerates the process of lipid oxidation in cold plasma treated samples. Nigiri and Hosomaki exposure to cold plasma for 300s resulted in the oxidation of lipids measured by elevation in TBARS value. The TBARS value of Hosomaki was much more than the TBARS value of Nigiri during storage [15]. The ratio of exposed surface to sample weight was higher in Hosomaki than Nigiri which resulted in higher oxidation in Hosomaki. Further, Hosomaki fatty acid composition showed the presence of higher unsaturated fatty acids, which are unstable and highly prone to oxidation, causing an increase in TBARS value of Hosomaki than Nigiri. Exposure of blackmouth angler fish to dielectric plasma resulted in a decrease in the *Staphylococcus aureus* and *Bacillus cereus* population with an increase in the exposure time. But increase in exposure time adversely affected the quality of lipids present in dried blackmouth angler fish [4]. The presence of unsaturated fatty acid in the dried blackmouth resulted in a higher degree of lipid oxidation which increased the TBARS value to 1.66 mg MDA/kg sample for treated and which was 1.04 mg MDA/kg sample for untreated sample after 30 days of storage. The increase in the TBARS value was within the limit safe for human consumption. 6 mg MDA/kg is accepted as safe in fish samples for human consumption [11]. The drying process also results in the formation of free fatty acids by breaking of ester linkage in triglyceride structure, and these free fatty acids act as a primary oxidation product that reacts with the free radicals and reactive species produced during cold plasma leading to an increase in the lipid oxidation process in fish. Choi *et al.* (2017) reported a similar study for dried squid using corona discharge plasma treatment. The rapid increase in the TBARS value of fish samples after cold plasma exposure is attributed to a higher percentage of polyunsaturated fatty acids present in fish which are more rapidly oxidized, leading to the formation of secondary oxidation products. Proper packaging in airtight packages or vacuum packaging after cold plasma treatment may reduce oxidative damage to lipids in fish products. The studies reported in the literature revealed that the cold plasma treatment to fish results in an increase in secondary oxidation products due to higher unsaturation present in fish. But by optimizing the process parameters like reduction in exposure time, low applied voltage, the gas composition of carrier gas, vacuum packaging immediately after treatment can greatly reduce the oxidative deterioration of lipids in fish.

Conclusion

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 starch 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.

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