The potential of cold plasma applications in food safety

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Review

SUMMARY

Cold plasma is becoming ever more appealing for use in food hygiene and packaging. It is especially interesting because it involves a dry disinfection process which does not leave any residue in the environment, but at the same time enables the disinfection of food in a way that does not seem to cause significant side effects. The processing of food involving cold plasma and packaging materials treated with cold plasma may improve food safety and prolong shelf life. The fact that various foods have different needs for hygiene and that it is possible to use various forms of plasma generation, further contributes to the convenience of using cold plasma in food processing. Despite promising results of cold plasma applications in food hygiene, it is still necessary to verify the effect of cold plasma on the quality and organoleptic properties of foods.

Key words: *cold plasma, food, hygiene, safety*

Plasma, cold plasma, physical and chemical properties of plasma

Cold plasma is used in plasma televisions, fluorescent lamps, various surface application of substances, cleaning of surfaces, computer parts, textiles, polymers and sterilisation of medical devices made from heat sensitive polymers, as well as wound healing (Stoffels et al., 2008; NATO, 2013; Nettesheim et al., 2015). It is also used for coatings in jet engines, cleaning of toxic gases and hazardous waste. However, it has a negligible effect on materials and releasing residue in the environment (Griffits, 2014; Thirumdas et al., 2015).

Recently, a lot of attention has been paid to the use of cold atmospheric plasma for the non-thermal treatment of foodstuffs and packaging materials, in particular RTE (Ready to Eat) products that do not need to be precooked before ingestion (European Commission, 2015; Ariette, 2018). An important requirement of consumers is healthful, nutritious and less processed food with long shelf life in which the composition and quality of food stays unchanged (Misra et al., 2011; Kenner and Jense, 2011). Given the growing food market, the food of local origin and the food from distant foreign countries is transported for a rather long time, thus proper handling of foods is required. The provision of adequate food safety is ensured by specific food processing and packaging. Food processing involves different methods and various techniques such as thermal treatment, gamma and beta-irradiation, ultrasound, ozonation, pulsed light, UV treatment, pulsed electric field (PEF) and high hydrostatic pressure that enable microbiological food safety (Misra et al., 2011; Kenner and Jense, 2011). Even in principle, these methods are appropriate methods for trea-

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ting foodS. However, their effectiveness is limited in terms of safety, influence on food products, type of method and antibacterial efficiency.

What is cold plasma?

Irving Langmuir was in 1928 among first pioneers who studied the emission of charged particles in ionised gases (Thirumdas et al., 2015). The universe contains almost 99.9 % of plasma, called the fourth aggregate state, generated by the additional energy input (heat, electrical tension, microwave radiation, light) into gas, causing the splitting of gas molecules into electrons, ions and partially stable particles (Kenner and Jense, 2011; Griffits, 2014; Thirumdas et al., 2015). There are only few natural sources of plasma found on the Earth, among which aurora borealis and lightning are the best-known. Cold plasma appears in nature as polar glow, that is, St. Elmo's fire, which is an electrical weather phenomenon in which plasma glare is caused by discharge, i.e. due to the ionisation of air molecules in the vicinity of sharp points noticeable as strong light blue or purple annealing. Namely, the input of energy into gas turns into plasma, that is, ionised gas consisting of photons, ions, free electrons and atoms in their elementary or excited condition characterised by the "avalanche effect", which means that numerous highly reactive, short-term chemical substances are formed in gas (Thirumdas et al., 2015; Misra et al., 2011; Ariette, 2018). Their excitation is triggered by the electric field which accelerates the dissociation of gas molecules into ions and electrons in an excited state, causing the loss of their internal energy in collision with other particles, surface, or by the emission of UV photonS. For example, the input of energy in the air creates the Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNO), hydroxyl (OH), oxygen ions, and UV photons (Griffits, 2014; Ziuzina et al., 2013; Ariette, 2018; Misra et al., 2011; Rød et al., 2012; Noriega et al., 2011; Fernández et al., 2012). The types of plasma with bactericidal properties consist of a lot of ozone, nitrogen oxides and peroxides (Keener and Jense, 2011). The fundamental principle of plasma treatment therefore refers to ionisation, followed by the distribution of electron energy (Thirumdas et al., 2015). There are two types of plasma: thermal and cold plasma. Due to the relative energy level of electrons, cold plasma is formed at atmospheric pressure (0.1-1 atm) (Thirumdas et al., 2015) by the discharge of gas in frequency ranges between 1 kHz and 103 MHz, or at microwave frequencies (GHz), but always under the influence of direct (DC) or alternating electric current (AC) at voltages of 1-100 kV (Griffits, 2014; Thirumdas et al., 2015).

Generation and use of cold plasma

Typical forms of generation of cold plasma at atmospheric pressure include corona discharge, dielectric barrier discharge (DBD), radiofrequency plasma (RFP) and discharge of sliding arcs (Thirumdas et al., 2015; Ariette, 2018; Conrad and Schmidt, 2000; Ziuzina et al., 2015). For example, plasma generated by DBD plasma generator can be generated by ionisation of gas in high-voltage field between dielectric electrodes representing cathode and anode (Keener and Jense, 2011, Thirumdas et al., 2015). If the space between the electrodes is narrow, the pressure of the gas increases, resulting in plasma jet. As long as such plasma jets are microscopically small (microplasma), it is possible to create micro-jet systems that can handle large surfaces. In the case of corona discharge in plasma formation, high-voltage strimmers are formed as a type of splash, such as miniature high-temperature lightning (Keener and Jense, 2011; Conrads and Schmidt, 2000). Cold plasma is produced at low energies (at only a few hundred watts of power), while thermal plasma is generated by high energy and at high pressures (Misra et al., 2011; Keener and Jense, 2011). Cold plasma is mainly applied in industrial use. Depending on the purpose and treated products, different types and plasma generators are used. Plasma intensity, dispersion and operating time has to be adapted to the requirements of production processes (Keener and Jense, 2011). It has been shown that DBD plasma, as a diffused plasma gas or gas jet, is most suitable for the processing of food, whereas plasma energy can also be used for the ionisation of gases within sealed food packages (Misra et al., 2011; Keener and Jense, 2011; Song et al., 2012; Nijboer, 2017; Vleugels et al., 2005). Since the selection of plasma carrier gases is optional, different gases and their mixtures or, usually, common air can be used (Misra et al., 2011; Keener and Jense, 2011; Vleugels et al., 2005). When oxygen is used as a carrier gas, within a few seconds, a variety of microbiocidal reactive oxygen and nitrogen ions can be generated, including allotropic ozone (Keener and Jense, 2011). Cold plasma is a dry surface treatment process unlike many "wet" treatments that are considered not suitable in many environments where the moisture is not recommended. However, plasma also has the property of changing the surface tension of often hydrophobic packaging materials that become hydrophilic after plasma treatment, which enables additional functions of packaging materialS. For example, the uniform application of bactericidal substances such as nanoparticles on the surface of

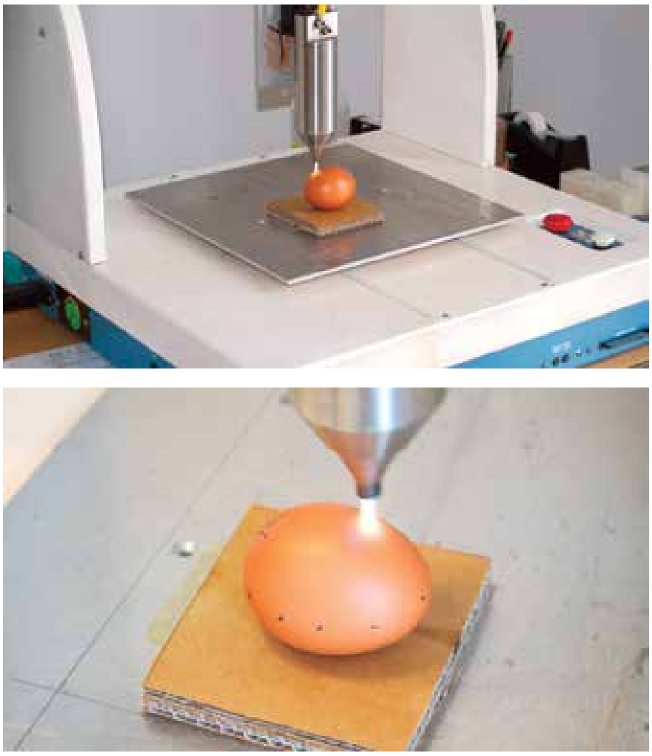

Figure 1: *Cold plasma jet directed at an egg (University of Ljubljana, Slovenia, Veterinary Faculty Ljubljana, Plasmatreat GmbH®, Rogač Plus d.o.o.)*

packaging material protects the food from recontamination (Misra et al., 2011). The special feature of plasma is that, despite its primary surface treatment properties, the materials can also be processed with plasma through pores, which is an important advantage over bactericidal irradiation with UV rays, which only works on exposed parts of surfaces but not in the »shadows« (Griffits, 2014).

Cold plasma applications in food safety

The main advantage of plasma treatment is that plasma gas quickly returns to the original carrier gas, leaving almost no residue (Keener and Jense, 2011). In addition, due to the short duration of plasma surface treatment, low energy and, above all, low released temperature, cold plasma can also be used directly on food. Namely, it does not pose a great risk from changes and affecting the quality of food, such as change in colour and texture, loss of nutrients, change of acidity and pH, effect on proteins, carbohydrates, enzymes, vitamins and fats (Keener and Jense, 2011; Vleugels et al., 2005). All of the above mentioned properties of food are considered important factors influencing the organoleptic properties and product quality.

Nevertheless, even though cold plasma shows great potential for the use in food hygiene, it is very difficult to determine plasma's effect on food, which can cause more than 100 chemical reactions and changes in the chemical quality of processed food, mainly due to the action of reactive substances that largely depend on the gas used for plasma generation (Vleugels et al., 2005). Namely, it has been established that when cold plasma is working in moist environments, it can contribute to the acidification of food due to the formation of nitric acid, caused by the content of reactive nitrogen species acting as carrier gas in the air. This is even more evident in the treatment of liquids (Vleugels et al., 2005). The reactive substances in plasma may also affect the denaturation of proteins, mainly due to interaction with amino acids, degradation of carbohydrates, especially starch and sugars, and lipid oxidation caused by the action of free radicals, that contrary positively influence antioxidant activity in food (Vleugels et al., 2005).

However, the potential of plasma use in food processing is nonetheless very interesting, primarily due to biocidal activity, whereby reactive ions react with almost all cellular components of microorganisms that significantly contribute to food safety. At the same time, these processes are running at temperatures that do not affect the composition and quality of food (Ariette, 2018; Laroussi et al., 2003; Ross et al., 2016, Ziuzina 2014; Bermúdez et al., 2013; Fernández et al., 2013). In comparison with the heat treatment of food, the use of cold plasma thus represents an important new approach in food processing technology (Ulbin-Figlewicz et al., 2015). The new technology can inactivate microorganisms at ambient or sublethal temperatures (Fernández and Thompson, 2012). In order to determine possibilities of using cold atmospheric plasma in food industry, the factors influencing microbial decontamination were assessed (Han et al., 2016).

Cold plasma treatment is a dry surface microbicidal treatment of packaging and processing surfaces, as well as foods such as meat, milk, vegetables, fruits, nuts, herbs, seeds and liquids (e.g. oil, wine, milk, contaminated water) (Ariette, 2018; Misra and Jo, 2017; Schnabel et al., 2015). *Escherichia coli*, *Salmonella* enterica serotype Typhimurium, *Staphylococcus aureus* and *Listeria monocytogenes* are the most significant microorganisms in food industry subject to the desired effect of cold plasma (Vleugels et al., 2005; NATO, 2013; Han et al., 2016; Ziuzina et al., 2015; Misra et al., 2011; Ziuzina et al., 2013; Keener and Jense, 2011; Griffits, 2014; Surowsky et al., 2013; Thirumdas et al., 2015). Though mechanisms of plasma bacterial action have not yet been fully explained, the mechanism of plasma action on cells was determined to be only a function of molecular ozone and free radicals that, at the least, causes irreversible lesions on the surface of cells (Smet et al., 2017; Laroussi et al., 2002). Bacterial cells electroporation and cell membrane disorders were manifested (Bermúdez et al., 2013). In the cell, cold plasma can cause damage on proteins, vital cell macromolecules and nucleic acids (Ziuzina et al., 2013; Yu et al., 2006; Laroussi et al.; 2003, Misra et al., 2011; Rød et al., 2012; Noriega et al., 2011; Fernández et al., 2012). The plasma microbicide efficiency depends on the distance between electrodes, distance between the source of plasma and treated surface (Gap (cm)), the type of carrier gas (air, He, O_2, ...), gas stream (L/min), electric voltage (kV), frequency (kHz), temperature, duration and the type of bacterial population (Ziuzina et al., 2013). Namely, different types of microorganisms have different resistance to plasma treatment, which is still the subject of research. Recently, more and more alternative forms of red and poultry meat disinfection were investigated. The purpose of food treatment is to ensure the durability of products while, at the same time, preventing the growth of spoiling and pathogenic microorganisms (Ahmad et al., 2016; Schluter et al., 2013). Many studies of direct cold plasma surface treatment on food matrices for the prevention of contamination with bacteria such as *Campylobacter*, *Listeria*, *Salmonella*, *Campylobacter*, *Streptococci*, *E. coli* and *Lactobacillus*, have already been conducted (Thirumdas et al., 2015; Shashi et al., 2018; Isbary et al., 2013; Bermúdez et al., 2013; Misra et al., 2011). The study of cold plasma bactericidal efficiency on test microorganism from the genus *Salmonella enterica* serotype Typhimurium found that the degree of bacterial inactivation is inversely proportional to the initial concentration of bacteria. However, it was found that multilayered bacterial structures and a higher amount of biomass significantly reduced plasma's effect on bacteria (Fernández et al., 2012). Another study of cold plasma effect on the survival of bacteria from the genus *Salmonella* on surfaces that come in contact with food demonstrated the reduction of colony bacteria of 1.57 log in 5 sec, 1.82 log in 10 sec and 2.13 log in 15 sec, which means that the time of action significantly affects the bactericidal effect of plasma (Niemira et al., 2014). The study in which effects of cold plasma generated on the dielectric barrier (DBD) of *E. coli* ATCC 25922 bacterial culture were suspended in liquid medium in closed container found that cold plasma treatment resulted in the inactivation of bacteria (7 log CFU ml-1), both in direct treatment lasting 20 sec and indirect treatment lasting 45 sec (Ziuzina et al., 2013). The use of DBD plasma on the surface of chicken breasts without skin and chicken thighs with skin inoculated with bacterial cultures *S. enterica* serotype Typhimurium and *Campylobacter jejuni* showed bactericidal effects in the range of 1.25-3.11 log, depending on the exposure time (20 seconds - 3 minutes) and the concentration of bacterial inoculum (10^2-10^4) (Brian et al., 2012). The poultry meat and skin treatment with atmospheric cold plasma resulted in 3 log reduction of bacteria from the genus Listeria innocua. A study showed a significant effect of surface roughness on the reduced bactericidal effect of plasma (Noriega et al., 2011). Cold plasma was also tested for bactericidal effects in sealed packaging on the inoculated bacterium from the genus *L. innocua* on sliced meat intended for immediate consumption without prior cooking. In the period between 2 to 60 sec, bacterial colony reductions ranged from 0.8 ± 0.4 to 1.6 ± 0.5 log (Rød et al., 2012). Bactericidal effects of cold plasma on the survival and persistence of bacteria from the genus *E. coli*, *S. enterica Typhimurium* and *L. monocytogenes* were also tested on cherry tomatoes and strawberries in closed container *s*. After treatment with plasma at exposure times of 10, 60 and 120 sec, the bacterial colony reductions for 3.1, 6.3, and 6.7 log (Ziuzina et al., 2014) were observed. Also, the susceptibility of bacteria from the genus *E. coli* after exposure to cold plasma was tested on tomatoes, carrots and green salad. The rate of reduction of bacterial cultures was 1.6 log. In addition, it was found that plasma disinfects smooth surfaces (tomatoes) better and faster than rough surfaces (carrots). Cold plasma was also tested for use in the decontamination of salads, strawberries and potatoes contaminated with *S. enterica* serovar typhimurium (*S.* Typhimurium) cultures. The test showed that under optimum conditions on membrane filters, a 2-minute of plasma exposure resulted in 2.71 log-reduction of *S.* Typhimurium, while it took 15 minutes to reach 2.72, 1.76 and 0.94 log-reduction in salads, strawberries and potatoes (Fernández et al., 2012). In our study (Dobeic et al., 2016) we investigated the effect of atmospheric cold plasma in the dry disinfection of the surface of egg shells. Egg shells of table eggs were exposed to individual or multiple effects produced by atmospheric plasma jet for 10-60 seconds. In such treatment of egg shells, it was found that the reduction of *S. aureus* (NCTC 8325) on polyethylene terephthalate (PET) plates was > 3 log (99.9 %, $P < 0.05$). A reduction in the total number of aerobic mesophilic bacteria and *S. aureus* on the surface of plasma treated egg shells ranged between 1.8 and 2.5 log (Fig. 1). Despite physical and ionising properties of plasma gas, egg shell cuticles remained functionally undamaged. Plasma-treated eggs were not changed in terms of sensory and physicochemical properties, while aging process remained identical to untreated eggs. The results of the experiment show that the treatment of an egg shell with a jet-stream of atmospheric plasma has a positive effect on the decontamination of eggs in the shell and no negative effects on the quality and aging of eggs, which is important in terms of food safety and quality (Dobeic et al., 2016).

CONCLUSION

The use of cold plasma for the inactivation of microorganisms is a relatively new field of research, with particular attention being given to the possibility of preventing the contamination of food. The use of cold plasma by generating short-lived highly reactive chemical free radicals shows great potential in the disinfection of food and packaging. Cold plasma has bactericidal efficiency, but it does not leave any residue, which is important in terms of healthy food and environmental protection. The advantages of using cold plasma for treating foods are its high efficiency at low temperatures (mostly < 70 °C), the treatment and possibilities of adjusting the method of plasma generation for certain purposes, relatively low tissue damage and bactericidal action achieved without using water and other solventS. Nevertheless, it is necessary to further investigate sensory and nutritional effects of plasma-treated food, although no major organoleptic changes have been observed in previous studieS. In particular, it is necessary to emphasize that the use of cold plasma is a method of decontamination alternative to chemical (e.g. chlorine), physical (e.g. ionisation, pulsed electric fields, pulsed illumination) and some biological methods of decontamination (e.g. macrophages). The future use of cold plasma in food processing shows promise, but further investigations are needed (Schluter et al., 2013).

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Mogućnosti primjene hladne plazme u sigurnosti hrane

SAŽETAK

Hladna plazma postaje sve privlačnija za uporabu u području higijene i pakiranja hrane. Posebice je zanimljiva jer podrazumijeva postupak suhe dezinfekcije koji ne ostavlja ostatke u okolišu, a omogućava dezinfekciju hrane bez uzrokovanja značajnijih nuspojava. Obrada i pakiranje hrane primjenom hladne plazme mogli bi omogućiti veću sigurnost hrane, a vjerojatno i dulji rok trajanja. S obzirom na različite higijenske potrebe različitih vrsta hrane, postoji mogućnost uporabe različitih oblika proizvodnje plazme, što dodatno pridonosi praktičnosti uporabe hladne plazme. Unatoč obećavajućim rezultatima istraživanja hladne plazme u higijeni hrane, još je uvijek potrebno provjeriti njene učinke na kvalitetu i organoleptička svojstva hrane.

Ključne riječi: *hladna plazma, hrana, higijena, sigurnost*

Möglichkeiten der Anwendung von Kaltplasma im Bereich der Lebensmittelsicherheit Übersichtsarbeit

ZUSAMMENFASSUNG

Kaltplasma ist im Bereich der Hygiene und Lebensmittelverpackungen immer gefragter. Sie ist insbesondere interessant, weil sie ein Trockendesinfizierungsverfahren umfasst, das keine Rückstände in der Umwelt hinterlässt und zugleich die Desinfizierung von Lebensmitteln ohne bedeutende Nebenerscheinungen ermöglicht. Die Behandlung und Verpackung von Lebensmitteln durch Anwendung von Kaltplasma könnte nicht nur eine höhere Lebensmittelsicherheit sondern wahrscheinlich auch eine längere Haltbarkeitsdauer sicherstellen. In Anbetracht der diversen hygienischen Anforderungen von unterschiedlichen Lebensmitteln gibt es verschiedene Herstellungsverfahren von Plasma, was zusätzlich zur praktischen Anwendbarkeit von Kaltplasma beiträgt. Trotz der versprechenden Forschungsergebnisse in Bezug auf Kaltplasma im Bereich der Lebensmittelhygiene, ist es immer noch erforderlich, seine Auswirkungen auf die Qualität und die organoleptischen Parameter von Lebensmitteln zu prüfen.

Schlüsselwörter: *Kaltplasma, Nahrung, Hygiene, Sicherheit*

Las posibilidades de la aplicación del plasma frío en el campo de la seguridad de la comida artículo de revisión

RESUMEN

El plasma frío se está volviendo más atractivo para el uso en la higiene y envasado de la comida. Su característica de desinfección en seco la hace muy interesante porque no perjudica el medio ambiente y en el mismo tiempo facilita la desinfección de comida sin efectos secundarios significativos. La preservación y el envasado de la comida por plasma frío podrían permitir una seguridad más alta de la comida y posiblemente la extensión de las fechas de caducidad. Teniendo en cuenta diferentes requisitos higiénicos de diferentes tipos de comida, existe la posibilidad del uso de diferentes tipos de producción del plasma, lo que añade a la utilidad del uso del plasma frío. A pesar de los resultados prometedores de las investigaciones del plasma frío en el campo de la higiene de la comida, todavía hay que averiguar sus efectos sobre la calidad y sobre las propiedades organolépticas de la comida.

Palabras claves: *plasma frío, comida, higiene, seguridad*

Le possibilità applicative del plasma freddo nel campo della sicurezza alimentare

RIASSUNTO

Il trattamento al plasma freddo acquisisce sempre maggior appeal per le sue possibilità applicative nel campo dell'igiene e del confezionamento alimentare. È particolarmente interessante perché sottintende il procedimento della disinfezione a secco che non lascia tracce nell'ambiente e consente una disinfezione senza significative controindicazioni. La lavorazione e il confezionamento degli alimenti con l'applicazione del trattamento al plasma freddo potrebbero garantire maggior sicurezza alimentare e, probabilmente, un termine di scadenza più lungo. In considerazione delle differenti esigenze igieniche dei diversi tipi di cibo, esiste la possibilità di impiegare differenti forme di produzione del plasma, il che aumenterebbe le possibilità d'impiego del trattamento al plasma freddo. Nonostante i buoni risultati ottenuti nella ricerca svolta sul plasma freddo nel campo della sicurezza alimentare, è necessario approfondire le verifiche sui suoi effetti riguardo alla qualità e alle proprietà organolettiche del cibo trattato.

Parole chiave: *plasma freddo, cibo, igiene, sicurezza*