Review Article

Microbial Decontamination of Spices Using Cold Plasma

Hadi Bagheri[1*], Sepideh Abbaszadeh[1, 2]

1-Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

2 -Department of Nutrition and Food Hygiene, Faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran

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ABSTRACT

Most of spices are produced using traditional systems. In unsanitary conditions, spices can contain large numbers of pathogenic microbes such as bacteria, molds and yeasts. Some microorganisms are known as human pathogens, which need disinfection mechanisms that minimize their potential harms to active substances in spices. Use of contaminated spices in foods can significantly decrease the shelf life of food products and may include health hazards to consumers. In recent decades, various technologies such as fumigation (e.g., ethylene oxide, propylene oxide and methyl bromide), steam heating and gamma radiation have been used to eliminate pollutions. However, these technologies include disadvantages. Therefore, researchers eagerly investigate novel methods of disinfection that do not include the highlighted disadvantages. This study has reviewed conventional methods for the sterilization and decontamination of spices, focusing specifically on cold plasma as an alternative technique and its uses in microbial inactivation of spices. Cold plasma is a novel food processing technology which uses energetic reactive gases for the inactivation of contaminating microbes in spices. Decontamination spices with cold plasma is safe and much more effective than previous methods. Furthermore, effects of cold plasma on bioactive ingredients are negligible and almost final quality of the products after processes are constant.

Keywords: Microbial decontamination, Spices, Non-thermal, Cold plasma

Introduction

Spices are widely used in various food products to create favorite flavor, aroma and color. Most of spices and herbal products are often produced using traditional methods (e.g., drying at room temperature). Under poor sanitary conditions, these food products may contain high numbers of pathogenic and spoilage organisms such as bacteria, fungi and insects (1). The primary microbial contamination is strongly linked to the hygienic conditions at harvest time, drying and milling stages (2). Therefore, large numbers of these organisms in spices can cause food poisoning and corruption of the foods (3). Increases in foodborne diseases and food poisoning caused by contaminated plants and spices in the last decades of the twentieth century have been reported by Buckenhuskes and Rendlen (4), mostly associated to the hygienic handling during the harvest and processing. Reproduction of microorganisms in spices is limited due to the low a_w of spices. Once contaminated spices are added into water-rich food products, microorganisms begin to multiply (5). Resistance of microorganisms in dry media (low a_W) is higher than that in water-rich media (high a_W); therefore, decontamination of herbs and spices is difficult (6). Contamination of spices and herbs with fungi is common and up to 100% of the samples may be contaminated, ranging 9.07×10^3 to 2.2×10^4 CFU/g (7, 8). Demands for healthy food products with minimal processing have increased significantly since the last decades. This is due to increased consumer desire to use top-quality nutritional foods with novel sensory properties and lower preservatives and chemicals *(9, 10)*. Regarding contaminated samples of spices, it is essential to find appropriate methods to inactivate microorganisms in spices. Therefore, spices should undergo sterilization or decontamination processes. Unfortunately, a very few studies have been carried out to inactivate microorganisms in spices and no reviews have been published on microbial inactivation of spices using cold plasma. Thus, the major objectives of this review were 1) introducing various techniques for the sterilization of spices and 2) assessing cold plasma for the sterilization of spices and herbal products.

Methodology

In the present review, three electronic databases of Google Scholar, PubMed and Science Direct were searched to find eligible studies published 2000–2020. Keywords included spices, sterilization, chemical decontamination and cold plasma. Totally, 120 articles were identified. Inclusion criteria included empirical studies assessing effects of cold plasma as a novel method of non-thermal

***Address for correspondence:** Hadi Bagheri, Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
E-mail address: bagherihadi51@yahoo.com

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processing for the decontamination of spices and the method effects on final quality of spices.

Sterilization of spices using various techniques

Chemical decontamination techniques

Use of chemical materials such as ethylene oxide, propylene oxide and methyl bromide significantly decreases the initial microbial population in several spices. However, use of this method is difficult due to the potential health risks to operators as well as risks of environmental contamination. Several mixed spices can be disinfected by ethylene oxide (11), except spices containing salt, because salt in spices can reacts with ethylene oxide and toxic chlorohydrin is formed. Propylene oxide is not as effective as ethylene oxide. Based on the US regulations, the ethylene oxide residue after use should not exceed 50 ppm. However, European countries include stricter regulations and do not allow use of ethylene oxide to disinfect spices due to potential health risks (11). The residue tolerance for propylene oxide in spices is 300 ppm. However, this gas is considered as a carcinogen and mutagen, especially when enters the body through inhalation (11). Use of propylene oxide is permitted in several countries and prohibited in others due to residues in spices (12). Methyl bromide is sometimes used to disinfect spices. Methyl bromide is a natural chemical and manufactured as a fumigant to control microbial population in dry foods such as spices (12). Use of chemicals to kill germs in foods has been prohibited in several regions of the world due to their possible toxic residues and carcinogenic properties after the process (13).

Steam sterilization

Steam sterilization is an effective disinfection method that involves high temperature steam and is usually accompanied with color changes and decreases in volatile oils in processed products (14). Steam is one of the best ways to sterilize spices because of no chemical residues in processed products. Steam sterilization can be used to whole and milled herbs and spices. However, special equipment is needed because steam must be used under pressure. The pressure must be maintained at fixed values; otherwise, temperature of the products increases and their essential oils are lost. As soon as the steam treatment is complete, the moisture of steam in spices should completely be removed. Presence of the moisture in spices results in agglomeration phenomena and growth of microorganisms. A 3-log CFU/g decrease in microbial loads has been seen in black peppers after steam treatment of 120 ℃ for 20 s. Under these conditions, no significant changes in volatile oils have been reported. However, volume of the moisture in black peppers have increased, which can decrease shelf-life of these food products (15). In general, use of high-temperature steam can decrease volatile materials and increase moisture contents of dried spices, leading to decreases in their shelf life (15).

Irradiation

Irradiation is considered as an effective method for the spice decontamination. Due to destroy of microorganisms, spices are exposed to ionizing radiation (e.g., gamma ray, electron beam and x-ray) (16). Despite confirmation of ionizing radiation in terms of its efficiency, eco-friendly nature and low-energy consumption, it is rarely used due to its poor consumer acceptance. In addition, minor changes in food sensory and antioxidant properties have been observed (17). However, exposure to high doses can adversely affect flavor of spices. In special cases where spices have been exposed to irradiation inside packages to prevent secondary contaminations, harmful compounds of the package materials have migrated into the spices. Sensory properties of most spices are well preserved at 7.5–15 k Gy (18). Various studies have shown that radiation preserves sensory properties of the spices better than that the chemical methods do (19, 20).

High hydrostatic pressure

The high hydrostatic pressure (HHP) is a valuable tool for disinfecting and stabilizing fruits and vegetables. Usually, pressures of 100–1000 Mpa are used for this purpose (21). Inactivation of microorganisms by HHP method highly depends on a_w. Therefore, HHP treatment is an inappropriate sanitation method in spice production. Samples of spices with aw below 0.66 have not shown decreases in the microbial count after treatment with HHP (22). Windyga et al. (23) have reported microbiological decontamination of coriander and caraway using half-hour treatment of HHP at 800 and 1000 MPa, used within increased temperatures of 60–121 °C under helium atmosphere. A 2-log decrease of aerobic mesophilic bacteria has been observed in this study and all coliforms, yeasts and molds were completely destroyed. Results of this study have shown that the microbiological quality of spices can be improved under high pressure and helium atmosphere using appropriate combinations of pressure and time. However, high dependence of inactivation of microorganisms on aw is still a significant challenge for the use of HHP as a hygienic method in spice production.

Trends and state-of-art on sterilization of spices

Cold plasma

Conventional methods such as fumigation with chemical materials, treatment with super-heated steam and HHP are used for the decontamination of spices. However, limitations such as low antibacterial activity, pH dependence, aw dependence, oxidation of most aromatic components of spices, affection of sensory parameters (e.g., loss of flavor and color) and decreased quality of fatty acids and vitamins are reported for the conventional methods (24). Irradiation with rays to remove contamination is a quiet efficient process but the consumer acceptance is relatively low. Fumigation with chemicals is prohibited due to their carcinogenic potentials for humans (13). Steam sterilization, as a thermic disinfectant method, is widespread. However, use of steam in heat sensitive food products can cause changes in their flavor and taste as well as decreases in volatile compounds, especially when it is used in milled herbs and spices (13). Due to the disadvantages and problems described previously (Fig. 1) (2, 13, 25), novel processes need to be developed and replaced with the old traditional methods for decontamination of spices. Relatively, non-thermal technologies are promising alternatives. Cold plasma technology, as a novel processing technology, has attracted much attentions (26). This emerging technology is used to decontaminate a wide variety of heat sensitive instruments. Cold plasma is a novel non-thermal method that can be used for the microbial decontamination of foods as an alternative to current thermal, chemical and physical technologies (27). This method is appropriate to decontaminate microorganisms in heat-sensitive products due to generation of cell-lethal reactive species (2, 3, 5, 9). Generally, plasma is grouped into two major categories of thermal and non-thermal (cold) plasmas. Cold plasma technology is a relatively novel method with a purpose of improving microbiological safety of foods, usually associated to the preservation of sensory properties of the treated foods (28).

Cold plasma is a state of ionizing gas, including ions, electrons, ultraviolet rays and highly reactive species such as gas molecules, charged particles, free radicals, electrons, atoms, excited molecules and photons (Fig. 2), which are able to deactivate and kill bacteria, viruses and other microorganisms with no significant temperature effects (29). The non-chemical, economical (low-energy consumption), eco-friendly and design versatility of cold plasma are unique advantages of this technology, compared to traditional technologies (29). Cold plasma can be produced by excitation of gas molecules through the electrical discharges (30). The decontamination result of cold plasma is due to charged particles and reactive species in the plasma that can damage DNA molecules, break chemical bonds and damage cell membranes (10). Furthermore, plasma ions can accelerate processes such as oxidation and peroxidation inside the cell, leading to the inactivation of microorganisms (31). Effectiveness of cold plasma depends on biological parameters such as types of the substrate and characteristics of the microorganism (32). Table 1 illustrates effects of various cold plasma methods on the sterilization of spices. Spices are widely used as food additives in the world and valuable due to their special flavor and aroma as well as their medicinal properties (25, 33). The process of producing spices powder involves several steps, including harvesting, drying, grinding, packaging and storage. Spices may be contaminated during these steps (34).

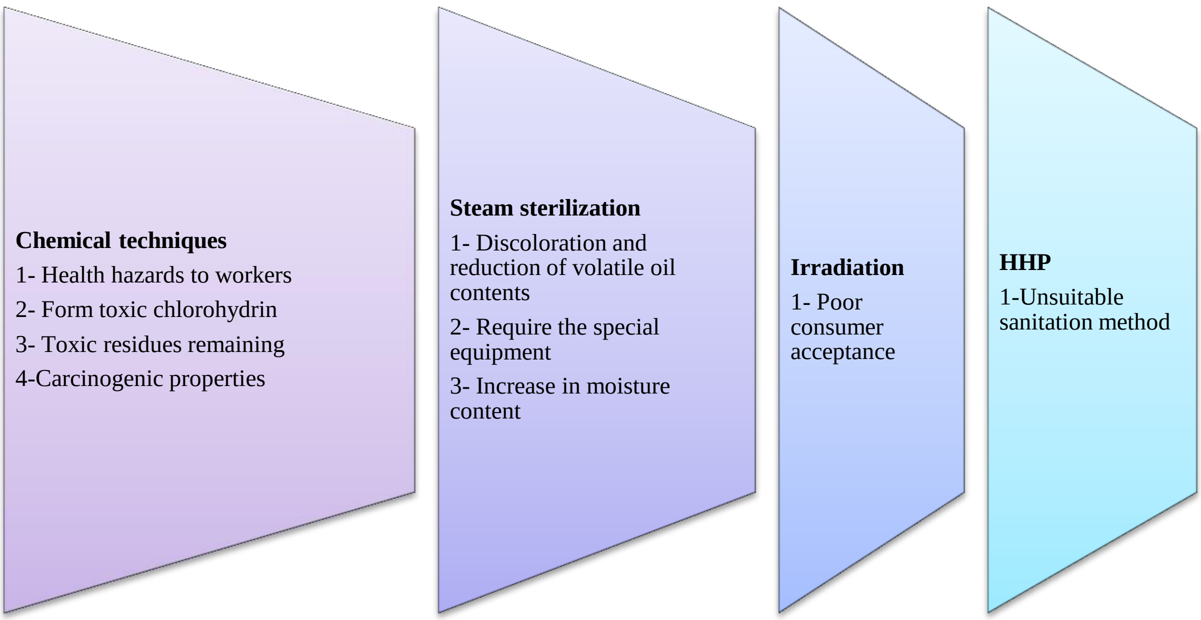

Figure 1. Disadvantages of conventional methods for

Table 1. Use of various cold plasma methods for the sterilization of spices

Spice	Cold plasma type	Salient result	Reference
Red Pepper	Atmospheric pressure – dielectric	Complete decontamination of *E. coli*, *B. cereus* and *A. flavus* were achieved within 20 min	Abdi et al. (3)
	Microwave-powered	Microwave-powered cold plasma were effective on *A. flavus* inactivation	Kim et al. (25)
Dried peppermint	Radiofrequency-low pressure	LPCP process had a significant effect on the removal of *E. coli O157:H7* at 50 and 60 W	Kashfi et al. (1)
Black pepper	Direct plasma jet	The study Showed promising results for the inactivation of both *Bacillus* spores on the surface of black pepper	Hertwig et al. (2)
Onion powder	Microwave power cold plasma	Cold plasma at 400 W for 40 min, determined as the optimum conditions for *B. cereus* spore inhibition	Kim et al. (36)
Lemon verbena	Low-pressure cold plasma	The LPCP treatment had a positive effect on the essential oil content of lemon verbena leaves	Ebadi et al. (9)
Whole black pepper Red paprika powder Oregano	Cold atmospheric pressure plasma- remote plasma	The cold plasma technology inactivate the spores, bacteria, molds and yeast in various types of herbs and spices	Hertwig et al. (5)

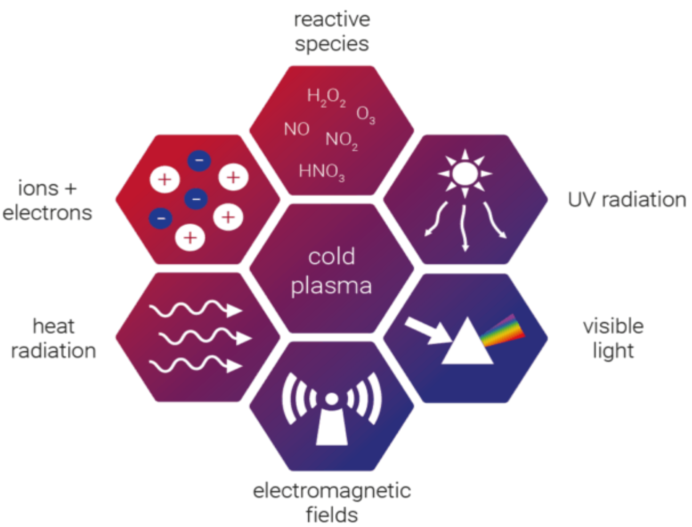

Figure 2. Cold plasma decontamination technology

High levels of contamination with microorganisms can be detected in spices. Pathogenic microorganisms as an Escherichia (E.) coli, Aspergillus (A.) flavus, Bacillus (B.) cereus, Clostridium (C.) perfringens and Staphylococcus (S.) aureus are sometimes found in spices (35). Potentially, this microorganisms can create public health risks. To prevent or decrease outbreaks of foodborne diseases, use of effective decontamination methods with no changes in food quality are necessary. Several decontamination methods have been used to control microorganisms in spices to ensure the food safety (11, 14, 23). One of the best methods used to ensure that spices are hygienically acceptable is cold plasma treatment (Fig. 3) (3). Microbial decontamination of A. flavus and B. cereus spores in red pepper powders has been investigated by Kim et al. using cold plasma (25). Based on their results, microwave-powered cold plasma systems effectively inhibit A. flavus and B. cereus spores in red pepper powders. In fact, 2.5-log spores/g of A. flavus and 1-log CFU/g total aerobic bacteria have decreased using cold plasma with nitrogen at 900 W and 667 Pa for 20 min. Moreover, their results have shown that a_W of the powders decreases using cold plasma, which might be due to water evaporation. Cold plasma at 900 W for 20 min does not significantly increase temperature of the powders, indicating the potential of cold plasma in non-thermal decontamination of red pepper powders. Furthermore, results have demonstrated that color properties of the red pepper powders have not significantly been changed by cold plasma.

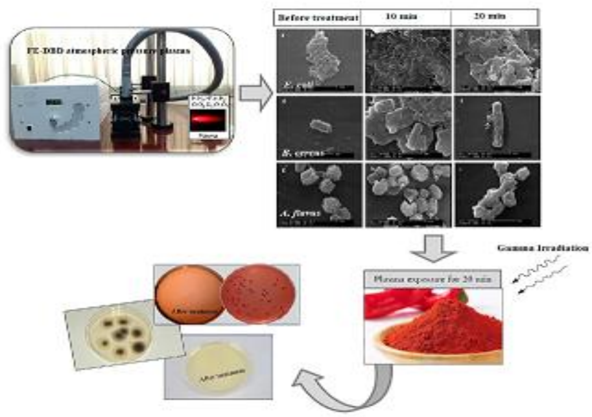

Figure 3. Decontamination of red pepper using cold atmospheric pressure plasma

Kashfi et al. (1) have simultaneously investigated physicochemical and microbial properties of dried peppermints using non-thermal plasma. In this study, effects of radiofrequency low-pressure cold plasma (LPCP) on microbial properties, antioxidant compounds, total phenolic contents and color changes of dried peppermints (Mentha piperita) have been investigated (1). Results have shown that use of LPCP at 50 and 60 W can destroy E. coli O157: H7 in dried peppermints. At 20 W, the method does not show significant effects on the elimination of the bacteria ($p > 0.05$). Furthermore, the total phenolic content significantly has increased ($p < 0.05$). Results also have revealed that LPCP darkens the color of dried peppermints. In summary, cold plasma can destroy microorganisms similar to that pasteurization can. Due to the absence of waste production, low-energy consumption and low-costs as well as nutrient preservation and improvement, cold plasma is an appropriate alternative for the product decontamination of spices, medicinal plants and other heat-sensitive foods. Hertwig et al. (2) have used two various atmospheric pressure cold plasma methods, including direct plasma jet and MW-driven remote plasma, for the destruction of B. subtilis, B. atrophaeus and Salmonella spp. in black peppers. The study also included effects of this method on food sensory characteristics, essential oils and levels of piperine. Direct plasma jet has inactivated 0.8, 1.2 and 2.5 log CFU of B. subtilis, B. atrophaeus and Salmonella spp., respectively, whereas MW-driven plasma has inactivated approximately 4.1, 2.4 and 2.8 log CFU of the highlighted bacteria, respectively. Results have shown higher effects of MW-driven remote plasma on inactivation of the bacteria, compared to those of direct plasma jet. Differences in inactivation levels are possible due to differences in inactivation mechanisms and complex surface structures of the peppercorns.

Effects of microwave-integrated cold plasma on inactivation of the spores from B. cereus and A. brasiliensis and E. coli O157:H7 in onion powders have been investigated by Kim et al (36). Onion powders have been treated using cold plasma in low microwave density at 170 $mW \cdot m^{-2}$ and high microwave density at 250 $mW \cdot m^{-2}$. Effects of high microwave density cold plasma treatment (HMCPT) on inactivation of the spores have been more than those of low microwave density cold plasma treatment (LMCPT). The microwave-integrated CPT includes potentials for non-thermal decontamination of onion powders with no alterations in their sensory properties and harmful effects on their physicochemical properties (36). Inactivation of microbial flora from paprika using remote plasma treatment have been investigated by Hertwig et al. (8). After 5-min use of remote cold plasma, native spores in paprika powders have been inactivated, which is linked to the low initial contamination with bacterial spores. The highest inactivation of total mesophilic aerobic (approximately 3 log CFU/g) has been achieved after 60 min of remote plasma treatment. When the treatment time exceeded 5 min, color of the red paprika decreased due to the destruction of carotenoids (8). This review purpose was to study the cold plasma effect on the spices microbial and quality properties. In general, results of this review have indicated that plasma can inactivate microorganisms. Therefore, cold plasma can be used as a promising method to improve safety and extend shelf-life of the spices and other food products.

Conclusions and future prospective of cold plasma

In future, cold plasma technology can play significant roles in food industries. Cold plasma is one of the newest technologies in food decontamination. This technology has emerged as a novel, non-thermal, contact-free, water-free antimicrobial intervention that can eliminate food pathogens. The feasibility of microbial decontamination in spices by cold plasma has been investigated in this review (Fig. 4).

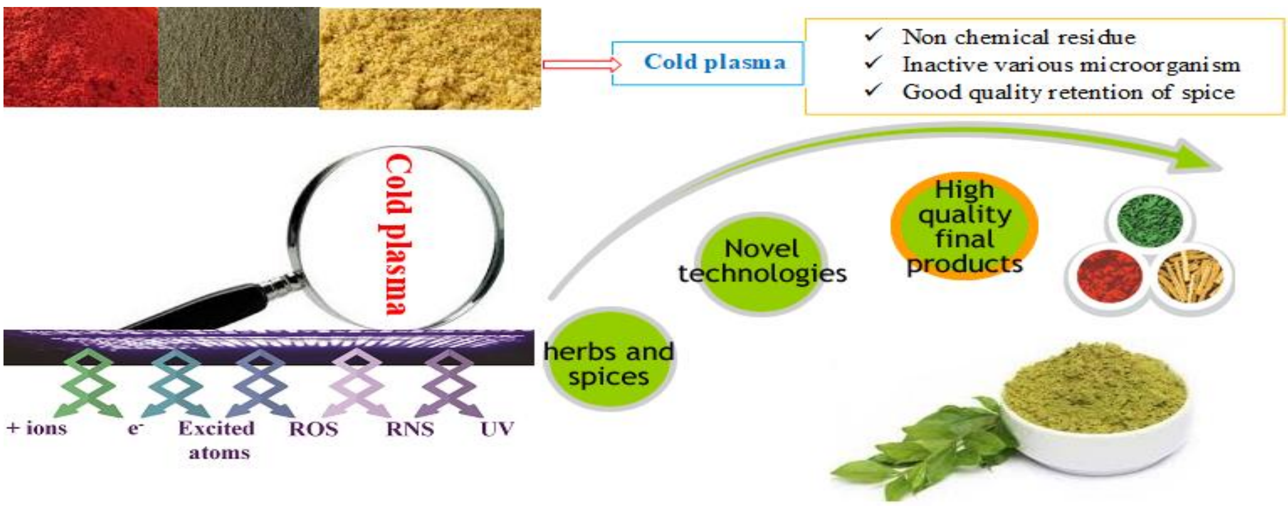

Figure 4. A graphical abstract, representing feasibility of microbial decontamination of spices using cold plasma

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This method can be used for a wide range of spices (e.g., red pepper, black pepper, peppermint, onion powder and paprika) and has been proven to be efficient in removing microbial contaminations from spices. Results of the cold plasma study showed that final quality of spices is better preserved, compared to other methods. In addition to sterilizing the product, decreases of nutrients in this method is less than that in other traditional methods. Findings of this review have shown that cold plasma can be used for the sterilization of inactivate microorganisms, including spores, bacteria, molds and yeasts. Furthermore, the current method is a good option for the disinfection of heat-sensitive products such as spices and medicinal plants due to lack of waste generation, lower energy consumption and low costs as well as nutrient preservation and improvement. However, further studies on cold plasma process optimization, safety of the process and economical assessment of large-scales are necessary.

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