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Non-thermal microbial decontamination of onion and dehydrated onion products

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Onions are a major component of recipes from all-over the world. Apart from fresh onions, dehydrated onions in forms of powder, flakes and shreds are available in the market. These are used as seasonings, flavors or as condiments. Dehydrated flakes and shreds are also reconstituted and used. Dehydration is mainly done for increasing the shelf-life of the product, as well as to reduce the water weight for ease of transportation. Reduction in water activity during dehydration helps inhibit microbial growth. Nevertheless, bacterial and fungal contamination has been reported. These contaminations cause various food-borne illnesses and health hazards. Targeting spoilage and pathogenic microorganisms would not only help prevent wastage and food-borne illnesses, respectively, but also improve the quality standards as per regulations. The use of non-thermal technologies has a great potential not only in decontamination but also in the preservation of phytochemicals and volatile compounds present in onions.

1. Introduction

Onion contribution in the export market is the highest in India amongst the fruits and vegetables. It is also of the utmost importance in the country's own consumption. The world-wide comparison of onion producers in 2011 shows that even though India is the second highest producer of onions, after China, its productivity has a good scope of improvement. This is established by the fact that China produced 20 million tons from 9,30,000 ha, while India produced 15 million tons from 10,64,000 ha, showing lower productivity. Amongst the Indian states, Maharashtra produced the highest amount (5,000 thousand tons), followed by Karnataka, Gujarat and, Bihar [1].

It is important to understand that during dehydration, microbial decontamination gets tougher due to low moisture and stress caused by drying. Water molecules in the food matrix are an important factor for decontamination. For instance, during irradiation, water molecules are the major dipole molecules that cause volumetric heating in the food matrix due to the molecular alignment in presence of the electromagnetic wave. Once this available moisture is removed or reduced, the effect of electromagnetic radiation for decontamination is compromised. On the other hand, removal of water molecules causes a stressful condition in the microbial environment of the food matrix. Ergo, the spore forming microorganisms form spores for survival. Once the spores are formed, it becomes challenging to eliminate the viable microorganisms.

Water removal through drying helps in easy bulk handling and cheaper transportation with a limited requirement of space. Another advantage of dehydrated products is their very low moisture content (from 84-90% in fresh onions to ~8-12% in dehydrated onions), which retards or inhibits microbial growth. Water activity is an intrinsic property of the food, and all sorts of biochemical changes, as well as microbial growth in food products, occur beyond a certain threshold value of water activity [2]. The water activity denotes the amount of free water available for microbial growth and other biochemical reactions. This is measured as the ratio of the partial pressure of water vapor present in the food to the vapor pressure of pure water at that condition [3]. The water in food material may be present in a physically bound form that is adsorbed into either proteins, carbohydrates, or hydrophilic colloids. In contrast, the chemically bound water is present typically as the water of hydration. Free water is also present in the food matrix. Generally, the free and physically bound forms of water are removed during dehydration, and sometimes chemically bound water can also be removed [4]. However, the microorganisms generally acquire the water available in the food, which is believed to be the free water inherent to the system [3]. Therefore, the water activity in the food plays a crucial role in controlling the microbial population.

The microbial growth is dependent on the pH of the food matrix. Microorganisms can survive in a specific pH range, and they have a tolerable pH range as well. Lower pH is responsible for microbial enzyme denaturation. On the other hand, H^+ ions permeate the microbial cell and hamper the pH balance of the cell cytoplasm. This change in pH affects the microbial cell's metabolism and the microorganism starts producing alkaline products in the food matrix. This is how the microorganisms acclimatize themselves to the changing pH surroundings [5]. The optimum pH range for microbial growth (mainly bacteria) is generally pH 6-7, and minimum tolerable pH is 4 while the maximum tolerable pH is around 11. The pH range of fruits and vegetables is 3.3-7.1 and 4.0-7.0, respectively [6]. Amongst bacteria, neutrophiles (such as *E. coli, Staphylococci, and Salmonella*)

show an optimal growth at pH 7.0. Pathogenic species such as *E. coli* and *S. typhi* can survive at acidic pH values. Acidophiles can grow at pH < 5.55, and *Lactobacillus* can grow at pH values of 3.5-6.8. Alkaliphiles (*Vibrio cholera, Bacillus firmus*) can grow between pH 8 and 10.5. Fungi can tolerate a pH of 5.0-6.0 and can grow at lower pH values as well. There are also chances of occurrence of sporing anaerobes like *Clostridium botulinum*, *Salmonella*, *Staphylococci* and *Streptococci*; and sporing aerobes like flat sour organisms and thermophiles. This is due to the stress-induced by the modulated water activity and pH of the food matrix during dehydration [7]. Onions contain a bioflavonoid, quercetic, which has antibacterial and anti-fungal properties. Additionally, a thiosulfinate, allicin, is antibacterial in nature. On comparing the shelf-life of onions with other fruits and vegetables, their shelf-life is longer. This is due to the presence of the flavonoids in onions [8]. Despite of having such compounds, reports of bacterial and fungal contamination can be found in the state of art.

It is well known that water removal or dehydration serves as a long-term preservative step for dehydrated onions. However, there is enough literature showing concern about the microbial contamination in the fresh and dehydrated onions products. This review covers the non-thermal technologies that have been explored for the decontamination of the same. Non-thermal technology is focused here as the bio actives of onions would be potentially preserved. This article discusses the scope of using dehydrated onions with extended shelf-life for local as well as export market.

2. Spoilage and pathogenic micro-organisms in onion bulb.

The survival of microorganisms in dehydrated F&V is influenced by the presence of biomolecules such as sugars and polypeptides. Some acidic fruits, such as berries, show lower microbial growth. According to the nutrient availability the type of species of microorganisms that are able to thrive can be found. As discussed above, dehydration stimulates the bacteria and fungi to form spores for survival purposes. These remain dormant until favorable conditions, water and nutrients, are present again. Such favorable conditions include water and nutrients, in the presence of which these survive and continue with the log phase of their growth. This is also a major cause of spoilage as the spoilage organisms start proliferating once the dehydrated food gains some moisture through the jacketing packaging material. Pathogens such as *Bacillus cereus*, *Salmonella*, *Clostridium perfringens*, and Hepatitis A virus have been reported to survive in dehydrated food products [9]. Aerobic spores can cause souring when reconstituted in a product [7].

The contamination by bacteria and fungi is introduced from soil, air and during handling and processing. On isolating microbial species from onions harvested in metropolis, Nigeria, the spoilage was found to be caused by *Bacillus*, *Staphylococcus*, *Erwinia*, *Pseudomonas sp.* and *Xanthomonas compestris*. The most common microorganism is *E. coli* that enters the system through water during harvesting. Spores of *Staphylococcus* and *Aspergillus* sp. contaminate the onions when carried through air. Presence of *Pseudomonas* sp., yeast and mold occur during handling, processing and storage. Fungi and *Aspergillus* sp. cause ring worm and aspergillosis. Blue mold rot is caused by *Penicillium allii* post-harvest [10]. Bacteria such as *Staphylococcus*, *Bacillus*, *Pseudomonas* and *E. coli* also cause food poisoning in humans [11]. During storage *Fusarium oxysporum*, *Penicillium sp.*, *Botrytis sp.*, *Aspergillus awamori*, *Alternaria sp. and Rhizopus oryzae* have been found to cause spoilage of fresh onions [12]. Co-existence and co-occurrence of pathogenic species increases the severity of plant diseases. *Pantoea* sp. have been found to co-exist in onion bulbs and co-express virulent genes in onion tissues [13].

3. Non-thermal treatment

Microbial inactivation by nonthermal treatment basically refers to replacement of thermal energy by any other form of energy, which can cause similar stress to microorganisms as in thermal techniques. For instance, in the case of UV irradiation, the photo-energy creates stress on microorganisms without increasing the temperature in the sample. For the pulsed electric field, the electrical energy is serving the same. The different non-thermal technologies applied for microbial decontamination in onions and dehydrated onion products have been discussed briefly in the following section.

3.1. Gamma Irradiation

Electromagnetic waves penetrate the food product where they are converted into heat energy depending on the dielectric property of the sample. The polar molecules align according to the electric field and ionic conductivity in presence of electromagnetic waves, hence causing movement of molecules. This molecular movement causes volumetric heating in the food matrix and denatures the proteins, enzymes and nucleic acids in the microorganisms, leading to the microbial killing [14]. Gamma, an electromagnetic wave, is an ionizing radiation. The maximum permissible dose for food products is 10 kGy as set by the Codex Alimentarius General Standard for Irradiated Foods. For decontamination of dehydrated food products, dosage nearing to 10 kGy is required, which is lower for fresh fruits or vegetables (1-3 kGy) [15].

Pezzutti et al., (2005) treated onion flakes at dosages of 7, 9, 11, 15, 18 and 23 kGy. Dose of 7 kGy diminished the aerobic plate count by 3 log cycles and for yeast and mold by 2 log cycles. It was also concluded that flavors were not affected, based on the enzymatic production of pyruvic acid. On comparing the aerobic plate count (APC) and yeast mold count (YMC) in the imported and locally available onion flakes in Argentina, local products contained 4.6 log CFU/g of APC and 4.4 log CFU/g of YMC. On the other hand, the 5.3 log CFU/g of APC and 3.3 log CFU/g of YMC were reported in the imported onion flakes were found. Contamination by *Clostridium* within a range of 1-3.6 log CFU/g was also reported. The researchers found the presence of mesophilic bacterial spores in onion powders, flakes and shreds, (4.5 – 5.6 log CFU/g) in both packed and unpacked samples procured from the market. It was concluded that 10 kGy of radiation was required for to reduce the bacterial spore count to an undetectable level. The dehydrated onion samples were found to have a significant number of coliforms (3.04 log CFU/g) [16].

3.2. Cold plasma

Atmospheric air or gas is brought in contact with electrical, radioactive or thermal energy to generate ions, atoms and free electrons. Cold plasma is a non-thermal technique, in which these reactive species attack the biomolecules of the microbial cells and disintegrate the integrity of the DNA, RNA, proteins and the cell membrane [17]. Corona discharge air plasma (CDAP) is a non-thermal treatment under atmospheric pressure. Chemically active species such as oxygen ions, NO^+, NO^-, $OH^{\cdot}$, HO_2, H_2O_2 are generated using air, Ar, Ar/O2, He/O_2 or N_2 during this treatment. These species act as surface oxidizers on the cell membrane of the microorganisms and cause microbial killing [18].

CDAP was conducted by Chang and coworkers to assess effect of reactive species on spoilage causing fungi including *Fusarium sp. Alternaria sp. and Botrytis sp*. found in fresh onions stored for 10 months. The major antimicrobial reactive species found were O_3 and NO_2, whereas NO was poorly generated and undetected. It was observed that treatment at 2-2.6 ppm concentration of O_3 triggered the mycelial growth of *Alternaria* sp. Higher concentrations of O_3 (20-24 ppm) results in impeding the growth of the same. Alternatively, longer treatment time (16 hours) showed inhibitory effects as compared to shorter treatment time (4 hours). However, the mycelial growth pattern of *Botrytis* sp. did depend on the O_3 concentration. Rather, it depended on the treatment duration. Treatment time of 4 hours inhibited the mycelial growth, while longer treatment duration supported the mycelial growth. On studying the conidia gemination pattern due to the treatment, 2 hours of treatment showed inhibition in germination with increasing concentration of O_3 in case of both *Alternaria* sp. and *Botrytis* sp. [12].

Kim et al., (2017) experimented with microwave-powered cold plasma for decontamination of onion powder. High microwave density cold plasma (HMCPT) is generated using electromagnetic waves having 100 MHz frequency. Ramifications of this treatment on the spores of *Bacillus cereus* and *Aspergillus brasiliensis* were studied. Around 0.5 log reduction in *B. cereus* after 40 min of treatment at 900 W was achieved. During a storage period of 60 days, the growth of B. cereus, *A. brasiliensis* and *E. coli* were seen to be retarded. They also concluded that minimal loss in volatiles were observed in terms of quercetin [19].

3.3. Low Energy Electron Beam

Electron beam consists of a group of ionizing radiations. It uses high energy electrons through electricity for microbial inactivation. Low energy electron beam (LEEB) has energies below 300 keV, while high energy electron beam (HEEB) has energies above 300 keV [18]. The penetration depth of these electrons depends on the energy and the density of the food sample. On penetration into the food matrix the kinetic energy is lost with the particle collisions. High energy electrons have shown to extend the shelf life of fresh melons and blueberries [18, 20]. Penetration of LEEB is in the micrometer scale while that of HEEB is in the centimeter scale. Hence, we can say that LEEB is a surface treatment as it targets the microorganism on the surface of the food samples [18].

LEEB is generated by using tungsten filament inside an ultrahigh vacuum. Due to low energy, minimal heat is generated and the product quality is expected to be preserved. LEEB causes DNA and RNA strand breaks and base modifications, and also proteins and enzymes degradation. Indirectly, LEEB ionizes water and O_2 molecules resulting in generation of reactive species such as OH^- and O^{2-}. These in turn affect the DNA and cell membrane. Electron beam of energy 200 keV and 300 keV were used to treat onion flakes to eliminate total aerobic bacteria. Around 1 log reduction was achieved at both energies. The penetration ability of electron beam at 200 and 300 keV was 8 mg cm^{-2} and 32 mg cm^{-2}, respectively [21]. There is scope to increase the efficiency of LEEB for decontamination since it has good penetration.

3.4. Ultraviolet Light

Another electromagnetic radiation is ultraviolet light having wavelength between 100-400 nm. The mechanism of ultraviolet light in microbial inactivation is well-known. These radiations cause pyrimidine base dimerization in the DNA of the microorganisms. This dimerization causes kinks in the single stranded DNA which hinder DNA replication [22]. UV radiation is a surface treatment and it was used to reduce the *E. coli* load on fresh onions by Rodov et al., (2010). About 3 log CFU cm^{-2} of reduction in *E. coli* was observed after treatment of 1.2 kJm^{-2} [10]. To achieve an effective decontamination, UV can be hurdled with heat or could be given in pulses. These would provide an added advantage of a deeper penetration than just the surface treatment provided by UV.

4. Future scope

Thermal technologies have been explored enough for decontamination of products in general. Non-thermal techniques are being explored for their quality preservation properties. UV treatment is a surface treatment which could be further exploited by addition of electron sources and a wider range of wavelengths for deep penetration. It is also the need of the hour to produce shelf-stable as well as sensorially acceptable onion and dehydrated onion products. Commonly, the onions that are available in the market are often found to have mold growth. Therefore, a post-harvest treatment could help reduce the potential mold growth. Increasing the shelf-life and reducing the spoilage and pathogenic microorganisms would help meet the export standards.

5. Conclusion

Onions are the highest exported agri-products and hence it becomes critical to match the international quality standards. Dehydration of onions expands the scope of onion usage to a higher extent, which include onion powder, flakes and shreds. Reduction of microbial contamination would improve the safety standards. Non-thermal technology would help maintain the sensorial properties of onions such as volatiles and flavonoids.

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