Cold Plasma Treatment on Biopolymer-Based Films

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Biopolymers, like polysaccharides and proteins, are sustainable and green materials with excellent film-forming potential. Bio-based films have gained a lot of attention and are believed to be an alternative to plastics in next-generation food packaging. Compared to conventional plastics, biopolymers inherently have certain limitations like hydrophilicity, poor thermo-mechanical, and barrier properties. Therefore, the modification of biopolymers or their films provide an opportunity to develop packaging materials with desired characteristics. Among different modification approaches, the application of cold plasma has been a very efficient technology to enhance the functionality and interfacial characteristics of biopolymers. Cold plasma is biocompatible, shows uniformity in treatment, and is suitable for heat-sensitive components.

cold plasma biopolymers packaging antimicrobial biodegradable

1. Impact of Cold Plasma on Packaging Properties

1.1. Surface Roughness

The application of cold plasma increases the surface roughness of biopolymer films and can be attributed to the etching effect phenomena. This is caused by the bombardment of high-energy plasma species like electrons, photons, positively or negatively charged ions, free radicals, and gas molecules or atoms in their excited or fundamental states [1][2]. The etching effect in a film's surface may arise due to chemical processes such as cleavage of chemical bonds, scission of polymer chains, or chemical degradation of film components by the influence of the free radicals. It can also be caused by a physical process like the removal or re-aggregation of low molecular weight components/fragments on the surface of the film. The other possible reason for the change in the surface roughness by the application of cold plasma may be due to its micro-discharge filaments [3][4][2].

The extent of roughness may vary depending on the power supplied (voltage), exposure time, and uniformity of the exposed energy of the plasma species onto the film surface [4][5]. The etching effect phenomenon is noticed commonly with the application of DBD plasma, radiofrequency (RF) generated plasma, microwave (MW)-driven plasma with different gases like air, argon, O_2, N_2, and CO_2.

1.2. Contact Angle

Contact angle indicates the tendency of a liquid drop to spread out and adhere to the surface of materials, reflecting its wettability and hydrophilicity [5][6]. The higher the contact angle values, the higher the tendency of the surface to repel water. In general, the application of cold plasma onto film surfaces decreases the contact angle values indicating an increase in the hydrophilicity and the wettability of the films. Application of cold plasma onto the film surface generates reactive species and free radicals resulting in the amplification of several polar groups like -COOH, -OH, and CO leading to increased polarity, surface tension, and surface free energy, eventually increasing the hydrophilicity and wettability of the film surface [4][7][8]. Another probable reason is the surface roughness caused by the etching effect that aids in the spreading of liquid onto the surface [9][7][10]. As reported in the published literature, the water contact angle is the function of plasma treatment time and voltage. Plasma treatment at higher voltages and with longer exposure times increases the surface roughness and polar groups, resulting in hydrophobicity and wettability of the film surface. In contrast to the general trend where the application of cold plasma decreases contact angle, DBD plasma-treated starch film showed an increase in the contact angle values. This could be due to the oxidation of the hydroxyl group into carbonyl groups, resulting in the formation of the new hydrogen bonds. These hydrogen bonds may have decreased the availability of polar groups on the surface, leading to the enhancement in hydrophobicity of the film surface [11]. Similarly, contact angle values increased for corn starch thermoplastic films treated with SF_6 plasma [12]. Overall, it can be concluded that the contact angle is a factor of plasma gas composition, as well as voltage and exposure time. The contact angle of a film is an important criterion to decide its suitability in packaging applications [13][14]. The wettability of a film affects the coating, printing, absorbance, adhesion, and frictional properties of the film surface [14][15].

1.3. Molecular Properties of the Film

Fourier Transform Infrared (FTIR) Study

A microstructural study of the biopolymer-based film provides insight into the molecular interactions of the different components present in the film network. The understanding of molecular interactions is important as it influences the physical, thermal, and mechanical properties of the film. Cold plasma treatment is a novel technique used to enhance the structural features of the biopolymer-based film. One of the most prominent methods to investigate the structural changes in the biopolymer film is Fourier transform infrared (FTIR). It has been reported that plasma treatment induces a number of chemical reactions like molecular rearrangements, dehydration, and the hydrogenation of molecules [5].

Overall, it was observed that the application of plasma influences the structure of the biopolymer-based film by various mechanisms like creating a reactive site, crosslinking, reordering, and enhancing the structural arrangement of the polymeric network. These structural modifications affect the various properties like mechanical, thermal, and barrier properties of the biopolymer films.

1.4. Mechanical Properties

Mechanical properties such as tensile strength (TS) and elongation at break (EAB) of the films are key parameters in packaging applications to preserve food quality and integrity during handling, storage, and transportation [3][4][10].

The application of plasma to film induces physicochemical phenomena like etching, degradation, and cross-linking altering the mechanical properties of the films [4][12]. The bombardment of plasma (ionic species) on the film surface cleaves C-C and C-H bonds and generates free radicals that create linkage with the surface radicals or participate in chain reactions resulting in the significant improvement in mechanical properties of the films [16][17]. Dong, Guo [17] reported that atmospheric cold plasma treatment for 45 s significantly increased the TS and EAB of a zein film. However, increasing the treatment time to 60 s was ineffective in improving the mechanical properties. The increase in TS and EAB of the zein film could be due to the cross-linking that occurred during plasma treatment. The plasma discharge could have oxidized oxygen into an ozone that transformed the free S-H group to S-S. Cross-linking by strong intermolecular and intramolecular S-S in the zein matrix may have resulted in the higher TS and EAB. Such findings for zein/chitosan film, zein, and zein/polylactic acid films were reported in the literature [18][7].

Overall, it was observed that increases or decreases in the mechanical properties of biopolymer-based films treated with cold plasma are a function of various factors including the type of polymer (protein or polysaccharide), types of gases used to generate plasma reactive species, processing time, and voltage.

1.5. Thermal Properties

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) are the two most important approaches in understanding the thermal properties of biopolymers and their films. Thermal studies not only provide information on the changes in heating/cooling behaviors but also scrutinize the thermodynamic and thermophysical properties [4]. TGA shows the thermal stability of the samples and the DSC techniques determine the melting temperature (T_m), glass transition temperature (T_g), denaturation temperature (T_d), and enthalpy (ΔH). The T_g is the temperature at which the polymer chains become more mobile, meaning that they have more freedom of movement, whereas the melting temperature is the temperature at which crystalline structures are lost and the polymer chains become a disordered liquid [19]. Thermal parameters are important in determining the processing conditions of the biopolymers and their applications in packaging, as heat sealing is a critical point in the packaging [5][20].

1.6. Water Barrier Properties

Water vapour permeability (WVP) of packaging material is an important parameter in determining the moisture barrier property of the packaging materials that affect the resulting shelf life and quality of the packaged food. The poor moisture barrier property of biopolymer-based materials is one of the major shortcomings in packaging applications [21][22]. Different modification techniques are adopted to enhance the barrier property or at least avoid any drastic change in the permeability in order to augment the usage of biopolymers in food packaging applications.

1.7. Oxygen Permeability (OP)

OP is another key parameter of packaging materials that determines the oxygen transport across the film to enhance shelf life and quality of the packaged food. The presence of oxygen modulates the development of the different reactions like oxidation involving components responsible for the color and aroma of the food product [21][16]. When packaging oxygen-sensitive products, the materials with the least amount of oxygen permeability are preferred. Biopolymer-based packaging materials have lower oxygen barrier properties when compared to conventional plastic packaging. Therefore, some modifications of biopolymers are necessary to restrict oxygen permeability to enhance its application in the food packaging industry.

1.8. Antimicrobial Properties

A novel cold plasma technique is applied to modify polymer surface attributes, the decontamination of packaging surfaces and food processing instruments, and to enhance the safety level of several food products [4][23]. The active packaging films loaded with bioactive compounds like essential oils, peptides, or functional components when exposed to cold plasma show higher antimicrobial activity.

It is observed that plasma treatment facilitates the coating of functional components on the polymer layer and thereby increases antimicrobial efficacy. For instance, Wong, Hou [24] prepared gallic acid (GA) coated polyethylene (PE) film applying plasma treatment (30 W for 60 s). The PE/GA active film reduced the growth of *E.coli* and *S. aureus* by 0.5–1.1 log reduction at a concentration above 1.0%. Such antimicrobial activity was also reported for plasma-treated polylactic acid (PLA) film coated with nisin where the active film showed a log reduction of 3.23 against *Listeria monocytogene*, whereas pristine PLA film could not inhibit its growth. It was observed that with the increase in plasma treatment time (0–60 s) the microbial reduction increased and this could be ascribed to the content of nisin absorbed on the surface of the PLA. This indicates that cold plasma treatment influences the absorption capacity of PLA film and thus the content of nisin thereby affecting the antimicrobial activity of the film [25].

Some studies demonstrated that plasma treatment had no significant effect on the inhibition of microbial growth. Chen, Ali [26] showed that cold plasma had no significant effect on chitosan/ciprofloxacin hydrochloride antimicrobial film and further coating with zein had drastically reduced the release of ciprofloxacin hydrochloride resulting in reducing the antimicrobial effect. This could be attributed to the fact that plasma treatment may have enhanced intermolecular interaction (crosslinking) leading to firm adhesion between chitosan and zein, resulting in the increase in a barrier for the diffusion of ciprofloxacin hydrochloride [26]. Such reduction in the release of the antimicrobial compound due to cross-linking was also reported for sodium tripolyphosphate crossed linked chitosan film loaded with ciprofloxacin [27].

Overall, the impact of plasma treatment on the antimicrobial activity of the film containing functional compounds is critical. The strategic combination of polymers with antimicrobial substances treated with cold plasma can be a potential approach when preparing antimicrobial packaging materials suitable for food packaging applications.

1.9. Biodegradability

Biodegradable materials are degraded by the enzymatic action of living microorganisms such as bacteria, yeast, and fungi. Biodegradation is investigated in various environments such as soil burial, landfill and compost simulations, and microorganisms [6][28][29]. Arolkar, Salgo [6] investigated the degradation of corn starch/poly(ε-caprolactone) films following soil burial method and the extent of degradation was reported in terms of an alteration of TS and EAB. It was observed that the rate of degradation was higher for air plasma treated films as compared to untreated film and it increased with increase plasma treatment time. In another study, Chen, Chen [18] reported that the biodegradability of the plasma-treated zein-PLA composite film was higher than that of untreated films. The higher degradation of the plasma-treated film was attributed to the increase in the surface area of the porous structure that enhanced the accessibility to the microbes present in the compost into the film resulting in the higher degradation. A similar result was reported for plasma-treated defatted soybean meal film, where the plasma treatment increased the surface area of the film by increasing the roughness of the film, facilitating microorganisms in the compost in faster degradation [28]. In another study, Song, Oh [8] investigated the biodegradation of PLA sachets for a period of 0 to 35 days and reported that at the end of 28 days, the extent of degradation of plasma-treated PLA sachets was higher than in untreated sachets.

2. Safety Concerns Relating to the Application of Cold Plasma for the Modification of Food Packaging Films

The application of plasma in food processing/packaging generates reactive species that come in direct or indirect contact with food surfaces. The interaction between plasma reactive species and the matrices of food or biopolymers is complicated and limited studies have been carried out investigating the potentially detrimental effects to human or animal health [4][30]. Chen, Lin [31] reported that He gas-plasma treatment of di-ionized water for 30 min generated ROS (reactive oxygen species) and RNS (reactive nitrogen species) caused significant apoptosis [31]. In another study, Heslin, Boehm [32] assessed the cytotoxic and mutagenic potential of DBD plasma-treated iceberg lettuce in an in vitro CHO-K1 (mammalian cell) model and short-term toxic effects in an in vivo *Galleria mellonella* larva model. The results showed a low in vitro cytotoxic effect and spontaneous mutations, however reported a strong in vivo toxicity with less than a 10% larva survival when injected with lettuce broth treated for 5 min [32]. Few studies have been found to evaluate the toxicology of plasm-treated food constituents. Some studies have reported that the plasma reactive species induces alterations in food constituents, like modification of amino acids in proteins, oxidation of high molecular weight compounds to organic acids, and peroxidation of lipids resulting in undesirable metabolites like short-chain aldehydes, keto-acids, hydroxyl acids, and short-chain fatty acids [33][30][34]. Other effects of the application of cold plasma on food commodities include a reduction in firmness of fruits and vegetables, enhanced discolouration, and surges in acidity content [4].

Some studies showed that the application of cold plasma in food products caused no acute toxicity, although the work conducted on this has been limited. For instance, Kim, Sung [35] evaluated the mutagenicity and immune toxicity of sausage prepared with plasma-treated water as a nitrite source and reported that mutagenicity and inflammatory response was negative in mice fed with the plasma-treated sausage [35]. Similarly, Jo, Lee [36] reported that atmospheric plasma-treated winter mushroom powder caused no mutagenicity or acute toxicity in rats fed with 5000 mg/kg body weight [36]. A few studies have been found to investigate the toxicity of biopolymer-based films treated with plasma. In one of these studies, Han, Suh [37] investigated the safety of plasma-treated soya film by determining the acute and subacute oral toxicity in a rat model. It was reported that the rat fed with 5000 mg/kg body weight (Single-dose acute) or subacute 1000 mg/kg body weight/day for 14 days showed no acute toxicity resulting in the death of the rats. However, a change in blood components (like hematocrit, hemoglobin, bilirubin, creatinine, and aspartate aminotransferase) was observed. These changes were irrelevant to toxicity as their level were within acceptable physiological ranges [37].

Overall, it can be seen that the application of plasma in food processing or in the modification of biopolymers is a novel technique that still requires approval. The Food and Drug Administration (FDA) has not approved any guidelines on the application of plasma in food or on food contact surfaces [30][38]. Research on cold plasma optimization has to be performed specifically for each product. A safety and risk assessment should be comprehensively carried out in both an in vitro and in vivo environment to address the potential toxicity of food or food contact surfaces such as biopolymer films treated with cold plasma.

3. Conclusions

The commercial application of biopolymer-based films is limited due to their poor physical, structural, mechanical, thermal, and barrier properties, as well as poor ink printability and adhesion features. Herein was to investigate the impact of cold plasma used for the modification of the critical properties of packaging films prepared from protein, polysaccharides, or their combinations. Different factors that influence the effect of plasma treatment include the internal structure of biopolymers, types of plasma gas generating reactive species, and processing conditions (voltage, and treatment time). The application of cold plasma efficiently improved the physical, structural, and thermomechanical properties of the packaging films in most cases. The application of cold plasma modified surface properties enhancing the diffusion rate of functional components absorbed on the surface of the biopolymer. It also enabled adhesion between polymers facilitating the development of multilayer films and increased ink printability. The cold plasma treatment also enhanced the antimicrobial efficacy by increasing the diffusion rate, and retention of the volatile functional components. In addition, the plasma treatment augmented the biodegradability of the biopolymer-based films. Overall, the application of cold plasma treatment is a cost-effective approach to modifying the packaging properties of biopolymer-based films, as it is a simple inline process with an easy instrumental setup and no waste generation. However, comprehensive research is needed to understand the complex interaction between the plasma reactive species and components of the polymer, as well their migration into food concerning the safety of human and animal health, before cold plasma can be applied commercially.

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