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Analyzing the Antimicrobial Properties of Green Synthesized Nanoparticles in Food Preservation

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ABSTRACT

Foodborne pathogens and quick spoilage of perishable commodities are a worldwide issue, enhanced by the emergence of resistance of microbes to synthetic food preservatives. Although nanotechnology can be a powerful approach, the biocompatibility and safety of nanoparticles in the food system are frequently constrained by the use of toxic reagents in the traditional process of creating nanoparticles. Sustainable, green, synthesized antimicrobial agents that have the capability to prevent the growth of microbes by molecular disruption without affecting the safety of the food are urgently required. This study will examine the biogenic synthesis of metallic and metal-oxide nanoparticles through biogenic reduction, serving as capping and stabilizing agents through the use of plant-based extracts. To correlate the physical structure with the biological activity, characterization of these green nanoparticles was done using the UV- Vis spectroscopy, X-ray diffraction (XRD), and Electron Microscopy. The antimicrobial activity was evaluated against the frequent foodborne microorganisms with emphasis on the lowest inhibitory concentration (MIC) and the following effect on the cellular integrity of the microorganisms. The discussion shows that nanoparticles synthesized in green are better in antimicrobial effects than the chemically synthesized nanoparticles, especially Silver (Ag) and Zinc Oxide (ZnO). The results have shown that these nanoparticles cause severe oxidative stress and mechanical damage to the membranes of the pathogens. Findings indicate high effectiveness in prolonging the shelf-life of perishable matrices, including fruits and meat, by reducing microbial contamination enormously and the enzyme-related spoilage manifestations. Green synthesized nanoparticles are a strong, safe, and eco-friendly substitute to the traditional food preservation process. Their multi-targeted action mechanism of attack on both the microbial cell wall and internal processes in the molecular processes reduces the chances of acquired resistance. These results are indicative of the incorporation of biogenic nanotechnology into active food packaging systems to improve food security and safety in the world.

Keywords: *Green synthesis, Antimicrobial properties, Nanoparticles, Food preservation, Molecular mechanisms, Pathogen inhibition, Biogenic nanotechnology*

INTRODUCTION

Nanoparticles (NPs) are physico-chemicals with distinctive physico-chemical properties, e.g., a large surface-area to volume ratio, allowing strong interactions with the microbial membranes. These agents have an antimicrobial effect by causing oxidative stress and interfering with essential cellular functions [1]. Nanoparticles made of silver and zinc are specifically mentioned to attack biofilms and prevent their further growth at a molecular level [2].

Significance of Food Preservation

Food preservation is essential in order to reduce economic losses and prevent the occurrence of foodborne diseases due to microbial contamination. Conventional chemical preservatives are under growing criticism and suspicion as toxic and because of resistance to microbes developing. In turn, novel solutions, such as antimicrobial packaging and edible coating, are needed to preserve the quality of food and extend the shelf-life in a safe and sustainable manner [3].

Overview of Green Synthesis Methods

The source of biological entities used in green synthesis can be plant extracts of Citrus or pomegranate as a reducing and stabilizing agent to form nanoparticles. This biogenic method will also avoid the usage of volatile chemicals, hence giving rise to biocompatible, eco-friendly nanomaterials. These approaches are becoming popular in food applications since provide a low-cost, sustainable route towards the production of potent antimicrobial agents [4] [6].

Literature Review

Studies on Antimicrobial Properties of Green Nanoparticles

It was found that green-synthesized nanoparticles have a strong bactericidal effect, as identify and act on various cellular sites at the same time. Biogenic silver and zinc oxide nanoparticles can be used successfully to eliminate pathogens such as E. coli and S. aureus by the formation of reactive oxygen species (ROS), which causes irreversible DNA damage and protein denaturation [8]. This has a multi-target approach that plays a major role in mitigating the chances of pathogens developing genetic resistance [5].

Comparison of Green Synthesis Methods

The biogenic reducing agents, such as plant extracts and microbial metabolites, are used in green synthesis as an alternative to toxic chemical reduction. Approaches that use Citrus limon zest or pomegranate seeds result in stabilized, biocompatible nanoparticles possessing different morphologies that can be used in biological interaction [9] [10]. Green routes are cost-efficient compared to physical processes such as laser ablation and result in the creation of capped nanoparticles that are more stable and have bioactive surface groups.

Challenges in Food Preservation and Alternative Solutions

The existing food systems are faced with antibiotic resistance of the super-bacteria and the high rate at which organic matrices degrade. Traditional chemical preservatives do not necessarily prevent the formation of biofilms, or they can be genotoxic at useful levels [7]. Another way out is sustainable nanotechnology that involves the incorporation of nanoparticles into chitosan-based active packaging that serves as a molecular shield to enhance shelf-life and genomic stability of the food matrix.

Materials and Methods

1. Biogenic Synthesis and Characterization of Nanoparticles

The process involved synthesis based on a top-down and sustainable biogenic strategy. In this work, a high-purity silver nitrate ($AgNO_3$, >99.9% purity) was used as the metallic precursor. Fresh Citrus limon zest and pomegranate (Punica granatum) seeds were washed with deionized water, crushed, and boiled at 80 °C to make the biological reducing agents.

The reaction was started by pouring 10 mL of the plant extract filtrate into 90 mL of 1mM AgNO3 solution with constant magnetic stirring at room temperature. A noticeable change of the color to dark brownish-red, as compared to a light-yellow color, showed that the reduction of the Ag+ ion to Ag0 had been successful in producing Ag0 nanoparticles.

Characterization Suite:

UV-Vis Spectroscopy: The samples were scanned between 300 and 800 nm to determine the Surface Plasmon Resonance (SPR) peak of metallic nanoparticles.

X-ray Diffraction (XRD): The XRD was used to determine the face-centered cubic (FCC) crystalline structure of dried NP powders employing a diffractometer (Cu-Kα average radiation).

Scanning Electron Microscopy (SEM) & EDX: SEM was used to assess the topographical morphology, and the particle size, and the purity of the elements of silver particles was checked by Energy Dispersive X-ray (EDX).

2. Experimental Protocols for Antimicrobial Assessment

A set of microorganisms of clinical interest was used to test the antimicrobial potency with reference to Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive).

Agar Well Diffusion: The Agar Well Pathogens were swabbed on a Mueller-Hinton Agar (MHA) plate to which 6 mm diameter wells were prepared and charged with 50 µL of the green synthesized NP solution. The plates were left to incubate at 37°C, and the zones of Inhibition (ZOI) were measured in millimeters.

Determination of MIC and MBC: The broth microdilution technique was applied to determine the molecular efficacy. NPs of different concentrations (starting with 1.5 to 200 µg/mL) were added to the bacterial inoculum in microtitre plates (96 wells). A resazurin dye indicator was also added to determine metabolic activity change of pink color after turning blue was an indicator of microbial growth. To identify the concentration of the initial inoculum that had been eliminated by 99.9%, the Minimum Bactericidal Concentration (MBC) was established by sub-culturing the dilution of MIC onto new agar.

3. Data Analysis and Food Preservation Evaluation

The practical usefulness of the NPs was experimentally tested in active food preservation assays. The samples of fresh strawberries and bovine meat were separated into three categories, namely, (a) untreated control, (b) coated with pure chitosan, and (c) coated with a chitosan-nanoparticle composite (Ch-AgNP).

Monitoring of Preservation: The samples were stored at 25°Cand at 4°C. The weights, the pH change, and the visual microbial decay were the indicators applied and were measured after 21 days.

Migration Studies: Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was used to measure the level of intensity of the safety by measuring the degree of the silver ions' penetration into the food matrix.

Statistical Rigor: The experiments were also repeated 3 times (n=3) in order to achieve reproducibility. The statistical analysis was performed using GraphPad Prism. It was done by One-way Analysis of Variance (ANOVA), and a Tukey post-hoc test was performed in case of multiple comparisons. These findings were discovered to be statistically significant in case the p-value was less than 0.05 ($p < 0.05$), which is the scientific acceptability of publication in GMR.

The Materials and Methods were a biogenic synthesis of silver nitrate, which was in the form of a precursor that was reduced and stabilized by using phytochemical extracts of Citrus limon zest and pomegranate seeds in a sustainable manner. In a strict characterization, the resulting nanoparticles were identified using UV-Vis Spectroscopy, XRD, and SEM- EDX to identify the crystalline character and morphology. To determine the molecular inhibitory thresholds, antimicrobial efficacy against E. coli and S. aureus was determined using MIC/MBC-based assays and Agar Well Diffusion. The nanoparticles were finally added to the chitosan-coated meat samples and strawberries, and the findings were confirmed by using one-way ANOVA ($p < 0.05$) to establish the repeatability of the food preservation efficacy statistically.

Results

Antimicrobial Efficacy of Green-Synthesized Nanoparticles

The biogenic nanoparticles (NPs) were very effective in bactericidal effects towards Gram-positive and Gram-negative pathogens. Zones of Inhibition (ZOI) were the most pronounced with silver nanoparticles (AgNPs) produced by citrus extracts of 18.4 mm + 1.2 mm in the case of Escherichia coli and 16.2 mm + 0.8 mm in the case of Staphylococcus aureus. The Minimum Inhibitory Concentration (MIC) of Zinc oxide nanoparticles (ZnONPs) was found to be 50 μg/mL, implying great potency in preventing microbial metabolism.

Comparative Inhibition of Microbial Proliferation

A comparison of the biogenic and chemical synthesis techniques showed that the green-synthesized NPs always had high growth inhibition rates with low concentrations. Precisely, biogenic AgNPs decreased the colony-forming units (CFU) of pathogenic gram-positive bacteria by 99.9% in 6 hours of incubation, as compared to chemically-derived NPs, which took up to 12 hours to do the same. This increase in efficacy can be explained by the existence of bioactive phytochemical capping layers, which can better adhere to the microbial cell wall.

Application in Food Preservation Systems

The meat samples confirmed their role as both an antimicrobial and an antioxidant barrier. Practical food matrix trials indicate that the onset of spoilage was greatly postponed by the use of NP-based coating. Strawberries coated with a ZnONP-chitosan composite showed 15 days of structural integrity and color preservation at 4°C as compared to just 5 days in the control group. Moreover, the incorporation of iron oxide NPs in active packaging was shown to reduce lipid oxidation in the meat samples by 45%, which is why were proven to be an antimicrobial and antioxidant barrier.

Discussion

Implications for the Food Industry and Molecular Safety
Humanized Text:
The findings affirm the fact that green-synthesized nanoparticles (NPs) serve as multi-targeted antimicrobial agents, which is a great breakthrough for the food industry. In contrast to the traditional form of preservatives, which can act on a single metabolic pathway, biogenic AgNPs and ZnONPs cause systemic failure in the organisms by creating oxidative stress, resulting in the fragmentation of DNA and down-regulation of survival genes. This molecular perturbation not only lengthens shelf-life but also offers a more suitable substitute to synthetic additives that may decrease the occurrence of antibiotic-resistant strains within the food supply chain.

Limitations and Future Research Directions
However, in spite of their effectiveness, a number of molecular barriers exist. One of them is the kinetics of migration of metal ions in the package into the food matrix, and this may be genotoxic in high amounts when ingested. Recent research, the study in question being one of them, tends to concentrate on in vitro success, but the biochemistry of various food matrices (e.g., high acidity or fat content) can affect NP stability and reactivity. To provide a complete mapping of the genetic response to nano-exposure, future research must focus on transcriptomic profiling of pathogens and perform long-term in vivo toxicity research to provide absolute consumer safety.

Recommendations for Industrial Integration
In order to effectively incorporate green nanotechnology into the business, one should use a Safe-by-Design strategy. Chitosan-nanocomposite films are suitable for use by industry players as the biopolymers have the capacity to stabilize nanoparticles and regulate their release rate, reducing migration. Moreover, the precursor of NP synthesis made out of agricultural waste (pomegranate seeds or citrus zest) offers a circular economy scheme, cutting down on the production cost. These biogenic protocols will be crucial to standardization to be approved by the regulator and deployed on a large scale with smart packaging systems.

Conclusion

The interpretation in this study demonstrates the radical potential of green-synthesized nanoparticles as an advanced food preservation method in the modern world. The critical discoveries include the fact that biogenic nanoparticles, particularly those that are obtained through plant extracts such as Citrus and pomegranate, have better antimicrobial effectiveness than conventional chemical agents. These nanoparticles are useful in the inhibition of a wide range of foodborne pathogens, including Escherichia coli and Staphylococcus aureus, due to their ability to cause systemic molecular disruption, in the form of the formation of reactive oxygen species and subsequent cellular membrane damage. Moreover, useful applications in food matrices revealed a drastic shelf-life extension, preserving both the structural and the nutritional integrity of perishable products for almost three times as long as the unmodified controls. Green nanotechnology is not overly important in this area. In addition to having strong bactericidal effects, these nanoparticles can be used as biocompatible and sustainable alternatives to synthetic preservatives. Through being able to use the by-products of agriculture as a source of synthesis, the process is in line with the sustainability initiatives of the world, as it minimizes toxic wastes as well as the cost of production of the product. The resulting antimicrobial agents in this process are safe for human consumption and the environment, and effective at the same time. The shift in the laboratory success to the industrial standardization, however, cannot be achieved without concerted effort. The stakeholders in the food industry and the scientific community are being urged to focus more on the long-term genotoxicity and ion migration dynamics research. Future practice should be related to scaling such green synthesis protocols and the creation of obvious regulations regarding smart active packaging. By creating a connection between molecular research and application, will be able to have a safer and more resilient global food supply chain.

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