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Silver Nanoparticles Loaded Active Packaging of Low-Density Polyethylene (LDPE), A Challenge Study against *Listeria Monocytogenes, Bacillus Subtilis* and *Staphylococcus Aurerus* to Enhance the Shelf Life of Bread, Meat and Cheese

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ABSTRACT

Nanotechnology plays an important role in every field of science. Silver nanoparticles (Ag NPs) show the best antibacterial activity when utilized in the active packaging of food. In this study silver nanoparticles were synthesized using the green synthesis method. Characterization of the synthesized silver nanoparticles was conducted through Ultra Violet (UV) visible spectroscopy, Fourier Transform Infra-Red spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). The solvent evaporation method was employed to successfully prepare a low-density polyethylene-based film containing silver nanoparticles (LDPE/Ag nanocomposite). To evaluate the antibacterial activity of the synthesized silver nanoparticles and LDPE/Ag nanocomposite, the disc diffusion method was employed against food-borne pathogenic bacteria including *Listeria monocytogenes* (*L. monocytogenes*), *Bacillus subtilis* (*B. subtilis*), and *Staphylococcus aureus* (*S. aureus*). The LDPE/Ag nanocomposite demonstrated effective performance as an active packaging material, thereby enhancing the shelf life of bread, chicken, and cheese, as assessed in the challenge study.

Key words: Silver nanoparticles (Ag NPs), Silver nanocomposite, Low density poly ethylene (LDPE), Active packaging of food.

Graphical Abstract:

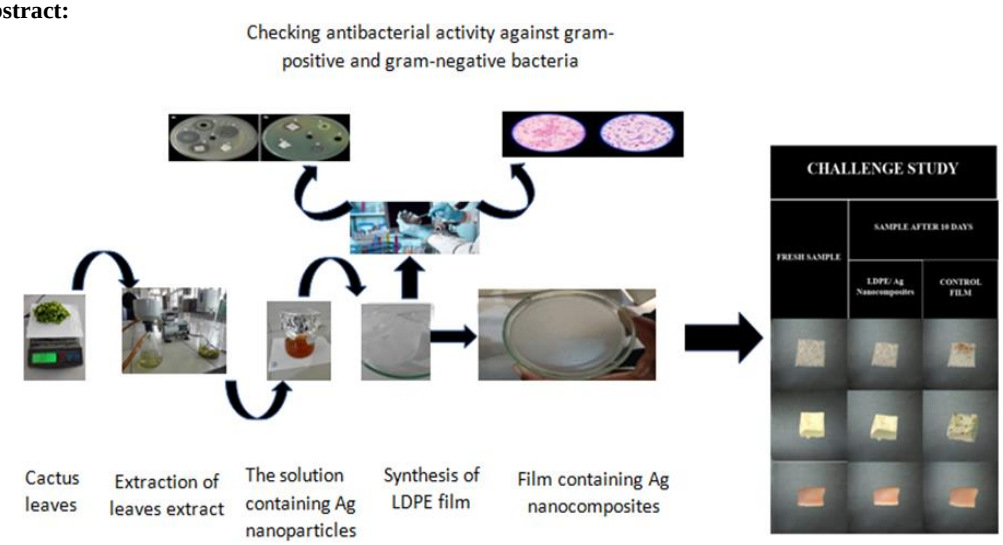

Fig. 1: Graphical demonstration of abstract.

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INTRODUCTION

Packaging materials are designed to protect food from decay and oxidation processes caused by various forms of contamination (including chemical, physical and biological factors) to ensure the quality of food over a long period, from storage to final consumption (Wróblewska-Krepsztul et al., 2018). Technological advancements in multifunctional packaging are aimed at minimizing food contamination, especially microbial contamination thereby enhancing food safety and preservation (Brody et al., 2008). Microbial growth is one of the main reasons for the deterioration of food quality. It can lead to discoloration, odor development, texture changes, loss of nutrients, shorten the shelf life of food and increase the risk of foodborne diseases (Bibi et al., 2017). Antimicrobial-based Active packaging of food extends the lag period and slows the growth of microorganisms to extend the shelf-life and maintain food quality and safety (Gorrasi et al., 2020).

To reduce the microbial burden of food, a new generation of active smart packaging has emerged, combining nanostructures and packaging containing metal nanoparticles (Sonkaria, Ahn, & Khare, 2012). The high surface-to-volume ratio of metal nanoparticles contributes to an enhanced antibacterial effect, among which silver nanoparticles (AgNPs) showed the strongest antibacterial activity (Hamouda, 2012). The antibacterial application of Silver (Ag) nanoparticles in food packaging brought a revolution in the active packaging of food and minimizes food waste (Kowsalya et al., 2019).

AgNPs may be incorporated into biodegradable and non-biodegradable polymers for the production of food packages with antimicrobial properties, leading to greater safety and longer shelf life (Siddiqi, Husen, & Rao, 2018). They are also ideal for incorporation into polymer-based materials due to their unique qualities not found in other antimicrobials, such as excellent thermal stability and chemical compatibility with the more common low-density polyethylene (LDPE) polymer matrix. LDPE is frequently used in several economic sectors of the food industry due to its flexibility, transparency, processability, and thermal stability (Carbone et al., 2016).

There are significant differences in the use of antimicrobial agents on a laboratory scale and in real-time applications. These differences, as well as regulatory issues and technical limitations, are the main factors that hinder the commercialization of antimicrobial packaging systems (Malhotra, Keshwani, & Kharkwal, 2015).A study using Japanese Industrial Standard (JIS) tests showed that silver nanocomposite low-density polyethylene films had better antibacterial effects against *Staphylococcus aureus* and *Escherichia coli* (Becaro et al., 2016). In another study, fresh-cut carrots coated with silver nanoparticles in LDPE significantly inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* in a disc diffusion test (Jokar et al., 2012). Furthermore, AgNPs have shown antibacterial properties against mesophilic aerobic bacteria and coliforms at lower doses. Additionally, the films lost little weight while maintaining the ascorbic acid levels of freshly cut carrots (Becaro et al., 2016). A study of orange juice encapsulated in silver nanocomposite sheets found lower total fungal and bacterial counts, a lower browning index, and better ascorbic acid retention (Emamifar et al., 2011).

The focus of this study is the green synthesis of AgNPs and their incorporation in LDPE film to prepare LDPE/ Ag nanocomposite having the strong antibacterial potential to enhance the shelf life of a variety of food products. Simple bread, chicken, and cheese with a high portion of carbohydrates, protein, and fat respectively are used in the study.

MATERIALS AND METHODS

Synthesis and Characterization of Silver Nanoparticles

Silver nanoparticles were prepared through the green synthesis method using plant extract as a capping and reducing agent by following the method of (Angamuthu et al., 2023). Fresh leaves of cactus (Opuntia ficus-indica) plant were collected from a local botanical farm in Lahore, Pakistan. The leaves were washed with distilled water and cut down into small pieces with the help of a sharp blade. 50 grams of weighed cactus leaves were taken into 200 ml of distilled water in a beaker and boiled for 15 minutes. The hot solution is filtered through Whatman filter paper no.1 with the help of a suction pump. The aqueous cactus extract was then stored at 4ºC in a tightly air-sealed container for further use. 0.17g of silver nitrate (Sigma-Aldrich) was dissolved in 1000ml of distilled water to prepare a 1mM solution of silver nitrate. Equal concentrations of 1 mM silver nitrate and cactus extract were mixed in a conical flask. The pH of the mixed solution was found to be 5.5. The pH of the solution was adjusted in the range of 7.5-8 with the help of concentrated sodium hydroxide. The color of the mixed solution was light green. Then the solution was incubated at 25ºC for 24 hours. The color of the solution was changed from light green to brown. The color change indicated the formation of silver nanoparticles. The synthesized silver nanoparticles were then centrifuged at 4000 rpm for 2 hours. After centrifugation, the AgNPs were washed with ethanol and distilled water twice to remove the impurities. Then washed silver nanoparticles were dried in the oven at 60 ºC. Powdered AgNPs were then stored for further analysis. The flow line of nanoparticles synthesis is given in Fig. 2.

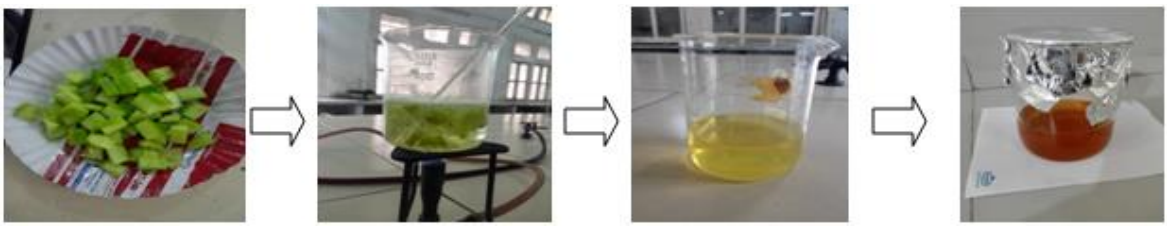

Fig. 2: Flow line of nanoparticles synthesis.

Characterization of synthesized silver nanoparticles was done by performing Ultra Violet (UV) visible spectroscopy, Fourier Transform Infra-Red spectroscopy (FTIR), and scanning electron microscopy (SEM). A BMS UV-1900 spectrophotometer with a wavelength of 300-650 nm was used for the UV-visible spectroscopy of silver nanoparticles. FTIR-4100 spectrophotometer was used for the FT-IR spectroscopy of silver nanoparticles. Scanning electron microscopy was performed by MIRA3 TESICAN under various magnifications of 5K X, 10K X, 25K X, 50K X, 100K X, and 200K X.

Preparation of Low-Density Polyethylene (LDPE)/ Ag Nanocomposite Film

Low-density polyethylene/Ag nanocomposite films containing 3% wt. were prepared by the solvent evaporation method. Control film (LDPE film without AgNPs) was prepared by preparing a 3% solution of low-density polyethylene. LDPE and toluene were purchased from Sigma Aldrich. 0.75g of LDPE was mixed in 25 ml of toluene with the help of a magnetic stirrer at 70 ºC. The hot solution was then poured into glass Petri dishes and kept for settling at room temperature. Toluene evaporates at room temperature. After 2 hours toluene solution evaporates and low-density polyethylene film is prepared. In the formation of LDPE/Ag nanocomposite prior to pouring LDPE in a petri dish AgNPs were dispersed in the solution with the help of temperature controlled ultrasonic bath sonicator by following the method of (Lomate, Dandi, & Mishra, 2018). Then the solution was poured into a petri dish and after two hours films start detaching the glass Petri dishes. Control and LDPE/ Ag nanocomposite film were then placed in a dry place for further analysis. The image of synthesized LDPE film is shown in figure 3.

Antibacterial Activity

The antibacterial activity of Ag NPs and LDPE/ Ag nanocomposite was determined through the Agar Disk diffusion method. The antibacterial activity was evaluated against food-borne pathogenic gram-positive, *Listeria monocytogenes* (*L. monocytogenes*), *Staphylococcus aureus* (*S. aureus*), and gram-negative, *Bacillus subtilis* (*B. subtilis*) *bacteria.* A solution of bacteria (100µl containing 10^2–10^4 CFU/ml) was evenly distributed over the surface of a nutrient agar plate. Then the discs were placed on the agar surface (including a control) and refrigerated for 2 h to allow disc expansion. The plates were then incubated at 37 °C for 24 h. Once the incubation was complete, the sizes of the inhibition zones were measured.

RESULTS AND DISCUSSION

UV- Visible Spectroscopy of Silver Nanoparticles

In the green synthesis of silver nanoparticles change in color indicates the formation of silver nanoparticles while UV-vis spectroscopy confirmed the formation of nanoparticles synthesis. During UV- vis spectroscopy Silver nanoparticles show an absorbance peak between the ranges of 370-500 nm (Seifipour, Nozari, & Pishkar, 2020). When the mixed solution of cactus extract and silver nitrate changes color from light green to dark brown it gives a strong idea about the formation of AgNPs. Fig 4. shows the result of UV-vis spectroscopy of silver nanoparticles. AgNPs show a sharp peak at 400 nm and according to the studies (Seifipour et al., 2020) it clearly indicates the formation of silver nanoparticles.

FTIR-Spectroscopy of AgNPs

FTIR spectroscopy reveals the chemical bonding present in a molecule. Fig 5 showed FTIR spectroscopy of AgNPs. According to the studies (Desai et al., 2012) the FTIR spectra show a sharp peak at 1600cm which indicates the presence of a carbonyl group, the sharp peak at 3400 cm confirms the existence of a hydroxyl group and the little sharp peaks at 2100 confirm the presence of aromatic compounds. FTIR spectra confirm the presence of OH, Carbonyl, and aromatic compounds in silver nanoparticles solution.

Scanning Electron Microscopy of AgNPs

According to the studies (Puchalski et al., 2007) scanning electron microscopies provide us with information about the morphology (shape and size) of synthesized silver nanoparticles. Figure 6 showed that all silver nanoparticles are in the range of 20-40 nm with a spherical shape. The complete formation of Ag NPs was seen in 100 X magnification.

Fig. 3: Image of synthesized LDPE film.

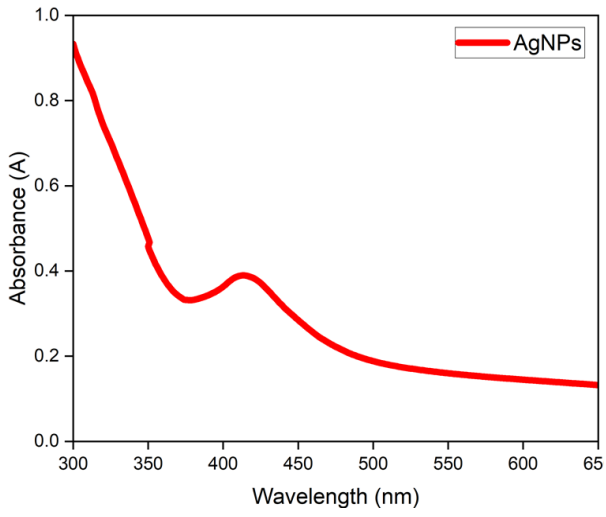

Fig.4: Result of UV-vis spectroscopy of silver nanoparticles.

Antibacterial Activity of AgNPs and LDPE/ Ag nanocomposites Film

According to the (Aziz et al., 2016) studies, AgNPs have broad antibacterial spectrum against a wide range of bacteria. Therefore, synthesized Ag NPs and LDPE/Ag nanocomposite film show good antibacterial activity against all food-borne pathogenic bacteria. Table 1 showed the antibacterial activity results.

Challenge Study

The antibacterial activity of LDPE/ Ag nanocomposite film was tested over a variety of fat, protein, and carbohydrate-based food. Raw chicken meat, bread, and cheese samples were used to determine the antibacterial potential of LDPE/ Ag nanocomposite film. All food samples (Bread, Cheese and Meat) were purchased from local grocery stores and cut down into pieces and exposed to UV light for two hours from each side along with LDPE/Ag nanocomposite and control film to ensure the elimination of unwanted bacteria before performing practical application. The graphical demonstration of challenge study is represented in Fig. 7.

According to the studies (Azlin-Hasim et al., 2015), the utilization of Ag/LDPE nanocomposite films prolonged the freshness of chicken breast fillets and considerably improved their resistance to oxidation when compared to the standard films ($p < 0.05$). The findings suggest that LDPE nanocomposite films incorporating Ag nanoparticles have the potential to serve as effective antimicrobial packaging in the field of food applications. Simple uncooked raw chicken has a shelf life of 2 hours at room temperature. Samples of raw chicken meat were sprayed with 50 ml of *L. monocytogenes* bacteria with hand operated spray bottle to get a rough $10^6 – 10^7$cfu/g of bacteria. One piece of chicken meat was wrapped in a simple LDPE film used as a controlled sample and the other piece of meat was wrapped in LDPE/Ag nano-composite primarily exposed to UV light. After wrapping

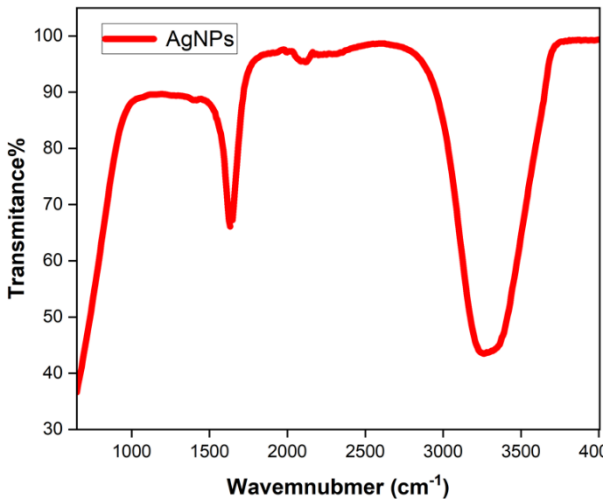

Fig. 5: FTIR spectroscopy of AgNPs.

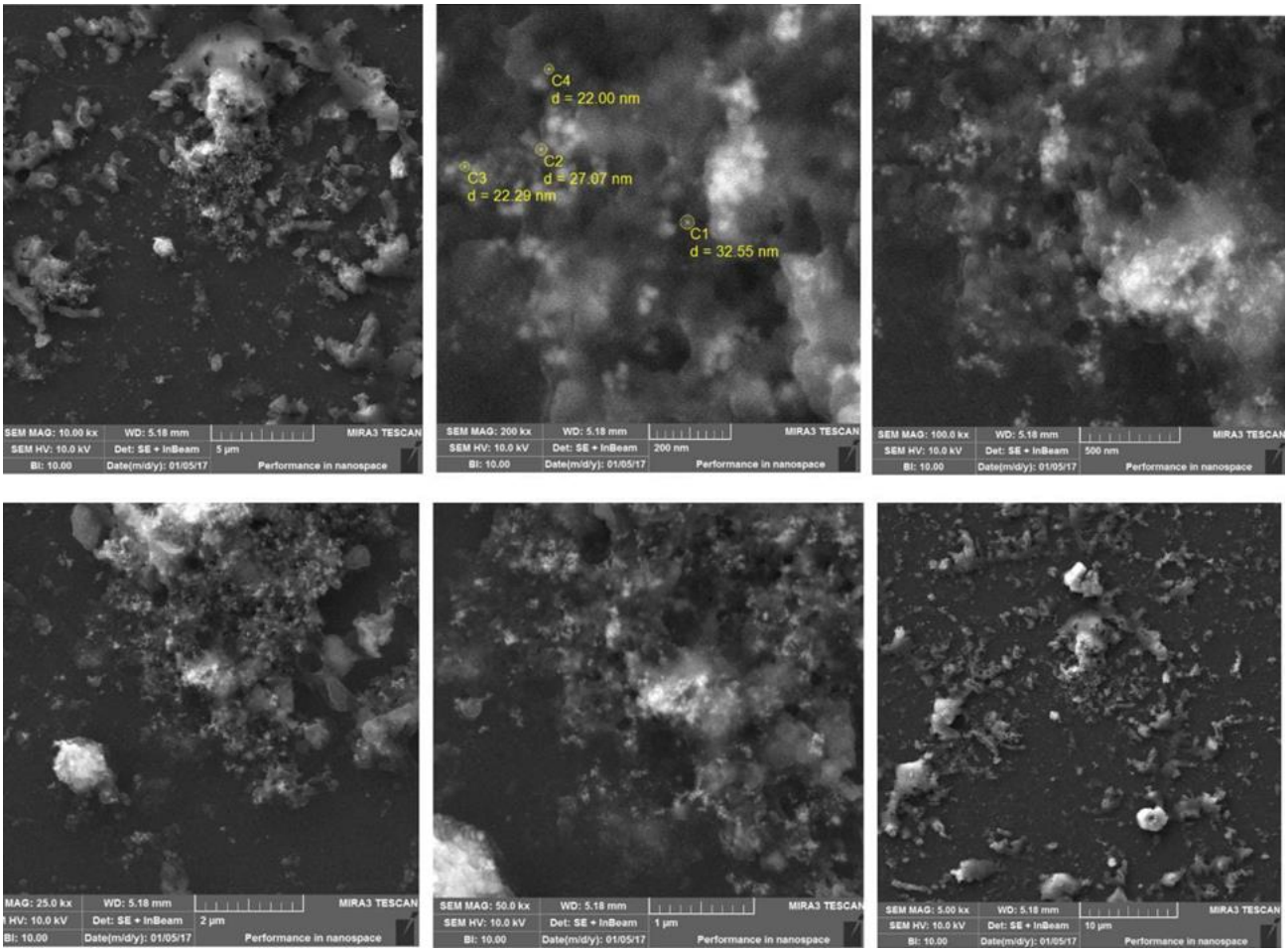

Fig. 6: Images of synthesized silver nanoparticles.

both samples were placed at refrigerated temperature (8º C) and then the rate of bacterial growth was monitored at an interval of two days through (Standard Plate Count) SPC method from day zero to day ten. The number of cfu/g start decreasing from day two and no bacteria were reported at day eight in the tested (LDPE/Ag nanocomposite) film while in the control film (LDPE without AgNPs) number of cfu/g bacteria remains fluctuated and 10^5cfu/g were reported at day ten. The results are shown in figure 8.

According to the studies (Braga et al., 2018), PVC-based films containing silver nanoparticles (AgNPs) can be conveniently produced to impart antimicrobial properties to the film. These films have the potential to extend the shelf life of bread and can be used for food storage under normal atmospheric conditions without the need for modified atmospheres. Simple bread has a shelf life of 7 days at room temperature. Samples of bread were sprayed with 50 ml of *B. subtilis* bacteria with hand operated spray bottle to get a rough 10^6 – 10^7cfu/g of bacteria. One piece of bread was wrapped in a simple LDPE film used as a controlled sample and the other piece of bread was wrapped in LDPE/Ag nanocomposite primarily exposed to UV light. After wrapping both samples were placed at room temperature and then the rate of bacterial growth was monitored at an interval of two days through the SPC
method from day zero to day ten. The number of cfu/g start decreasing from day two and no bacteria were reported on day eight in the tested (LDPE/ Ag nanocomposite) film while in the control film number of cfu/g bacteria remains fluctuated and the 10^5cfu/g was reported on day ten. The results are shown in figure 9.

According to the studies (Motelica et al., 2021), the incorporation of silver nanoparticles (Ag NPs) and lemongrass essential oil endowed the biodegradable alginate films with superior antimicrobial properties. The antimicrobial agents showed synergistic effects against

Table 1: Antibacterial activity of the cactus extract, silver nanoparticles and LDPE/Ag nanocomposites.

Sample	*L. monocytogenes* (Zone of inhibition in mm)	*B. subtilis* (Zone of inhibition in mm)	*S. aurerus* (Zone of inhibition in mm)
Cactus Extract	1.0	1.2	1.1
Ag NPs	7.6	6.9	7.1
LDPE/Ag nanocomposites	8.4	8.0	7.7

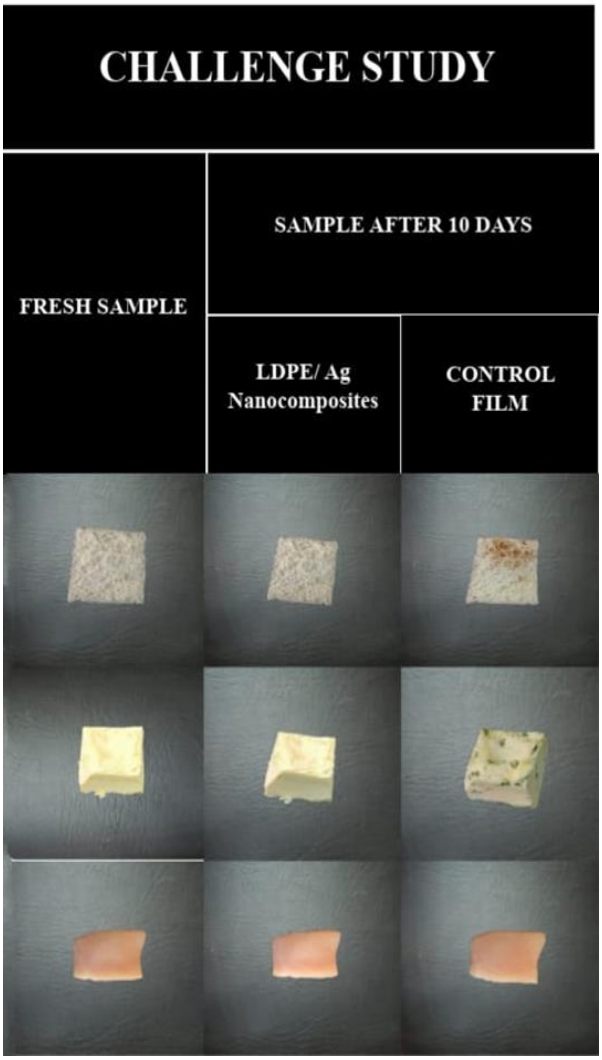

Fig. 7: The graphical demonstration of challenge study.

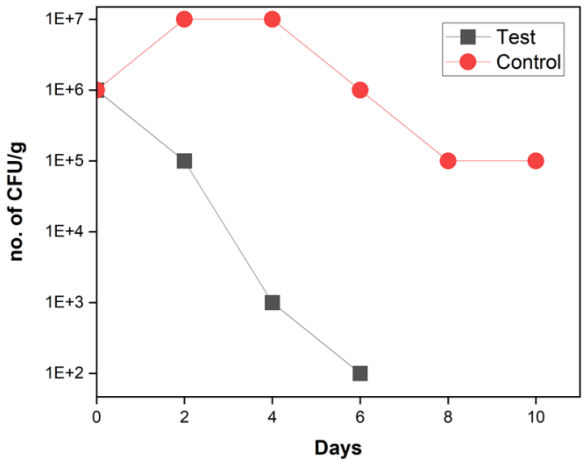

Fig. 8: Antibacterial activity of test and control film against *L. monocytogenes* on meat sample.

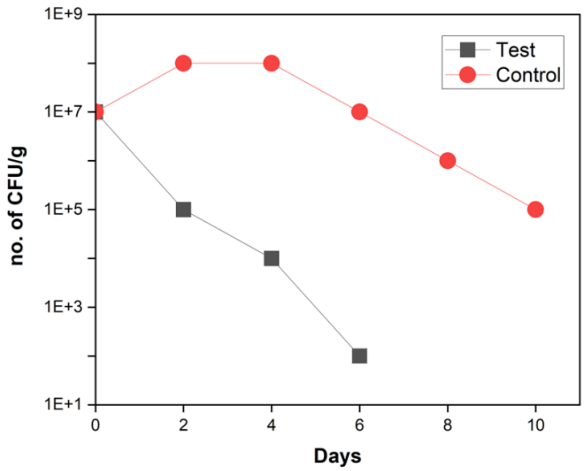

Fig 9: Antibacterial activity of test and control film against *B. subtilis* on bread sample

both Gram-positive and Gram-negative bacterial strains, with the most striking results against Bacillus cereus. These antimicrobial films are being evaluated as a wrapping material for soft cheeses, with preliminary tests showing they can effectively preserve cheese for up to 14 days. Soft cheese has a shelf life of 7-14 days at refrigerated temperatures. Samples of cheese were sprayed with 50 ml of *S. aurerus* bacteria with hand operated spray bottle to get a rough 10^6 – 10^7cfu/g of bacteria. One piece of cheese was wrapped in a simple LDPE film used as a controlled sample and the other piece of cheese was wrapped in LDPE/Ag nanocomposite primarily exposed to UV light. After wrapping both samples were placed at room temperature and then the rate of bacterial growth was monitored at an interval of two days through the SPC method from day zero to day ten. The number of cfu/g start decreasing from day two and no bacteria were reported at day ten in the tested (LDPE/ Ag nanocomposite) film while in the control film (LDPE without AgNPs) number of cfu/g bacteria remains fluctuated and 10^4cfu/g were reported at day eight 10. The results are shown in Figure 10.

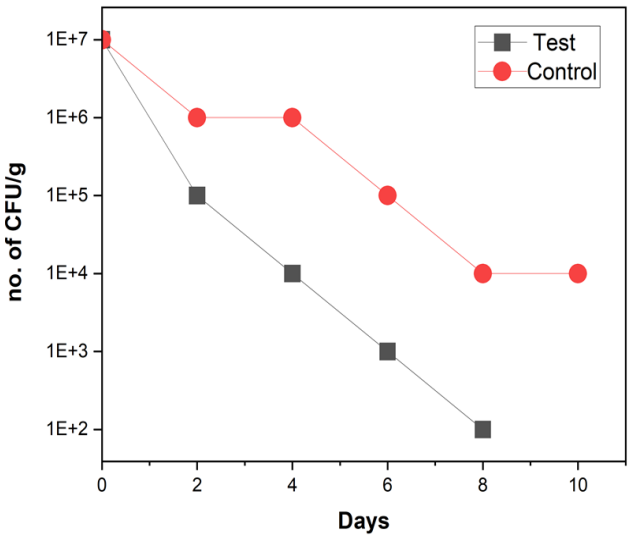

Fig. 10: Antibacterial activity of test and control film against *S. aurerus on* cheese sample.

Conclusion

Silver nanoparticles of 20-30 nm with spherical shape were successfully synthesized by the green synthesis method. Their characterization was done with UV-Vis spectroscopy, FITR spectroscopy, and Scanning Electron Microscopy. Simple LDPE film and LDPE/ Ag nanocomposites were synthesized with the solvent evaporation method. Synthesized Ag NPs and LDPE/Ag NPs composite show good antibacterial activity against all food-borne pathogenic bacteria, *Listeria monocytogenes* (*L. monocytogenes*), *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. subtilis*) tested in the study. LDPE/Ag nanocomposites act as active packaging and enhanced the shelf life of a variety of food items. The result of studies demonstrated that LDPE/Ag nanocomposite has higher antibacterial in descending order for bread, chicken, and cheese correspondingly carbohydrates, proteins, and fats-based food.

Conflicts of Interest

The authors declare no conflict of interest.

Author's Contribution

All authors contributed equally in this research.

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