Physicochemical characteristics and anti-oxidant capacity of pullulan active packaging containing green synthesised nanoparticles

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RESEARCH ARTICLE

Abstract

The effects of incorporating green-synthesised silver nanoparticles (AgNPs) on oxidative stability, antioxidant activity, chromatography, chroma, hue, depth (D), pH, volume, moisture sorption, solubility and density of pullulan active packaging were examined here. The impact of various concentrations of AgNPs (0.5%, 1% and 2% v/v) on four groups of pullulan active packaging (PF-CTRL, PF-C-AgNPs, PF-P-AgNPs and PF-M-AgNPs) was also determined. During 14 days of storage, pullulan active packaging incorporating 2% (v/v) curcumin-stabilised AgNPs (PF-C-AgNPs) had significantly reduced transparency, pH and film density, as well as significantly better oxidative stability and antioxidant activity than the other groups. The integration of green AgNPs into edible pullulan films did not significantly affect film D, moisture sorption or film solubility. These results suggest that pullulan active packaging, especially PF-C-AgNPs, can resist oxidation and degradation and maintain better quality and tactile characteristics during refrigerated storage.

Keywords: Biopolymer; Electrolytic matrix; Oxidative stability; Polysaccharide packaging; Refrigerated storage

Introduction

Pullulan is an extracellular polysaccharide that was isolated from the culture broth of the fungus *Aureobasidium pullulans* in 1958. It is now considered one of the best biopolymers for synthesising active packaging (Hassan and Cutter, 2020; Trinetta and Cutter, 2016).

In terms of its chemical structure, pullulan consists of repeating maltotriose units with and linkages, with the chemical formula $C_6H_{10}O_5$ (Dewan and Islam, 2024; Khan *et al*., 2020; Trinetta and Cutter, 2016). These linkages in pullulan mimic the amylose and dextrin groups of polysaccharides, with a higher degree of solubility in water but no solubility in organic solvents (alcohols,

ethers, acetones and oils) (Farris *et al*., 2014; Mohammed *et al*., 2023; Simões *et al*., 2024). The dry powder of pullulan is tasteless, colourless, odourless, edible, environmentally friendly, non-mutagenic and non-carcinogenic and can be degraded at 250–280°C (Ogzhan and Yangilar, 2013; Trinetta and Cutter, 2016). The chain with and linkages also confers exceptional flexibility on this exopolysaccharide, which is necessary for the formation of edible and transparent films and coatings for food preservation (Dewan and Islam, 2024; Trinetta and Cutter, 2016).

Edible pullulan films can be prepared using aqueous solutions containing pullulan at 1% to 20% w/v (Trinetta and Cutter, 2016). These films are clear, glossy, tasteless, odourless and water-soluble, with excellent gas barrier and mechanical properties (Hassan and Cutter, 2020; Khan *et al*., 2020; Simões *et al*., 2024; Trinetta and Cutter, 2016). By mixing pullulan with various plasticisers, including xanthan gum and glycerol, at suitable ratios, film integrity, mechanical properties, transparency, consumer acceptance and storage time can be enhanced (Trinetta *et al*., 2011).

The use of 'pullulan active packaging' incorporating any active antioxidant substance can improve the quality and safety of food via active release and/or absorption (Farris *et al*., 2014; Khan *et al*., 2022; Khan *et al*., 2024; Simões *et al*., 2024). Metal nanoparticles (Ag, ZnO, Cu and Au), plant extracts and essential oils can be used as active substances to prolong the quality and shelf life of food items (López-Mata *et al*., 2015; Wang *et al*., 2015). For example, edible pullulan films incorporating silver nanoparticles (AgNPs) were reported to exhibit better depth (D)/thickness, more tensile strength and low water absorption (Mohammed *et al*., 2023; Mousavi *et al*., 2018; Trinetta *et al*., 2011). It seems that AgNPs and the hydroxyl group (OH) of pullulan interact with each other, creating better cross-links with the matrix of the exopolysaccharide, which in turn result in improved mechanical strength (Bahrami *et al*., 2018; Mohammed *et al*., 2023). It has also been reported that the inclusion of glycerol and xanthan gum in edible pullulan films, along with AgNPs, amplifies their antioxidant and physiochemical properties for prolonged storage (Khalaf *et al*., 2013; Morsy *et al*., 2014; Trinetta *et al*., 2011). Furthermore, researchers have investigated the better antioxidant capacity of edible films incorporating green synthesised AgNPs compared with those containing other active substances (Khan *et al*., 2022; Mulla *et al*., 2023; Wang *et al*., 2015). These unique properties of pullulan active packaging have led to its use in the biomedical sector as anticancer, antimicrobial, antifungal and wound healing material. Similarly, the pronounced antioxidant and antimicrobial characteristics of pullulan active packaging have attracted interest in the food industry (Khan *et al*., 2024; Mulla *et al*., 2023; Wang *et al*., 2017). However, to the best of our knowledge, pullulan active packaging containing AgNPs as an active substance has yet to be explored in terms of its physicochemical characteristics and antioxidant behaviour to preserve food items for longer storage periods (Khan *et al*., 2022; Khan *et al*., 2024; Mulla *et al*., 2023).

Against this background, the present study was planned to investigate the effects of incorporating AgNPs on the physiochemical characteristics (appearance, chromatography, pH, transparency, density, etc.) and antioxidant capacity of pullulan active packaging. Specifically, the antioxidant capacity was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid]) and TBARS (thiobarbituric acid reactive substances) assays. The effects of various concentrations of green-synthesised AgNPs (0.5%, 1% and 2%) along with the impact of storage time (0, 7 and 14 days) on the oxidative stability (malondialdehyde (MDA) concentration) of edible pullulan films were also determined.

Materials and Methods

Materials

Pullulan (97.0% pure, food grade, obtained from *A. pullulans*), glycerol (anhydrous, Glycerine), xanthan gum (99.0% pure), 2-thiobarbituric acid (GR grade), butylated hydroxytoluene (≥ 99% pure, BHT), sodium dodecyl sulphate (SDS), potassium chloride (KCl), ethanol (99% pure), 1,1,3,3-tetraethoxypropane, ABTS, DPPH and deionised water (DW) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium peroxodisulphate (potassium persulphate, $K_2S_2O_8$) and glacial acetic acid (99.9%) were supplied by HmBG Co. Inc. (Hamburg, Germany) (Khalaf *et al*., 2013; Khan *et al*., 2022; Morsy *et al*., 2014; Simões *et al*., 2024).

Formulation of pullulan active packaging integrating green AgNPs

The synthesis of curcumin AgNPs (C-AgNPs) and pullulan-mediated AgNPs (P-AgNPs) was conducted as per our previous reports using both the chemical and physical reduction of $AgNO_{3(aq)}$, respectively (Khan *et al.,* 2019a; Khan *et al.,* 2019b). For the synthesis of edible pullulan films, aqueous dispersions of pullulan (5%, 10%, 15% and 20% w/v) were prepared by dissolving pullulan powder in DW and stirred at 80 ± 1°C and 300 rpm for 2 h as per a slightly modified version of the methods reported by Simões *et al*. (2024), Khan *et al*. (2022), Morsy *et al*. (2014) and Khalaf *et al*. (2013). Glycerol (1% v/v) and xanthan gum (0.5% w/v) were added slowly into the pullulan dispersion as plasticiser

and homogenised at 300 rpm until gelatinisation occurred. Gelatinised pullulan dispersions were autoclaved at 121 ± 1°C and 15 psi for 20 min to reduce the microbes and then left at room temperature (24–25°C) for 3 h. After cooling, green-synthesised AgNPs (C-AgNPs, P-AgNPs) were incorporated into the edible pullulan film, except for in the control treatment, at concentrations of 0.5%, 1% and 2% (Khan *et al*., 2022; Morsy *et al*., 2014; Khalaf *et al*., 2013). Gentle agitation was performed to ensure complete mixing. A total of four treatment groups were generated as follows: (i) PF-CTRL (control, without AgNPs), (ii) PF-C-AgNPs (pullulan active packaging containing C-AgNPs), (iii) PF-P-AgNPs (pullulan active packaging containing P-AgNPs) and iv) PF-M-AgNPs (pullulan active packaging containing both C-AgNPs and P-AgNPs; Khan *et al*., 2022). The mixed filmogen material was poured into plastic Petri dishes of 10 mm in diameter and dried for 48 h at 25 ± 2°C and 45 ± 5% relative humidity in a laminar air flow cabinet (Khalaf *et al*., 2013; Khan *et al.*, 2022; Pattanayaiying *et al*., 2015). After drying, pullulan active packaging incorporating green AgNPs was harvested as long strips measuring 8 × 4 cm or 8 × 2 cm and stored in the dark at 25 ± 1°C and 45 ± 5% relative humidity for further characterisation.

Characterisation of pullulan active packaging containing AgNPs

Appearance and colour

The appearance of the active packaging was recorded after drying and peeling off the strips from Petri dishes at 25°C (Khalaf *et al*., 2013). The colour of the pullulan active packaging was measured by placing square pieces (measuring 3 × 3 cm) into a colour meter (CR-300 Minolta Chroma Meter; HunterLab, Osaka, Japan) as per a slightly modified version of the procedure reported by Bahrami *et al.* (2018). Three observations were recorded per sample at random in the standard cup with a white background. The lightness (L*), redness (a*) and yellowness (b*) of the samples were recorded against the standard values. The chromatographic mean (ΔE) was calculated as per Equation (1):

$$\Delta E = \sqrt{(L^* - L^s)^2 + (a^* - a^s)^2 + (b^* - b^s)^2} \quad (1)$$

L^s, a^s and b^s were taken as standard values (93.91, –1 and 0.61, respectively) after calibration of the equipment. In addition, the chroma and hue of pullulan active packaging were recorded as per Equations (2) and (3), respectively (Vital *et al*., 2016):

$$\text{Chroma (C) of pullulan film} = \sqrt{(a^*)^2 + (b^*)^2} \quad (2)$$

$$\text{Hue (H)} = \tan^{-1}(b^*/a^*) \quad (3)$$

Antioxidant activity

The antioxidant activity of pullulan active packaging was determined by three assays, namely, TBARS, DPPH and ABTS assays (Dai *et al*., 2023; Gehrcke *et al*., 2022; Ramiah *et al*., 2014). Specifically, oxidative stability was measured using the TBARS assay. A total of 750 mg of pullulan active packaging, incorporating 0.5%, 1% or 2% AgNPs, was homogenised in an aqueous solution (1.15% w/v) of KCl at 15,000 rpm for 3 min. A total of 200 µL of this homogenised film sample was then mixed with 300 µL of DW + 35 µL of BHT (7 mM) + 165 µL of SDS (8.1% w/v) solution + 2 mL of TBA (0.8%) solution, followed by incubation for 3 min at 25 ± 1°C for 5 min. Next, this mixed solution was heated at 95 ± 1°C for 60 min using a water bath (WNB-14; Memmert GmbH, Schwabatch, Germany) and then cooled using running tap water. The sample solutions were centrifuged at 1000 rpm for 10 min at 25 ± 1°C with the help of a refrigerated centrifuge (5810 R; Sigma-Aldrich, Inc., St. Louis, MO, USA), after which the obtained supernatant was collected. The absorbance of the film supernatant was recorded at a wavelength of 532 nm by a UV-VIS spectrophotometer (UV-1800; SHIMADZU Corp., Kyoto, Japan), and the results are expressed as mg of MDA/kg measured against a standard curve of 1,1,3,3-tetraethoxypropane (Dai *et al*., 2023; Ramiah *et al*., 2014).

DPPH and ABTS assays were also employed to analyse the antioxidant activity of pullulan active packaging incorporating 2% green AgNPs, in accordance with a slightly modified version of the method reported by Ferreira *et al*. (2014). For the DPPH assay, 135 mg of each film sample, measuring 10 × 10 mm, was immersed in 3 mL of methanolic DPPH solution (0.16 mM, adjusted with methanol to produce absorbance between 7.0 and 8.0 to maximise the generation of free radicals). The DPPH solution containing packaging samples was stirred gently at 150 rpm and 25 ± 1°C for 60 min, vortexed by VTX-3000L (LMS. Co., Japan) for 1 min and incubated at 25 ± 1°C for 30 min in the dark. The absorbance of the final solution was determined at a wavelength of 517 nm using a UV-VIS spectrophotometer (UV-1800; SHIMADZU Corp., Kyoto, Japan) against the DPPH solution, and the free radical scavenging activity was calculated by Equation (4):

$$\text{DPPH scavenging activity \%} = [1 - (Abs^a - Abs^e) / Abs^c)] \times 100 \quad (4)$$

where Abs^a is the absorbance of the DPPH solution with the packaging sample, Abs^e is the absorbance of the packaging and Abs^c is the absorbance of the DPPH methanolic solution.

To determine the ABTS free radical scavenging activity of pullulan active packaging, 5 mL of ABTS aqueous solution (7 mM) was mixed with 100 mL of $K_2S_2O_8$ solution (2.45 mM) and kept in the dark at 25 ± 1°C and 45% relative humidity for 16 h to generate free radical ions. The absorbance of the mixed solution was adjusted between 7.0 and 8.0 with 80% (v/v) ethanol. A 10 × 10 mm packaging sample (containing 2% AgNPs) was immersed in 4 mL of adjusted ABTS solution and stirred at 150 rpm for 60 min in the dark. The solution was vortexed for 1 min using a vortex mixer (VTX-3000L; LMS. Co., Japan) and incubated for 30 min at 25 ± 1°C in the dark. After incubation, the absorbance was determined at a wavelength of 734 nm using a UV-VIS spectrophotometer (UV-1800; SHIMADZU Corp., Kyoto, Japan) against a blank (adjusted ABTS + $K_2S_2O_8$ solution). ABTS free radical scavenging activity was calculated using Equation (5):

$$\text{ABTS free radical scavenging activity \%} = [(Abs^c - Abs^a) \times 100] / Abs^c \quad (5)$$

where Abs^c is the absorbance of adjusted ABTS + $K_2S_2O_8$ solution, and Abs^a is the absorbance of adjusted ABTS + $K_2S_2O_8$ solution with pullulan active packaging sample.

Transparency and pH

Transparency was measured using a slightly modified version of the procedures reported by Simões *et al.* (2024) and Khalaf *et al.* (2013). Filmogen material weighing 250 ± 5 mg (3 × 3 cm) incorporating 2% green-synthesised AgNPs was diluted with 2 mL of DW. The solution was vortexed for 1–2 min until complete homogenisation, and the pH was determined using a digital benchtop pH meter (WD-35413-20; Oakton Instruments, IL, USA). The homogenised solution was then centrifuged at 2000 rpm and 30°C for 10 min using a refrigerated microcentrifuge (5810 R; Sigma-Aldrich, Inc., St. Louis, MO, USA). The supernatant was carefully separated, and its absorbance was measured at 550 nm with a UV-1800, UV-VIS spectrophotometer (SHIMADZU Corp., Kyoto, Japan). The absorbance was measured in triplicate per film sample (n = 3), and the film's transparency was measured using Equation (6):

$$\text{Transparency (T)} = A_{bs} / D \quad (6)$$

where A_{bs} is the absorbance, and D is the depth of pullulan active packaging (mm).

Active packaging D and volume

The D of pullulan active packaging was measured at 10 different locations selected at random by using a digital carbon fibre micrometre with a measuring range of 0–200 mm, accuracy of 0.01 mm and operating temperature range of 0–40°C (Syntek Technologies, Arlington, VA, USA), as per the procedures described by Simões *et al.* (2024) and Khalaf *et al.* (2013). The area was calculated in triplicate for each sample (n = 3), and the mean ± standard deviation (S.D.) was reported.

Volume (F_V) was calculated three times per sample as per Equation (7), and the mean ± S.D. was reported according to the method of Saberi *et al.* (2016).

$$F_V = F_a \times D \quad (7)$$

where F_a is the film area and D is the depth of pullulan active packaging (mm).

Active packaging density

The weight (F_W) was measured in triplicate (n = 3) for each active packaging sample, and density (F_D) was calculated in triplicate for each sample using Equation (8), in accordance with the method reported by Saberi *et al.* (2016).

$$F_D = F_W / F_V \quad (8)$$

Solubility

To determine solubility in water, the percentage of soluble matter (SM%) was calculated in accordance with a slightly modified version of the procedures reported by Simões *et al.* (2024) and Khalaf *et al.* (2013). A total of 250 ± 5 mg of pullulan active packaging (3 × 3 cm) incorporating 2% green-synthesised AgNPs was dried in a hot air oven (UF-260; Memmert Co. GmbH, Germany) at 110°C for 12–16 h to obtain dried flakes as a film sample. These dried flakes of pullulan active packaging were dissolved in 30 mL of DW at 25°C for 10 min. The obtained syrup was then placed at a temperature of 110°C in the hot air oven until its weight stabilised. Film solubility was calculated using Equation (9), and the experiment was conducted in triplicate (n = 3) for each film sample.

$$SM\% = \frac{IW - FW}{IW} \times 100 \quad (9)$$

where IW is the initial dry weight and FW is the final dry weight.

Moisture content and moisture sorption

The moisture sorption and moisture content of the pullulan active packaging were determined in triplicate using

a slightly modified version of the method reported by Saberi *et al*. (2016). Briefly, a 50 × 20 mm piece of packaging was cut, and its initial weight (M_i) and final weight (M_f) were recorded after oven drying at 90 ± 2°C using a hot air oven (UF-260; Memmert Co. GmbH, Germany) for 36 h. The moisture content was calculated using Equation (10):

$$MC\% = \frac{Mi - Mf}{MiMf} \times 100 \quad (10)$$

For the determination of moisture sorption, pieces of pullulan active packaging (50 × 20 mm^2) were placed on glass bottles (7 mL) filled with a saturated salt (NaCl) solution (6% w/v). The initial weights (M_0) were recorded, and the glass bottles were placed in a glass desiccator at 25 ± 2°C and 45 ± 5% relative humidity. The weights were continually recorded at 24 h intervals until the film samples (M) reached equilibrium. Moisture sorption (M_{oisorp}) was determined by Equation (11) (Saberi *et al*., 2016):

$$M_{oisorp}\ \% = [(M_0 - M) / M_0)] \quad (11)$$

Fourier transform infrared (FT-IR) spectroscopy

The involvement of the functional groups of pullulan and green AgNPs during the synthesis of the active packaging was investigated by FT-IR spectroscopy (IRTracer 100; SHIMADZU Corp., Kyoto, Japan). A 10 × 10 mm piece of each active packaging sample containing 2% AgNPs was used during the analysis (n = 3), and FT-IR spectra were recorded from 400 to 4000 cm^{-1}(Bahrami *et al*., 2018; Tang *et al*., 2024).

Field emission scanning electron microscopy

To assess the surface topography of pullulan active packaging and the distribution of green AgNPs in the film, field emission scanning electron microscopy (FESEM) was performed (JSM 7600 F FESEM; JEOL Ltd., Tokyo, Japan) (Bahrami *et al*., 2018; Shahhosseini, 2023; Tang *et al*., 2024). The dried active packaging samples (10 × 10 mm, containing 2% AgNPs, dried at 45°C for 6 h) were mounted over the FESEM carbon specimen holder with a transparent coating using a rotary pumped coater (Q 150 RS; Quorum Technologies Ltd., Laughton, UK) with a vacuum pressure of 1 × 10^3 to 1 × 10^{-5} mBar for 10 min (n = 3). This transparent coating on the pullulan active packaging incorporating AgNPs improved the stability and visibility under the FESEM LaB_6 electron gun.

Statistical analysis

All of the experiments were performed in triplicate with three samples (n = 3) per treatment (Khalaf *et al*., 2013; Khan *et al*., 2022; Saberi *et al*., 2016). A completely randomised design (CRD) was applied to all of the treatments, and the obtained results are reported as mean ± S.D. The reported results were statistically analysed by one-way analysis of variance (ANOVA) using SPSS software (v.20.0; IBM Corp., Armonk, NY, USA). Tukey's multiple comparison test (HSD) was applied as a post hoc test to detect the similarities or differences among the calculated mean ± S.D. at a significance level of $p < 0.05$.

Results

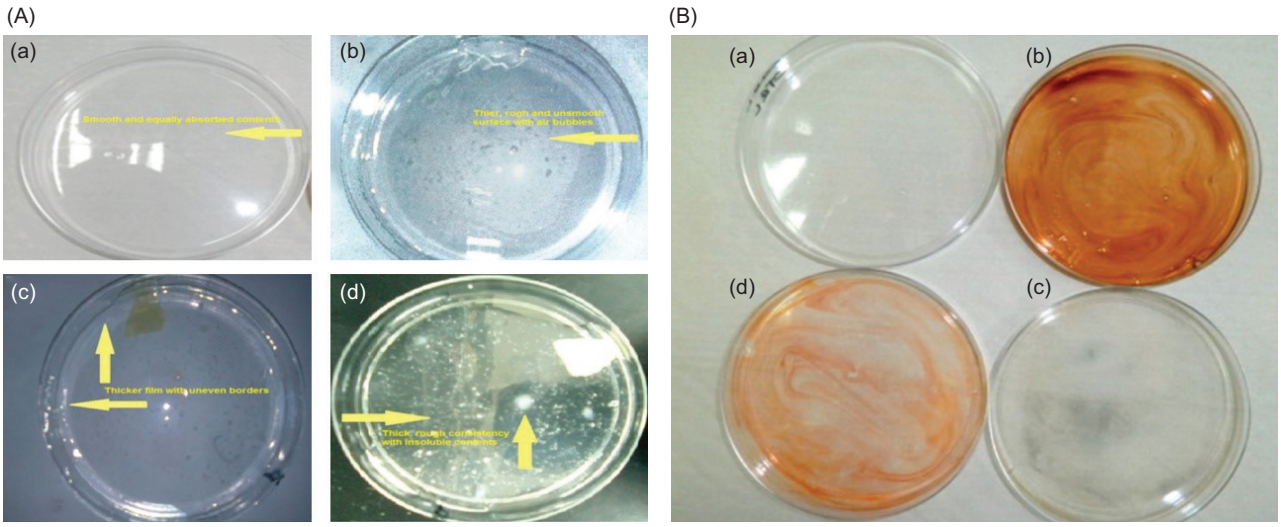
In this study, pullulan active packaging incorporating green AgNPs was successfully synthesised using pullulan powder with 5%, 15%, 15% and 20% concentrations (w/v). Similarly, the green AgNPs were incorporated at rates of 0.5%, 1% and 2% with respect to the total concentration of the filmogen materials.

Appearance and colour of pullulan active packaging

Pullulan is increasingly being used to synthesise edible films in the food and meat industries because of its unique film-forming characteristics for prolonged preservation (Liu *et al*., 2019; Morsy *et al*., 2014). The surface qualities and colour of pullulan edible films are presented in Figure 1.

Edible films synthesised from pullulan at a concentration of 5% (w/v) were relatively homogeneous, smooth and equally absorbed contents compared with the films synthesised from pullulan at concentrations of 10% (thicker, rough and non-smooth surface with air bubbles), 15% (thicker, with uneven surface and borders) and 20% (insolubility of filmogen material, thicker and rough consistency) (Figure 1A). These changes were recorded after autoclaving the filmogen contents to destroy any microbial contamination in the gelatinised pullulan dispersion.

The incorporation of green AgNPs altered the appearance and colour of the edible pullulan films (Figure 1B). Pullulan active packaging, incorporating C-AgNPs (PF-C-AgNPs) or mixed AgNPs (PF-M-AgNPs), showed a darker appearance than the control packaging (PF-CTRL) and the active packaging incorporating pullulan-mediated AgNPs (PF-P-AgNPs), as shown in Figure 1B. The colour attributes of the edible pullulan



(a) Pullulan 5% (w/v)
(b) Pullulan 10% (w/v)
(c) Pullulan 15% (w/v)
(d) Pullulan 20% (w/v)

(a) Pullulan films without AgNPs (PF-CTRL)
(b) Pullulan films with curcumin AgNPs (PF-C-AgNPs)
(c) Pullulan films with pullulan mediated AgNPs (PF-P-AgNPs)
(d) Pullulan films with mixed AgNPs (PF-M-AgNPs)

Figure 1. (A) Pullulan edible films synthesised from various pullulan concentrations (aqueous; w/v). (B) Pullulan active packaging with 5% concentration (w/v) containing green silver nanoparticles (AgNPs).

films are listed in Table 1, presenting overall lightness (73.14 ± 16.57), redness (3.32 ± 5.11) and yellowness (8.35 ± 5.82) in a suitable range.

No significant differences ($p < 0.05$) were recorded between PF-CTRL and PF-P-AgNPs in terms of their colour attributes (transparency, redness, yellowness, chroma and chromatography). The results of our study are in good agreement with those of Bahrami *et al.* (2018), who described the reduction of transparency or lightness (L*) and increases of redness (a*) and yellowness (b*) of edible films prepared from hydroxypropyl methylcellulose/beeswax incorporating 2% AgNPs.

Table 1. General colour attributes of pullulan active packaging containing AgNPs.

	L*	a*	b*	ΔE
N	36	36	36	36
Mean	73.14	3.32	8.35	22.87
Std. Dev.	16.57	5.11	5.83	17.98
Minimum	39.27	–1.62	2.27	4.64
Maximum	89.61	12.90	18.88	57.64
Skewness	−0.828	0.632	0.423	0.725
Kurtosis	−0.827	−1.137	−1.378	−1.026

PF-C-AgNPs expressed significantly ($p < 0.05$) reduced transparency (47.16 ± 4.18), with significantly ($p < 0.05$) higher redness (10.77 ± 1.65), yellowness (15.93 ± 2.42), chroma (19.23 ± 2.92) and chromatographic character (50.74 ± 3.20) as compared to PF-CTRL, PF-P-AgNPs, whereas PF-M-AgNPs and PF-C-AgNPs reflected significantly higher ($p < 0.05$) 'hue' (67.21 ± 3.24; 55.94 ± 0.69), respectively (Table 2).

Antioxidant capacity of pullulan active packaging containing green AgNPs

The incorporation of green AgNPs (0.5%, 1% and 2 % v/v) into edible pullulan films (5% pullulan; w/v) was performed in this study to synthesise active packaging. The pullulan active packaging containing 2% green AgNPs exhibited tremendous antioxidant potential.

2-Thiobarbituric acid reactive substances (TBARS) assay

In this study, the impact of incorporating green AgNPs on the oxidative stability of pullulan active packaging was investigated during 14 days of storage at 25 ± 2°C. It was observed that the formation of aldehyde compounds, especially mg MDA/kg, in the active packaging increased with prolonged storage (Figure 2). The higher concentration of green AgNPs (2%) significantly ($p < 0.05$) reduced the formation of mg MDA/kg at 0, 7 and 14 days of storage compared with that for the pullulan active packaging incorporating 0.5% and 1% green AgNPs (Figure 2).

Lower concentrations of MDA were generally recorded on day 0 (0.5278 ± 0.3354) than on day 7 (0.8596 ± 0.6908) and day 14 (1.359 ± 0.5683) of storage (Figure 2).

Table 2. Comparative colour attributes of pullulan active packaging containing AgNPs.

	PF-CTRL	PF-C-AgNPs	PF-P-AgNPs	PF-M-AgNPs	*p*-value
L*	88.53 ± 0.73[a]	47.16 ± 4.18[c]	82.32 ± 4.27[a]	74.54 ± 5.77[b]	0.001
a*	−1.44 ± 0.10[c]	10.77 ± 1.65[a]	−0.51 ± 0.82[c]	4.48 ± 2.0[b]	0.001
b*	2.73 ± 0.37[b]	15.93 ± 2.42[a]	4.52 ± 2.52[b]	10.22 ± 3.47[a]	0.000
Chroma	2.32 ± 0.43[b]	19.23 ± 2.92[a]	4.37 ± 2.65[b]	11.18 ± 3.96[a]	0.001
Hue	−61.81 ± 3.02[c]	55.94 ± 0.69[a]	−17.09 ± 72.58[b]	67.21 ± 3.24[a]	0.001
ΔE	5.80 ± 0.77[c]	50.74 ± 3.20[a]	12.29 ± 4.89[c]	22.67 ± 6.51[b]	0.001

*Within the rows, the means with different superscripts are significantly different ($p < 0.05$).
*Means were calculated in triplicate ± Std. Dev (n = 3).
*PF-CTRL = controlled, without AgNPs; PF-C-AgNPs = pullulan active packaging containing curcumin AgNPs; PF-P-AgNPs = pullulan active packaging containing pullulan-mediated AgNPs; PF-M-AgNPs = pullulan active packaging containing mixed AgNPs.

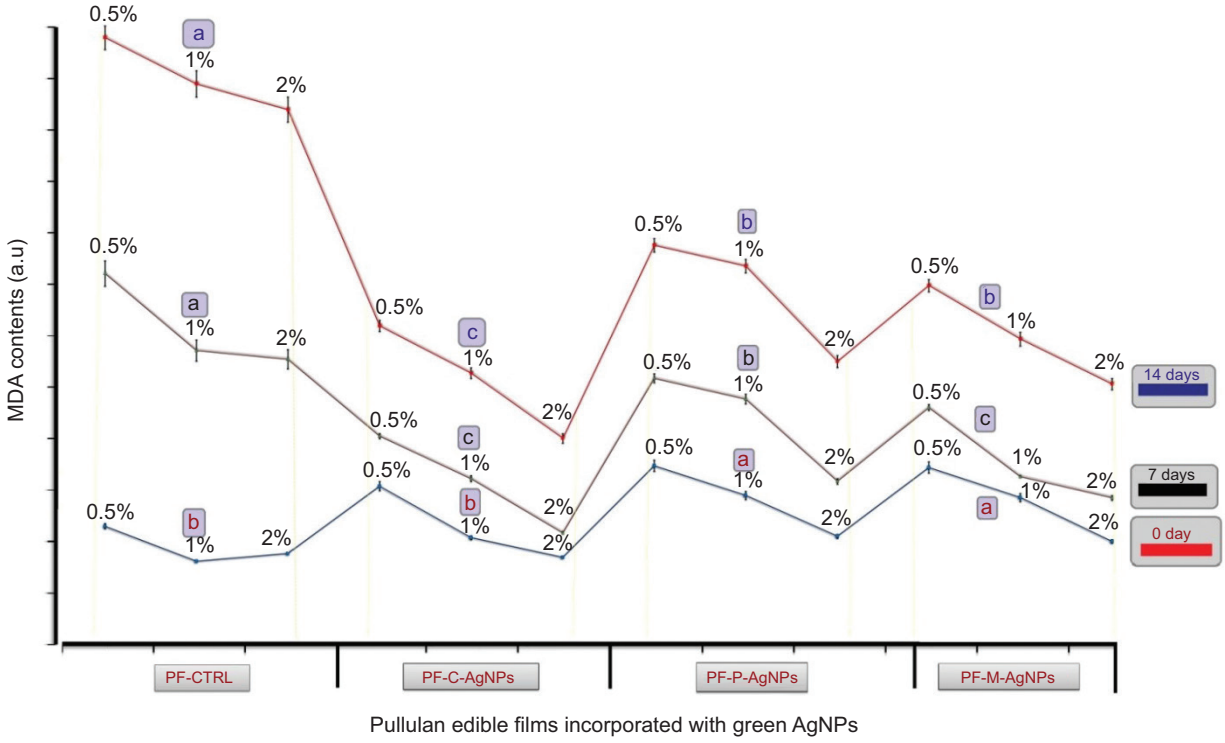


Figure 2. Oxidative stability of pullulan active packaging containing different AgNP concentrations along with impact of storage period on melanodialdehyde contents (mg MDA/ kg).

Similarly, the PF-C-AgNP active packaging was associated with significantly better ($p < 0.05$) oxidative stability, with the lowest mg MDA/kg value (0.5821 ± 0.3085), followed by PF-M-AgNPs (0.7204 ± 0.3786), PF-P-AgNPs (0.8552 ± 0.3270) and then PF-CTRL (1.4938 ± 0.9274). These results suggest that the incorporation of green C-AgNPs can reduce the likelihood of periodic oxidation of edible pullulan films, with lower concentrations of aldehydes, especially MDA, upon storage at 25°C for 14 days. The results of our study are in good agreement with the findings of Dai *et al.* (2023), Liu *et al.* (2015) and Spatareanu *et al.* (2014), who showed elevated MDA contents of edible films containing tea polyphenol nanoparticles with an increasing storage period (up to 6 weeks) at room temperature.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The scavenging of DPPH free radicals was used here to represent the antioxidant capacity of the active packaging, as shown in Table 3. PF-C-AgNP active packaging

Table 3. Anti-oxidant capacity of pullulan active packaging containing 2% green AgNPs.

p-value	Storage days	PF-CTRL	PF-C-AgNPs	PF-P-AgNPs	PF-M-AgNPs
			Mg MDA/ Kg		
0.002	0	0.21 ± 0.167^{Ab}	0.175 ± 0.053^{Ab}	0.36 ± 0.037^{a}	0.32 ± 0.12^{Aa}
0.000	7th	1.73 ± 0.11^{a}	0.22 ± 0.068^{c}	0.49 ± 0.27^{b}	0.39 ± 0.11^{bc}
0.001	14th	2.23 ± 0.025^{a}	0.845 ± 0.045^{c}	1.07 ± 0.25^{b}	1.02 ± 0.095^{b}
			DPPH radical scavenging activity %		
0.001	0	36.37 ± 7.33^{b}	60.43 ± 2.20^{a}	52.87 ± 0.42^{a}	32.83 ± 3.50^{b}
0.022	7th	32.68 ± 8.22^{a}	47.68 ± 6.14^{Aa}	42.67 ± 0.98^{a}	28.23 ± 9.78^{ab}
0.000	14th	24.30 ± 2.86^{c}	47.57 ± 0.40^{a}	36.19 ± 1.45^{b}	22.37 ± 0.55^{c}
			ABTS radical scavenging activity %		
0.001	0	60.42 ± 0.76^{c}	78.60 ± 1.80^{a}	71.91 ± 0.83^{b}	54.80 ± 1.29^{d}
0.000	7th	46.51 ± 0.33^{c}	65.32 ± 2.45^{a}	60.85 ± 0.45^{b}	40.87 ± 0.50^{d}
0.000	14th	22.55 ± 5.43^{c}	52.41 ± 3.30^{a}	41.54 ± 2.43^{b}	37.87 ± 1.86^{b}

*Means ± Std. Dev. were compared at $p < 0.05$ (n = 3).
*The values with different superscripts are significantly different ($p < 0.05$) within the same rows.
*PF-CTRL = controlled positive; PF-C-AgNPs = pullulan active packaging with curcumin AgNPs; PF-P-AgNPs = pullulan active packaging with pullulan-mediated AgNPs; PF-M-AgNPs = pullulan active packaging with mixed AgNPs.

exhibited higher free radical activity at days 0, 7 and 14 of storage compared with the control (PF-CTRL), PF-P-AgNPs and PF-M-AgNPs (Table 3). The incorporation of green AgNPs (C-AgNPs, P-AgNPs) was associated with significantly ($p < 0.05$) better free radical scavenging activity at day 0 (60.43%, 52.87%) and day 14 (47.57%, 36.19%) of storage than the control (PF-CTRL) and PF-M-AgNPs (Table 3). Meanwhile, no significant differences ($p < 0.05$) in the rates of DPPH free radical scavenging activity were observed for the pullulan active packaging at day 7 of storage at room temperature (25 ± 2°C).

2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay

The free radical scavenging ability of PF-C-AgNPs incorporating green C-AgNPs was significantly better ($p < 0.05$) than that of the other materials at day 0 (78.60%), day 7 (65.32%) and day 14 (52.41%) of storage, followed by the ability of PF-P-AgNPs incorporating P-AgNPs, control pullulan film (PF-CTRL) and PF-M-AgNPs incorporating mixed AgNPs (Table 3).

In addition, a gradual decrease in the scavenging activity (ABTS assay) of edible pullulan films was noted with prolongation of the storage period. The same pattern was recorded in the DPPH free radical scavenging capacity of edible pullulan films, confirming the improved antioxidant efficacy of green C-AgNPs and P-AgNPs. Similar reports have been presented by Gehrcke *et al*. (2022) and Ferreira *et al*. (2014) with respect to the equivalence of DPPH and ABTS radical scavenging abilities of edible chitosan films incorporating grape pomace extract. Depending on the colour, appearance and antioxidant capacities, the physiochemical characteristics of pullulan active packaging were subjected to further assessment based on samples of active packaging containing 5% pullulan (w/v) and 2% green AgNPs.

Transparency of pullulan active packaging

The transparency of pullulan active packaging decreased with the incorporation of green AgNPs in the following order: PF-CTRL > PF-P-AgNPs > PF-M-AgNPs > PF-C-AgNPs (Figure 3). A significant difference was recorded ($p < 0.05$) in terms of the transparency of pullulan active packaging measured at 24–25°C and 55%–60% relative humidity (Table 4). Notably, PF-CTRL and PF-P-AgNPs were relatively transparent compared with the PF-M-AgNP and PF-C-AgNP active packaging. The incorporation of C-AgNPs into edible pullulan films not only reduced the film transparency but also provided the active packaging with a darker appearance (Figure 3b). Similar findings have been reported by Khan *et al*. (2024) and Khalaf *et al*. (2013), who observed the enhanced optical density of edible pullulan films incorporating 2% essential oils and AgNPs.

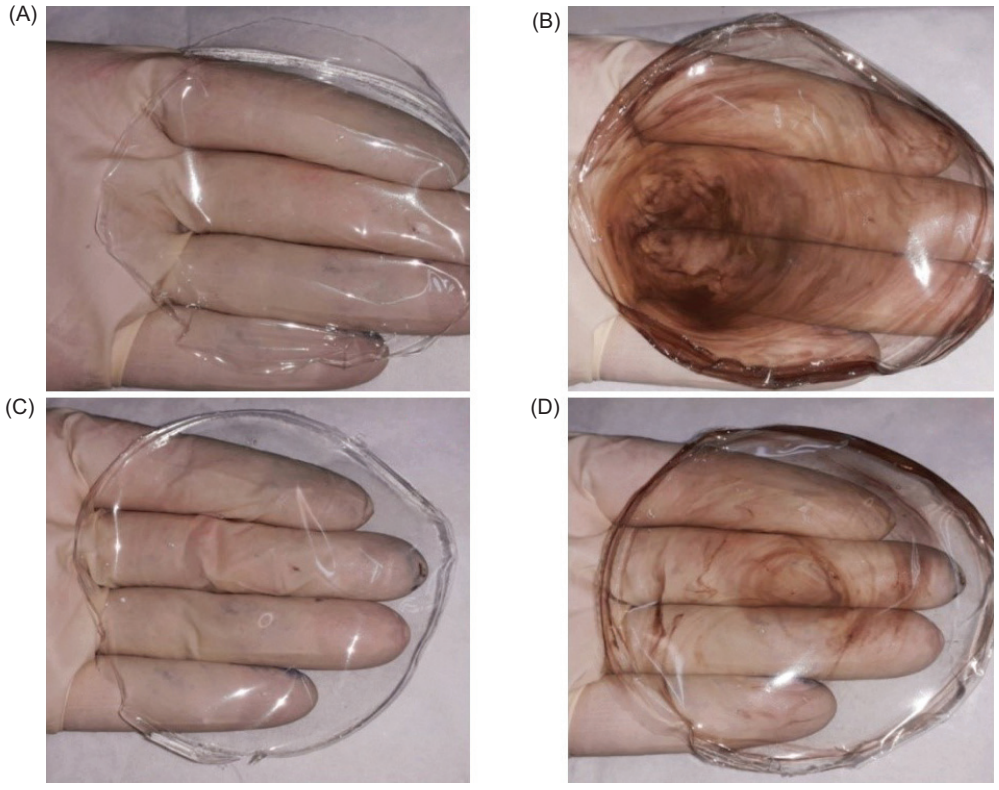


Figure 3. Transparency expressions. (A) Pullulan film with no AgNPs (PF-CTRL), (B) Pullulan active packaging with C-AgNPs (PF-C-AgNPs), (C) Pullulan active packaging with P-AgNPs (PF-P-AgNPs), (D) Pullulan active packaging with mixed AgNPs (PF-M-AgNPs).

Table 4. pH and transparency of pullulan active packaging containing green AgNPs.

pH (p-value = 0.001)				Transparency (p- value = 0.001)			
PF-CTRL	**PF-C-AgNPs**	**PF-P-AgNPs**	**PF-M-AgNPs**	**PF-CTRL**	**PF-C-AgNPs**	**PF-P-AgNPs**	**PF-M-AgNPs**
7.15 ± 0.06^{b}	6.18 ± 0.06^{d}	7.14 ± 0.13^{c}	8.28 ± 0.44^{a}	4.97 ± 0.87^{a}	0.49 ± 0.13^{d}	2.79 ± 0.27^{b}	1.65 ± 0.14^{c}

*Means were calculated in triplicate ± Std. Dev (n = 3).
*Means were significantly different with different superscripts ($p < 0.05$).
*PFCTRL = controlled, without AgNPs; PF-C-AgNPs = pullulan active packaging containing curcumin AgNPs; PF-P-AgNPs = pullulan active packaging containing pullulan-mediated AgNPs; PF-M-AgNPs = pullulan active packaging containing mixed AgNPs.

pH of pullulan active packaging containing green AgNPs

It was noted that the PF-C-AgNP active packaging was associated with a significantly ($p < 0.05$) lower pH (6.18 ± 0.06) than the other materials: PF-P-AgNPs (7.14 ± 0.13), PF-CTRL (7.15 ± 0.06) and then PF-M-AgNPs (8.28 ± 0.44; Table 4). Our results suggest that the improved physiochemical properties and modification of pullulan active packaging involving the incorporation of green AgNPs, especially C-AgNPs, can provide enhanced outcomes (Hassan and Cutter, 2020; Singh *et al*., 2008; Šuput *et al*., 2016).

D/thickness of pullulan active packaging

The incorporation of green AgNPs reduced the D of pullulan active packaging from 0.328mm (PF-CTRL) to 0.302mm (PF-P-AgNPs). Although certain physical

changes in the colour, transparency and appearance of the packaging were recorded in association with the incorporation of these nanoparticles, no significant differences ($p < 0.05$) in D/thickness were observed (Table 5). The results of our study are similar to the findings reported by Gehrcke *et al*. (2022) and Khalaf *et al*. (2013) regarding changes in the D or thickness of edible pullulan films after the inclusion of AgNPs. Bahrami *et al*. (2018) also found that the incorporation of 2% AgNPs significantly improved the D or thickness of edible packaging because of the improved solid contents.

Pullulan active packaging density

The details of the density (g/cm^3) of the different types of active packaging are given in Table 5. The results reveal that the inclusion of green AgNPs significantly ($p < 0.05$) altered the density. Specifically, the density of PF-P-AgNPs (0.58 ± 0.011) was significantly ($p < 0.05$) higher than that of PF-CTRL (0.46 ± 0.011), PF-C-AgNP (0.43 ± 0.010) and PF-M-AgNP active packaging (0.42 ± 0.010). These results demonstrated that the incorporation of P-AgNPs elevated the density of edible pullulan films, whereas C-AgNPs maintained the density in PF-C-AgNP and PF-M-AgNP active packaging (Table 5).

The results of our study are analogous to the findings of Wang *et al*. (2015), who confirmed that the inclusion of *Lycium barbarum* fruit extract altered the density of edible chitosan active coatings at various concentrations. In our study, pullulan active packaging with a higher density (PF-P-AgNPs) exhibited a significantly ($p < 0.05$) lower thickness. Similarly, Gniewosz *et al*. (2022) and Saberi *et al*. (2016) demonstrated that increasing concentrations of glycerol in edible coatings of pea starch not only reduced the density but also enhanced the D and moisture content of pea starch films.

Solubility percentage

No significant differences ($p < 0.05$) in the water solubility of the pullulan active packaging were identified, but its numerical value decreased in the following order: PF-CTRL > PF-M-AgNPs > PF-C-AgNPs > PF-P-AgNPs (Table 5). All types of active packaging showed high water solubility (i.e. > 86%), which is in close agreement with the studies by Kücüközet and Uslu (2018), who confirmed a solubility range of 71.06 ± 1.69% to 95.98 ± 2.27% for sodium caseinate edible coatings, and by Wang *et al*. (2015), who described that the water solubility of chitosan edible films was in a similar range.

Moisture content/moisture sorption percentage

The PF-M-AgNP film, incorporating mixed AgNPs, exhibited significantly higher ($p < 0.05$) moisture content than did PF-C-AgNPs, PF-CTRL and PF-P-AgNPs. Furthermore, the moisture content

Table 5. Physicochemical characteristics of pullulan active packaging.

Pullulan active packaging	Film depth (mm)	Film volume (cm^3)	Film density (g/cm^3)	Film area (cm^2)
PF-CTRL	0.328±0.147^{NS}	1.82±0.016^{b}	0.46± 0.01^{b}	5.25±0.005^{d}
PF-C-Ag NPs	0.328±0.118^{NS}	2.16±0.016^{a}	0.43± 0.010^{c}	6.43±0.037^{a}
PF-P-Ag NPs	0.302± 0.064^{NS}	1.73±0.009^{d}	0.58±0.011^{a}	6.02±0.033^{b}
PF-M-AgNPs	0.305± 0.071^{NS}	1.82±0.012^{c}	0.42±0.010^{c}	5.57± 0.035^{c}
p-value	0.929	0.001	0.001	0.001
	Moisture contents (%)	**Solubility (%)**	**Moisture sorption (%)**	
PF-CTRL	25.23±1.50^{b}	93.97±6.90^{NS}	2.56±0.11^{NS}	
PF-C-Ag NPs	29.78±2.67^{b}	90.72±6.72^{NS}	2.57±0.05^{NS}	
PF-P-Ag NPs	17.92±1.65^{b}	86.24±7.51^{NS}	2.63±0.037^{NS}	
PF-M-AgNPs	44.52± 11.26^{a}	93.67± 6.58^{NS}	2.55± 0.016^{NS}	
p-value	0.012	0.672	0.534	

*Means were calculated in triplicate ± Std. Dev (n = 3).
*Means with different superscripts were significantly different ($p < 0.05$) within the column.
*NS= Means were not significantly different ($p < 0.05$) within the column.
*PFCTRL = controlled, without AgNPs; PF-C-AgNPs = pullulan active packaging containing curcumin AgNPs; PF-P-AgNPs = pullulan active packaging containing pullulan-mediated AgNPs; PF-M-AgNPs = pullulan active packaging containing mixed AgNPs.

of PF-P-AgNPs (17.92 ±1.65) was lowest among the different types of pullulan active packaging, confirming our assertion that there is a negative correlation between density and moisture content, as discussed earlier (Table 5).

No significant differences ($p < 0.05$) in the moisture sorption percentages of pullulan active packaging were observed, although the PF-P-AgNP film was associated with higher values (2.63 ± 0.037) than PF-CTRL, PF-C-AgNPs and PF-M-AgNPs (Table 5).

Furthermore, the moisture sorption percentage (2.55 ± 0.016) and density (0.42 ± 0.010) of the PF-M-AgNP active packaging reached their lowest levels with increased moisture content (44.52 ± 11.26). The results of our study are similar to the findings in reports by Saberi *et al.* (2016) and Wang *et al.* (2015) with respect to there being negative correlations between the moisture content and the density of edible films incorporating glycerol and natural extract, respectively.

FT-IR spectroscopy

The results of the FT-IR spectral analysis of edible and active pullulan packaging, before and after the incorporation of green C-AgNPs and P-AgNPs, are provided in Figure 4. It was observed that the incorporated AgNPs exhibited almost the similar transmittance during FT-IR spectroscopy, along with some modifications in the involvement of functional groups, as highlighted in Figure 4. PF-M-AgNP active packaging, incorporating mixed AgNPs, generated more prominent and identical spectra compared with PF-C-AgNPs, PF-P-AgNPs and PF-CTRL; however, PF-C-AgNPs, PF-P-AgNPs and PF-CTRL exhibited dissimilar FT-IR spectra.

The PF-CTRL exhibited almost the same spectrum as reported by Khan *et al.* (2019b), reflecting that pullulan maintained not only its originality but also its chemical integrity during the formation of edible packaging (Pinto *et al.*, 2013). Pullulan active packaging was associated with FT-IR spectral peaks from the wavenumber

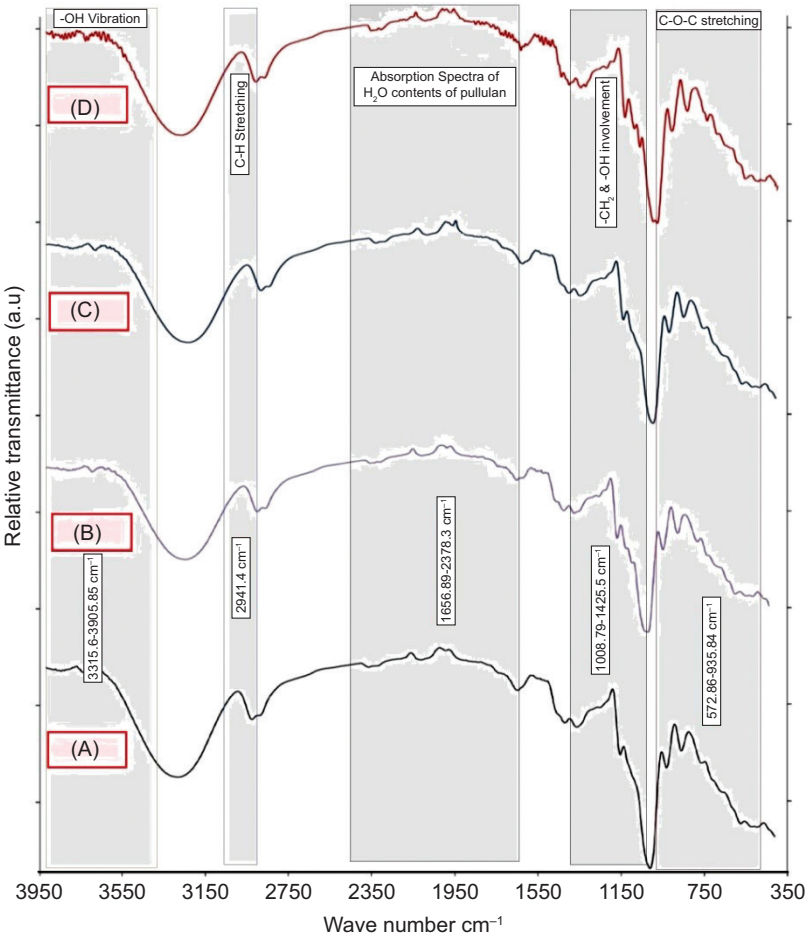


Figure 4. Fourier transform infrared spectra of pullulan active packaging with AgNPs: (A) PF-CTRL, (B) PF-C-AgNPs, (C) PF-P-AgNPs and (D) PF-M-AgNPs.

of 399.26 cm^{-1} to 3998.43 cm^{-1} (Figure 4). Seventeen prominent FT-IR spectral peaks were observed for each packaging type, with the exception of PF-M-AgNPs (Figure 4). The reflection of the FT-IR spectral peaks is provided in Table 6. Our FT-IR spectral results are in reasonable agreement with the findings in studies by Simões *et al*. (2024), Tang *et al*. (2024), Spatareanu *et al*. (2014) and Varaprasad *et al*. (2011) regarding the strong electrolytic behaviour and pullulan–AgNP network during the formation of active packaging.

Field emission scanning electron microscopy (FESEM)

The morphological monographs of pullulan active packaging by FESEM revealed that PF-CTRL exhibited a homogeneous surface morphology as compared to the active packaging incorporated with green AgNPs with some depressions on it (Figure 5 A). PF-C-AgNPs (Figure 5 B) and PF-P-AgNPs (Figure 5 C) reflected the through distribution of green AgNPs along with a stronger and smoother surface as compared to PF-CTRL and PF-M-AgNPs, which reflected 'cracks' on the surface (Figure 5 D).The results of our study are in agreement with those reported by Gehrcke *et al.* (2022), Shahhosseini (2023), Bahrami *et al.* (2018), Martelli *et al.* (2017), and Djerahov *et al.* (2016) for the enrichment of surface morphology and strength of the edible coatings with an efficient dispersion and absorption of nanocapsules, tragopogon graminifolius, nano silver, α-tocopherol and phayom wood extract, respectively.

Discussion

Pullulan films are considered to be strong, smooth, tasteless, odourless and colourless media that can be used as active packaging while exhibiting an improved capacity to act as a barrier to oil, gas and water (Khalaf *et al*., 2013; Trinetta and Cutter, 2016). It has been reported that edible pullulan films having homogeneous, smooth, shiny and clear surfaces exhibit greater strength and elasticity, along with being less permeable to oxygen (Khalaf *et al*., 2013). Furthermore, edible pullulan films synthesised from 5% pullulan concentration (w/v) had smoother and shinier surfaces, reflecting good interaction between the filmogen materials and green AgNPs (Khalaf *et al*., 2013; Khan *et al*., 2024). It has also been reported that the incorporation of C-AgNPs not only decreases the lightness (L^*) of pullulan active packaging but also can enhance its redness (a^*) and yellowness (b^*; Bahrami *et al*., 2018). It is evident that AgNPs incorporated into edible pullulan films reduce transparency, with the films being increasingly red and yellow because of resistance to the

Table 6. Fourier transform infrared spectral peaks of pullulan active packaging.

FT-IR spectral peaks	Pullulan active packaging					Functional group Involvement
	Wave No.	PF-CTRL	PF-C-AgNPs	PF-P-AgNPs	PF-M-AgNPs	
1st	572.86 cm^{-1}	Present	Present	Present	Present	*C – O – C* linkage at *α-(1→4)* point of pullulan
2nd	766.32 cm^{-1}	Present	Present	Present	Present	
3rd	856.39 cm^{-1}	Present	Present	Present	Present	
4th	935.84 cm^{-1}	Present	Present	Present	Present	
5th	1008.79 cm^{-1}	Present	Present	Present	Present	
6th	1082.1 cm^{-1}	Absent	Present	Absent	Present	Involvement *of – CH_2* and *OH* functional groups
7th	1109.1 cm^{-1}	Absent	Present	Absent	Present	
8th	1151.6 cm^{-1}	Absent	Present	Absent	Present	
9th	1369.5 cm^{-1}	Present	Present	Present	Present	
10th	1425.5 cm^{-1}	Present	Present	Present	Present	
11th	1656.89 cm^{-1}	Present	Present	Present	Present	Absorption spectra of pullulan water contents
12th	2137.3 cm^{-1}	Present	Present	Present	Present	
13th	2378.3 cm^{-1}	Present	Present	Present	Present	
14th	2941.4 cm^{-1}	Present	Present	Present	Present	Stretching of *C – H* functional group of pullulan
15th	3315.6 cm^{-1}	Present	Present	Present	Present	Vibration of *– OH* functional group of pullulan
16th	3755.4 cm^{-1}	Present	Present	Present	Present	
17th	3905.85 cm^{-1}	Absent	Absent	Absent	Present	

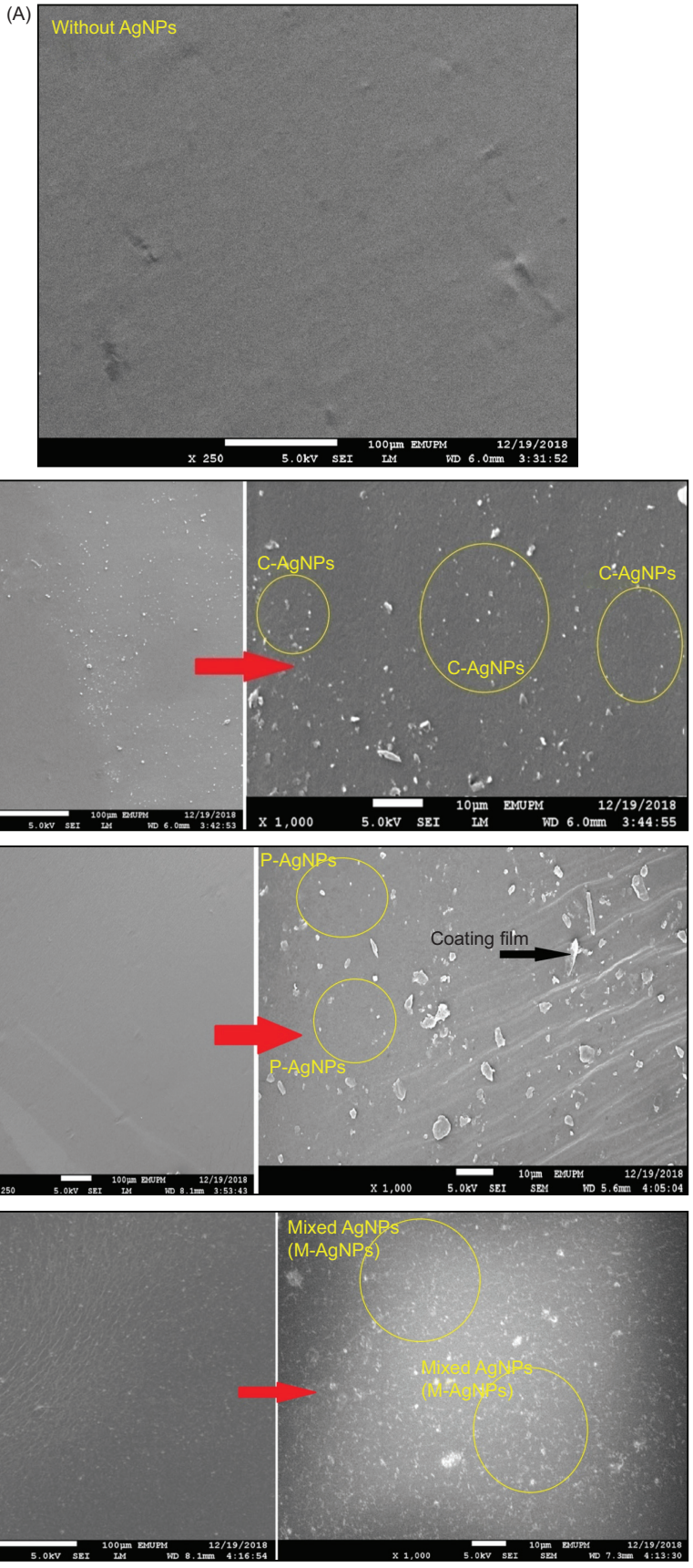


Figure 5. Field emission scanning electron microscopy micrographs of pullulan edible films containing green AgNPs: (A) PF-CTRL, (B) PF-C-AgNPs, (C) PF-P-AgNPs and (D) PF-M-AgNPs.

passage of light through these films (Bahrami *et al*., 2018; Khan *et al*., 2024). In addition, the opacity of edible pullulan films is mainly associated with the type and nature of the AgNPs incorporated into them (Khalaf *et al*., 2013; Simões *et al*., 2024).

The mechanical strength of edible pullulan films can be amplified by incorporating AgNPs, which also leads to a change in their colour or appearance (Bahrami *et al*., 2018; Khan *et al*., 2024). This factor improves the light resistance and mechanical strength of pullulan active packaging declining the utilisation of clear edible films in the food industry. Our study proved that the incorporation of curcumin-mediated AgNPs not only maintained the surface and texture of pullulan active packaging but also altered its colour characteristics (Tables 1, 2). The mechanical strength of active packaging determines its oxidative stability under conditions of higher temperature and humidity because of the supplementary sugar content in their medium (Dai *et al*., 2023; Hassan and Cutter, 2020; Singh, 2015; Trinetta and Cutter, 2016). The repeating maltotriose units of pullulan with and linkages induce periodic oxidation, resulting in the formation of various dialdehydes during storage (Hassan and Cutter, 2020; Trinetta and Cutter, 2016). These dialdehydes are created during the oxidative reduction and degradation of long-chain pullulan molecules (Spatareanu *et al*., 2014). As used in this study, measurement of the level of mg MDA/kg (TBARS assay provides an overview of the oxidative and thermal stability of pullulan active packaging during storage, whereas determining its free radical scavenging ability (DPPH and ABTS assays) provides an indication of its antioxidant capacity (Dai *et al*., 2023; Hassan and Cutter, 2020; Martelli *et al*., 2017; Trinetta and Cutter, 2016).

Within pullulan active packaging, chemically semirigid compounds, namely, hemi-acetals and hydrates, form the main detectable aldehydes because of the moisture content of this packaging (Khan *et al*., 2024). Moreover, the oxidative mechanism in active packaging is directly associated with their 'oxygen barrier' capacity, which gradually decreases with prolongation of the storage period and with increased MDA concentration (Liu *et al*., 2015). Similarly, it was reported that the DPPH free radical scavenging activity of polysaccharide active packaging was enhanced with the incorporation of nanoparticles, α-tocopherol and tea polyphenols, as also presented in our study (Liu *et al*., 2015; Martelli *et al*., 2017). It is believed that the free functional groups of polysaccharide active packaging fetch free hydrogen ions (H^+) from DPPH methanolic solution, with the formation of relatively stabilised 'macromolecules' expressing their scavenging power or activity (Ferriera *et al*., 2014; Khan *et al*., 2024; Liu *et al*., 2015; Martelli *et al*., 2017). The higher scavenging activity of the active packaging upon the incorporation of any antioxidant material (AgNPs, essential oils, etc.) reflects a better antioxidant capacity (Ferriera *et al*., 2014; Šuput *et al*., 2016).

The results of the current study demonstrate that the incorporation of green-synthesised AgNPs (C-AgNPs, P-AgNPs) into pullulan active packaging can act as a substitute for any synthetic 'antioxidant' in order to preserve food items via improved antioxidant capacity. The results of the TBARS, DPPH and ABTS assays on pullulan active packaging in this study confirmed that the incorporation of 2% AgNPs, especially C-AgNPs, can reduce the likelihood of oxidative rancidity and can sustain the antioxidant ability by creating stronger electrostatic interactions between AgNPs and the pullulan matrix. These interactions in turn increase the strength and oxygen barrier function compared with those of edible pullulan films (PF-CTRL) (Bahrami *et al*., 2018; Khan *et al*., 2024; Trinetta and Cutter, 2016; Trinetta *et al*., 2011).

Transparent active packaging would undoubtedly be more acceptable to consumers than darker active packaging. However, darker pullulan active packaging is more light-resistant with better oxygen barrier function because of the strong interaction between its filmogen contents and active compounds (Khalaf *et al*., 2013; Khan *et al*., 2024; Trinetta and Cutter, 2016). The resultant lower transparency of active packaging incorporating green C-AgNPs (PF-C-AgNPs) coincides with improved antioxidant capacity. With respect to the pH of pullulan active packaging, the appropriate range is considered to be 5 to 7, somewhat acidic to neutral (Gniewosz *et al*., 2022; Liu *et al*., 2019; Oğuzhan and Yangılar, 2013; Shahhossaini, 2023). A suitable and active biopolymer such as pullulan can respond to external or environmental factors including pH, temperature and relative humidity during film formation (Farris *et al*., 2014). The viscosity of pullulan aqueous solution is sustained by these factors during the synthesis process; otherwise, it can be decomposed either by the environment or by microbes (Farris *et al*., 2014; Han, 2014; Han *et al*., 2015; Singh, 2015). It appears that the incorporation of C-AgNPs into pullulan filmogen aqueous solution has the ability to lower its pH. In contrast, the incorporation of P-AgNPs significantly raised the pH of the solution. Moreover, the addition of metal nanoparticles (Ag, Au and ZnO) into pullulan active packaging can not only alter the physiochemical properties but also affect the efficacy of mixing and the absorption of solutes during the synthesis process (Farris *et al*., 2014; Šuput *et al*., 2016; Trinetta and Cutter, 2016).

The mechanical strength and water permeability of the pullulan active packaging are the major properties exhibited by such packaging, which are duly affected by the incorporation of nanomaterials (Gniewosz *et al*., 2022; Hassan and Cutter, 2020; Khalaf *et al*., 2013; Liu *et al*., 2019;

Trinetta and Cutter, 2016; Wang *et al*., 2017). The changes in the D/thickness of pullulan active packaging (incorporating AgNPs) as identified in this study can be defined by the 'swelling index', which is strongly associated with their mechanical strength and flexibility (Gehrcke *et al*., 2022; Tang *et al*., 2024). Owing to the interaction between the pullulan matrix and the incorporated nanoparticles (Ag, Au and ZnO), the availability of OHs in the aqueous solution of pullulan decreases the interaction between these groups and water in the environment (Wang *et al*., 2015; Wang *et al*., 2017). The resultant interaction can not only modify the density but also change the water retention abilities in the form of lower or higher moisture content (Othman *et al*., 2017; Trinetta and Cutter, 2016; Wang *et al*., 2015). This was also observed in our study in that the PF-M-AgNP active packaging had a significantly lower ($p < 0.05$) density reflecting increased moisture content. This higher moisture content causes serious handling and packaging issues during food preservation because of the lower mechanical integrity of the material (Trinetta and Cutter, 2016; Trinetta *et al*., 2011). In this context, it seems that there may be a negative correlation between the density-thickness and the density-moisture content.

The solubility of pullulan active packaging can potentially explain its flexibility and uniform distribution of antioxidants, which are key features of its active packaging functions (Khan *et al*., 2019a; Noori *et al*., 2018). Generally, pullulan exhibits high water solubility because of its hydrophilicity; this is a rather necessary characteristic for food packaging because it helps maintain the quality of the packed product and pullulan simultaneously (Gniewosz *et al*., 2022; Liu *et al*., 2019; Wang *et al*., 2017). Hence, the moisture content and moisture sorption of active packaging detect the quality parameters (Gniewosz *et al*., 2022; Liu *et al*., 2019; Trinetta *et al*., 2011). Saberi *et al*. (2016) reported that these quality attributes (moisture content, moisture sorption percentage) are mainly governed by glycerol, included as a plasticiser during the synthesis process, which develops an active collaborative matrix through OHs and hydrogen bonding. This collaborative matrix between glycerol and the filmogen contents retains moisture from the atmosphere and interacts with the relative humidity. It has been proven that lower moisture content and lower moisture sorption percentage improve permeability to water vapour and vice versa (Liu *et al*., 2019; Wang *et al*., 2015; Wang *et al*., 2017). In our study, a similar pattern was noticed in that the PF-P-AgNP active packaging exhibited higher moisture sorption (2.63 ± 0.037) and density (0.58 ± 0.011) with lower moisture content (17.92 ± 1.65) and D (0.302 ± 0.064). Here, it was also noted that the pullulan active packaging, with its lower moisture content, exhibited higher moisture sorption with enhanced water-holding capacity and thus showed a greater capacity to retain water. This trend is a primary factor affecting the quality of preserved food treated with pullulan active packaging under refrigerated storage (Liu *et al*., 2019; Tang *et al*., 2024; Trinetta and Cutter, 2016).

The involvement of the active components of a polysaccharide and the incorporated substance can be confirmed by FT-IR, which provides a comprehensive overview of their interactions during edible film formation (Djerahov *et al*., 2016; Simões *et al*., 2024; Varaprasad *et al*., 2011). The peak transmittance of FT-IR spectroscopy in the wavenumber region below 1000 cm^{-1} reflects the stretching of a C – O – C linkage at the identical α-(1→4) region of pullulan (Dewan and Islam, 2024; Spatareanu *et al*., 2014). Similarly, the spectral peaks around 1458 cm^{-1} indicate the vibration, bending and involvement of $–CH_2$ and –OH functional groups (Spatareanu *et al*., 2014; Varaprasad *et al*., 2011). In addition, the spectral peaks of pullulan active packaging around 1656 cm^{-1} are because of the absorption of water contents, but the stretching of the C–H functional groups of pullulan is represented by spectral peaks around 2918 cm^{-1} (Djerahov *et al*., 2016). Moreover, FT-IR spectral peaks at the wavenumber region of 3000–3600 cm^{-1} and above reveal the involvement and vibration of the –OH functional groups of pullulan (Nady and Kandil, 2018; Spatareanu *et al*., 2014; Varaprasad *et al*., 2011). It was observed in our study that the green AgNPs incorporated into the pullulan active packaging created a stronger 'nanocomposite' with the matrix of pullulan films via electrostatic interaction, which maintained the surface integrity during scanning electron microscopy. Moreover, the surface integrity promotes the mechanical strength and stability of pullulan active packaging during its handling, application and storage (Bahrami *et al*., 2018; Trinetta and Cutter, 2016). The distribution of AgNPs in the biopolymer matrix (pullulan) has already been reported in many studies and shown to reflect the conjugation between the pullulan filmogen matrix and nanoparticles (Bahrami *et al*., 2018). This prevents the active material (AgNPs) from being freely distributed in the pullulan fibre network, providing stiffness and water resistance to the active packaging (Bahrami *et al*., 2018; Gehrcke *et al*., 2022).

The modern trend of using pullulan active packaging in real food systems in order to preserve fruit, vegetables and meat products has been promoted by the consumer-driven shift towards biodegradable active packaging (Gehrcke *et al*., 2022; Khan *et al*., 2024). It has been reported that biodegradable active packaging (including that containing pullulan) incorporating nanoparticles, essential oils and natural nut extracts can remarkably delay 'lipid oxidation' in meat products in refrigerated storage with a cleaner label appeal

(Gómez-Estaca *et al*., 2014; Khan *et al*., 2022; Khan *et al*., 2024). This delay is reportedly governed by the moisture sorption, surface integrity and antioxidant capacity of pullulan active packaging incorporating AgNPs (Khan *et al*., 2022; Trinetta and Cutter, 2016). The pullulan active packaging developed in the current study was shown to exhibit good surface integrity, antioxidant capacity and moisture resistance, making it suitable as a material for packaging food items. Similarly, the composite and multilayered criss-cross surface arrangement of pullulan–xanthan gum–AgNP packaging can minimise the formation of mould in dairy products because of it exerting superior effects as a moisture barrier, as reported by Wu *et al*. (2019). These advances provide convincing examples of the growing importance of materials science for the modern-day food packaging industry. Nonetheless, for pullulan active packaging to receive approval from regulatory authorities and acceptance from consumers and industry, it needs to pass toxicological assessments and meet demands in terms of utility, scalability and cost-efficiency (Huang *et al*., 2019; Jafarzadeh *et al*., 2021; Sharma *et al*., 2020).

Conclusions

The physicochemical characteristics of pullulan active packaging were shown to be affected by the incorporation of AgNPs (C-AgNPs, P-AgNPs). The oxidative stability (mg MDA/kg) and antioxidant capacity (DPPH/ABTS free radical scavenging) of pullulan active packaging incorporating C-AgNPs (PF-C-AgNPs) were maintained during storage for 14 days at 24–25°C compared with those of PF-CTRL, PF-P-AgNPs and PF-M-AgNPs. Furthermore, the incorporation of C-AgNPs significantly minimised ($p < 0.05$) the film transparency, pH and density, while its mechanical strength was maintained. Interestingly, the incorporation of P-AgNPs not only influenced the colour and appearance of edible pullulan films but also significantly ($p < 0.05$) enhanced the density (0.58 ± 0.011) and reduced the moisture content (17.92 ± 1.65) of the pullulan active packaging (PF-P-AgNPs). The electrolytic behaviour and formation of a pullulan–AgNP network were also found to be similar in PF-CTRL and PF-P-AgNPs, as confirmed by FTIR spectroscopy.

In conclusion, this study revealed that the incorporated AgNPs can establish a remarkable networked matrix with pullulan films, resulting in improvements in physiochemical characteristics and antioxidant potential. These properties mean that pullulan active packaging has tremendous potential to prolong the shelf life and quality of various food products during refrigerated storage.

Limitations and Future Work

A bottom-up approach (chemical for C-AgNPs, biological for P-AgNPs) was used to synthesise AgNPs more securely, easily and cost-effectively (Khan *et al*., 2019b; Velidandi *et al*., 2020). Based on food safety considerations, the AgNPs were markedly reduced in size (i.e. from 12.6 nm to 6.02 nm) (Khan *et al*., 2019a; Khan *et al*., 2019b) to avoid any adverse impact on pullulan active packaging applications (Khan *et al*., 2024). Nevertheless, the toxicity of nanoparticles has been a leading concern for scientists, so a green bottom-up approach is valuable as a way of minimising the health risks associated with human consumption. Our study was conducted as a 'pilot project' at a temperature of 24–25°C, at a humidity of 55%–80% and with laboratory-grade equipment for the fabrication of pullulan active packaging. This can be considered the main limitation of the current study. Finally, the performance of real-time assays of the toxicity of AgNPs for human consumption (apoptosis assay, proliferation assay, necrosis assay, etc.) and under industry-based conditions is advisable to determine the commercial feasibility of pullulan active packaging incorporating green AgNPs.

Author Contributions

Suriya Kumari Ramiah and Muhammad Jamshed Khan did conceptualization, methodology, and validation. Muhammad Jamshed Khan, Suriya Kumari Ramiah and Kamyar Shameli did formal analysis. Suriya Kumari Ramiah, Muhammad Jamshed Khan and Muhammad Tariq Navid looked into investigation. Suriya Kumari Ramiah and Muhammad Jamshed Khan carried out data curation. Suriya Kumari Ramiah, Muhammad Jamshed Khan, Awis Qurni Shazili and Muhammad Tariq Navid were responsible for writing–review and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript.

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