



Original article

Selenium nanoparticles–enhanced potato starch film for active food packaging applicationBongekile K. Ndwandwe,¹  Soraya P. Malinga,² Eugenie Kayitesi³ & Bhekisisa C. Dlamini^{1*} ¹ Department of Biotechnology and Food Technology, University of Johannesburg, Doornfontein, South Africa² Department of Chemical Sciences, University of Johannesburg, Doornfontein, South Africa³ Department of Consumer and Food Sciences, University of Pretoria, Hatfield, South Africa

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Summary This work developed an active selenium nanoparticles-based potato starch film. The incorporation of selenium nanoparticles (SeNPs) improved the microstructure, physical and biological properties of the nanocomposite film. Scanning electron microscopy (SEM) showed a slight increase in surface roughness and heterogeneity of nanocomposite film. Addition of SeNPs resulted in an improvement in film thickness and density from 0.02 ± 0.01 to 0.04 ± 0.00 mm and 1.01 ± 0.12 to 1.31 ± 0.03 g cm⁻³, respectively, while water content, film solubility, swelling degree as well as water vapour transmission rate decreased. Integration of SeNPs into potato starch film caused a significant change ($P < 0.05$) of colour to red (a*) and yellow (b*). The tensile strength also improved with addition of SeNPs from 3.42 to 9.86 MPa. The presence of SeNPs in the potato starch film enhanced its antioxidant and antimicrobial activity. The overall migration and specific migration were within acceptable levels as stipulated in the EU regulations. The findings of this study present an alternative biodegradable biopolymer material that can be used as active food packaging material in replacement of nonbiodegradable synthetic polymer material.

Keywords active food packaging, antimicrobial activity, antioxidant activity, nanocomposite film, selenium nanoparticles.

Introduction

Microbiological safety and quality of food is generally achieved through prevention of contamination during food processing or reduction of food contaminants to acceptable levels using thermal methods and/ or addition of chemicals (Le *et al.*, 2021; Glicerina *et al.*, 2021; Nizam *et al.*, 2021; Wu *et al.*, 2021). However, preservation approaches that are more reliant on the addition of chemicals in food as preservatives may have detrimental effects on human health such as neurological damage, hypersensitivity, allergy and cancer (de Souza *et al.*, 2020; Le *et al.*, 2021; Mousavi *et al.*, 2021). Therefore, there is a need for finding alternative and effective food preservation methods.

Active food packaging effectively preserves or enhances the quality of packaged food by exhibiting active functions like prevention of moisture ingress and displaying antioxidant and antimicrobial activities (Schumann & Schmid, 2018; Yu *et al.*, 2019; Jamróz *et al.*, 2019a). Lately, research has proven that the addition of low quantities of nanoparticles to biopolymers improves their physico-chemical properties, enabling

the use of polymers in numerous application particularly in food packaging (Jafarzadeh *et al.*, 2017). Among the natural biopolymers, starch has been widely used to develop food packaging because it is regarded as economical and always available (Chaurasia & Lal, 2016; Feng *et al.*, 2018; Guz & Bernal, 2021). The inclusion of nanoparticles directly into the starch biopolymer augments its mechanical and barrier characteristics, and it can also improve food shelf-life by exhibiting antioxidant and antimicrobial properties (Peighamardoust *et al.*, 2019; Neda *et al.*, 2021). Different nanoparticles such as AgNPs (Mathew *et al.*, 2019; Guz & Bernal, 2021; Saravanakumar *et al.*, 2021), ZnO (Jayakumar *et al.*, 2019), SiO₂ (Zhang *et al.*, 2018), MgO (Negar *et al.*, 2021) have been studied as additives for improving mechanical, antimicrobial and general characteristics of starch polymer-based food packaging.

SeNPs have excellent antimicrobial, antioxidant activities and UV-barrier properties (Menon *et al.*, 2019; Jamróz *et al.*, 2019a). However, in majority of the investigations, the SeNPs are naked and interact directly with the test organism and/ or the oxidisable matter. Recently, there is an interest in the effect of SeNPs incorporated into food packaging material. There are few SeNPs-based packaging material

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reported (Jamróz *et al.*, 2018; Vera *et al.*, 2018; Jamróz *et al.*, 2019a; Jamróz *et al.*, 2019b). Nevertheless, the SeNPs were synthesised using physico-chemical methods, which compromises their suitability in food packaging due to possible chemical contamination. Plant extract-based SeNPs synthesis methods are preferred for application where human contact is envisaged, due to nontoxic nanoparticles produced (Murthy *et al.*, 2019; Garg *et al.*, 2021). There is inadequate information on the effectiveness of plant extract-mediated synthesised SeNPs-based active packaging. Furthermore, the application of nanoparticles in food packaging is still novel; therefore, information on the potential SeNPs migration from food packaging into food is still limited. Therefore, the aim of this study was to develop SeNPs/potato starch nanocomposite film with improved physical properties, antioxidant activity and antibacterial properties and determine its overall and specific migration to assess suitability of the SeNPs-based packaging as an alternative candidate for food packaging material.

Materials and methods

Chemical reagents

All reagents used in this study were purchased from Sigma-Aldrich and no additional refinement was done before usage. These were starch from potato (catalogue No. S 2004, density 0.14 g cm^{-3}), glycerol ($\geq 99.0\%$), sorbitol syrup (99%), methanol 99.9% HPLC grade, acetic acid (glacial), ethanol 99.9%, isooctane, anhydrous calcium chloride (CaCl_2), nitric acid 65% (HNO_3), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS).

Preparation of SeNPs-based starch films

Film development was carried out according to Zhang *et al.* (2018) with modifications. SeNPs were synthesised according to previously published method (Ndwandwe *et al.*, 2021). Potato starch, glycerol, and sorbitol were added into SeNPs solution (1 mg mL^{-1}) at 2, 0.5 and 0.5% (w/v of water), respectively. The blend was then maintained at $80 \text{ }^\circ\text{C}$ for 30 min and then cooled for degasification. The film formulation was transferred into a rectangular Pyrex glass utensil and allowed to dry at $25 \text{ }^\circ\text{C}$ for 24 h.

Film characterisation

Microscopy

Topographic and cross-sectional microstructure of potato starch and nanocomposite film was studied using

SEM (TESCAN Vega TC instrument, VEGA 3 TESCAN software) following the method outlined by Jayakumar *et al.* (2019). The equipment was operated at an acceleration current of 20 kV and it was equipped with EDX, which was used to determine the distribution of SeNPs in the surface of polymer matrix at 5 kV.

Film thickness and density

The width of films was ascertained by a Mitotuyo No. 7327 portable micrometre with a sensitivity of 0.001 mm. Five various points were measured and mean value reported as film thickness. Density was obtained by weighing 1 cm^2 of film specimens, which have been previously maintained at 0% relative humidity for 20 days. The density formula below applied.

$$\text{Density} \left(\frac{\text{g}}{\text{cm}^3} \right) = \frac{m}{A * \delta}$$

where A , δ and m are the area of film (1 cm^2), film width (cm) and film dehydrated mass (g), respectively.

Film water behaviour

A method by Jamróz *et al.* (2019a) was used with slight change. Firstly, 900 mm^2 of each film was weighed (W_1) followed by drying at $105 \text{ }^\circ\text{C}$ for 24 h and W_2 logged. Dried films were then submerged in 30 mL purified water at $23 \text{ }^\circ\text{C}$ for 24 h and W_3 recorded. The films were further dehydrated at $105 \text{ }^\circ\text{C}$ for 24 h and W_4 logged. The below equations were then applied to determine film water behaviour:

$$\text{Water content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

$$\text{Solubility (\%)} = \frac{W_2 - W_4}{W_2} \times 100$$

$$\text{Swelling degree} = \frac{W_3 - W_2}{W_2} \times 100$$

Water vapour transition rate of nanocomposite films

WVTR was measured according to Zhang *et al.* (2018). Film testers were tightly positioned on top of weighing flasks loaded with 3 g anhydrous CaCl_2 , and the flasks were then maintained at $25 \text{ }^\circ\text{C}$ and 90% RH for 24 h. WVTR was ascertained as follows:

$$\text{WVTR} = \frac{m_f - m_i}{D \times S}$$

where m_f , m_i , D and S are the weight of the final flask (g), initial flask (g), time (h) and efficacious part of the films (m^2).

Surface colour measurement of SeNPs based films

A hand-held CR-410 Chroma Meter was used for colour parameters (L^* -lightness, a^* - green/red, b^* - yellow). The overall colour transformation value (ΔE) was obtained by the equation

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

where ΔE signifies the colour differences between the standard film (starch only) and SeNPs/potato starch films.

Mechanical properties

Tensile solidity and ultimate elongation were established from film specimen ($25 \times 20 \text{ mm}^2$) using a tensile testing machine, which was equipped with 100 N force transducer and 10 mm min^{-1} piston-rod speed.

Antioxidant activity of nanocomposite films

DPPH radical scavenging assay

DPPH method as outlined by (Feng *et al.*, 2018) was adapted. Films (0.1 g) were immersed in 20 mL methanol and allowed to stand for 24 h. Equal volumes of film solution and 0.1 mM DPPH solution were mixed, vortexed and placed in the dark for 1 h. Thereafter, optical density was taken at 517 nm and methanol was used a blank. To obtain nanocomposite film scavenging characteristics, the following formula was applied:

$$\text{DPPH radical scavenging ability (\%)} = \frac{A_{\text{DPPH}} - A_{\text{film}}}{A_{\text{DPPH}}} \times 100$$

A_{DPPH} and A_{film} correspond to optical density of methanolic DPPH solution without films and with film testers, respectively.

ABTS assay

ABTS method, as stated by Nunes *et al.* (2013), was followed with minor changes. ABTS (8 mM) solution was mixed with 3 mM potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) and then left in the dark for 12 h at ambient temperature and used before 16 h. The ABTS• + formulation was then adapted to give an absorbance of 0.700 ± 0.01 at 734 nm. The films were then placed in ABTS• + solution (3 mL) and left in the dark for 48 h. Thereafter, the absorbance of the solution was read at 734 nm. Antioxidant efficacy of films was calculated as shown in the formula below:

$$\text{Inhibition ratio (\%)} = \frac{A_b - A_f}{A_b} \times 100$$

A_b and A_f correspond to optical density of blank ABTS and ABTS with film, respectively.

Overall migration and specific migration analysis

Determination of overall migration was in accordance with the European Union (EU) regulation No 10/2011 recommendation (European Commission, 2011) and a method stated by Moreno *et al.* (2017). A revised method by Polat *et al.* (2018) was used for specific migration. Films were placed in food simulators representing hydrophilic, hydrophilic acidic and lipophilic foods, with free fats on the surface in a $6 \text{ dm}^2 \cdot \text{kg}^{-1}$ contact ratio. After a contact time of 10 days at 25°C , films were removed, dried and then rehydrated in 2% nitric acid (HNO_3). A PerkinElmer Nexion 300X ICP-MS in KED mode Inductively coupled plasma mass spectrometry (ICP-MS) was used for determining selenium (Se) concentration in the final solution. Then a calibration curve was obtained by using Se of mass 78 in 2% HNO_3 .

Antimicrobial assay of SeNPs/potato starch nanocomposite film

Disk diffusion method

A disc agar diffusion method as stated by Narasagoudr *et al.* (2020) with modifications was used. Foodborne microorganism, Gram-positive (*Bacillus cereus* ATCC 10876, *Listeria innocua* ATCC 33090) and Gram-negative (*Salmonella typhimurium* ATCC 14028 and *Escherichia coli* ATCC 43888) bacteria were obtained from Thermo Fisher Scientific, Gauteng, South Africa. The test microorganisms were revived (37°C for 18 h) in Muller-Hinton (MH) broth and adjusted to 0.5 McFarland standard. Thereafter, 100 μL of each bacterium was spread-plated on nutrient agar (NA) followed by placement of sterile cellulose disks (5 mm diameter) impregnated with film forming solution. For negative control, disks impregnated with film formulation without SeNPs were used and streptomycin was used as positive control. All plates were maintained at 37°C for 24 h and the diameter (mm) of inhibition zones was measured.

Shake flask culture method

The shake culture method as outlined by Feng *et al.* (2018) was carried out with slight modification. Overnight cultures were adjusted to 0.5 McFarland standard and films (30 mm diameter) were placed in 10 mL MH broth containing 10 μL of test organism. The samples were kept at 37°C for 24 h. Thereafter, serially diluted overnight cultures were spread-plated on NA and incubated at 37°C for 24 h. The outcome was expressed as colony forming units (CFU). The antibacterial efficacy of the films was calculated by the following equation (Karkhanechi *et al.*, 2013).

$$\text{Bacteria killing ratio} = \frac{N_b - N_s}{N_b} \times 100$$

where N_b and N_s denote the number of bacteria in control film and nanocomposite film samples (CFU ml⁻¹), respectively.

Statistical analysis

The one-way MANOVA using GenStat statistical software 18th edition was used to analyse data statistical. Treatment comparison was determined at 95% confidence level by Fisher's least significant difference (LSD).

Results and discussion

Microstructure of SeNPs/potato starch nanocomposite films

The SeNPs nanocomposite film exhibited a slight rough surface and heterogenous structure (Fig. 1b) when compared to the control film which exhibited an acceptable structural integrity with a smooth surface (Fig. 1a). The slightly rough surface was possibly due to recrystallisation of starch molecules that might have occurred during the film drying process (Heung *et al.*, 2020). The SEM micrographs did not show obvious cracks on the surface of the nanocomposite film as well as on the control film. Their absence on the nanocomposite film indicates good affinity between the SeNPs and the starch polymer matrix (Feng *et al.*, 2018; Kritchenkov *et al.*, 2021). The cross section of nanocomposite film appeared smooth and more compact, possibly due to that SeNPs filled interspaces that existed in the starch polymer matrix (Wu *et al.*, 2018; Narasagoudr *et al.*, 2020; Kritchenkov *et al.*, 2021).

An EDX analysis of the nanocomposite film indicated a signal at 1.37 keV, corresponding to elemental Se (Fig. 1c), and this was in agreement with other reports (Bai *et al.*, 2017; Menon *et al.*, 2019; Dumore & Mukhopadhyay, 2020; Ndwandwe *et al.*, 2021). The presence of this peak proves that SeNPs were successfully incorporated in the potato starch film matrix. Oxygen and carbon were also detected, which may be attributed to the chemical structure of the potato starch. Elemental mapping (Fig. 1c corresponding micrographs) indicated that SeNPs were homogeneously dispersed in the starch nanocomposite film matrix.

Film thickness and density

A significant ($P < 0.05$) increase was observed in film thickness and density with assimilation of SeNPs into films from 0.02 to 0.04 mm and 1.01 to 1.31 g cm⁻³, respectively (Table 1). This trend may be because of increase in solid matter in the film formulation

attributed to the addition of SeNPs (Jamróz *et al.*, 2018; Praseptiangga *et al.*, 2021). Similar findings were documented by Guz *et al.* (Guz & Bernal, 2021) when silver nanoparticles were incorporated into cassava starch film. According to the ASTM standard method D6988-21 (, 2021), a film should be less than 0.25 mm thickness; therefore, the films developed in this study were within the criterion stipulated by the standard.

Film solubility in water, water content and swelling degree

Film solubility decreased significantly ($P < 0.05$) when SeNPs were added into the potato starch film (Table 1). This was attributed to reduced free -OH groups of starch due to SeNPs/ starch macromolecules interaction, hence the observed hydrophobicity of nanocomposite film (Nafchi *et al.*, 2012; Mathew *et al.*, 2019; Peighambardoust *et al.*, 2019). The moisture content of the potato starch film decreased from 4.02% to 2.67% (Table 1) after addition of SeNPs. Similarly, the nanocomposite film recorded less swelling degree (Table 1). The biopolymer moisture content is associated with the overall void space occupied by water molecules, and the film swelling degree is associated with water uptake ability of the films (Jamróz *et al.*, 2018). Therefore, behaviour by the SeNPs-based nanocomposite film here indicates that SeNPs replaced the water molecules in the starch matrix. A biopolymer with low sensitivity in water is desirable as moisture-sensitive films are more likely to negatively impact the quality of the packaged food. The fabricated SeNPs nanocomposite film in this study have exhibited enhanced moisture resistance suggesting their suitability for use in food packaging.

Water vapour transmission rate analysis

The hydrophilic characteristic of starch is one drawback that limits its usage in food packaging (Hassan *et al.*, 2020; Mehboob *et al.*, 2020). As anticipated, the control film exhibited higher water affinity (0.09 g m⁻² h⁻¹), while the film enhanced with SeNPs displayed significantly low water absorption capacity (0.04 g m⁻² h⁻¹) (Table 1). Restricted moisture movement between internal and external packaging environment is an important attribute for food packaging material. In this work, the addition of SeNPs contributed to the creation of a more solid structure and low swelling of the starch films, which subsequently decreased the WVT.

Physical appearance and mechanical properties of the film

The film colour and its physical appearance are the most determining properties that influence consumer

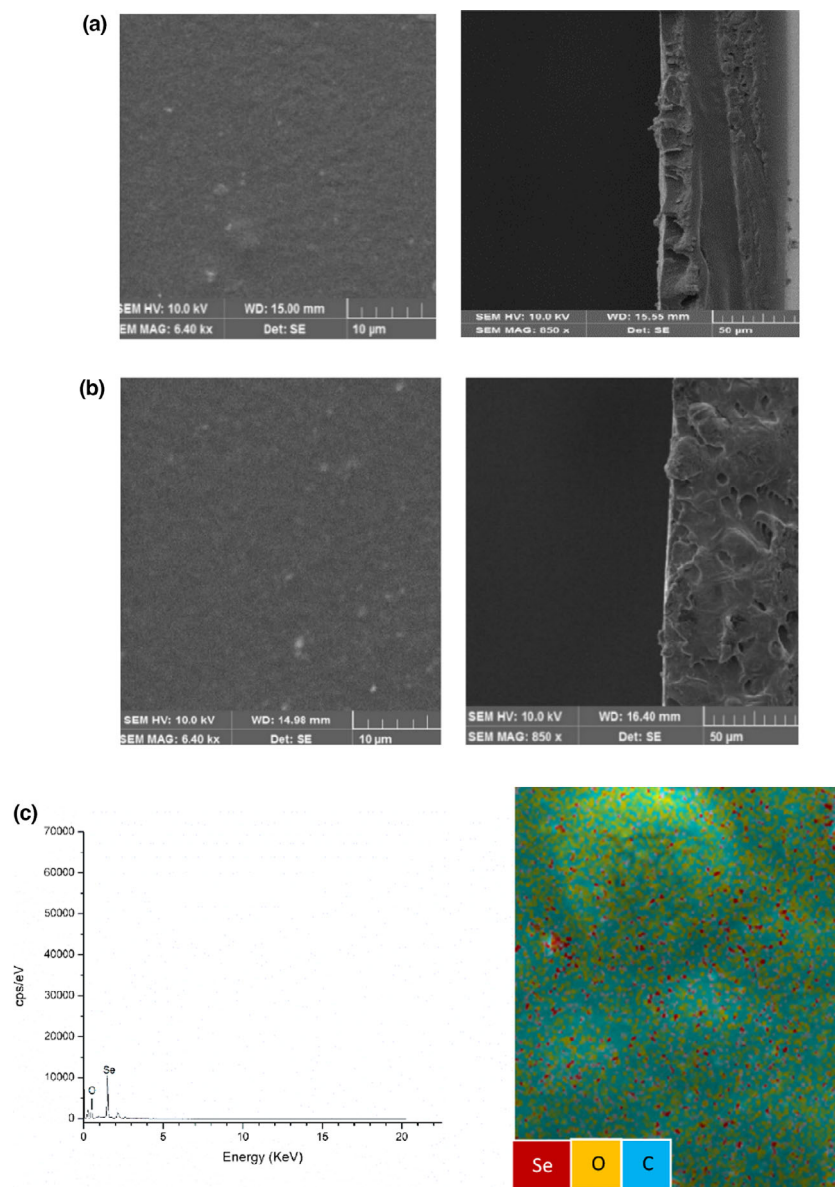


Figure 1 Surface and corresponding cross section SEM images of potato starch films (a) control film, (b) nanocomposite film, (c) EDX spectrum of nanocomposite film and corresponding elemental mapping.

acceptance of a food product (Jamróz *et al.*, 2018; Zhang *et al.*, 2019; Dai *et al.*, 2021). Figure 3 shows that the films were visually smooth and uniform and exhibited a homogenous flexibility nature without obvious pores and bubbles.

The colour parameters (L^* , a^* and b^*) and colour difference (ΔE) are displayed in Table 2. Incorporation of SeNPs caused a substantial ($P < 0.05$) change of colour to red (a^*) and yellow (b^*) and reduced the lightness (L -value). These results correlated with visual observations made after the development of the SeNPs/potato starch films as shown in Fig. 2. Jamróz *et al.* (2018) reported a similar colour change when SeNPs were incorporated in a furcellaran-gelatin films.

The colour changes observed here agree with EDX analysis (Fig. 1C), which indicated the presence of Se element in the nanocomposite film. Also, elemental mapping showed an even distribution of the SeNPs, which agrees with the uniform visual appearance of the films observed. The reddish colour of the developed films suggests that they could be appropriate for packaging food that is light sensitive.

The tensile strength and elongation were improved with addition of SeNPs concentration from 3.42–9.86 MPa and 13.48%–17.94%, respectively (Table 3). The enhancement of the mechanical attributes of the nanocomposite film indicates that incorporation of the SeNPs altered the structure potato starch matrix. This

Table 1 Thickness, water content, solubility, swelling degree and WVTR for SeNPs-based film

Film parameters	Control film	Nanocomposite film
Thickness (mm)	0.02 ± 0.01 ^a	0.04 ± 0.00 ^b
Density (g cm ⁻³)	1.01 ± 0.12 ^a	1.31 ± 0.03 ^b
Water content (%)	4.02 ± 0.56 ^a	2.67 ± 0.12 ^b
Solubility	2.20 ± 0.42 ^a	1.13 ± 0.12 ^b
Swelling degree	2.43 ± 0.41 ^a	1.37 ± 0.12 ^b
WVTR (g m ⁻² .h)	0.09 ± 0.02 ^a	0.04 ± 0.01 ^b

Note: Results are presented as average ± standard deviation ($n = 3$). Dissimilar letters in the same row specify significant variations ($P < 0.05$).

Table 2 Effect of SeNPs addition on the colour of potato starch film

Colour parameters	Control film	Nanocomposite film
Luminosity (L*)	50.28 ± 0.62 ^a	48.99 ± 0.02 ^b
Chromaticity parameter a*	0.44 ± 4.87 ^a	14.47 ± 2.15 ^b
Chromaticity parameter b*	-1.09 ± 4.95 ^a	6.00 ± 0.96 ^b
Colour difference (ΔE)	0.78	15.74

Note: Values are presented as average ± standard deviation. Dissimilar letters in the same rows specify significant variations ($P < 0.05$) ($n = 3$).

variation might probably be due to intra- and inter-molecular interactions among constituents of film matrix (Jamróz *et al.*, 2019a).

Antioxidant activity of SeNPs films

The control potato starch film exhibited no antioxidant activity, while the nanocomposite film's antioxidant activity improved to 9.26% and 5.86% for ABTS and DPPH, respectively (Table 3). It was noticed that the antioxidant activity determined with DPPH was

relatively low compared to that obtained with ABTS assay. This was probably because DPPH is organic in nature, which may limit its interaction with the hydrophilic starch polymer, thus negatively affecting the release of active sites of SeNPs (Priyadarshi *et al.*, 2021). Previous reports have indicated the antioxidant nature of SeNPs (Yan *et al.*, 2018; Rajagopal *et al.*, 2021). Therefore, the presence of SeNPs improved the antioxidant activity of the potato starch films. In a similar study by Vera *et al.* (2018), the antioxidant activity of a multilayer polymer was improved by incorporation of SeNPs. The nanocomposite biopolymer was further used as an antioxidant active packaging, and it effectively prolonged the shelf-life of packaged hazelnuts.

Overall and specific migration of starch-based film constituents into food simulants

The migration behaviour of the starch-film components was influenced by the presence of SeNPs, with the control film displaying the greatest migration with all simulants (Table 4). The release of starch films constituents was higher when they were placed in hydrophilic simulants as compared to lipophilic food simulant. The different degree of constituents' migration into the studied simulants could be due to differences in solubility, polarity and interaction between SeNPs, potato starch polymer and food simulant (Kuorwel *et al.*, 2013; Narasagoudr *et al.*, 2020; Le *et al.*, 2021). General, the migration of film constituents was below the Overall Migration Limit (OML) of 10 mg dm⁻² stipulated by Regulation EU No 10/201.

The Se content in the potato nanocomposite film was found to be 0.09 mg g⁻¹, and this was lower than Se concentration (1.0 mg g⁻¹) that was initially incorporated. The reduction may be attributed to the nanosize of the Se particles, which may have enhanced their absorption into the film matrix during the film

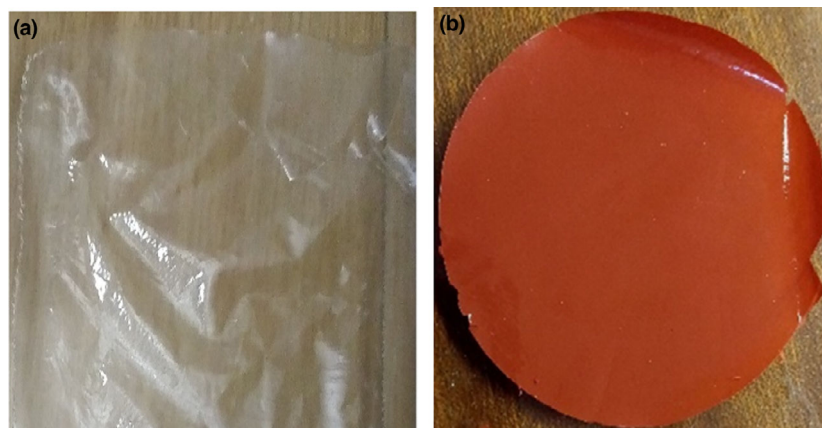
Figure 2 Physical appearance of the (a) control film, (b) nanocomposite film.

Table 3 The mechanical properties and antioxidant activity of control and nanocomposite film

	Mechanical properties		Antioxidant activity (%)	
	Tensile strength (MPa)	Elongation (%)	ABTS	DPPH
Control film	3.42 ^a	13.48 ^a	0.00	0.00
Nanocomposite film	9.86 ^b	17.94 ^b	9.26	5.86

Note: Values are presented as average \pm standard deviation. Dissimilar letters in the same rows specify significant variations ($P < 0.05$) ($n = 3$).

Table 4 Overall and specific migration of SeNPs film constituents into food simulants

Food simulants	Overall migration (mg dm ⁻²)		Specific migration (mg kg ⁻¹)
	Control	Nanocomposite	Nanocomposite
10% ethanol	9.45 ^b \pm 0.92	5.98 ^a \pm 0.19	0.26 ^c \pm 0.05
3% acetic acid	5.38 ^b \pm 0.69	3.41 ^a \pm 0.91	0.24 ^b \pm 0.04
Isooctane	3.30 ^b \pm 0.16	3.24 ^a \pm 0.09	0.01 ^a \pm 0.08

Note: Data represent the average \pm standard deviation ($n = 3$). Data in a row with differing superscript letters are significantly different ($P < 0.05$).

development processes (Shi *et al.*, 2021). As shown in Table 4, the highest (0.26 mg kg⁻¹) and least (0.01 mg kg⁻¹) Se migration occurred with the acidic hydrophilic food simulant and fatty food simulant, respectively. The migration of Se from the prepared film into the food simulants under study was below the acceptable limit of 60 mg kg⁻¹ (European Commission, 2011). However, migration of Se ions into the acidic food simulant was higher than both the aqueous and fatty food simulant. Increased migration of nanomaterials in acidic food simulant has been observed by other investigators (Echegoyen & Nerín, 2013; Yu *et al.*, 2021; Videira-quintela *et al.*, 2022). This observation may be due to the presence of hydrogen ions (H⁺) in the acidic hydrophilic simulants, which is believed to accelerate the weakening of the hydrogen bond between SeO in nanocomposites, hence causing an increased outflow of Se (Yu *et al.*, 2021).

Antibacterial activity of SeNPs/starch nanofilm

The addition of SeNPs into the potato starch film improved the antimicrobial property of the film against all the test microorganisms as opposed to control (film without SeNPs) (Table 5). The growth of

Table 5 inhibitory effect of SeNPs/potato starch nanofilm against selected bacteria

Name of microorganism	Inhibition diameter (mm)	Total bacteria killing ratio (%)
<i>S. typhimurium</i>	12.00 \pm 1.06 ^b	10.66
<i>E. coli</i>	11.00 \pm 0.56 ^b	11.29
<i>B. cereus</i>	6.67 \pm 1.61 ^a	8.35
<i>L. innocua</i>	NI	0.73

Note: Data represent the average \pm standard deviation of duplicates for every test. Data in a column with contrasting superscript letters significantly vary ($P < 0.05$). NI - No inhibition.

both Gram-negative (*S. typhimurium* and *E. coli*) bacteria was significantly ($P < 0.05$) inhibited by the nanocomposite film with 12.00 mm and 11.50 mm zones of inhibition, respectively. The Gram-positive *B. cereus* had a minimum growth inhibition (6.67 mm), while no inhibition occurred with *L. innocua*.

The highest total bacteria killing ratio occurred against *S. typhimurium* (10.66%), while *L. innocua* (0.73%) was the most resistant bacteria to the SeNPs potato starch films (Table 5). In literature, there is inadequate information on the antimicrobial efficiency of SeNPs/potato starch nanocomposite films evaluated using the shake method. Despite that, the total bacteria killing ratio results agree with the observation made on the specific migration analysis performed on the nanocomposite film, which indicated low migration of SeNPs. This behaviour might have played a role in the weak killing effect of the test bacteria by the nanocomposite films since there was minimal dissolution of film to release SeNPs to the surrounding media. Also, the contact time (24 h) between the nanocomposite film and test bacteria might have been inadequate for substantial bactericidal effect at low SeNPs concentration. The reason for the variation between Gram-negative and Gram-positive bacteria may be due to the differences in the structural composition of the two types of bacteria (Jamróz *et al.*, 2019b; Priyadarshi *et al.*, 2021). Antimicrobial efficacy is one of the desired characteristic in food packaging films (Zhang *et al.*, 2019; Helmiyati *et al.*, 2021). Therefore, antimicrobial activity exhibited by the nanocomposite film against two food pathogens suggests its potential for application as food packaging material.

Conclusion

This investigation led to the successful development of active SeNPs/potato starch film. The addition of SeNPs increases the thickness and density of fabricated nanocomposite film, while the solubility, swelling degree, film humidity and water vapour penetrability

of the SeNPs/potato starch film decrease. Incorporation of SeNPs into film results in a rough and heterogeneous surface when compared to film without SeNPs. With EDX, uniform distribution of SeNPs in the nanocomposite film was detected. The addition of SeNPs in the film improves the tensile strength as well as the elongation. The overall and specific migration of Se ions from the film to the food simulants was acceptable. In addition, The SeNPs film exhibits antibacterial efficacy against *S. typhimurium* and *E. coli*. Therefore, the developed SeNPs/potato starch nanocomposite film can potentially be used for active food packaging. Furthermore, the low overall and specific migration of film constituents suggest that it can be suitable for packaging of acidic, hydrophilic, as well as fatty food.

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Author contributions

Bongekile Khanyisile Ndwandwe: Conceptualization (equal); investigation (lead); methodology (equal); writing – original draft (equal). **Soraya Malinga:** Funding acquisition (equal); supervision (equal); writing – review and editing (equal). **Eugenie Kayitesi:** Supervision (equal); writing – review and editing (equal). **Bhekisisa Dlamini:** Conceptualization (lead); methodology (equal); writing – review and editing (equal).

Conflict of interest

The authors declare they have no conflict of interest.

Ethics statement

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at <https://publons.com/publon/10.1111/ijfs.15990>.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

References

- ASTM, D6988-21, (2021). Standard guide for determination of thickness of plastic film test specimen. In: Annual Book of ASTM of Standards, 06.03. American Society for Testing and Materials.
- Bai, K., Hong, B., He, J., Hong, Z. & Tan, R. (2017). Preparation and antioxidant properties of selenium nanoparticles-loaded chitosan microspheres. *International Journal of Nanomedicine*, **12**, 4527–4539.
- Chaurasia, S. & Lal, A. (2016). Development of biodegradable packaging film using potato starch. *International Journal of current Engineering and Technology*, **6**, 915–925.
- Dai, L., Yu, H., Zhang, J. & Cheng, F. (2021). Preparation and characterization of cross-linked starch nanocrystals and self-reinforced starch-based nanocomposite films. *International Journal of Biological Macromolecules*, **181**, 868–876.
- de Souza, A.G., NMA, D.S., da Silva Torin, R.F. & Dos Santos Rosa, D. (2020). Synergic antimicrobial properties of carvacrol essential oil and montmorillonite in biodegradable starch films. *International Journal of Biological Macromolecules*, **164**, 1737–1747.
- Dumore, N.S. & Mukhopadhyay, M. (2020). Antioxidant properties of aqueous selenium nanoparticles (ASeNPs) and its catalytic activity for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) reduction. *Journal of Molecular Structure*, **1205**, 127637.
- Echegoyen, Y. & Nerin, C. (2013). Nanoparticle release from nano-silver antimicrobial food containers. *Food and Chemical Toxicology*, **62**, 16–22.
- European Commission (2011). Commission regulation (EU) no 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Official Journal of the European Union*, **L12**, 1–89.
- Feng, M., Yu, L., Zhu, P. *et al.* (2018). Development and preparation of active starch films carrying tea polyphenol. *Carbohydrate Polymers*, **196**, 162–167.
- Garg, R., Kumari, M., Dhiman, S. & Garg, R. (2021). Green synthesis of calcium carbonate nanoparticles using waste fruit peel extract. *Materials Today: Proceedings*, **46**, 6665–6668.
- Glicerina, V., Siroli, L., Canali, G. *et al.* (2021). Efficacy of biodegradable, antimicrobial packaging on safety and quality parameters maintenance of a pear juice and rice milk-based smoothie product. *Food Control*, **128**, 108170.
- Guz, L. & Bernal, C. (2021). Effect of green synthesized silver nanoparticles on the properties of active starch films obtained by extrusion and compression molding. *Carbohydrate Polymers Journal*, **261**, 117871.
- Hassan, M.M., Tucker, N. & Le Guen, M.J. (2020). Thermal, mechanical and viscoelastic properties of citric acid-crosslinked starch/cellulose composite foams. *Carbohydrate Polymers*, **230**, 115675.
- Helmiyati, H., Shabira, Z., Hidayat, Z. *et al.* (2021). Antimicrobial packaging film based on biodegradable CMC/PVA-zeolite doped with noble metal cations. *Polymer Testing*, **104**, 107412.
- Heung, J., Young, S., Duck, G., Lee, M.H. & Park, H.J. (2020). Effect of moisture content on the heat-sealing property of starch films from different botanical sources. *Polymer Testing*, **89**, 106612.
- Jafarzadeh, S., Alias, A.K., Ariffin, F. *et al.* (2017). Characterization of a new biodegradable edible film based on semolina loaded with nano kaolin. *International Food Research Journal*, **24**, 304–309.
- Jamróz, E., Kopel, P., Juszcak, L. *et al.* (2018). Development and characterisation of furcellaran-gelatin films containing SeNPs and AgNPs that have antimicrobial activity. *Food Hydrocolloids*, **83**, 9–16.
- Jamróz, E., Kopel, P., Juszcak, L. *et al.* (2019a). Development of furcellaran-gelatin films with se-AgNPs as an active packaging system for extension of mini kiwi shelf life. *Food Packaging and Shelf Life*, **21**, 100339.
- Jamróz, E., Kulawik, P., Kopel, P. *et al.* (2019b). Intelligent and active composite films based on furcellaran: structural

- characterization, antioxidant and antimicrobial activities. *Food Packaging and Shelf Life*, **22**, 100405.
- In this article, furcelleran nanocomposite films enhanced with either green tea of yerba mate extracts or nanoparticles (SeNPs or ZnONPs) or a combination of a plant extract and nanoparticles were developed. The films were evaluated for their antimicrobial activities against two food pathogens, and it was found that the nanocomposite film enhanced with SeNPs exhibited increased antibacterial activity against Gram-negative bacteria (*E. coli*) as compared to Gram-positive bacteria (*S. aureus*). This article is relevant to the present article as it demonstrated the potential of SeNPs-based nanocomposite film as an antibacterial active food packaging.
- Jayakumar, A., Heera, K.V., Sumi, T.S. *et al.* (2019). Starch-PVA composite films with zinc-oxide nanoparticles and phytochemicals as intelligent pH sensing wraps for food packaging application. *International Journal of Biological Macromolecules*, **136**, 395–403.
- Karkhanечи, H., Razi, F., Sawada, I., Takagi, R., Ohmukai, Y. & Matsuyama, H. (2013). Improvement of antibiofouling performance of a reverse osmosis membrane through biocide release and adhesion resistance. *Separation and Purification Technology*, **105**, 106–113.
- Kritchenkov, A.S., Egorov, A.R., Volkova, O.V. *et al.* (2021). Novel biopolymer-based nanocomposite food coatings that exhibit active and smart properties due to a single type of nanoparticles. *Food Chemistry*, **343**, 128676.
- Kuorwel, K.K., Cran, J., Sonneveld, K., Miltz, J. & Bigger, S.W. (2013). Migration of antimicrobial agents from starch-based films into a food simulant. *LWT - Food Science and Technology*, **50**, 432–438.
- Le, T.D., Phasupan, P., Visaruthaphong, K., Chuwatwat, P., Thu, V.T. & Nguyen, L.T. (2021). Development of an antimicrobial photodynamic poly (3-hydroxybutyrate-co-3-hydroxyvalerate) packaging film for food preservation. *Food Packaging and Shelf Life*, **30**, 100749.
- Mathew, S., Snigdha, S., Mathew, J. & Radhakrishnan, E.K. (2019). Biodegradable and active nanocomposite pouches reinforced with silver nanoparticles for improved packaging of chicken sausages. *Food Packaging and Shelf Life*, **19**, 155–166.
- Mehboob, S., Ali, T., Sheikh, M. & Hasnain, A. (2020). Effects of cross linking and / or acetylation on sorghum starch and film characteristics. *International Journal of Biological Macromolecules*, **155**, 786–794.
- Menon, S., Shrudhi, S., Agarwal, H. & Venkat Kumar, S. (2019). Efficacy of biogenic selenium nanoparticles from an extract of ginger towards evaluation on anti-microbial and anti-oxidant activities. *Colloids and Interface Science Communications*, **29**, 1–8.
- Moreno, O., Cárdenas, J., Atarés, L. & Chiralt, A. (2017). Influence of starch oxidation on the functionality of starch-gelatin based active films. *Carbohydrate Polymers*, **178**, 147–158.
- Mousavi, Z., Naseri, M., Babaei, S., Hosseini, S.M.H. & Shekarforoush, S.S. (2021). The effect of cross-linker type on structural, antimicrobial and controlled release properties of fish gelatin-chitosan composite films incorporated with ϵ -poly-L-lysine. *International Journal of Biological Macromolecules*, **183**, 1743–1752.
- Murthy, A.H.C., Abebe, B., Prakash, C.H. & Shantaveerayya, K. (2019). A review on green synthesis and applications of Cu and CuO nanoparticles. *Material Science Research India*, **15**, 279–295.
- Nafchi, A.M., Alias, A., Mahmud, S. & Robal, M. (2012). Antimicrobial, rheological, and physicochemical properties of sago starch films filled with nanorod-rich zinc oxide. *Journal of Food Engineering*, **113**, 511–519.
- Narasagoudr, S.S., Hegde, V., Chougale, R., Masti, S.P. & Dixit, S. (2020). Influence of boswellic acid on multifunctional properties of chitosan/poly (vinyl alcohol) films for active food packaging. *International Journal of Biological Macromolecules*, **154**, 48–61.
- Ndwandwe, B.K., Malinga, S.P., Kayitesi, E. & Dlamini, B.C. (2021). Solvothermal synthesis of selenium nanoparticles with polygonal-like nanostructure and antibacterial potential. *Materials Letters*, **304**, 130619.
- Neda, S., Daneshvar, H., Saeed, M., Ghasempour, Z., Panahi-Azar, V. & Ehsani, A. (2021). Starch/alginate/cu-g-C₃N₄ nanocomposite film for food packaging. *Materials Chemistry and Physics*, **267**, 124583.
- Negar, S., Pirsá, S. & Farzi, J. (2021). Biodegradable nano composite film based on modified starch-albumin/MgO; antibacterial, antioxidant and structural properties. *Polymer Testing*, **97**, 107182.
- Nizam, N.H.M., Rawi, N.F.M., Ramle, S.M.M. *et al.* (2021). Physical, thermal, mechanical, antimicrobial and physicochemical properties of starch based film containing aloe vera: a review. *Journal of Materials Research and Technology*, **15**, 1572–1589.
- Nunes, C., Maricato, E., Cunha, A., Nunes, A., Silva, J.A.L. & Coimbra, M.A. (2013). Chitosan – caffeic acid – genipin films presenting enhanced antioxidant activity and stability in acidic media. *Carbohydrate Polymers*, **91**, 236–243.
- Peighambardoust, S.J., Peighambardoust, S., Pournasir, N. & Pakdel, P.M. (2019). Properties of active starch-based films incorporating a combination of Ag, ZnO and CuO nanoparticles for potential use in food packaging applications. *Food Packaging and Shelf Life*, **22**, 100420.
- In this study, starch films enhanced with different nanoparticles (Ag, ZnO and CuO NPs) single or in combination were prepared. It was observed that regardless of the type of nanoparticles used, adding the nanoparticles into starch matrix led to a significant ($P < 0.05$) decrease in the solubility of the developed films. This article is relevant to the present article as it verified that addition of nanoparticles into a starch biopolymer improves its hydrophobicity, which is a desired property in food packaging material.
- Polat, S., Fenercioglu, H. & Güçlü, M. (2018). Effects of metal nanoparticles on the physical and migration properties of low density polyethylene films. *Journal of Food Engineering*, **229**, 32–42.
- Praseptiangga, D., Mufida, N., Panatarani, C. & Joni, I.M. (2021). Enhanced multi functionality of semi-refined iota carrageenan as food packaging material by incorporating SiO₂ and ZnO nanoparticles. *Heliyon*, **7**, e06963.
- In this article, SiO₂ and ZnO nanoparticles were incorporated into semi-refined iota carrageenan to develop a food packaging material. It was observed that the addition of the nanoparticles into the polymer improved its thickness and density. This article is relevant to the present study as it confirmed that addition of nanoparticles into a biopolymer improves its physical properties.
- Priyadarshi, R., Kim, S. & Rhim, J. (2021). Carboxymethyl cellulose-based multifunctional film combined with zinc oxide nanoparticles and grape seed extract for the preservation of high-fat meat products. *Sustainable Materials and Technologies*, **29**, e00325.
- Rajagopal, G., Nivetha, A., Ilango, S., Muthudevi, G.P., Prabha, I. & Arthimanju, R. (2021). Phytofabrication of selenium nanoparticles using *Azolla pinnata*: evaluation of catalytic properties in oxidation, antioxidant and antimicrobial activities. *Journal of Environmental Chemical Engineering*, **9**, 105483.
- Saravanakumar, K., Sriram, B., Sathiyaseelan, A. *et al.* (2021). Synthesis, characterization, and cytotoxicity of starch-encapsulated biogenic silver nanoparticle and its improved anti-bacterial activity. *International Journal of Biological Macromolecules*, **182**, 1409–1418.
- Schumann, B. & Schmid, M. (2018). Packaging concepts for fresh and processed meat – recent progresses. *Innovative Food Science and Emerging Technologies*, **47**, 88–100.
- Shi, Y.J., Wrona, M., Hu, C. & Nerin, C. (2021). Copper release from nano-copper/polypropylene composite films to food and the forms of copper in food simulants. *Innovative Food Science and Emerging Technologies*, **67**, 581.
- Vera, P., Canellas, E. & Nerin, C. (2018). New antioxidant multi-layer packaging with nanoselenium to enhance the shelf-life of market food products. *Nanomaterials*, **8**, 837.
- In this article, laminates incorporated with SeNPs were prepared. The antioxidant activity of the laminates was evaluated and it

exhibited efficient radical scavenging ability. Furthermore, the antioxidant packaging was used to monitor the shelf-life of packaged hazelnuts, and it was concluded that the developed antioxidant active packaging can effectively extend the shelf-life of the packaged food. This article is relevant to my study as it demonstrated the antioxidant potential of selenium-based active food packaging.

Videira-quintela, D., Francisco, G., Martin, O. & Montalvo, G. (2022). Antibacterial LDPE films for food packaging application filled with metal-fumed silica dual-side fillers. *Food Packaging and Shelf Life*, **31**, 772.

Wu, M., Zhou, Z., Yang, J., Zhang, M., Cai, F. & Lu, P. (2021). ZnO nanoparticles stabilized oregano essential oil Pickering emulsion for functional cellulose nanofibrils packaging films with antimicrobial and antioxidant activity. *International Journal of Biological Macromolecules*, **190**, 433–440.

Wu, Z., Huang, X., Li, Y., Xiao, H. & Wang, X. (2018). Novel chitosan films with laponite immobilized ag nanoparticles for active food packaging. *Carbohydrate Polymers*, **199**, 210–218.

Yan, J.K., Qiu, W., Wang, Y., Wang, W.H., Yang, Y. & Zhang, H.N. (2018). Fabrication and stabilization of biocompatible selenium nanoparticles by carboxylic curdlans with various molecular properties. *Carbohydrate Polymers*, **179**, 19–27.

Yu, F., Fei, X., He, Y. *et al.* (2021). Poly (lactic acid) -based composite film reinforced with acetylated cellulose nanocrystals and ZnO nanoparticles for active food packaging. *International Journal of Biological Macromolecules*, **186**, 770–779.

Yu, Z., Wang, W., Kong, F., Lin, M. & Mustapha, A. (2019). Cellulose nanofibril/silver nanoparticle composite as an active food packaging system and its toxicity to human colon cells. *International Journal of Biological Macromolecules*, **129**, 887–894.

Zhang, R., Wang, X. & Cheng, M. (2018). Preparation and characterization of potato starch film with various size of Nano-SiO₂. *Polymers*, **10**, 1172.

Zhang, X., Liu, Y., Yong, H., Qin, Y., Liu, J. & Liu, J. (2019). Development of multifunctional food packaging films based on chitosan, TiO₂ nanoparticles and anthocyanin-rich black plum peel extract. *Food Hydrocolloids*, **94**, 80–92.