Effects of Nanocomposite Based Nano-Silver and Nano-Titanium Dioxideon Food Packaging Materials

Amal M. Metak
School of Science & Engineering
Teesside University
Middlesbrough, TS1 3BA
UK

Abstract

Commercial antimicrobial nano-silver food packaging containers were characterised using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). The presence of nanoparticles consistent with the incorporation of 1% nano-silver (Ag) and 0.1% titanium dioxide (TiO_2) nanoparticles into polymeric materials formed into food containers was confirmed. Both nano-materials used in this type of packaging appear to be embedded in a layered configuration within the bulk polymer. The dimensions of the incorporated nanoparticles were investigated using X-ray diffraction (XRD) and were determined by calculation using the Scherrer Formula; these were consistent with Ag and TiO_2 nanoparticles in the size range of 20-70 nm, both were of spherical shape nanoparticles. Migration assessments were performed in a wide range of food matrices to determine the migration of nanoparticles from the packages. The analysis was based upon the relevant European Safety Directives and involved the application of inductively coupled plasma mass spectrometry (ICP-MS) to identify the range of migration risk. The data pertain to insignificance levels of Ag and TiO_2 nanoparticles and the results confirmed the antimicrobial activity of Ag and TiO_2 nanoparticles in food packaging containers.

Keywords: Nano-silver, antimicrobial food packaging, migration, titanium dioxide

1. Introduction

The application of nanotechnology to the food sector offers significant potential benefits, including enhancements in production and processing - suggested improvements being consonant with the following preferences; superior food contact materials, quality and freshness monitoring, traceability and product security, sensation, consistency, fat content and nutrient absorption (Mihindukulasuriya, & Lim, 2014; Espitia, Soares, Coimbra, De Andrade, & Medeiros, 2012). Nanocomposite food packaging comprises the largest contribution of this technology in the food sector market; and the application is predicted to increase over the next two decades (Peelman *et al.*, 2013; Murali*et al.*, 2010).

Silver is well-known for its wide-ranging antimicrobial activity against Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains, fungi and certain viruses. The application of silver as an antimicrobial agent has also proved effective when incorporated into a variety of materials such as polymers (Cushen, Kerry, Morris, Cruz-Romero, & Cummins, 2012).

A variety of nanotechnology-derived food ingredients and their additives, as well as materials intended to be placed in close contact with foodstuffs, are available in many countries and the market for these products is expected to have substantial future growth (Calzolai, Gilliland, & Rossi, 2012; Chaudhry *et al.*, 2008). The antimicrobial material used for food packaging is classified into two types: organic and inorganic. Organic antimicrobial materials are frequently less stable at high temperatures compared to that of inorganic agents. Inorganic materials, for example, metal and metal oxides, have the ability to withstand exacting processing conditions and have attracted the attention of scientist over the last decade (Reig, Lopez, Ramos, &Ballester, 2014 ; Zhang, Wa, & Sen, 2012). A great amount of research has been conducted on the antimicrobial activity of nano-material against different types of microorganisms (De Azeredo, Agroindustry, &Mesquita, 2013). It would appear that the research investigation of antimicrobial activity is mostly conducted with antimicrobial packaging against live bacteria as opposed to real-food matrices.

Investigations into Ag nanoparticles film bioactivity have been undertaken for more than two decades, but the detailed mechanism of Ag nanoparticles as an antimicrobial agent remains unclear. Several authors have suggested that the interaction of Ag nanoparticles with oxygen results in enhanced antimicrobial activity compared with that of Ag oxide or Ag metal (Silvestre, Duraccio, &Cimmino, 2011). It has been proposed that the oxidation state of the Ag atom in Ag nanoparticles is fundamental to achieve its antimicrobial properties. However, it seems that antimicrobial activity is mediated only by Ag^+ chemisorbed on the surface of Ag nanoparticles, and therefore, zero-valence Ag nanoparticles do not display appreciable biological activity (Lok*et al.*, 2007). Combinations of more than one antimicrobial incorporated into packaging have been used in many packaging materials.

Microbial growth, enzyme, water activities and change in pigments are the most important factors that affect the quality of fresh fruit and vegetables. By enhancing the packaging material, problems relating to these damaging factors are reduced. Lloret, Picouet, & Fernandez (2012) studied the suitability of cellulose Ag nanoparticles as an antimicrobial packaging. Meat and fruit samples were stored for 10 days at 4°C and the total viable count of microorganisms, yeasts and moulds in kiwi and melon juices were reduced by 99.9%. The total viable count and lactic acid bacteria in drips from poultry and beef samples stored in nanocomposite packaging were consistently 90% below that of the controls. Whilst this research tested the meat dripping and juices from the examined samples, it did not test the sample matrices themselves. Only a few authors have studied the antimicrobial activity of nanomaterials directly in real-food matrices. Motlagh, Mosavian, Mortazavi, &Tamizi (2012)concluded that low-density polyethylene-silver (LDPE-Ag) packages with >1% concentration of Ag nanoparticles preserve the quality and prolong the shelf-life of barberries compared with that of LDPE alone. The authors recommended the study of possible human health problems caused by Ag^+ nanoparticles penetrating into the barberries before any commercialisation. Zinc oxide nanoparticle (ZnO) packaging was synthesised by coating polyvinyl chloride (PVC) with ZnO nanoparticle powder of 200-400 nm particle size. Samples were used to monitor the antimicrobial activity in sliced apples. The results of fruit decay rates were significantly lower in sliced apples stored using ZnO-NP packaging at 4°C. The fruit decay rate of 21.5% was significantly lower than in the control sample of 42.4% on Day 12; in contrast, samples stored using conventional packaging as the control began to decay on Day 1 (Li *et al*., 2011).

Several studies have been conducted on Ag nanoparticles polymer coatings (An, Zhang, Wang, & Tang, 2008). The authors confirmed that Ag nanoparticles coating was effective in decreasing microbial growth, thus increasing the shelf-life of asparagus, and reported that the efficiency of smaller Ag nanoparticles was greater than larger-dimensional Ag nanoparticles due to the greater surface area available for interaction with the microbial cells. Nanocomposite film synthesised with Ag nanoparticles based polyethylene (PE) retarded the senescence of Chinese jujube (Feng, Wang, Sheng, Xin, Zhao, & Xiao, 2009). Research by Emamifar, Kadivar, Shahedi, &Soleimanian-Zad (2010) revealed the extension of the shelf-life of orange juice samples to 56 days at 4°C, by storing in nanocomposite LDPE films loaded with Ag nanoparticles. The total plate count was significantly decreased in the Ag nanocomposite film compared with that of LDPE film. A different method of measuring the antimicrobial activity of nanoparticles was applied by Chadeau, Oulahal, Dubost, Favergeon, &Degraeve (2010) who demonstrated that two types of textiles using Ag nanoparticles, presented a strong antimicrobial activity against *Listeria Innocua* (*L.Innocua*), and that thinner Ag layers were equally effective. Likewise, absorbent hybrid pads based on Ag nanoparticles developed by physical methods were optimised by Fernandez, Soriano, Carballo, Picouet, &Lloret (2009). Their study showed that Ag^+ delayed microbial growth in unsanitary absorbent pads loaded Ag nanoparticles used to preserve poultry, and that the highest concentrations tested corresponding to 6.7 $mgmL^{-1}$ were efficient in reducing Gram positive and Gram-negative loads in poultry exudates. Similarly, Damm, &Munstedt (2008)reported that Ag nanoparticles-filled acrylate photopolymer layers displayed greater efficiency against *Escherichia coli* (*E. coli)* compared with unfilled polymer layers. Their paper focused on determining the percentage of Ag nanoparticles that were able to eliminate more than 90% of *E. coli*. The Ag content in the photopolymer layers corresponding to $\geq$ 0.1% was achieving the required level of antimicrobial activity, to eliminate all the bacteria, within the range of 5 to 24 hours.

Concomitantly, there is dearth of information about the type, actual use and quantity of nanomaterials in food products, utilised by the food industry. Additionally, there has been limited publication concerning the risks to humans associated with the in-gestation of these materials, (Sayes, & Santamaria, 2014; Beer, Foldbjerga, Yuya, Sutherland, &Autrup, 2012).

Material in nano-scale has a high surface to volume ratio which has significant consequences in relation to the enhancement of physical and chemical properties of the nanocomposite products. The enhanced physical properties of nanoparticles occur due to an increase in the percentage of atoms at the surface which are available for reaction and bonding with the surrounding environment. This therefore increases the opportunity for the migration of nano-sized particles into external matrices in close contact with a nanomaterial (Muncke, 2009).By way of approaching the health and safety consequences of the use of nanocomposite materials in food sector this work attempts to characterise nanocomposite containers which have been specifically manufactured to incorporate nanoparticle polymers. In the first place, it is important to determine the size of the nanoparticles involved and their distribution within the packaging material, primarily to establish whether these match the manufacturers' claims. Furthermore, independent study is initially and specifically required to establish the efficacy of these nanocomposite products. In this work, the effect of Ag and TiO_2 nanoparticles in Polyethylene (PE) packaging were investigated in order to reveal some information about their benefit to the food packaging.

2. Material and Methods

All chemicals and reagents used were of analytical grade and high purity. Nonmetallic certified nitric acid (HNO_3) was supplied by Fisher Scientific, UK. Nano-silver containers marketed as antimicrobial containers under commercial name Fresh Box based PE were purchased from Blue Moon Goods, USA and the food samples were purchased from local UK supermarkets. The water was purified using a SG Millipore system, manufactured by Triple Red Laboratory Technology, UK.

2.1 Characterisation Method

The structure and the surface morphology of randomly selected nano-silver containers were examined; **Table 1** shows the treatment of the samples. Some packaging materials were analysed without calcination. In these analyses, some samples were cut into small sections to analyse the sample from each side; others were cut into small pieces, and ground to a powder; and some were weighed and heated at 500-600°C to determine the ash residue. The obtained ash was stored in sealed vessels for further analysis (Khanna, Narendra, &Shobhit, 2007). The morphology, size and shape of particles were determined using a scanning electron microscope (SEM) equipped with EDAX, S-3400N, manufactured by Hitachi High-Technologies Corporation, Japan. SEM images were obtained at a voltage 20 kV and pressure of 1 torr. The elemental mapping was performed using EDX in environmental scanning mode, coupled with a Thermo-Noran Vantage light element energy dispersive X-ray detector. Characterisation of all the elements was obtained by X-ray spectroscopy under electron flux. The X-ray diffraction (XRD) analysis was performed using Siemens D500 diffract meter with CuKα radiation at 20 mA° and 40 kV, supported with HBX software to determine the particle size. Diffraction data were collected in the range of 2θ from 20 to 80° at step size of 0.02° and step time of 1s. Thermo-gravimetric (TGA) STA 1500 with Infinity pro-thermal Analysis software was used. Information about the composition of multi-components, the thermal stability of materials and the amount of moisture in the samples was obtained. In this case, the sample analysis was conducted by raising the temperature of the sample gradually and plotting percentage weight against temperature. The container sample (23 mg) was heated by raising the temperature every 5 minutes up to 600°C (Xie, &Ziegmann, 2011). Transmutation electron microscopy (TEM) attached to Oxford Instruments INCA EDX system at University of Oxford was used to measure the particle size and the particle size distribution was obtained by using the software package SPSS Inc., Chicago USA. The ash sample was prepared by diluting in Ultra purification water (UPW), then, (2-3 drops) of the solution were injected to a copper grid and dried at room temperature, after the removal of excess solution using a filter paper.

2.2 Migration Assays

Experiments were performed in a temperature and humidity controlled laboratory environment.
Agilent 7500 series, inductively coupled plasma mass spectrometer (ICP-MS) instrumentation with Octoped Reaction System (ORS), high-purity grade (99.99%) Argon used as plasma gas supply.

2.2.1 Samples

Six food samples were selected with a range of compositions which included: solid, liquid, high fat and high acidity. The samples were: fresh apples (A), white sliced bread (BR), fresh carrots (C), pre-packed soft cheese (CH), modified atmosphere packaging (MAP) milk powder (MP) and fresh orange juice (OJ). Each of the six sample type were randomly selected, apples samples and carrot samples were cleaned.

The solid samples were cut into pieces 2 cm^2 x2 cm^2, and all samples were weighed into portion sizes of 20 g. Each sample category was individually mixed before placing in a dedicated container and sealed using the provided lid, then stored to eliminate light variation in thermostatically controlled oven capable of maintaining a temperature of 40°C ±1°C, for either 7 or 10 days following the migration test procedure of Regulation (EU) No. 10/2011(Granda-Restrepo *et al*., 2009).

For verification the migration, identical tests were conducted using conventional containers as controls for each sample, as appropriate, purchased from local UK supermarkets. The conventional containers were used to compare the migration with the antimicrobial nano-silver containers (Zerdin, Rooney, &Vermue, 2003). During each measurement, random packaged samples of all six food types were prepared for the migration analysis, as detailed in the following section.

2.2.2 Extraction Procedure

The migration measurements were carried out in two stages of 7 and 10 days, following the incubations of the samples in sealed containers at 40°C, including the controls. The packaged samples were removed from the incubator and left to cool for few minutes at room temperature (Avella, De Vlieger, Errico, Fischer, Vacca, & Volpe, 2005).

Each individual food material (whole sample) was weighed and heated to dryness at 105°C for several hours in an electric fume furnace. This was followed by cooling in a dry atmosphere for 40 minutes. The sample was carbonised using a Bunsen burner, before heating again to 550°C for several hours in a muffle oven furnace until ash formed. The sample was cooled in a dry atmosphere for 40 minutes and stored under aseptic conditions. Triplicate and certified reference samples were prepared at the same time including the controls (Sahan, Basoglu, &Gucer, 2007). Food samples were subject to SEM and EDX analysis in ash form due to the higher amount of water activity of the raw food samples. Whilst, samples subject to ICP-MS require acid digestion as follows.

2.2.3 Acid Digestion Method

The obtained food sample ash (3.0 g) was dissolved in (20 mL) non-metallic concentrated HNO_3 solution with anti-bumping chips and heated slowly in water bath to constant volume (5-10 mL) and filtered. The samples were diluted to 1 μgL^{-1} using UPW and later with 2 % HNO_3. To avoid contamination, all glassware (vessels and flasks) were immersed in freshly prepared 2% v/v HNO_3 for 24 hours, prior to rinsing thoroughly with UPW and dried in a dust free area before use (Brody, Bugusu, Han, Sand, & McHugh, 2008).

2.3 Antimicrobial Assessment Procedure

The antimicrobial containers used in migration assays were investigated in order to determine the limit of the nanoparticles as antimicrobial agents. Clean containers, subject to examination (antimicrobial containers and conventional containers) were sprayed with 70% ethanol, then rinsed thoroughly with sterilised UPW and dried at 60°C. The samples of cheese, carrot and apples were subjected to a total plate count (TPC) and pathogenic bacteria assessments. Solid samples were cleaned, peeled, washed with sterilised UPW, dried, and cut into small pieces. Each sample was weighed (20 g) before being placed either in an antimicrobial container or in a conventional container. Packaged samples were sealed with the provided lids. All packaged samples were incubated for 10 days: the packaged samples of carrot and apples at a temperature of 35°C (Chau, Wu, & Yen, 2007).

Daily visual observation was performed on packaged samples stored in the antimicrobial containers and conventional containers at the selected temperatures for each type, over 10 days. The samples were weighed every day, and changes in colour, odour and yield loss were documented during the period of testing and the packages used in the measurements were discarded after each inspection. The fruit decay rate was calculated according to the following formula:

$$\text{Sample decay rate \%} = \frac{\text{the weight decayed x100}}{\text{the total weight of sample}}$$

Weight decayed was calculated as:

$$\text{Weight decayed} = \text{initial sample weight} - \text{weight of stored sample}$$

Total count plate was employed every 24 hours. Each sample was weighed (10 g) and were homogenised in (90 mL) of saline primary 1/10 dilution.

A standard method protocol of ISO 4833 (2003) was followedwith the primary dilution (1 mL) being serially diluted to 10^{-5} and included in the nutrient agar, 5 different dilutions and 2 plates per dilution being prepared. Duplicate samples were prepared for all assessments and incubated at 37°C ±1°C for 72 hours.

Colonies were counted using Quebec colony counter and further by visual inspection (Aycicek, Oguz, &Karci, 2006). Results were converted to their base 10 logarithm. Plates with 25-250 colonies were counted. Plates with more than 250 colonies were deemed to be too numerous to count (TNTC). Plates with less than 25 colonies were deemed to be too few to count (TFTC) (Khamis& Hafez, 2011).

3. Main findings and Discussion

3.1 Morphology Structure

The information provided by the supplier is presented in **Table 2** with a photographic image of the commercial box is shown in **Figure 1**, indicating the nano-size of silver used in the antimicrobial containers. The technical information provided by the manufacture regarding the amount of nanomaterial, its shape and size used in the product was limited; therefore, an investigation was carried out to identify the element composition of the product, the particle size, and the amounts of nanoparticles as well as whether the nanoparticles coated onto the surface or embedded inside the polymer matrix. The characterisation of the container revealed that the nanomaterials used as antimicrobial agents were of two types: Agand TiO_2 nanoparticles. The container samples without calcinations were characterised using EDX and SEM. TiO_2 nanoparticleswere identified as flake-like structures spread out within the polymer matrix, and Ag nanoparticles were observed in other places, as shown in **Table 3** spectrum 1 and 2 prior calcinations. Qualitative analysis confirmed the presence of Ag and TiO_2 nanoparticles on the bulk of the polymer by EDX spectrum as shows in **Figure 2**. EDX results indicate that the percentage of Ag nanoparticles was 10 times that of the TiO_2 nanoparticles present in the bulk polymer. Correspondingly, ICP-MS analysis of container ash sample showed that the concentration of Ag was 1 mgL^{-1}, and the Ti concentration was 0.1 mgL^{-1}.

The structure of Ag nanoparticles in the containers after calcinations was observed using a SEM analysis, the sample scanned to confirm the presence of Ag using EDX analysis, both analyses were run concurrently and the image of that region of the nanoparticle was obtained.

TiO_2 nanoparticles appear to be integrated into the Ag nanoparticles layers present at some locations in the nanocomposite polymer, and also occurred on their own in other regions. The particle size measured at 20-70 nmas shows in **Figure 3.a.** Following numerous examinations, focusing on many different sides on the surfaces of the nano-composite before calcination, Ag and TiO_2 nanoparticle layers were found incorporated within the bulk polymer and neither Ag nanoparticles nor TiO_2 nanoparticles were applied as a coating on the container polymer surface. The polymer layers were characteristic of randomly distributed Ag and TiO_2 nanoparticleswith some evidence of aggregation of these particles were apparent. This suggests the uncontrolled nature of the process by which nanoparticles have been incorporated into the bulk polymer. TEM analysis confirmed the particle size and the shape of both nanoparticles with an image of the frequency distribution of particles size measurement was obtained as displays in **Figure3.b**.

XRD patterns of nano-silver container as presented in **Figure 4,** revealed a characteristic of small particle size within the range 20-70 nm. However, aggregation was apparent due to thesize of the particles were around100 nm as observed following XRD and SEM characterisation; particle size was calculated also using Scherrer Formula (Pereira de Abreu, PaseiroLosada, Angulo, & Cruz, 2007).

$$t = \frac{k\lambda}{\beta \cos\theta}$$

Where t, is the averaged dimension of crystallites; K is the Scherrer constant, somewhat arbitrary value that falls in the range 0.87-1.0 (it is usually assumed to be 1); λ is the wavelength of X-rayCuKα = 0.15406nm and B is the integral breadth of a reflection (in radians 2θ)is the diffraction angle and convection of the unit of θ to degrees by the following equation.

$$\text{Degrees} = \frac{\text{radians} \times 180}{\pi}$$

XRD pattern also, indicated intercalation of nanoparticles in the nanocomposite polymer. The three major characteristic peaks of nanocomposite container was within 2θ value of 38°, 44° and 64° with spaces as 134, 329 and 508 which was corresponding to the crystal face of (111), (200) and (220) of Ag nanoparticle, while before calcination, the nanoparticle peaks are interacted with polymer matrix only the (200) peak appeared. The other peaks are related to the TiO_2 nanoparticle, which interacted with Ag nanoparticle peak at 27° and 36° indicating TiO_2 in the rutile phase (Kasetsart, Limsuwan, &Ngotawornchai, 2008). The results provide supporting evidence for the polymer captured Ag and TiO_2 nanoparticle structure.

TGA analysis was performed to obtain: the thermal stability, the moisture content of the commercial antimicrobial containers and to confirm the decomposition of the polymer matrix, as shown in **Figure 5**, the polymer did not decompose before reaching a temperature of 250°C and the mass loss was small. The nanocomposite polymer started to decompose at temperatures around 400°C. The residual mass was that of silver oxide (Ag_2O) and TiO_2 indicating that Ag and TiO_2 nanoparticle encapsulated in containers had reacted with oxygen. When the polymer decomposes at about 400°C, the nanoparticles in the polymer matrix were released and reacted with amount of oxygen quickly generating an increase of the heat resulting by more than 75% in the polymer weight loss at 450°C.

3.2 Migration Assessments Results

ICP-MS results for all samples incubated at 40°C and examined after 7 and 10 days, respectively, are presenting in **Figure 6**. These results indicate that insignificant levels of Ag nanoparticles were released from antimicrobial containers. For example, the highest level of Ag nanoparticles released from the packaging into food materials after both 7 and 10 days was from the orange juice sample at a value of 5.7± 0.02 μgL^{-1}. In contrast with the orginal Ag content in the control sample 0.16 ± 0.01 μgL^{-1} and the same amount was recorded also from the food samples stored in conventional containers. Similarly for the Ti, the highest migration levels were reported from the orange juice samples 2.5 ±0.03 μgL^{-1} as shows in **Figure 7**. The second highest migration rate of Ag nanoparticle released was that of the cheese followed by the apples samples, wherease, the bread samples showed the lowest migration level of the tested samples.However, migrating of Ag and TiO_2 nanoparticles from the antimicrobial containers into food samples were less than the permitted European Union standard of a concentration of 10 mgL^{-1} (Appendini & Hotchkiss, 2002). Therefore, the amount of Ag and TiO_2 nanoparticles were found in the selected food samples at concentrations as low as 6 μgL^{-1}. Detecting nanoparticles in foodstuffs is one of the major challenges in determining the risks associated with them. For this reason, many articles relating to the migration of nanoparticles avoid dealing with the difficulty of their analysis in real-food matrices. A small number of articles have assessed migration into real-food matrices, for example the work of Cushen, Kerry, Morris, Cruz-Romero, & Cummins (2013) who evaluated the migration of Ag nanoparticle from synthesised nanocomposite-based PVC and applied migration tests to chicken breasts at storage temperatures between 5°C and 20°C. The results showed levels of migration corresponding to 0.03-8.4 $mgkg^{-1}$.

Method detection limits (MDL) was measured using spiked samples of individual concentrations solution of Ag and Ti near the expected detection limit and was calculated each time using the standard deviation for the seven replicate. MDL was recorded as 0.040859 $\mu gL^{-1}$and limit of quantifications of 0.13μgL^{-1}. Samples were carefully prepared, a background calculation was instituted at each time of analysis, and triplicate samples were performed throughout. Certified reference samples were used in all experiments to identify any errors.

3.3 Antimicrobial Activity of Ag and TiO_2 nanoparticles

The aim of antimicrobial assay is to investigate the activity of Ag and TiO_2 nanoparticles as antimicrobial agents in this type of commercial containers. The information provided by the supplier, claimed that their product was up to 99.9% antimicrobial effective with an expiration period extending to as long as three years. The labelling of the product, gave no indication as to whether TiO_2 nanoparticles were present.

The effect of Ag and TiO_2 nanoparticles on the microbial growth over time of food samples compared with food samples stored in conventional containers was determined. Antimicrobial packaging exhibited antifungal activity as demonstrated by inhibition of the growth of *Penicillum* on the raw samples tested.

Food samples stored using conventional packaging started decaying on day 1 and reached a complete decaying on day 5 at 35°C storage. Conversely little microbial growth was observed during the first six days of storage under identical conditions in antimicrobial containers.

A significantly lower rate of deterioration was observed up to day 10, due to the antibacterial activity of Ag and TiO_2 nanoparticles. The reason for selecting 35°C as a storage temperature for the food samples tested was that some fruit and vegetable are eaten fresh; this temperature is common in many countries, and hence was applied to the test as an ambient storage temperature.

The results of the total plate count of food samples, stored in antimicrobial containers, were performed to determine the extent of Ag and TiO_2 nanoparticles as antimicrobial agents and presented in **Figure 8**. Samples stored in antimicrobial containers show significant activity against the growth of microorganisms compared with the samples stored in conventional food containers, where the samples stored in conventional food containers produced similar results to that of the control samples in their original packaging. Samples were compared with their controls over the course of 10 days. The safety and stability of food depends on the microorganisms initially present, and on their being unable to overcome various biologically undesirable factors in order to multiply.

Fruit decay rate was hardly detectable in freshly sliced samples comprising of apples and bread stored in antimicrobial packaging for the first five days at 35°C; the decay rate measured was 12% and 8% respectively for antimicrobial container-stored samples representing 82% and 80% less decomposition compared with the control by Day 10 as presented in **Figure 9**. Sliced samples were subjected to lactic acid fermentation through the action of a starter culture, *Lactobacillus*, with changes in the amount of both the naturally present microorganisms and developmental growth in samples stored using different packaging types recorded. The antimicrobial packaging investigated in this study clearly possessed effective antimicrobial activity. The sample stored in the antimicrobial containers showed signs of gradual decay commencing on Day 6. The Ag and TiO_2 nanoparticles were able to retard the decay only on the top surface of the samples, in places where there were no contact with the nanoparticles as presented in **Figure 10,** photographic images taken of freshly sliced carrot samples stored using antimicrobial packaging and conventional packaging at 35°C for 10 days. *Staphylococcus* (*S. aureus*), *Coliforms* and *E. coli* were not observed in any of the samples investigated, this is considered normal, as these samples are meant for human consumption and must be free of any pathogenic bacteria.

The reference standards for *E. coli* and *Coliforms* are shown in **Figure 11**. Cheese samples stored in antimicrobial packaging and conventional packaging at 35°C for 10 days were tested for *Listeria* using the Compact Dry-LS system. Once more, the results were negative for the different types of packaging assessed, including the control, which indicates that the original sample was free from pathogenic bacteria. A great amount of research has been conducted on the antimicrobial activity of nanoparticles mostly against different types of microorganism (De Azeredo, Agroindustry, &Mesquita, 2012). Therefore, this study focused on examining the selected real-food samples packaged in commercial packaging, in order to report the effects of the antimicrobial agent material on food samples and the decay rates in real matrix. Samples held in antimicrobial containers, were found to have a limited amount of microorganism growth, compared with those samples held in conventional containers, indicating a prolonged shelf-life of sliced apples samples up to 10 days. Therefore, antimicrobial packaging provided the unique conditions that controlled the growth of microorganisms, mainly due to the inhibition of spoilage microorganisms, thus maintaining the quality of food products during storage (Espitia, Soares, Coimbra, De Andrade, & Medeiros, 2012 ; Kheybari, Samadi, Hosseini, Fazeli, &Fazeli, 2010). Samples stored in antimicrobial containers showed little decay during the first six days of storage, and on Day 7 the decay rate was significantly lower than that of the control, due to the antibacterial activity of Ag and TiO_2 nanoparticles. It is important to note that the decay started mainly on the top surface of the samples (Jokar, Abdul Rahman, Ibrahim, Abdullah, & Tan, 2012). The rate and extent of antimicrobial activity is impacted by temperature and the contact time. In addition, several authors have reported that the smaller particle size of nanoparticles are more efficient than bigger ones because of the larger surface area available for interaction with microbial cells, and also a correlation has been also found between the aggregation stability and antimicrobial activity (An, Zhang, Wang, & Tang, 2008). Combinations of more than one antimicrobial have also been incorporated into many packaging materials.

However, decay is a complex natural phenomenon that is driven by countless conditions. Fruit and vegetable have high water content in proportion to their weigh. Moisture loss due to transpiration is a major factor in fruit and vegetable decay and is one of the main factors that drive changes in their internal structure. Microbial growth, enzymes and changes in pigments are important factors that affect the quality of fresh fruit and vegetables.
The enhancement of the packaging material, problems related to food damaging factors can be solved (Motlagh, Mosavian, Mortazavi, & Tamizi, 2012).

The need to package foods in a multipurpose form, for transportation and storage, along with the increasing consumer demand for fresh, convenient and safe food products, presages a bright future for the use of nanoparticles in impregnated polymer packaging forms. In this study, the inhibition of microbial growth was measured using several methods. Impregnation of Ag and TiO_2 nanoparticles in container packaging displayed a significant difference from the control packaging, suggesting that impregnation packaging could provide a vital service to consumers and a reduction in food yield degradation.

4. Conclusion

This study demonstrated that nano-silver antimicrobial food packaging applications are a novel approach toward the preservation of foods and the extension of their shelf-life.

Characterisation of the commercial nano-silver containers revealed, contrary to the company's literature, that, two types of nano-materials were used as antimicrobial agents, namely: Ag and TiO_2 nanoparticles. The structural morphology showed the intercalation of Ag and TiO_2 nanoparticles in the 20-70 nm range within the bulk polymer and this can explain the significant antimicrobial effect observed. Some aggregation was apparent, due to the random incorporation of nanoparticles in the composite within the polymer. Concentration of the nanoparticles was obtained by ICP-MS analysis of 1% of Ag nanoparticles and 0.1% of TiO_2 nanoparticles. Verification of the antimicrobial effect of nano-silver containers was effected. The investigation carried out on the Ag and TiO_2 nanoparticles used in this nanocomposite indicate that these are distinctly layered and embedded within the bulk polymer and not as a coating on the polymer surface. The work performed complete surface intercalations of the food matrices into antimicrobial containers have been achieved and used in the migration assessment; data pertain to insignificance levels of Ag and TiO_2 nanoparticles in the selected food matrices which is a far lower than the acceptable levels at 0.01 mgL^{-1}.

5. Future Work

The determination of the shelf-life of foodstuffs contained in nanocomposite packaging and the effect of nanoparticles migration into food, in order to ascertains the safety of these packaging on everyday use. There is a need to identify the nature of the interactions between nanoparticles and their effects on the fundamental cellular processes.

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Conflict of Interest

The author has declared no conflict of interest.

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Figure 1:Commercial Antimicrobial Containers Label

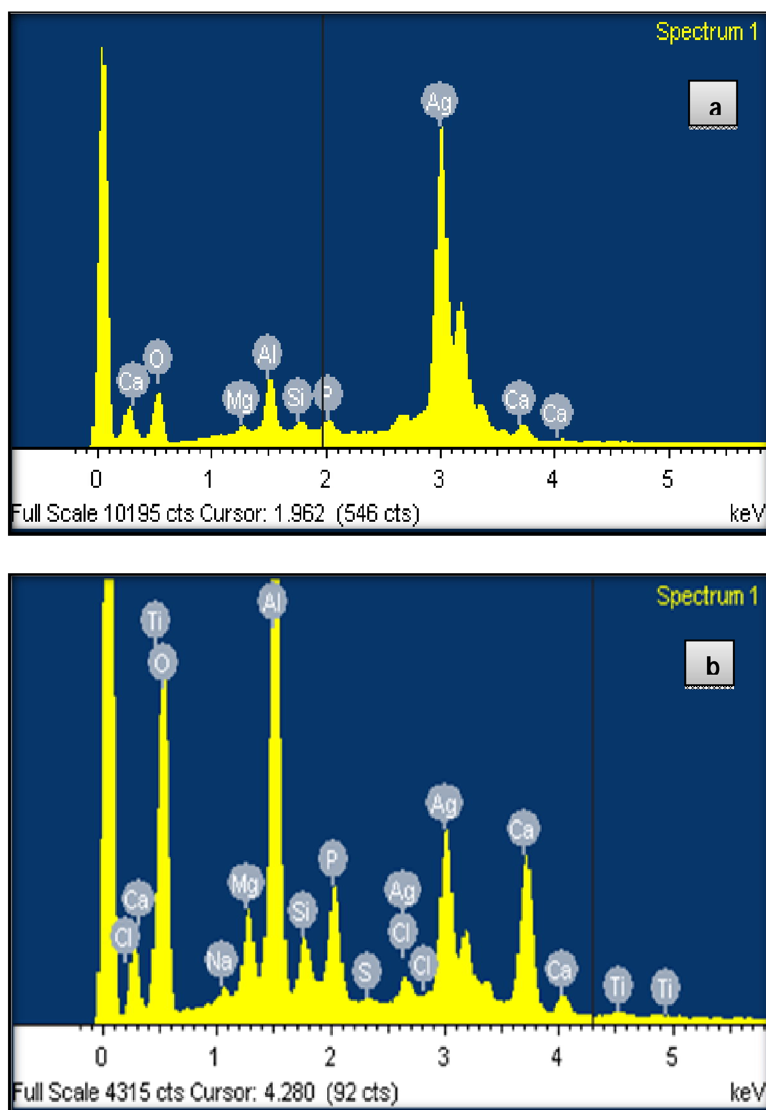

Figure 2: EDX Spectrum of elements Present in Antimicrobial Container, (a)Ag Nanoparticles only before Calcination and (b) Ag Nanoparticles Integrated with TiO_2Nanoparticles after Calcination

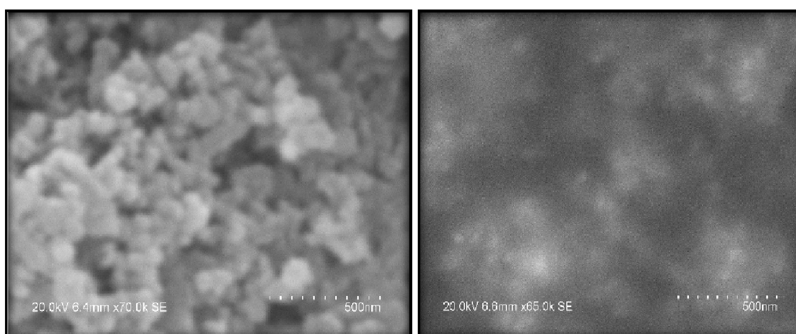

Figure 3.a: SEM images of Ag and TiO_2Nanoparticles in Antimicrobial Container at 500nm Magnifications

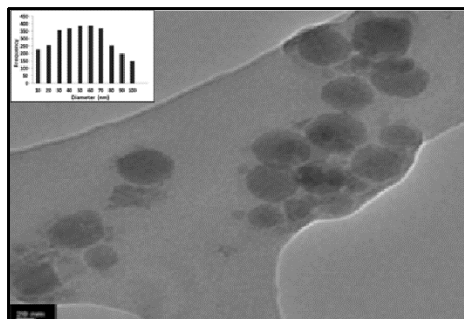

Figure 3.b: TEM image of Ag Nanoparticles (lighter colour) and TiO_2Nanoparticles(dark colour) in Antimicrobial Container at 30nm Magnifications with Particle Distribution Histogram

TEM	Transmutation electron microscopy
SEM	Scanning electron microscopy
Ag	Silver
TiO_2	Titanium dioxide
nm	Nanometre

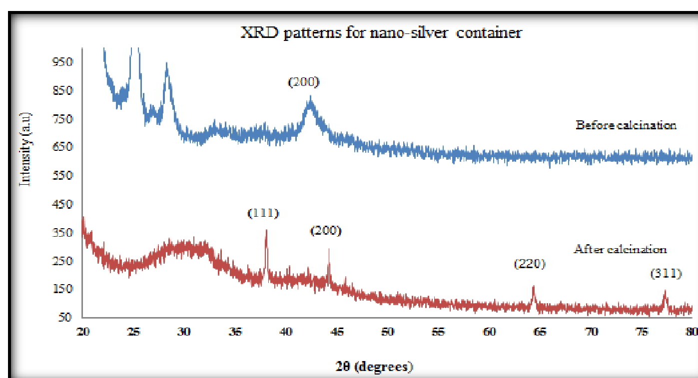

Figure 4: XRD Patterns for Ag and TiO_2Nanoparticles in Antimicrobial Container Polymer

XRD	X-ray diffraction
TiO_2	Titanium dioxide
Ag	Silver

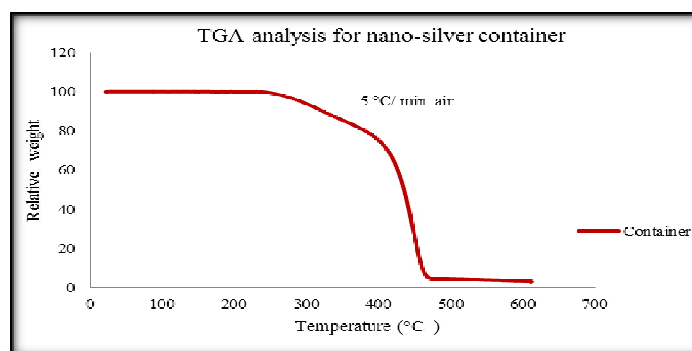

Figure 5: TGA analysis for Antimicrobial Container Polymer

CTGA	Thermo-gravimetric analysis
min	Minute

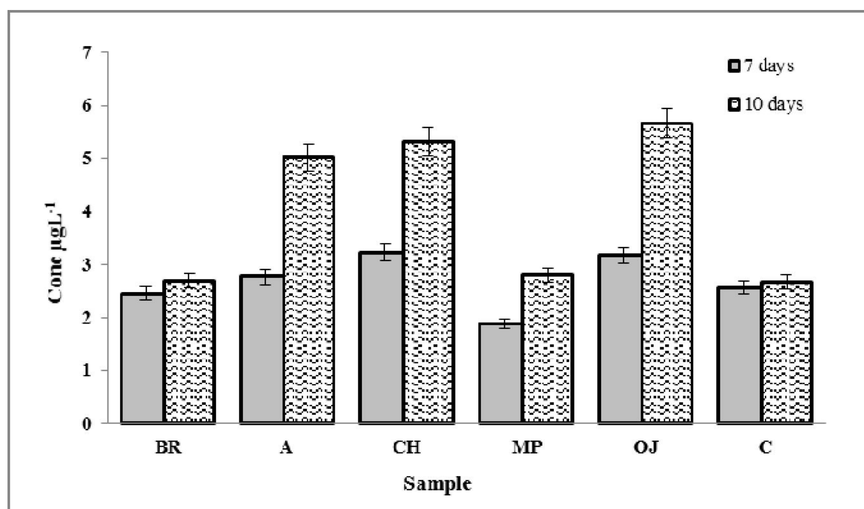

Figure 6: ICP-MS Determination of Ag Migration from AntimicrobialContainers into Specified Foodstuffs for 7 and 10 days at 40°C

ICP-MS	inductively coupled plasma mass spectrometry
Ag	Silver
A	Apples
BR	Bread
CH	Cheeses
C	Carrot
MP	Milk powder
OJ	Orange juice

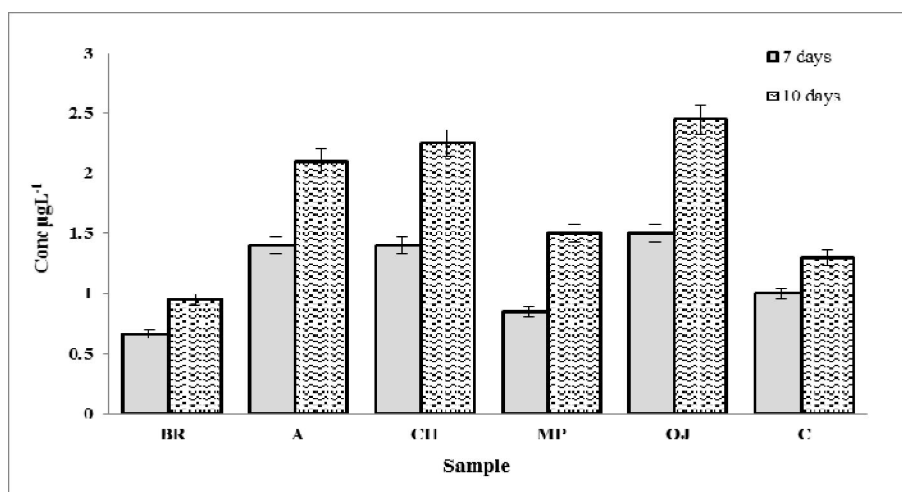

Figure 7: ICP-MS Determination of Ti Migration from AntimicrobialContainers into Specified Foodstuffs for 7 and 10 days at 40°C

ICP-MS	Inductively coupled plasma mass spectrometry
Ti	Titanium
A	Apple
BR	Bread
CH	Chesses
C	Carrot
MP	Milk powder
OJ	Orange juice

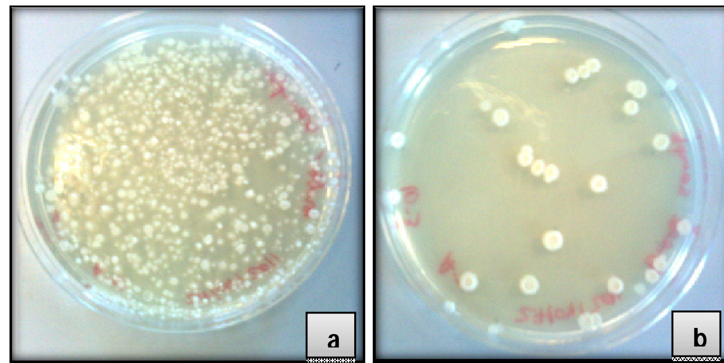

Figure 8: Total Count for Carrot Sample Stored for 10 days at 35°C (a) Conventional Containers Packaging (b) Antimicrobial Containers Packaging

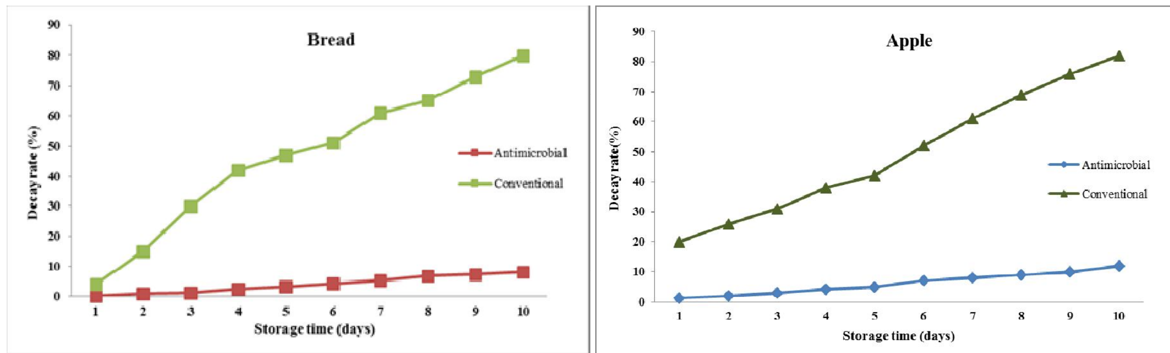

Figure 9 Decay Rate of Sliced Samples Stored in Antimicrobial Containers and Conventional Containers at 35°C for 10 days, values are mean ± SE (n=3) with Statistical Significance corresponding to: $p \leq 0.001$

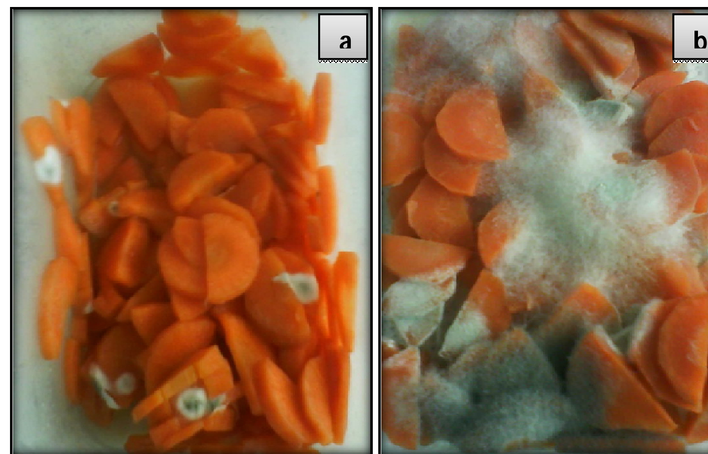

Figure 10: Photographic images for Carrot samplesStored (a) in Antimicrobial Containers Packaging (b) in Conventional Containers Packaging, at 35°C for 10 days

a

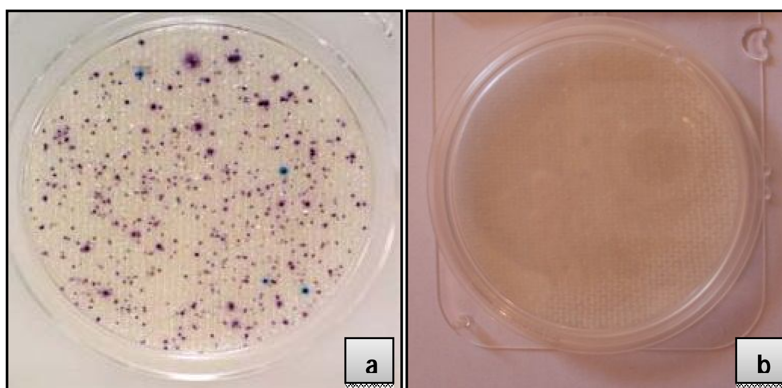

Figure 11: Compact Dry-EC for *E. coli* and Coliform (a) Reference Standard (b) Negative Result from food Samples Stored in AntimicrobialPackaging and Conventional Packaging at 35°C for 10 days

Table 1: Sample Preparation Procedures for Packaging Materials

Sample	Treatment 1	Treatment 2
Sample 1	Cut from different sides	No treatment
Sample 2	Cut from different sides	Grounded to powder
Sample 3	Cut from different sides	Calcination

Table 2:Commercial Property for Antimicrobial Containers

Property	Container
Material	PE
Type	Fresh Box
Usage	Home food storage
Feature	Odour and air impermeable
Hardness	Solid
Processing type	Unknown
Transparency	Semi transparent
Place of origin	USA
Brand name	Antimicrobial
Model number	Unknown
Colour	Transparent
Particle size	Unknown
Conc. of nano Ag	Unknown
Rate of Ag^+ release	Unknown
Structure of particle	Unknown
Classification	Nano
Product testing Information	KOTRIC-CC

Table 3: Elements Present within Antimicrobial Container Prior and Post Calcination (wt %)

Elements	Prior calcinations		Postcalcinations	
	Spectrum 1	Spectrum 2	Spectrum 1	Spectrum 2
Ti	0.11	-	-	0.46
Ag	-	1.20	4.98	4.97