

Review Article

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Nutritional Changes in stored Tomatoes (*Solanum lycopersicum L.*) as influenced by Silver Nanoparticles Coating

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ABSTRACT

This study evaluated the influence of silver nanoparticle (AgNP) coating on the nutritional content of stored tomato fruits. Silver nanoparticles were fabricated via the green method of synthesis using ethanolic extract of *Moringa oleifera* leaves as reducing, capping and stabilizing agent. The antimicrobial efficacy was evaluated at various concentrations (10 – 100 ppm) against *Aspergillus niger*, *Aspergillus parviticus*, *Aspergillus flavus* and *Fusarium oxysporum*. Silver nanoparticle coating was prepared from a mixture of silver nanoparticles, carboxyl methyl cellulose (CMC) and sodium caseinate with glycerol as plasticizer. Fresh tomato fruits were treated with AgNP coating and observed for 20 days with chemical analyses carried out every 5 days. Results showed that AgNP was able to inhibit *A. niger* from 40 ppm with 0.72 cm inhibition zone, *A. parviticus* and *A. flavus* at 80 ppm with 0.72 and 0.95 cm inhibition zones respectively. No inhibition was observed for *Fusarium oxysporum*. There was an observed increase in the ascorbic acid, lycopene and β -carotene contents over the storage period, showing AgNP coating was able to effectively retain the nutrients better than the other treatments. Potassium was well preserved by the application of AgNP coating while the control samples retained calcium and sodium better than the coated fruits. Silver was not detected in any of the fruits. The findings suggest that silver nanoparticle coatings can effectively inhibit microbial growth, thereby preserving the nutritional integrity of stored tomato fruits. However, further research is recommended to evaluate the long-term effects and stability of silver nanoparticle coating.

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Introduction

Tomato (*Solanum lycopersicum L.*) is a highly nutritious climacteric fruit and very important horticultural crop, grown globally. It can be consumed both fresh and cooked but maximum nutrients are derived from the fruit when consumed fresh. Tomatoes have been reported to be a rich source of polyphenols, lycopene, ascorbic acid and other phytonutrients. Tomato fruits suffers a short shelf-life due to several factors which include environmental condition such as temperature and relative humidity, poor post-harvest management, fruit softening and senescence. All these contributes to increased pathogenic infection which compromise the quality and availability of the fruit. Cold/low temperature storage of tomato fruits has been a prevalent means of preservation of fruits due to reduced rate of ripening and low risk of microbial infection. However, the high risk of chilling injury and its effects have called for alternative storage technique which is cost effective, ecofriendly and biodegradable [1-4].

The application of edible coatings for the preservation of fruits has been reported to be a promising technique that improves the appearance, retains the natural volatiles of fruits, and provides the fruits with excellent mechanical strength in addition to the control of gaseous exchange in and out of the fruit [5-8].

The use of nanomaterials on edible coatings is on the increase due to its high surface area to volume ratio, low use of chemicals and low energy consumption. Silver nanoparticles, among other nanomaterials, has been widely used due to its notable high antimicrobial property and low toxicity to human cells. Its synthesis using the green technique (use of bio-based compounds as reducing, stabilizing and capping agents) has contributed extensively to the low toxicity, less cost effectiveness, biodegradability and high eco-friendliness of the product. The use of silver nanoparticle coating for preservative purpose extends from food to medical instrument as well as personal care products [9-19].

In a bid to reduce unprecedented challenges that are affecting the sustainability of food and agriculture systems, there is a need to increase the pace of agricultural innovation to overcome the challenges of 21st century post production system. One of such innovations is the use of edible coatings and nanotechnology, which has been introduced in post-harvest management of agricultural commodities. This study therefore aims to evaluate the influence of silver nanoparticle as coating on the nutritional properties and sensory attributes of stored tomato fruits.

Materials and Methods

Preparation of Leaf Extract

Fresh *M. oleifera* leaves were collected from the University of Ilorin, Ilorin, Kwara State premises at coordinates and identified at the University Herbarium with herbarium number

UILH/001/559/2021. The collected leaves were destalked, washed in distilled water and air dried under shade. The dried leaves were blended using an electric blender, and was stored in an air tight container for further use. Ethanolic extract of the leaves was prepared according to the method explained by Coelho with modification, where 10 g of the blended sample was soaked in 250 mL ethanol and shaken for 4 hours using an orbital shaker (Stuart SSL1 R160001264). The solution was filtered and the filtrate was taken for further use [20].

Synthesis and purification of AgNPs

The ethanolic extract was mixed with silver nitrate solution in ratio 1:5 and the solution was agitated constantly using with a magnetic stirrer at room temperature until a colour change from bright green to brownish green was observed. The colour change indicates the reduction of silver ion and thus, the formation of the silver nanoparticle. The colour change was observed till maximum and the solution was centrifuged using Centurion Scientific Benchtop Centrifuge (ProResearch K2015R) at 5000 rpm for 15 min at a temperature of 4°C. The supernatant was discarded and the sediment was washed by adding distilled water, shook vigorously and then centrifuged at the same condition. This wash step was done twice and the resulting sediment was collected and dried at 37°C for 1hr to obtain the silver nanoparticle [21].

Microbiological Analyses

Media preparation and microbial isolation was carried out following the methods described by Fawole and Oso, Oyeleke and Manga, and FAO. Appropriate quantity of the commercial Potato Destrose Agar (PDA) media was dissolved in distilled water contained in a conical flask; the mixture was homogenized on a magnetic stirrer hot plate then sterilized in the autoclave at 121°C for 15 mins and allowed to cool. Streptomycin was added at a concentration of 35 mg/L to restrict bacterial growth [22-24].

Microbial isolation was done using standard pour plate technique. Specific quantity of tomato fruit, subjected to spoilage, was homogenized separately in a sterile glass beaker with sterile peptone water as diluent and the plates were observed for 48 – 72 hours for fungal growth. Observed microorganisms were isolated to get a pure culture of each isolate. Purified microbial isolates via streaking and subcultures was kept on appropriate agar slants and broths; which was further utilized for biochemical characterization of isolated microorganisms.

Microscopic examination was carried out using the method described by Fawole and Oso. The mycelia of the fungi was picked with the aid of an inoculating pin which has been flamed red hot and allow to cool, was placed on a glass slide, 1-2 drops of lactophenol in cotton blue was dropped on to the slide and then the fungi mycelia was teased with the inoculating needle in order to macerate the mycelia for clear view on the microscope, a cover slip was placed gently on the slide to avoid bubble formation. The slide was then viewed under a light microscope at different magnifications; the characteristics observed on the microscope were recorded. The identity of the isolate was partially discovered by comparing their microscopic morphology with the ones found in fungal atlas [25-26].

Antimicrobial efficacy (In-vitro) of the synthesized AgNPs was tested against the isolated microorganisms at concentrations 100, 80, 60, 40 and 20 ppm to determine its inhibitory effect which was achieved using disc method.

Preparation of Coating

The coating was prepared with modification to the method described by Jafarizadeh. Carboxymethyl cellulose (1.32 % w/w) and sodium caseinate (0.4 % w/w) was dissolved in 100 mL distilled water, with 0.86 % w/w glycerol as plasticizer and AgNPs, at 80 mg/L. The solution mixture was stirred over a magnetic stirrer at 70°C till a clear solution was obtained. Another coating solution was separately prepared as earlier explain but without AgNP. Both solutions were allowed to cool and kept for further use [27].

Coating of Tomato Fruits

Fresh tomatoes were harvested at turning stage. They were sorted for the wholesome ones free of infestation and mechanical injuries, washed and surface disinfected by immersing in 0.1% Sodium hypochlorite (NaOCl) for 2 minutes. The tomato fruits were weighed and divided into three (3) lots. The first lot was treated with AgNP coating (nanocoated), the second lot was treated without AgNP coating (treated control), while the third lot was not coated, as control. Each treatment was allowed to dry, kept in perforated cartons and stored at ambient monitored with a thermohygrometer. The stored tomatoes were observed for 20 days and analyses were carried out at 5 days interval.

Determination of Vitamin C (Ascorbic Acid)

Vitamin C content was determined using a titration method as explained by Ndawula with slight modification. Two (2) g of tomato taken from each lot was properly homogenized with 10 mL of 0.5% oxalic acid and then later made up to 100 mL in a volumetric flask for extraction. The solution was filtered and aliquot of 10 mL of each mixture was titrated against freshly prepared and standardized 2,6-dichlorophenol indophenol solution (with 10 mL oxalic acid titrated appropriately as blank) [28].

Determination of Carotenoids

The lycopene and β -Carotene contents of the tomatoes were determined following the method as reported by Fashanu et al. One-gram (1 g) of well homogenized tomato fruits were extracted with 16 mL of Acetone: Hexane (4:6) solvent. The mixture was allowed to extract for 15 min and an aliquot of the upper layer was collected and the absorbance measured at four different wavelengths (663, 645, 505 and 453 nm), using the UV-Visible spectrophotometer (Shimadzu UV1902PC). The lycopene and β -Carotene contents were then estimated using the equation stated below [29]:

$$\text{Lycopene (mg/100mL)} = -0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453} \quad \text{eqn. 1}$$

$$\beta\text{-Carotene (mg/100mL)} = 0.216 \times A_{663} - 1.22 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453} \quad \text{eqn. 2}$$

Where A stands for Absorbance at the respective wavelengths.

Estimation of Mineral Contents

Dry digestion methods as described by AOAC was adopted in this study. One gramme (1g) of homogenized sample was weighed into a crucible and placed in a muffle furnace at 450°C for 8 h to ash and then transferred into desiccators to cool. The ash was digested with 2 mL of 6M HCl, evaporated to dryness, dissolved and made up to 100 mL with 0.1M Nitric acid. From the digest, various elements were determined; Sodium (Na) and Potassium (K) was measured with the use of Jenway digital flame photometer while Calcium (Ca), Silver (Ag), Magnesium (Mg), Iron (Fe), Copper (Cu) and Zinc (Zn) was measured using Atomic Absorption Spectrophotometer [30].

Statistical Analyses

The experiments were arranged in Completely Randomized Design (CRD) with three replicates each and data was subjected to Analysis of Variance (ANOVA) and tested for significant difference among treatments by New Duncan's Multiple Range F-Test (DMRT) at ($p < 0.05$) using SPSS software package version 20.0.0 (IBM Statistics).

Results and Discussions

Antimicrobial Efficacy and Inhibitory Effect of Silver Nanoparticles
The observed and isolated microorganisms from the tomato specimen were identified via the microscopic technique at 400 magnifications (Focal lens x10, Objective lens x40). Their morphology was compared with those of fungal atlas and identified as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium oxysporum*. Similar fungal isolation carried out on tomatoes by Onuorah and Orji showed the action of *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium digitatum*lt, *Saccharomyces cerevisiae* among others. It was observed that the synthesized AgNPs had inhibitory effects at 80 and 100 ppm against *A. flavus* and *A. parasiticus* with their respective inhibition zones expressed in the Table 1. It was also observed that AgNPs inhibited *A. niger* at a minimum concentration of 40 ppm recording 0.72 cm inhibition zone. However, the silver nanoparticle concentrations (20 – 100 ppm) were found to have no inhibitory effect against *Fusarium oxysporum*, which materialized with no recorded inhibition zone [31].

Table 1: Zones of Inhibition of AgNPs at Various Concentrations against Isolated Microorganisms

Concentrations (ppm)	Zones of inhibition (cm)			
	<i>A. niger</i>	<i>A. paraciticus</i>	<i>A. flavus</i>	<i>Fusarium oxysporum</i>
100	1.21±0.01 ^d	0.71±0.01 ^a	1.48±0.01 ^b	-
80	1.13±0.02 ^c	0.72±0.01 ^a	0.95±0.00 ^a	-
60	0.86±0.02 ^b	-	-	-
40	0.72±0.01 ^a	-	-	-
20	-	-	-	-

Result shows mean±standard error of triplicate reading. Means on the same row with unshared superscript are significantly ($p < 0.05$) different

Vitamin C contents

Tomatoes are reported by Bakhy et al. to be a rich source of vitamin C with an average content of 25 mg/100g, however, variation in the values occur among cultivars. Result from this study revealed, in Figure 1, that AgNPs coating was able to retain the vitamin C contents of the stored tomato fruits better as the Lot recorded the highest vitamin C content at day 20 of the storage period. There was a general increase in the vitamin C contents of the coated tomatoes at storage day 20, this could be attributed to the increased ripening of the fruits as the storage period progresses, though, the vitamin C content of the control dropped significantly ($p < 0.05$) at day 20 from 53.75 mg/100g and 40.65 mg/100g recorded for days 10 and 15 respectively to 35.72 mg/100g. Vitamin C is a water soluble and heat sensitive compound, thus a reduction over time can be attributed to increased transpiration, respiration and oxidation of ascorbic acid to dehydroascorbic acid by the action of ascorbic acid oxidase. The result of vitamin C from this study has revealed that the coatings, especially the nanocoating, were good indices for the retention of vitamin C content of the fruits over the storage days [32-35].

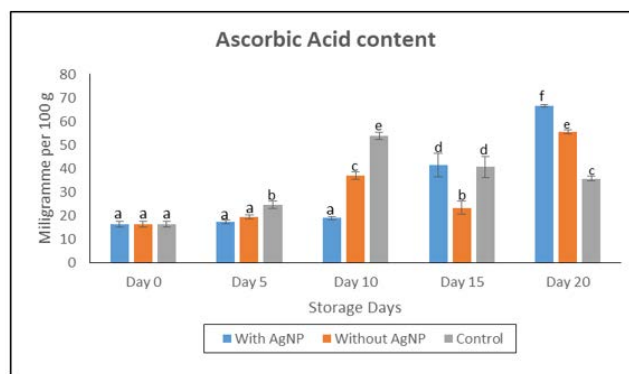


Figure 1: Effect of silver nanoparticle coating on ascorbic acid content of tomatoes after 20 days storage. Error bars represent standard error and bars with the same alphabets shares no significant difference at 95% confidence level ($p < 0.05$)

Lycopene and B-Carotene Contents

Lycopene is a pigment found to be responsible for the red colour of ripe tomatoes and it is the major pigment of tomato fruits, next is β -Carotene. The intensity of the colour increase as the fruit matures, i.e. matured green tomatoes are known to have a high amount of chlorophyll pigment and lower carotenoid contents which changes inversely as the fruits ripens. The result of lycopene and β -Carotene gotten from this study is as summarized in Figures 2 and 3. Silver nanoparticle coating further proved positive impact by keeping a good retention of the lycopene content of the stored tomato fruits. It was observed that fruits coated with silver nanoparticles had the highest lycopene contents which increased significantly ($p < 0.05$) over the storage period of 0 to 10 days from 30.30 to 101.49 $\mu\text{g}/100\text{g}$ respectively, though a significant reduction followed till day 20 from 101.49 to 90.31 $\mu\text{g}/100\text{g}$. Similar trend was observed for the treated control but the lot recorded significantly ($p < 0.05$) lower values compared to the nanocoated lot and the control [33,36].

As recorded for lycopene content, the nanocoated fruits were observed to have significantly ($p < 0.05$), the highest concentration of β -carotene over the storage days compared with other treatments. At day 20, the nanocoated fruits recorded 23.03 $\mu\text{g}/100\text{g}$ while the treated control had 21.82 $\mu\text{g}/100\text{g}$. The control lot particularly suffered a loss of the pigment as it recorded significantly reduced values as the storage period progresses. The β -carotene contents of nanocoated and treated control fruits ranged from 13.04 to 30.14 $\mu\text{g}/100\text{g}$ and 13.04 to 21.82 $\mu\text{g}/100\text{g}$, this showed that the coated fruits were able to retain a considerable amount of the pigment over the storage period [36].

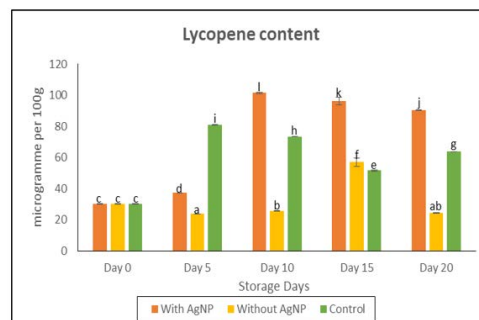


Figure 2: Effect of silver nanoparticle coating on lycopene content of tomatoes after 20 days storage. Error bars represents standard error and bars with the same alphabets shares no significant difference at 95% confidence level ($p < 0.05$)

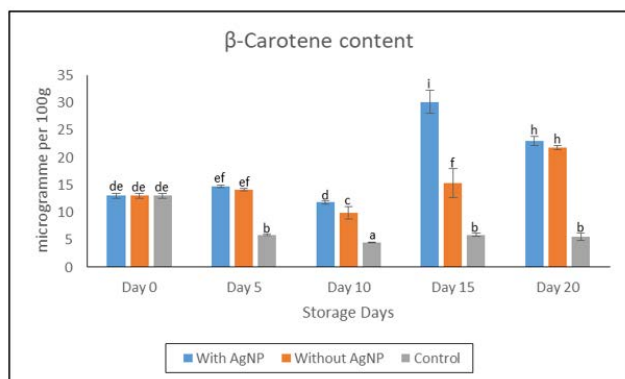


Figure 3: Effect of silver nanoparticle coating on β -Carotene content of tomatoes after 20 days storage. Error bars represent standard error and bars with the same alphabets shares no significant difference at 95% confidence level ($p < 0.05$)

Mineral Composition

As expressed in Table 2, the result of the mineral analyses revealed that Silver was not detected in any of the treated fruits, which explained that there exists no risk of contamination of silver in the nanocoated fruits. This explains that the ethanolic extract of *M. oleifera* leaves was able to reduce the cytotoxicity of Ag^+ in the synthesized nanoparticle as reported by Moodley et al. There was a trace amount of copper present in the fruits across all treatments ranging from 0.01 to 0.03 ppm, with treated control recording the highest value at day 15. Cu was observed to be reduced to 0.01 ppm at day 20, while, the metal was not detected in the treated control and control fruits [21].

Magnesium was not detected in the tomato fruits at day 0, which was later observed in the nanocoated fruits from day 5 over the storage period. The untreated fruits recorded 2.61 ppm at day 15, which further increased to 2.85 ppm at day 20. Calcium content of the nanocoated fruits were observed to decrease significantly ($p < 0.05$) from day 0 to day 20 with mean values of 13.15 to 8.20 ppm. Generally, at day 20, there was a decrease in the calcium content as well as Iron and sodium contents of all the fruits. The observed decrease in mineral contents could be attributed to the mineral being used in metabolic reactions as the fruit ripens over time. The study also revealed that potassium was abundant in the fruits recording 137.09 ppm at day 0 of storage, which further increased significantly ($p < 0.05$) at day 5 in all the treatments. However, this was followed by a general decrease as the storage period progresses, recording 158.35, 125.61 and 141.66 ppm for nanocoated, treated control and control fruits at day 20 respectively. Over the storage period, potassium content increased significantly but the treated control fruits recorded a decrease potassium content (125.61 ppm). Tomato fruits has been reported by Fashanu et al. to contain a decent amount of potassium and the result from this study is in conformity. Sodium contents was observed to be of a lower value ranging from 3.73 ppm at day 15 to 5.65 ppm at day 0 for the nanocoated fruits, 3.09 ppm at day 5 to 5.65 at day 0 for the treated control lot, while the control ranged from 2.78 ppm at day 15 and 8.14 ppm at day 10. This showed that there was a general significant ($p < 0.05$) decrease in the values of sodium across the treatment after storage [29,37,38].

Table 2: Effect of AgNPs Antimicrobial Coating on the Mineral Content of Tomato Fruits During Storage

Samples	Days	Cu (ppm)	Ag (ppm)	Ca (ppm)	K (ppm)	Na (ppm)	Fe (ppm)	Mg (ppm)	Zn (ppm)
With AgNP		0.017±0.01 ^{bc}	ND	13.15±0.05 ^k	137.09±0.21 ^b	5.65±0.15 ^h	9.32±0.11 ^j	ND	6.07±0.18 ^c
Without AgNP		0.017±0.01 ^{bc}	ND	13.15±0.05 ^k	137.09±0.21 ^b	5.65±0.15 ^h	9.32±0.11 ^j	ND	6.07±0.18 ^c
Control	0	0.017±0.01 ^{bc}	ND	13.15±0.05 ^k	137.09±0.21 ^b	5.65±0.15 ^h	9.32±0.11 ^j	ND	6.07±0.18 ^c
With AgNP		0.007±0.01 ^a	ND	10.12±0.12 ^g	197.69±0.26 ^m	5.16±0.15 ^f	8.93±0.04 ⁱ	1.72±0.01 ^b	6.08±0.06 ^c
Without AgNP		0.010±0.00 ^{ab}	ND	8.49±0.15 ^c	177.48±0.06 ⁱ	3.09±0.06 ^b	6.48±0.05 ^f	ND	8.38±0.09 ^d
Control	5	0.01±0.00 ^a	ND	7.19±0.06 ^a	178.02±0.12 ^j	6.28±0.07 ⁱ	6.20±0.01 ^c	ND	4.13±0.12 ^a
With AgNP		0.017±0.01 ^{bc}	ND	8.90±0.08 ^d	190.68±0.83 ^l	4.15±0.09 ^c	8.52±0.12 ^h	1.69±0.01 ^a	6.28±0.27 ^c
Without AgNP		0.013±0.01 ^{ab}	ND	12.04±0.08 ^j	175.04±0.15 ^h	3.19±0.06 ^{bc}	7.61±0.05 ^g	ND	6.26±0.19 ^c
Control	10	0.010±0.01 ^{ab}	ND	10.89±0.17 ⁱ	173.67±0.22 ^g	8.14±0.06 ^j	4.66±0.18 ^a	ND	5.24±0.21 ^b
With AgNP		0.023±0.01 ^{cd}	ND	8.29±0.05 ^b	179.30±0.22 ^k	3.73±0.10 ^d	10.15±0.13 ^k	1.92±0.02 ^c	6.14±0.12 ^c
Without AgNP		0.027±0.01 ^d	ND	9.81±0.11 ^f	164.12±0.31 ^f	3.30±0.19 ^c	5.75±0.18 ^d	ND	5.64±0.41 ^{bc}
Control	15	0.007±0.01 ^a	ND	10.57±0.05 ^h	156.74±0.55 ^d	2.78±0.09 ^a	4.92±0.06 ^b	2.61±0.05 ^c	6.74±1.45 ^c
With AgNP		0.010±0.00 ^{ab}	ND	8.20±0.08 ^b	158.35±0.17 ^c	4.17±0.09 ^c	7.47±0.20 ^g	1.79±0.01 ^b	6.39±0.56 ^c
Without AgNP		ND	ND	9.56±0.11 ^c	125.61±0.13 ^a	4.16±0.07 ^c	5.36±0.14 ^c	2.50±0.14 ^d	5.88±0.17 ^{bc}
Control	20	ND	ND	9.94±0.08 ^f	141.66±0.15 ^c	5.29±0.02 ^g	5.37±0.05 ^c	2.85±0.02 ^f	6.08±0.21 ^c

Result shows mean±standard error (SE) of triplicate readings. Mean with unshared superscript on the same row are significantly ($p < 0.05$) different.

Conclusion

The application of silver nanoparticle coating offers a viable way for extending the shelf life and enhancing the nutritional quality of stored tomato fruits. It has been revealed in this study that the nutritional contents of tomato fruits may be preserved efficiently with the use of silver nanoparticles through microbial growth inhibition and oxidative stress reduction during storage. Although further studies are required to clarify the long-term impact and improved application technique, the results thus far highlight the potential of silver nanoparticle coatings as a valuable tool in preserving nutritional value and extending the shelf-life of tomato fruits and other perishable crops.

Author Contributions

Israel Oluwasanmi Lawal conceptualized the research, carried out the experiments and wrote the original draft. Abiodun Adekunle Olapade supervised the research and proofread the first draft.

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Conflicts of Interest

The authors declare no conflict of interest

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