

Study on nanocoated pet bottles of tomato puree against Enteropathogenic bacteria

M. Ramya^{1*}, M. Sylvia Subapriya¹ and Nalini B²

¹Department of Food Science and Nutrition, Avianshilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu, India

²Department of Physics, Avianshilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu, India

Abstract

PET is globally recognized as safe, recyclable packaging, thermoplastic polymer, commonly used in beverage and food industries. It is a transparent, light-weight, strong, safe, shatterproof and recyclable packaging material with an inherent barrier used for wide range of product applications. The purpose of study is to assess the shelf-life of silver nanocoated (*Glycyrrhiz aglabra*) PET bottles of tomato puree for a period of ten days against enteropathogenic bacteria such as *E. coli* (MTCC40), *Salmonella enteric* (MTCC3219) and *Shigella dysenteriae* (PSGIMS & R). The maximum inhibition of antimicrobial activity of silver nanoparticle coated PET bottles of tomato from 0th day to 10th day, showed that 56.83 percentage against *E. coli* (MTCC 40), 67.67 and 87.67 percentage against *Salmonella enteric* (MTCC 3219) and *Shigella dysenteriae* (PSGIMS & R) respectively. Hence, different food packages can be tried out with coatings of silver-nanoparticles which could improve the shelf-life and antimicrobial property of food packages.

Introduction

The global production of plastics would reach about 227MT by 2015, with a growth rate increase of about five percent per year. PET is thermoplastic polyester widely used for production of beverage bottles. Plastic bottles made from polyethylene terephthalate (PET) are increasingly used in storage of beverages like softdrinks, mineral water, juices and beer, in comparison to other packaging plastic. PET is considered to be the most inert of polymers with good barrier properties against moisture, oxygen and carbon dioxide with a low migration tendency of its constituents [1]. According to National Association for PET Container Resources (NAPCOR, 2011), among 5,478 million pounds of PET bottles are available for recycling [2]. Different technologies can further enhance PET inherent barrier properties and offer great protection to natural properties of packaged products against oxygen migration and carbon dioxide. Food packed into PET containers is the main source of microorganisms causing diarrheal diseases [3,4]. Hence technology to minimize microbial health hazards is the need of the hour. Therefore barrier enhanced technology such as nanoparticle coatings on PET bottles was tried out in this study.

Materials and methods

The methodology adopted for the present study, 'Antimicrobial and Shelf-life Study of Nanocoated PET Bottles of tomato puree against *E. coli* (MTCC 40), *Salmonella enteric* (MTCC 3219) and *Shigella dysenteriae* (PSGIMS & R)', is as follows:

Preparation of the plant powder and extract

Fresh medicinal plants *Glycyrrhiz aglabra* were collected from Annaikatti Hills and were certified by Botanical Survey of India, Coimbatore. For the preparation of extract, 10 g of the plant powder was added to 100 ml sterile distilled water in a 250 ml Erlenmeyer flask and boiled for 5 mins. The boiled extract was filtered using three whatmann filter paper No.1. Further, the plant extract was subjected for synthesis of encapsulated silver nanoparticles.

Biosynthesis of nano-scale silver particles

The prepared medicinal plant extract of *Glycyrrhiz aglabra* was then centrifuged through GLC (1000 rpm for 45 minutes) and then added with 1 mM AgNO₃. Further, the filtrate was centrifuged through ultracentrifuge for 1 hr 10 minutes. The nanoparticle solution was used for coating PET bottles.

Preparation of tomato puree

Fresh tomatoes (*Solanum lycopersicum*) were boiled with ½ cup sugar and 1 tablespoon of salt. The puree was then stored in 100 ml PET containers (with and without nanocoat).

Testing highest antimicrobial activity of nanoparticles

The PET bottles were punched out into disc size for performing agar well diffusion method for determining the highest activity among the different concentration of nanoparticles. The antimicrobial activity of synthesized silver nanoparticles was done by the agar diffusion method. The log phase cultures were spread over the Nutrient agar medium plates using a sterile cotton swab in order to get a uniform microbial growth on test plates. Then approximately four wells of uniform sizes (0.65 cm) were made with a cork-borer, tested with nanoparticle solution at 10 µl, 20 µl, 30 µl, 40 µl and other petri plate tested with 50 µl, 75 µl, 100 µl and 125 µl of the encapsulated silver colloids were pipetted directly into the well against the test organisms of *E.coli* (MTCC-40), *Salmonella enteric* (MTCC 3219) and *Shigella dysenteriae* (PSGIMS & R). The plates were were incubated at 37°C for 24-48 hours.

Correspondence to: M. Ramya, Department of Food Science and Nutrition, Avianshilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu, India, **E-mail:** ramvindrheya@gmail.com

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Shelf-life study of nanocoated PET bottles with tomato puree

Further, PET bottles were made sterile under UV light, by swab method highest activity of freshly prepared nanoparticle solution were coated by swabbing and dried under the Laminar flow chamber for 40 minutes. After drying, the PET bottles were packed with tomato puree and inoculated with the test organism (in duplicates) *E. coli* (MTCC-40), *Salmonella enteric* (MTCC3219), *Shigella dysenteriae* (PSGIMS & R) and control (without nanocoating), tested for storage period of 10 days. For every two days interval, the sample was tested for enumeration of microbes.

Results and discussions

Highest antimicrobial activity of nanoparticle solution

Figures 1A, 1B and 1C present microbial activity of the three microbes against different concentrations of nanocoats. IA, IB and IC by Agar well diffusion method, with nanoparticle solution at 10 µl, 20 µl, 30 µl, 40 µl, 50 µl, 75 µl, 100 µl and 125 µl of the silver colloids tested against *E. coli* (MTCC 40), *Salmonella enteric* (MTCC3219) and *Shigella dysenteriae* (PSGIMS & R) showed that 30 µl had a maximum zone of inhibition. Hence, 30 µl was selected for coating the PET bottles.

From the Table 1, 30 µl nanoparticle solution showed less count of *E. coli* (MTCC 40) 33 counts, *S. enterica* (MTCC3219) 48 counts and *S. dysenteriae* (PSGIMSR) 42 counts. Since 30 µl nanoparticle solution

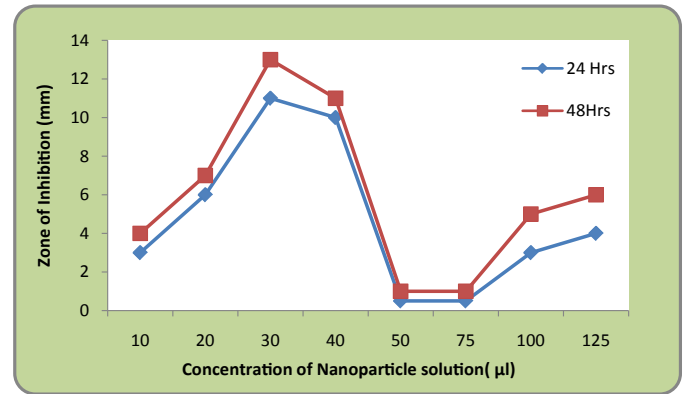


Figure 1c. Agar Well diffusion (*Glycyrrhiz aglabra*) nanoparticle solution *Shigella dysenteriae* (PSGIMSR).



Figure 2. Nanocoated PET bottles with tomato puree (*E. coli*, *S. enteric* & *S. dysenteriae*).

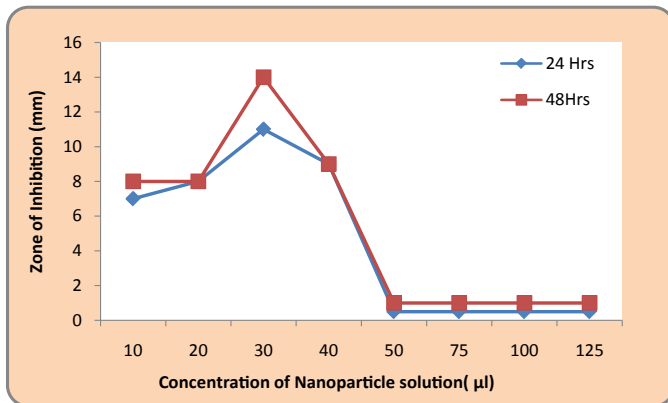


Figure 1a. Agar well diffusion (*Glycyrrhiz aglabra*) nanoparticle solution *E. coli* (MTCC 40).

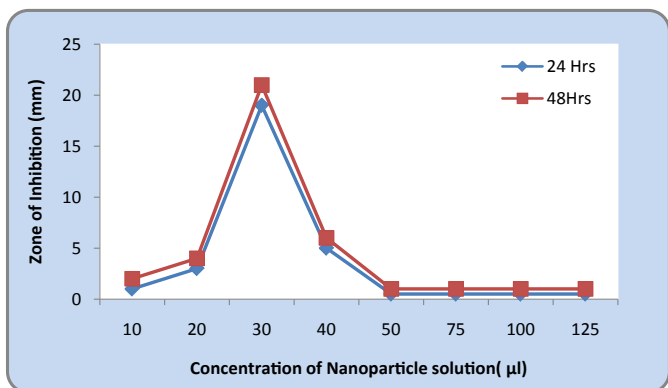


Figure 1b. Agar well diffusion (*Glycyrrhiz aglabra*) nanoparticle solution *Salmonella enterica* (MTCC 3219).

Concentration µl	<i>E. coli</i> (MTCC 40)	<i>S. enterica</i> (MTCC 3219)	<i>S. dysenteriae</i> (PSGIMSR)
10	36	80	55
20	28	74	50
30	23	48	42
40	37	66	49
Control	56	90	72
50	54	72	61
75	52	81	63
100	47	83	58
125	41	87	50
Control	64	93	76

Table 1. Enumeration of bacterial counts (*Glycyrrhiz aglabra*) nanoparticle solution.

showed maximum inhibition of microbial counts, 30 µl nanoparticle solution was used for further shelf-life study

Figure 2 and 3, shown the Shelf-life Study of tomato puree for PET bottles is taken for a period of ten days. The enumeration of bacteria was done periodically once in every two days. It was found that 0th day to 6th day there was no difference in microbial growth of all three species. But 0th day to 10th day nanoparticle coated showed that 56.83% difference against *E. coli* (MTCC40), 67.67 and 87.67% against *S. enterica* (MTCC 3219) & *S. dysenteriae* (PSGIMSR) respectively (Table 2).

Conclusion

The maximum inhibition of antimicrobial activity of silver

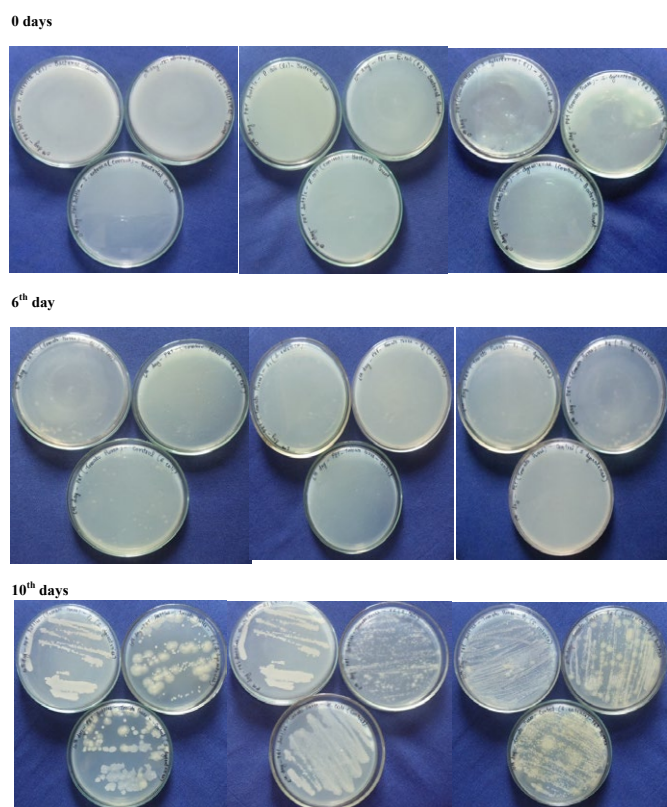


Figure 3. Shelf-life Study of tomato puree for PET bottles. Tomato Puree (*E. coli*, *S. enterica* and *S. dysenteriae*).

Table 2. Shelf-life Study of Tomato Puree–PET Bottles.

Day of Testing	<i>E. coli</i> (MTCC 40)			<i>S. enterica</i> (MTCC 3219)			<i>S. dysenteriae</i> (PSGIMR)		
	E1	E2	Control	E1	E2	Control	E1	E2	Control
0	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
2	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
4	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
6	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
8	46	50	113	89	93	162	24	27	TNTC
10	128	131	TNTC	96	98	TNTC	38	36	TNTC

nanoparticle coated in PET bottles of Tomato from 0th day to 10th day nanoparticle coated showed that 56.83% against *E.coli* (MTCC40), 67.67 and 87.67% difference against *S. enteric* (MTCC 3219) and *S. dysenteriae* (PSGIMSR) respectively. Feng *et al.* [5] stated that the microbial inhibition is due to effects of silver ions on gram-negative bacteria *Escherichia coli*, *Salmonella* and *Shigella dysenteriae* it was observed that microbial cells exposed to the Ag⁺ ions poses stress which led to the condensation of DNA. Therefore, these plant extracts of silver nanoparticles could be used as a coating in food packages as a value addition increasing the shelf- life.

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