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Comparative performance of nano-composite film and LDPE packaging for extending shelf-life of fresh Nagpur mandarin segments

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ABSTRACT

The ZnO nanocomposites film used for the study was characterized by 40 nm ZnO nanoparticle embedded in 50 nm thickness poly film with tensile strength of 3.35 ± 0.50 kg longitudinally and 1.67 ± 0.27 kg transversely average breaking load which was provided by CIRCOT, Mumbai. Microbial stability, total soluble solids, acidity, ascorbic acid (AA) content, browning index, colour value, limonin, carotenoids and sugars of Nagpur mandarin (*Citrus reticulate* Banco) segments packed in ZnO nanoparticle containing film and LDPE film were studied at monthly interval in refrigerated storage (4°C). Packaging containing ZnO nanoparticle was suitable to maintain the microbial load below the threshold population limit (6 log CFU/ml) till 30 days of storage. The segments had better colour in ZnO nanopackaging after 30 days of storage. The total soluble solids (TSS), vitamin-C and carotenoid contents were found better in ZnO nanopackaging than that of LDPE packaging. Relatively lesser value of browning was recorded in ZnO nanoparticle containing film (0.62 mg/l). The limonin content was recorded to be higher than the threshold limit in both the packaging. The changes in segments juice reducing, non-reducing and total sugars were found to be at higher side in LDPE packaging than that of nanopackaging. Study revealed that segments in nanopackaging had 30 days of shelf-life under refrigerated storage.

Key words: ZnO nanocomposite filmpackaging, LDPE packaging, mandarin segments, refrigerated storage.

INTRODUCTION

Citrus segment is one of the most globally accepted fruit products. Demand for natural loose jacketed orange segment having high nutritional, physico-chemical properties and sensory value with minimal or without heat treatment has increased considerably (Bull *et al*., 3; Souza*et al*., 18). Natural orange juice, even kept under refrigeration, has a short shelf-life due to increasing microbial spoilage (Souza *et al*.,18). Recently, nanotechnology introduced in the food packaging industry can potentially provide solutions to food packaging challenges such as shelf-life (Chaudhry *et al*., 4; Joseph and Morrison, 8), anti-microbial active packaging is a new development of nano food packaging based on metal nanocomposites, which are made by incorporating metal nanoparticles into polymer films. ZnO has found many applications in daily life such as in drug delivery, cosmetics (Yan *et al*., 22) due to its strong anti-microbial effect on a broad spectrum of microorganisms (Jones *et al*., 7).

Moreover, it is currently listed by FDA as a generally recognized as safe (GRAS) material (Jin *et al*., 6). Various methods have generally been used to produce antimicrobial polymer nanocomposites. The main objectives of this study is to evaluate the applicability of ZnO nanocomposite packaging as an new innovative approach for safe preservation with enhanced shelf-life of Nagpur mandarin segments.

MATERIALS AND METHODS

The present investigation was carried out at National Research Centre for Citrus, Nagpur during 2012. ZnO nanocomposites packaging (50 nm) and LDPE packaging (50 nm) were taken in completely randomized design with three replications. The ZnO nanocomposites film of 50 nm thicknesses was prepared by melt-blow method using 1:1 ratio of HDPE: LDPE and 1% ZnO nanocomposites provided by CIRCOT, Mumbai. The mixing time was 10 min. and the temperature was set at 200°C, to avoidthe starch degradation. The morphological analysis was carried out using both optical and scanning electron microscopy. For optical microscopy, direct examination of nanocomposites was done at the magnification of 400x. For scanning electron microscopy, the samples mounted on specimen stubs were coated with gold/palladium and examined under Philips XL 30 SEM at 10 KV with tilt angle of 45°.

The tensile strength of nanocomposites was measured in the Instron Automated Materials Testing System according to method ASTM D882-95[10].The specimens were conditioned at 65 ± 2% relative humidity and 27 ± 2°C before analysis. Initial distance of separation and velocity were fixed as 50 mm and 1 mm/s, respectively. For FTIR analysis, the spectra

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of the film (both control and nanocomposites) were recorded with an IRP Prestige-21 Fourier Transform Infrared spectrophotometer in transmission mode. For the antibacterial activity, evaluation was carried out with *Staphylococcus aureus* (ATCC 6538), a Gram-positive bacterium and *Klebsiella pneumonia* (ATCC 4352), Gram-negative bacterium. The assessment was carried out as per the procedures given in AATCC Test Method 147-2004. The ability of the nanocomposite to block UV light is measured UV-Visible spectrophotometer (Specord 50 Analytikjena®) using an Integrating Sphere loaded with sample from 280 nm at an interval of 2 h as reported by Vigneshwaran *et al*. (20).

Mature *Ambia* Nagpur mandarin fruits samples were collected from the farmers orchard at their maturity. They were brought to processing laboratory for washing and cleaning with tap water followed by distilled water to remove surface residues and segments of that fruits were separated and packed in nano zinc oxide composed packaging (50 nm) and LDPE packaging (50 nm) using heat sealing machine in aseptic condition using laminar flow. A total of 12 packets were kept under refrigerated storage at 4°C to investigate the shelf-life of fresh segments. Each packet containing 20 fresh segments. Observations were recorded periodically. The samples were evaluated in duplicate for their microbiological, physico-chemical characters immediately after packaging and thereafter at monthly interval under refrigerated storage. The microbiological evaluation of Nagpur mandarin segments stored in different packagings was done by serial dilutions, which were made from segment juice samples using sterile distilled water. One ml of segment juice sample was used. Total aerobic plate count was enumerated using 'spread plate technique' on sterile nutrient agar and potato dextrose agar. Plates were inoculated and incubated at 37°C for 48 h, and thereafter total aerobic count was taken and expressed in log CFU/ml (Aryou Emamifar *et al*., 2).

Colour was measured using a digital colorimeter model Lavibond RT series Reflectance Tintometer, which display L* - lightness/darkness, a* - Redness, b* - yellowness and a/b ratio – orange colour. A petri dish containing 25 ml of mandarin segments juice was placed into the lighting system of colorimeter. The TSS of the representative Nagpur mandarin segment juice was determined by using digital refractometer at temperature 25°C (A. Kruss Optronic, Germany). The titratable acidity and ascorbic acid were determined by the method given by AOAC (1). For ascorbic acid estimation, five ml of segment juice was mixed with 20 ml of 3% metaphosphoric acid as buffer which was titrated with 2,6 dichlorophenol indophenol dye till the light pink colour appeared as an end point. Limonin was estimated from segment juice by the method given by Wilson and Cruthchfield (21) and expressed in terms of ppm. Carotenoids were estimated from segment juice by the method given by Ting *et al*. (19). Mandarin segment juice sugars namely total, reducing and non-reducing were estimated by dinitro-salicylic acid (DNSA) method given by Miller (12).

To determine the browning index of the samples, 10 ml of the mandarin segment juice was centrifuged for 10 min of 7800 × g at 4°C, and 5 ml of ethyl alcohol (95%) was added to 5 ml of the juice supernatant followed by centrifuging the mixture again under the same conditions. The absorbance of the supernatant was read for browning index at 420 nm using a UV-spectrophotometer (UV-1650 PC Shimadzu) according to the method described by Meydav *et al*. (11). The experimental data was statistically analyzed by using the method of Panse and Sukhatme (13).

RESULTS AND DISCUSSION

Mandarin segment colour is mainly due to the presence of carotenoid pigments and is influenced by product ripening, processing treatments, storage conditions, and browning reactions. The colour of segment was found to be attractive in ZnO nanopackaging and in LDPE packaging after 30 days of storage. Weight of test packets were found to be reduced both in ZnO nanopackaging and LDPE packaging in refrigerated storage but was comparatively higher in many parameters of segments was maintained in nanopackaging than that of LDPE packaging of Nagpur mandarin fruits (Table 1). Similar findings on Nagpur mandarin were recorded by Ram and Kumar (17).

The juice acidity in Nagpur mandarin was recorded to be decreased from 0.59% to 0.41% and 0.48% in ZnO nanopackaging and LDPE packaging respectively, after 30 days of storage. (Table 2). The juice acidity was found to be decreased due to its conversion into sugar to maintain the original sweetness of juice even under refrigerated storage conditions. The ascorbic acid (AA) content of segments juice were found to be decreased as 17.60 mg/100 ml in nanopackaging and 17.05 mg/100 ml in LDPE packaging under 30 days of storage than that of initial value in fresh juice as 32.18 mg/100 ml (Table 2). The ascorbic acid content in the segments juice decreased during storage in all the experimental packages. Ascorbic acid is usually degraded by the oxidative process which is stimulated in the presence of light, oxygen, heat and enzymes (Plaza *et al*., 14).The rate of degradation was slower in ZnO nanopackaging than that of LDPE packaging, might be due to the non-barrier properties of packaging against oxygen Browning index of Nagpur mandarin segments juice were found to be lesser as 0.62 mg/l in ZnO nanopackaging as compared to 0.64 mg/l in LDPE packaging (Table 2).

Table 1. Nano zinc oxide embedded film and LDPE packaging effect on PLW, colour and quality of Nagpur mandarin segments during storage.

Parameter		Initial		30 days in refrigerated storage (4°C)		
		ZnO Nano packaging (50 nm)	LDPE (50 nm)	ZnO Nano packaging (50 nm)	LDPE (50 nm)	CD at 5%
Segment colour	*L	54.29	54.29	53.17	46.68	0.05
	*a	6.75	6.75	9.52	10.67	0.05
	*b	15.73	15.73	26.83	21.78	0.05
	*a/b	0.43	0.43	0.35	0.49	0.05
Weight of packet (g)		229.52	228.37	228.08 (0.62%)**	228.23 (0.06%)**	NS

*L : lightness/darkness, *a : Redness, *b : yellowness, *a/b : orange **Physiological loss in weight (%)

Table 2. Effect of different packaging on primary metabolites of Nagpur mandarin segments in storage.

Refrigerated storage period (4°C)	Package	Primary metabolite		
		Acidity (%)	Ascorbic acid (mg/100 ml)	Browning (OD)
Fresh juice (Initial)	ZnO Nano*(50 nm)	0.59	32.18	0.40
	LDPE** (50 nm)	0.59	32.18	0.40
30 days	ZnO Nano (50 nm)	0.41	17.60	0.62
	LDPE (50 nm)	0.48	17.05	0.64
	CD at 0.05%	0.05	NS	NS

*ZnO Nanoparticle containing film **Low density polyethylene film packaging

However, Leizerson and Shimoni (10) reported that increased values of browning index by up to 0.367 are still invisible. Increasing temperature has a major effect on the increased rate of browning reaction in fruit juice (Koca *et al.*, 9).

The limonin content dwindles to be higher of 10.72 ppm in ZnO nanopackaging and 10.78 ppm in LDPE packaging after 30 days of storage condition, which were recorded to be higher than that of fresh juice (6.01 ppm) (Table 3), which is well within the threshold limit. Similar finding in citrus juice powder under ambient storage condition was reported by Ram and Singh (16). Carotenoids of Nagpur mandarin segments was recorded to be decreased to 0.10 mg/100 ml after 30 days (Table 4) of storage. Orange juice colour is mainly due to the presence of carotenoid pigments and is influenced by product ripening, processing treatments, storage condition and browning reactions.

Table 3. Effect of different packaging on limonin content of Nagpur mandarin segments in storage.

Refrigerated storage period (4°C)	Package	Limonin (ppm)
Fresh juice (Initial)	ZnO Nano* (50 nm)	6.01
	LDPE** (50 nm)	6.01
30 days	ZnO Nano (50 nm)	10.70
	LDPE (50 nm)	10.78
	CD at 0.05%	NS

*ZnO Nano particle containing film **Low density polyethylene film packaging

Table 4. Effect of different packaging on carotene content of Nagpur mandarin segments in storage.

Refrigerated storage period (4°C)	Package	Carotenoids (mg/100 ml)
Fresh juice (Initial)	ZnO Nano* (50 nm)	0.18
	LDPE** (50 nm)	0.18
30 days	ZnO Nano (50 nm)	0.10
	LDPE (50 nm)	0.10
	CD at 0.05%	NS

*ZnO Nano particle containing film **Low density polyethylene film packaging

Total soluble solids were recorded to be all most on par to initial (natural) fresh segments (9.60%) both in ZnO nano packaging (8.30%) and LDPE packaging (8.10%) after 30 days of refrigerated storage (Table 5), but was higher in nanopackaging.

The slight changes in reducing sugar (1.10 mg/ml), total sugars (4.41 mg/ml) and non-reducing sugar (2.30 mg/ml) of LDPE packaging were recorded in comparison to initial fresh segment juice as 1.40, 5.23 and 3.64 mg/ml, respectively. The changes in juice reducing, non-reducing and total sugars were found to be at higher side in LDPE packaging than that of initial value and nanopackaging, respectively. The segments in both the packaging had 30 days of shelf-life under refrigerated storage (4°C) temperature possibly due to over lapping of segments in the test packages which become watery and lose the shelf-life within 30 days in refrigerated storage. Total sugars, reducing sugar and non-reducing sugar showed decreasing trend both in nano ZnO packaging film and LDPE normal film (Table 5). The change of sensory attributes of Nagpur mandarin segments packed in ZnO nanopackaging and LDPE packaging was observed after 30, days. The foul odour of stored segments was recorded after 30 days of refrigerated storage. The sensory panelists recognized that the nano ZnO packaging film found as the better packaging material in terms of overall acceptability of the segment. It is noteworthy that changing orange segments flavour during storage is not only due to the growth of microorganisms but also to heating, storage time and the common chemical interactions that occur in storage (Hangaord *et al*., 5).

Total aerobic count on nutrient agar from Nagpur mandarin segments juice after 30 day of storage was found to be 2.318 log CFU/ml (208 CFU/ml) in nanopackaging and 2.556 log CFU/ml (360 CFU/ml) in LDPE packaging. Total aerobic count on potato dextrose agar from Nagpur mandarin segments juice samples after 30 day of storage was found to be 2.274 log CFU/ml (188 CFU/ml) in nano packaging and 2.565 log CFU/ml (368 CFU/ml) in LDPE packaging, indicating that this total aerobic plate count is safe limit because the shelf-life of segment is defined as 'the time required to reach a microbial population of 6 log CFU/ml^- (Raccach and Mellatdoust, 15) (Table 6).

This study showed that application of film containing ZnO nano particles is a new approach for preserving and extending the shelf-life of fresh Nagpur mandarin segments under storage at 4°C temperature. The quality of the packaging film including good dispersion of nanomaterials in the polymer matrix free from agglomeration was shown to be very effective on the antimicrobial effects of these packaging materials. Application of packages containing nano-ZnO prolonged the shelf-life of Nagpur mandarin segments up to 30 days without any negative effects on sensorial attributes and physiological parameters.

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Table 5. Effect of ZnO nano particle containing film and LDPE packaging on sugarcontent of Nagpur mandarin segments in storage.

Quality parameter	Fresh juice (initial)		Refrigerated storage at 4°C		
	ZnO Nano* (50 nm)	LDPE** (50 nm)	ZnO Nano (50 nm)	LDPE (50 nm)	CD at 0.05%
TSS (%)	9.60	9.60	8.30	8.10	NS
Reducing sugar (mg/ml)	1.40	1.40	1.32	1.10	0.16
Non-reducing sugar (mg/ml)	3.64	3.64	3.10	2.30	0.16
Total sugars (mg/ml)	5.23	5.23	4.88	4.41	0.24

*ZnO nano particle containing film **Low density polyethylene film packaging

Table 6. Effect of ZnO nano particle containing film on total aerobic count of Nagpur mandarin segments juice under storage at 4°C.

Storage period	Total aerobic count log (CFU/ml) on nutrient agar		Total aerobic count log (CFU/ml) on potato dextrose agar	
	ZnO Nano packaging	LDPE packaging	ZnO Nano packaging	LDPE packaging
Initial	0.00	0.00	0.00	0.00
30 day	2.318	2.556	2.274	2.565

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