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Effect of biosynthesized zinc oxide nanoparticles coating on quality parameters of fig (*Ficus carica* L.) fruit

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Abstract
This study evaluated the effect of biosynthesized Zinc oxide (ZnO) nanoparticles coated on fig fruits to assess the quality parameters such as total soluble solids, pH, colour, weight loss, firmness and microbial population. The coating solution was prepared by mixing the ZnO nanoparticles in premixed chitosan-acetic acid solution. The fresh fig fruits were dipped in coating solution with different concentrations [T_1 (25), T_2 (50), T_3 (75), T_4 (100), T_5 (125), T_6 (150), T_7 (175) and T_8 (200) ppm] of ZnO nanoparticles. The coated and uncoated (control) fig fruits were stored at room temperature. The fig fruits coated with a concentration of 175 ppm ZnO nanoparticles delayed ripening, slowed down the weight loss, reduced the colour changes, increased the firmness of fruits and inhibited the growth of microorganism compared to uncoated fig fruits. Hence, it was concluded that the application of ZnO nanoparticles coating is a promising approach to extend the shelf-life of fig fruits.

Keywords: ZnO nanoparticles coating; fresh fig fruits; quality parameters; storage; shelf-life

Introduction
Fig (*Ficus carica* L.) fruit is one of the most ancient fruits known to mankind. It is reported to be under cultivation from 3000-2000 BC in the eastern Mediterranean region (Marpudi *et al.*, 2013) [13]. Fig (*Ficus carica* L.) is nutritious fruit rich in fiber, potassium, calcium, and iron with higher level than other fruit such as apples, grapes and strawberries. Additionally, figs are an important source of vitamins, amino acids and antioxidants. Fresh fig fruit is known as one of the most perishable horticultural commodities. The particular structure of the fruit as described by presence of an apical pore (ostiole) makes fig fruit very susceptible to a number of diseases caused by fungi and bacteria. The high metabolic activity and the easiness of pathogen development are the main causes of deterioration. The shelf life of fresh fig fruits harvested at fully ripe stage is limited to 2 days under the ambient temperature of the harvest season at the end of summer (Hung *et al.*, 2011) [8]. Thus, maintaining the freshness or fresh quality of fig is a pressing problem requiring urgent attention.

Although the microbial population could be reduced to some extent by washing, however, this does not satisfactorily arrest the microbial and non-microbial spoilage during storage. Thus, a treatment supplementing washing is required to keep the freshness of fig fruit. As one of multifunctional inorganic nanoparticles, ZnO nanoparticles are known to inhibit microbial growth. Because of their antimicrobial properties, ZnO nanoparticles have been increasingly applied in the food industry and it has been listed as generally recognized as safe by the U.S. Food and Drug Administration. However, there are no publications reporting the application of edible coating (containing ZnO nanoparticles) on fresh fig fruit. In this context, the objective of this research was to study the effect of biosynthesized Zinc oxide nanoparticles from Spinach (*Spinacia oleracea*) leaves on the quality parameters of fig (*Ficus carica* L.) fruit such as total soluble solids, pH, colour, weight loss, firmness and microbial population.

Materials and Methods
Raw Material
The fresh fig (*Ficus carica* L.) fruits were purchased from local market, Raichur. The fruits were carefully selected for uniformity in size, colour and absence of visual wounds and defects.

Biosynthesis of ZnO nanoparticles
The ZnO nanoparticles were synthesized from spinach (*Spinacia oleracea*) leaves with Zinc nitrate hexahydrate solution as a precursor. A change in the colour from dark green to pale

yellow indicated the formation of ZnO nanoparticles. The presence of ZnO nanoparticles were confirmed and characterized by Particle size analyzer, UV-Visible spectrophotometer and Scanning electron microscope.

Preparation of the coating solution

The coating solution was prepared as follows: firstly, chitosan solution was prepared by dissolving 5 g of chitosan in 300 ml of 1 % (v/v) acetic acid. The solution was continuously stirred at 60 °C for 6 h. The resultant chitosan coating solution was filtered through a Whatman No. 3 filter paper to remove undissolved particles. The ZnO nanoparticles (5 %) of different concentrations [T_1 (25), T_2 (50), T_3 (75), T_4 (100), T_5 (125), T_6 (150), T_7 (175) and T_8 (200) ppm] were added to the chitosan solution (95 %). The uncoated fig fruits were considered as control (T_0). The coating solution prepared was subjected to ultrasonication for 30 min (Meng *et al.,* 2014) [14].

Coating of ZnO nanoparticles on fig fruits

The fresh fig fruits of uniform size were selected and washed under running tap water. The selected fresh fig fruits were dipped in 100 ml prepared solution for coating with different concentrations of ZnO nanoparticles. The treated and untreated (control) samples were kept at room temperature.

Storage quality evaluation

Total soluble solids (TSS)

The total soluble solids of fig fruits coated with biosynthesized ZnO nanoparticles and uncoated fig fruits were determined by using digital refractrometer (Atago pocket refractrometer pal-1) at room temperature. A drop (~ 0.5 ml) of fruit pulp was placed on refractrometer and the reading was expressed in concentration per cent of total soluble solids as °Brix.

pH

The pH of fig fruits were measured using pH meter. Fruits were crushed and homogenized and the resultant pulp was filtered. The pH value of filtered juice was measured by using a pH meter (ECO pH2 tester) (Marpudi *et al.,* 2013) [13].

Colour

Colour of coated and uncoated fig fruit samples was measured using a Hunter's lab colourimeter (Colour Flex EZ, Hunter Associates Laboratory, Inc., United States). The colour was measured by using CIELAB scale at 10° observer at D_{65} illuminant. It works on the principle of focusing the light and measures energy reflected from the sample across the entire visible spectrum. It provides reading in terms of L^*, a^* and $b^*$where, luminance (L^*) forms the vertical axis, which indicates whiteness (100) to darkness (0). In the same way a^* indicates redness (+) to greenness (-) and b^* indicates yellowness (+) to blueness (-). The instrument was standardized before placing the sample, by placing black tile and white tile provided with the instrument and then standard tile. Once the instrument was standardized, each sample of fig fruit was measured for colour values of L^*, a^* and b^*(Hung *et al.,* 2011) [8].

Weight Loss

Weight loss in the fig fruit was determined as the difference in mass of the sample before and after storage and expressed as percentage of weight loss (Tsegay *et al.,* 2013) [18].

Firmness

Three samples of fig fruits per treatment were taken for measurement of firmness using a texture analyzer (TA.XT Plus, Stable micro system) fitted with a 2 mm cylinder probe. The fruit was punctured at a speed of 5 mm s^{-1} to a depth of 10 mm with a load cell of 5 kg. The peak puncture force (g) was used as a measure of firmness in fig fruits (Meng *et al.,* 2014) [14].

Microbiological Analysis

Ten grams of the minced sample was weighed and added with 90 ml of sterilized distilled water and blended for 15 min at room temperature. From this, 1 ml was accurately pipetted into test tube containing 9 ml of sterile distilled water (10^{-1}) and serially diluted until 10^{-3} dilution were reached. One ml aliquot each from 10^{-3} dilutions were transferred to the sterile petriplates for the enumeration of microbial load. Plates were triplicated for each dilution. Approximately 15-20 ml of cooled total plate count agar medium were added into the petriplates and the plates were rotated clockwise and anticlockwise directions on the flat surface to have a uniform distribution of colonies. After the solidification of agar, the plates were inverted and incubated at 30 ^{0} C for 2 days. Total plate counts were determined on plate count agar pour plates and enumerated after an incubation period of 48 h at 30 ^{0} C. The colonies were counted after the incubation period and the number of cfu/ml of sample were calculated by applying the following formula (Costa *et al.,* 2011) [5].

$$\text{No. of colony forming units/ml of sample} = \frac{\text{Mean number of cfu} \times \text{Dilution factor}}{\text{Volume of the sample}}$$

Where,

Dilution factor is the reciprocal of the dilution (e.g. 10^{-3}=10^3)

Statistical Analysis

Completely randomized design (CRD) was used to analyse the data. The statistical procedures for agricultural research given by Gomez and Gomez (1976) were referred.

Results and Discussion

Effect on TSS

The results showed that the initial TSS of fresh fig fruits was 11.90 ^{0} Brix and TSS of coated fig fruits ranged from 12.90 to 11.94 ^{0} Brix after first day of treatment. It is observed that in all the treatments there was an increase in the TSS with increase in storage period and decreased with increasing the concentrations of ZnO nanoparticles as shown in Fig.1. The maximum TSS after six days of storage was recorded as 14.43 ^{0} Brix in control, whereas the minimum was recorded as 12.23 ^{0} Brix in treatment T_8 which was statistically on par with treatment T_7 (12.80 ^{0} Brix). The increment in TSS might be due to disassociation of some molecules and structural enzymes in soluble compounds. This directly influenced the levels of TSS for stored fruits which was due to increase in respiration and metabolic activity (Tsegay *et al.,* 2013) [18]. Similar results were recorded by Ali *et al.* (2011) [1] who found that the higher respiration rate increased the synthesis and use of metabolites resulting in higher TSS due to the higher change from carbohydrates to sugars.

Effect on pH

As shown in Fig. 2, it is revealed from the results that there was an increase in pH with increasing storage days and decreased with increasing the concentrations of ZnO nanoparticles. Among all treatments, pH was lowest (4.79) in treatment T_8 and the highest (5.56) was in control compared to all treatments after 6 days of storage of fig fruits. These results might be due to respiration process, conversion of acids into sugars where organic acids were substrates for enzymatic reactions of respiration, so reduction of acidity that led to increase in pH of fruits (Marpudi *et al.*, 2013) [13]. These results are in agreement with the findings of Mahmud *et al.* (2008) [12] in papaya.

Effect on colour

The L^*, a^* and b^* values of fresh fig fruits were recorded as 41.14, 13.15 and 25.26 respectively. It was also observed that for all the treatments there was a decrease in L^* and b^* values and increase in a^* values with increase in storage days as shown in Fig. 3, 4 & 5. The reason might be due to reduction of total chlorophyll concentration in fig fruits during storage (Jianshen *et al.*, 2008) [10]. Similar results were obtained by Chuitchudet and Praist (2014) [4] in papaya fruits, Mohamed *et al.* (2015) [15] in mango fruits for all values of colour. Marpudi *et al.* (2013) [13] pointed out that low respiration activity of fig fruits lowered their rate of ripening and deterioration, hence a little change in colour was observed.

Effect on weight loss

Figure 6 shows the variation in weight loss of fig fruits throughout the storage period. The minimum weight loss after 6 days of storage was recorded as 19.01 % for treatment T_8 and the maximum was recorded in control (27.22 %). It is observed that the fig fruits coated with Zinc oxide nanoparticles recorded less weight loss compared to uncoated fig fruits. This might be due to the fact that the coating acted as a semi-permeable barrier against oxygen, carbondioxide, moisture and solute movement, thereby reducing respiration, water loss and oxidation reaction rates (Arowora *et al.*, 2013) [2]. The fruits coated with Zinc oxide nanoparticles and chitosan showed significantly lower weight loss. It was reported that chitosan was found to be more effective in reducing weight loss in banana and mango (Kittur *et al.*, 2001) [11] and for strawberries (Ribeiro *et al.*, 2007) [16]. Meng *et al.* (2014) [14] also reported on fresh cut kiwi fruit coated with nano ZnO which also contained the chitosan polymer, has found to restrict gas exchange on the surface of freshcut kiwifruit at 1.2 g/l.

Effect on loss of firmness

The results showed that the initial firmness of fresh fig fruits was 272.17 g. It is observed that on first day, the firmness of coated fig fruits ranged from 211.90 g to 258.23 g as compared to control (208.53 g). It is observed that there was a decrease in the firmness with increase in storage days and increased with increasing the concentrations as show in Fig. 7. The highest value of firmness after 6 days of storage was recorded as 215.03 g for treatment T_8, whereas the minimum was recorded as 168.83 g in control. From the figure, it is observed that the fig fruits coated with Zinc oxide nanoparticles had higher firmness than uncoated fig fruits. The apparent decline in fruit firmness with storage might be due to cell wall softening and activities of ripening related to enzymes such as pectin methylesterase which were largely responsible for fruit softening (Tsegay *et al.*, 2013) [18]. The activities of these enzymes in fig fruits might be retarded by the coating of Zinc oxide nanoparticles. These results are in good agreement with findings of Meng *et al.* (2014) [14] who reported that the fresh cut kiwifruit with combination of ultrasound treatment and nano Zinc oxide (ZnO) coating at 1.2 g/l exhibited similar firmness loss with increase in storage. Chen and Zhu (2011) [3] also reported that ultrasound treatment maintained higher firmness than untreated in plum fruit during storage.

Effect on microbial population

As shown in Fig. 8, the fig fruits coated with Zinc oxide nanoparticles recorded less microbial count compared to uncoated fig fruits and the microbial growth increased with the increase in storage period and decreased with increasing the concentrations. The minimum microbial count after 6 days of storage was recorded as 34.33×10^3 cfu/ml for treatment T_8 and maximum of 41.67×10^3 cfu/ml recorded in control. This might be due to the antimicrobial property of the coated biosynthesized Zinc oxide nanoparticles. The chitosan also could have improved the antimicrobial property as reported by Jeon *et al.* (2002) [9] and Tsai *et al.* (2002) [17]. Moreover, evaluation of microbial growth (both fungi and microbial) indicated that the coated fig fruits had extremely lower values of growth rate than those of uncoated fig fruits (Hajirasouliha *et al.*, 2012) [7]

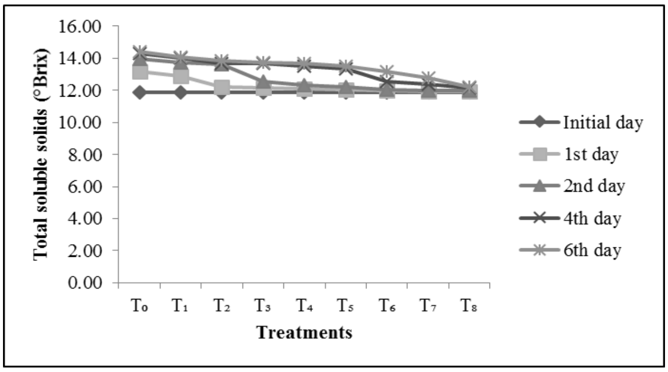

Fig 1: TSS of fig fruits coated with ZnO nanoparticles during storage

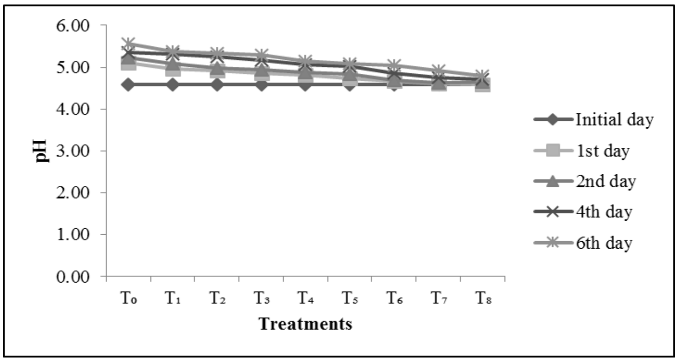

Fig 2: pH of fig fruits coated with ZnO nanoparticles during storage

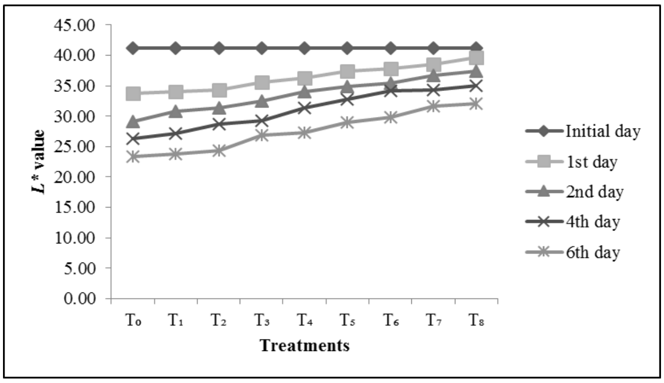

Fig 3: L^* value of fig fruits coated with ZnO nanoparticles during storage

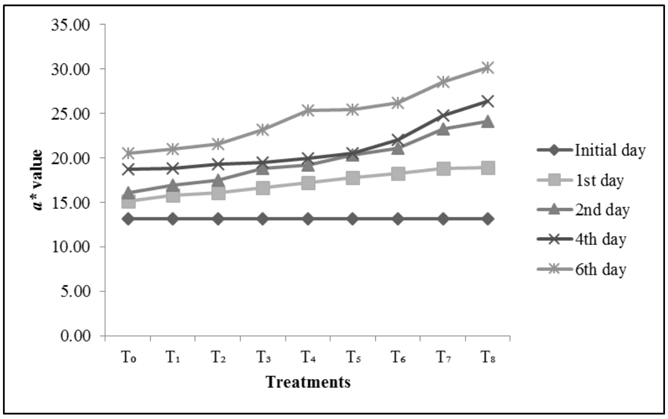

Fig 4: *a** value of fig fruits coated with ZnO nanoparticles during storage

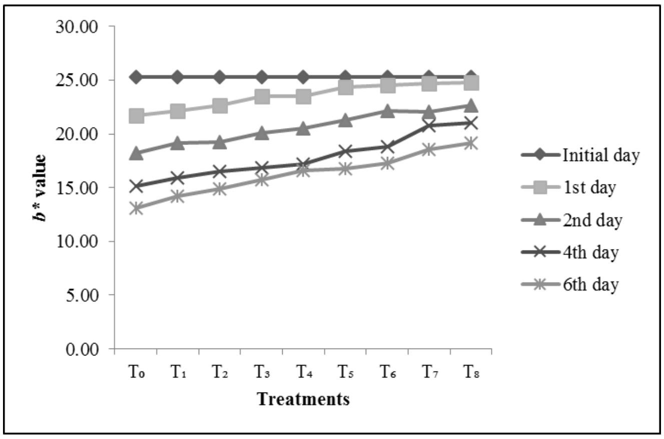

Fig 5: *b** value of fig fruits coated with ZnO nanoparticles during storage

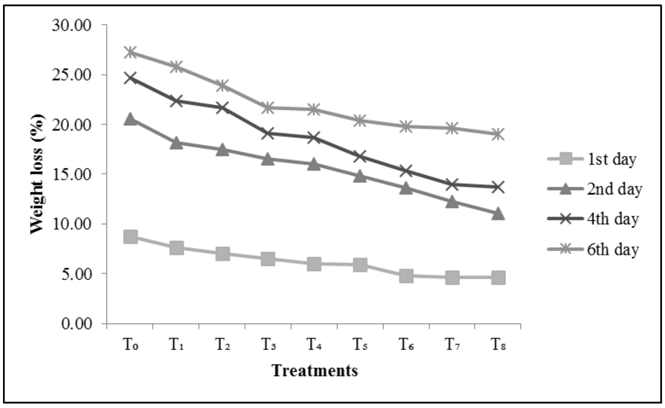

Fig 6: Weight loss of fig fruits coated with ZnO nanoparticles during storage

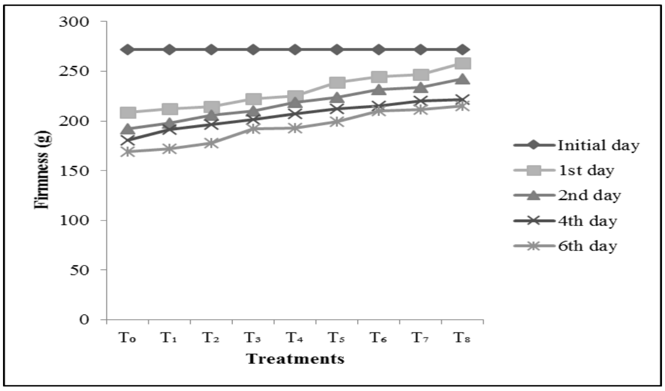

Fig 7: Firmness of fig fruits coated with ZnO nanoparticles during storage

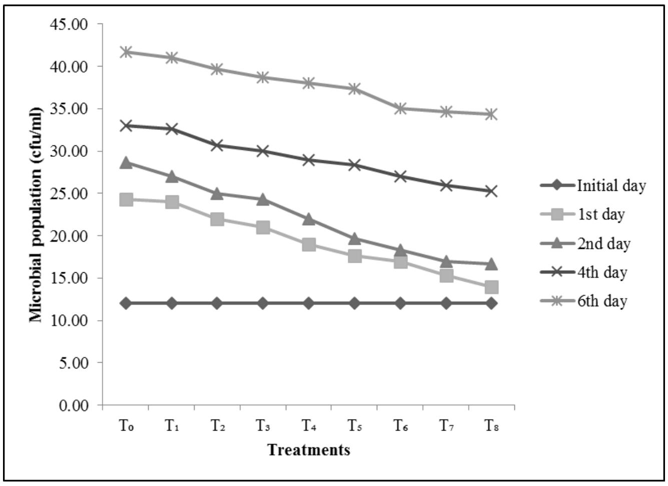

Fig 8: Microbial population of fig fruits coated with ZnO nanoparticles during storage

Conclusions

This study investigated the effect of biosynthesized Zinc oxide nanoparticles from Spinach (*Spinacia oleracea*) leaves on the quality parameters (total soluble solids, pH, colour, weight loss, firmness and microbial population) of fig fruit. However, 175 ppm is statistically on par with 200 ppm concentration, the combination of chitosan and ZnO nanoparticles coating at 175 ppm to fig fruits delayed ripening, slowed down the weight loss, reduced the colour changes, increased the firmness of fruits and inhibited the growth of microorganism compared to uncoated fig fruits. Application of Zinc oxide nanoparticles to fig fruits was observed to be beneficial in keeping the quality during storage.

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Compliance with Ethical Standards

No funding and during research there is no involvement of human participants and animals.

Conflict of Interest

No conflict of interest

Funding

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