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SYNTHESIS AND IMPREGNATION OF Fe_2O_3 NANOPARTICLES ON CELLULOSE PAPER AND SODIUM ALGINATE FILMS FOR THE PRESERVATION OF FRUIT AND VEGETABLES

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ABSTRACT

In this study, development of nanocoated paper and film to increase the shelf life of food has been demonstrated. Iron oxide (Fe_2O_3) nanoparticles were synthesized by neem leaves extract and characterized by UV-visible spectrophotometer, exhibited an absorption peak at 326 nm. Transmission electron microscope (TEM) showed the nanoparticles ranges between 5 nm to 50 nm and selected area electron diffraction (SAED) analysis revealed the amorphous in nature of nanoparticles. The biomolecules involved in the reduction of Fe_2O_3 nanoparticles were confirmed by Fourier transform infrared (FTIR) spectrum. The characterized nanoparticles were coated on cellulose paper, which was characterized by Scanning electron microscope (SEM). Coated paper was wrapped with fruit, and vegetables. Similarly, fruit and vegetables were also dipped in nanoparticle-incorporated sodium alginate solution. As a result, protein estimation and water loss were estimated on regular basis to determine the shelf-life of fruit and vegetables. The shelf-life of fruit and vegetables increases while evaluated with control and uncoated on weight loss and soluble protein content. Hence, the nanomaterial-coated paper and sodium alginate film prove its potential application as food packaging materials for longer shelf-life of food.

Keywords: Fe_2O_3 Nanoparticles, TEM, SEM, FTIR, Packaging, Shelf-life of food

INTRODUCTION

Nanotechnology is the field of study of matter at nanolevel ranges from 1 to 100 nm. Nanotechnology field is primarily concerned with the preparation of nanoparticles of variable size, shapes, chemical composition, control dispersity and their possible exploitation for human benefits. The exclusive size-dependent properties of nanoparticles make them indispensible and superior in many areas of human activities. The chemical composition, size, shape and texture will determine the potentiality of these nanoparticles. These particles have large surface-to-volume ratio which is responsible for wide spread use of these particles in electronics, optics, mechanics, biotechnology, microbiology, environmental remediation, medicine and food technology (**Eric, 1986**). Metallic nanomaterials such as silver, gold, zinc and iron are used in various fields because of their broad applications; among these nanoparticles, iron nanoparticle is preferred for the following reasons: cost effective, antimicrobial activity, high reactivity, smaller size; therefore, it gives high surface-area-to-volume ratio, which allows interact with different chemical species and also efficient in binding metal ions (**Huber, 2005**).

Iron oxides are familiar compounds that can simply be prepared by different methods in laboratory (**Wu *et al.*, 2015**) and it consists of magnetite and magnetite particle with diameter ranges from 1 to 100 nm. They are considered due to their super paramagnetic properties and their potential uses in many areas such as terabit magnetic storage devices, catalysis, drug delivery, and it has vast application in food technology which includes food processing, food packaging, etc. (**Laurent *et al.*, 2008**).

The main problem faced by many countries is keeping safe and fresh of fruits and vegetables until reach consumer's hand. Naturally, fruits and vegetables have coating but this is insufficient to offer complete defense against water and protein loss, hence resultant in decompose. Therefore, edible coatings and films are used on range of foods such as fruits, vegetables, chocolates, meats and candies. The functional properties of edible coating depend on film-forming materials used for preparation (**Cagri *et al.*, 2004**). The exploitation of defensive nanocoating and appropriate packaging has become an area of concern in nano-food technology. In current food packaging, these iron nanoparticles are used to impart antimicrobial function, thus extending shelf life and freshness of food.

There are few studies dealt with the synthesis of Fe_2O_3 nanoparticles by using extract of plant. For instance, iron oxide nanoparticles have been synthesized using aqueous extracts of *Hordeum vulgare* and *Rumex acetosa* plants, peel extract of plantain and *Tridax procumbens* leaf extract (**Makarov *et al.*, 2014**; **Venkateswarlu *et al.*, 2013**;**Senthil and Ramesh 2013**). However, there is a study on zero valent iron nanoparticles synthesized by neem extracts, reported by **Pattanayak and Nayak,** (**2013**).

Therefore, in this study, we have made an attempt on the synthesis of Fe_2O_3 nanoparticles using extract of neem leaves and incorporation of nanoparticles onto cellulose paper and sodium alginate solution for food packaging to extend the fruit and vegetables shelf-life.

MATERIAL AND METHODS

Preparation of extract and synthesis of Fe_2O_3 nanoparticles

Leaves of *Azadirachta indica* (neem leaves) extract were utilized as a reducing agent for the biosynthesis of Fe_2O_3 nanoparticles. De-ionized water was used to prepare all the aqueous solution and also for the synthesis of iron oxide nanoparticles. 10 mL of neem leaves extract were added with 90 mL of 1 mM ferric chloride solution. The mixture was subjected to 1-hour incubation in the boiling water bath at 90°C.

Characterization of nanoparticles

The formation of nanoparticles was monitored by UV-visible spectroscopy on a Shimadzu (UV-1800). Size and morphology of nanoparticles were determined by TEM with SAED pattern (TEM (Joel/Jem 2100)). The possible biomolecules involved in the reduction of Fe_2O_3 nanopartcles were identified by FTIR (Shimadzu, Japan).

Coating of Fe_2O_3 nanoparticles on paper

100 mL of synthesized Fe_2O_3 nanoparticles were poured into the gel tray and it was placed in a gel rocker. Time of 1 hour and speed limit of 70 rpm were set for coating. Then, equal size of cellulose-based paper (butter paper) was immersed in

iron oxide nanoparticle solution on gel tray. After 1 hour, the coated papers were removed and air dried for further characterization.
The Fe_2O_3 nanoparticles on the coated paper were characterized by scanning electron microscopy (JEOL Model JSM - 6390LV).

Apples storage by wrapping in Fe_2O_3 nanoparticle-coated paper

The paper coated with Fe_2O_3 nanoparticles using the gel rocker as well as uncoated paper was utilized to cover apples to confirm the efficiency of coated paper for storage of fruits. The experiment was carried out for 10 days.

Fe_2O_3 nanoparticles incorporated sodium alginate film preparation for the packaging of fruit and vegetables

Nanoparticle-based film preparation, film coating, weight loss determination and sensory analysis of experiments were carried out according to **Mohammed *et al*.** (**2009**) with some modifications. The Bradford method was used to estimate the soluble protein content (**Bradford**, **1976**). Experimental analysis were done in triplicates..

RESULTS AND DISCUSSION

Synthesis and characterization of iron oxide nanoparticles

The Fe_2O_3 nanoparticles were synthesized using extract of neem leaves and were confirmed by the change in color of the solutions. The freshly prepared extract of neem leaf was added to 1 mM Ferric chloride solution the change in color from yellowish brown color to black color was observed, which indicates the formation of the Fe_2O_3 nanoparticles as shown in Figure 1a. The change in color is the easiest and commonly used indication of the formation of the metal nanoparticles (**Toshima *et al*., 1998**). Figure 1b show the UV-visible spectra reading of the Fe_2O_3 nanoparticles synthesized using extract of neem leaves, which recorded the absorption peak at 326 nm. Similar observations were reported earlier (**Balamurughan *et al*., 2014**; **Guo *et al*., 2001**). Also, this UV-visible spectrophotometer has already confirmed to be especially helpful technique for the analysis of nanoparticles (**Mulvaney, 1996**).

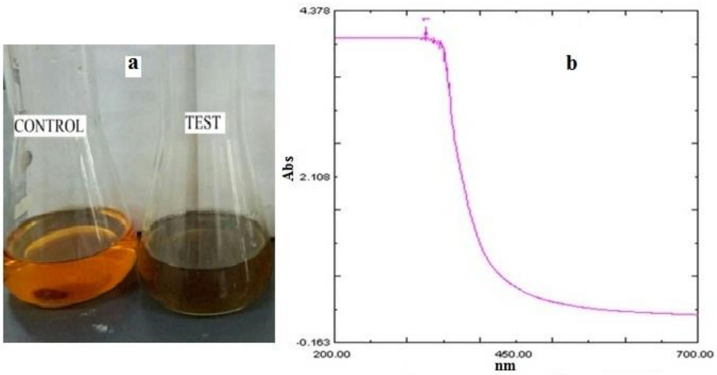

Figure 1 (a) Reaction vessel containing 10^{-3} M of ferric chloride solution, before reaction (Control) and after 1 hour of reaction at 90°C in water bath (Test). (b) UV-visible absorption spectra of Fe_2O_3 nanoparticles after 1 hour of reaction

Figure 2 proves the FT-IR spectrum of synthesized Fe_2O_3 nanoparticles. It exhibits three strong bands were around at 3339 cm^{-1}, 1636 cm^{-1} and 598 and 552 cm^{-1}. The observed bands are similar to previously reported for Fe_2O_3 nanoparticles (**Kumar and Singhal, 2007**). The vibration bands at 648 cm^{-1} are due to Fe–O stretch, which indicates the Fe_2O_3 nanoparticles (**Gotic *et al*., 2009**), at 1636 cm^{-1} is due to alkenyl C=C stretch and a broad peak at 3448 cm^{-1} is due heterocyclic amine NH stretch. The presence of organic molecules over the surface of Fe_2O_3 nanoparticles has the influence on the FT-IR peaks as previously reported (**Balamurughan *et al*., 2014**; **Lee *et al*., 1996**).

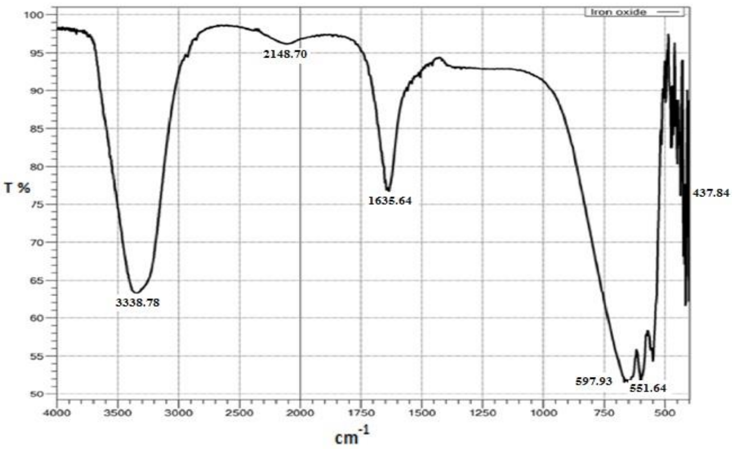

Figure 2 FTIR spectra of Fe_2O_3 nanoparticles synthesized by neem leaves extract

The powdered nanoparticles were analyzed for the determination of size and morphology of the prepared Fe_2O_3 nanoparticles using TEM at different magnification levels as shown in Figure 3a. TEM images showed that the prepared Fe_2O_3 nanoparticles were sphere shapes with little aggregation. The morphology of the nanoparticles consistently appears to be a squishy. The size of the nanoparticles ranges between 5 and 50 nm. According to selected area electron diffraction analysis (SAED), these nanoparticles had a diffraction pattern characteristic of amorphous nanoparticles (Figure 3b), which was consistent with the earlier published data (**Makarov *et al*., 2014**; **Kharissova *et al*., 2013**; **Njagi *et al*., 2010**; **Hoag *et al*., 2009**).

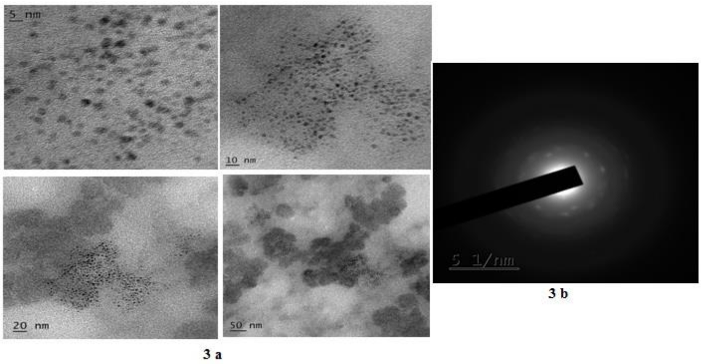

Figure 3 (a) TEM images of synthesized Fe_2O_3 nanoparticles ranging from 5 nm and 50 nm (b) SAED pattern

SEM figure of the blank paper (Figure 4a) showed the thick network of cellulose fibers with damaged surface which is already present in the paper that may be during the paper-making process. The SEM figure of Fe_2O_3 nanoparticle-coated paper taken after 1 hour of shaking on gel rocker and the nanomaterials were examined to be coated on the surface of the cellulose fibers that could be proved from the magnified figures of SEM (Figure 4b) as small spherical spots, which reveals the nanoaparticles were bound to the cellulose fibers.

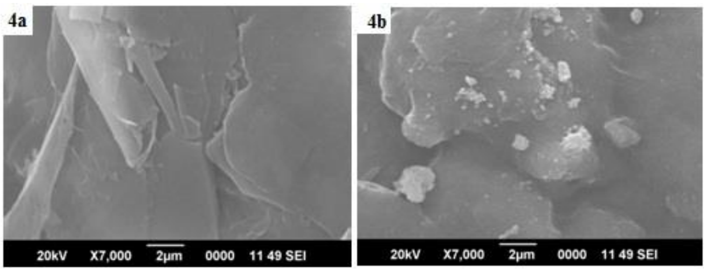

Figure 4 SEM images of Fe_2O_3 nanoparticles uncoated cellulose paper (a) and nanoparticles coated cellulose paper (b)

Measurement of protein content and weight loss on apple

The Fe_2O_3 nanoparticle-coated paper was used for covering of apples to increase its shelf-life. In general, 3–4 week is for shelf-life of apple, afterward it starts rotting by microorganisms. Taking into account of this reality, we have assessed the effect of nanoparticles on shelf-life of apple at 8^{th} day of studies (Figure 5a). No significant difference was observed during the storage period in soluble protein content. The preliminarily soluble protein content of nanoparticle-coated paper wrapped apple was 2.426 ± 0.02 mg/g of dry weight, which fallen accordingly and arrived at their lower values of 2.34 ± 0.05 mg/g of dry weight, respectively. Herein, the decline in soluble protein content between 2 and 8 days is due to consumption of soluble protein for metabolic activity such as substrate for respiration because of insufficient carbohydrate source (**King *et al*., 1999**).

Similarly, Fe_2O_3 nanocoated paper wrapped apples were monitored to have minimal weight loss while comparing to control and uncoated as revealed in Figure 5b. The water loss was in severe increase in the first 2 days, afterwards the water loss become stable and increased comparatively slow, until the eighth day. The loss of water is one of the usual processes of catabolism in fresh-cut fruits and is attributed to the respiration and other senescence-related metabolic processes during storage (**Watada and Qi, 1999**). Similarly, Bhople *et al*. (2016) have prepared a silver nanoparticle-coated butter paper and found that silver nanoparticle-coated paper significantly increases the shelf-life of apples.

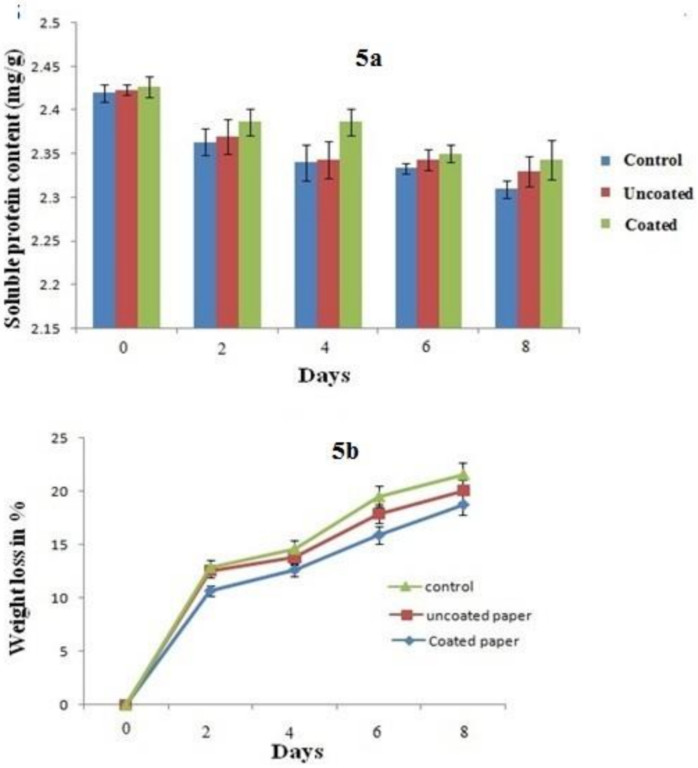

Figure 5 Soluble protein content and percentage of weight loss in control, apple wrapped by uncoated cellulose paper (a) and apple wrapped by coated cellulose paper (b)

Measurement of protein content and weight loss on carrot and brinjal

No significant difference was observed during the storage period in soluble protein content. The first soluble protein content of Fe_2O_3 nanoparticle incorporated sodium alginate film-coated carrot and brinjal were 2.13 ± 0.041 and 2.03 ± 0.045 mg/g of dry weight, respectively, which fallen significantly and arrived at their lowest values of 1.82 ± 0.054, 1.43 ± 0.02 mg/g of dry weight, respectively, as revealed in Figure 6a,b. Nanoparticle incorporated sodium alginate film-coated carrot and brinjal were monitored with minimal weight loss while comparing sodium alginate coated, uncoated and control, which is shown in Figure 6c,d. The water loss was in severe increase in the early 2 days, and afterwards the water loss stabilized and increased moderately slow, until the eighth day. A similar result has already been reported for silver nanoparticles incorporated sodium alginate film for vegetable and fruit preservation (**Mohammed *et al*., 2009**).

Sensory analysis

The overall acceptance was assessed for control, uncoated paper wrapped apple and Fe_2O_3 nanoparticle-coated paper wrapped apples as shown in Figure 7a. Similarly, control, sodium alginate-coated carrot and brinjal and nanoparticle-incorporated sodium alginate-coated carrot, brinjal are shown in Figure 7b and 7c. The acceptance was significant for Fe_2O_3 nanoparticle-coated paper wrapped apple and nanoparticle-incorporated sodium alginate-coated carrot, brinjal up to eighth day as judged by color, appearance and texture when compared to control, wrapped with paper and sodium alginate coated which was dropped on seventh and eighth day. This may be due to gradual increase microbial infection on control and sodium alginate-coated carrot, brinjal, which reduces their acceptance.

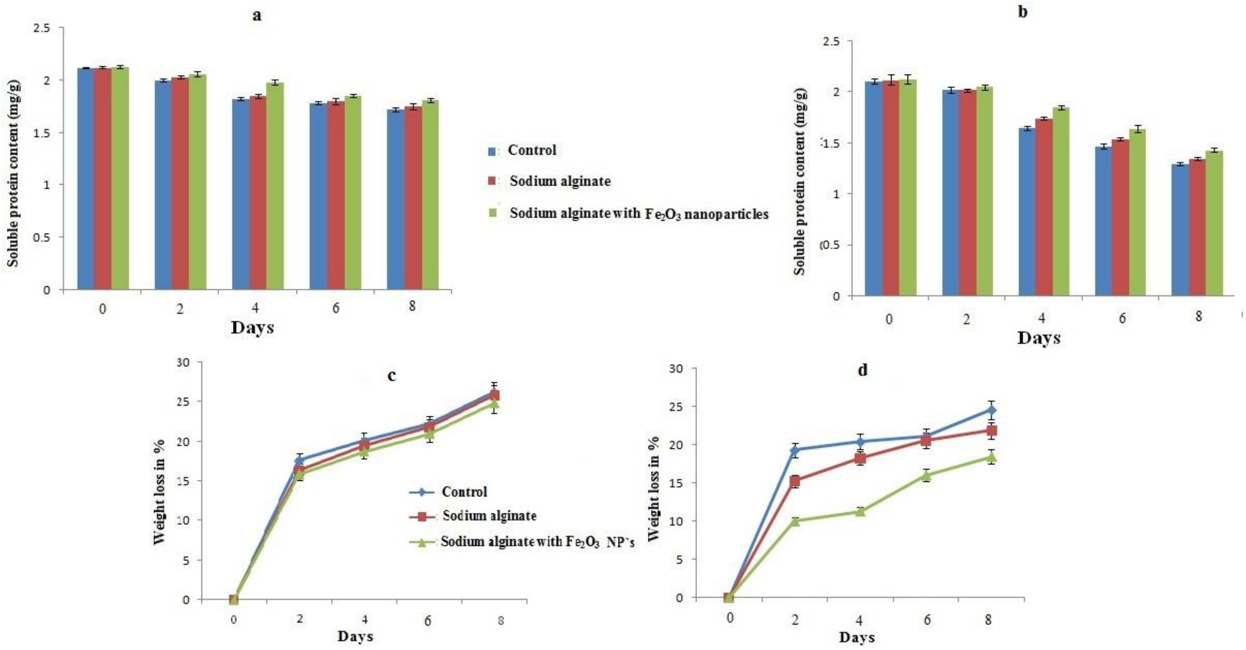

Figure 6 Soluble protein content (a, b) and percentage of weight loss (c, d) in control, sodium alginate film coated and sodium alginate with Fe_2O_3 nanoparticles coated on (a, c) carrot and (b, d) brinjal

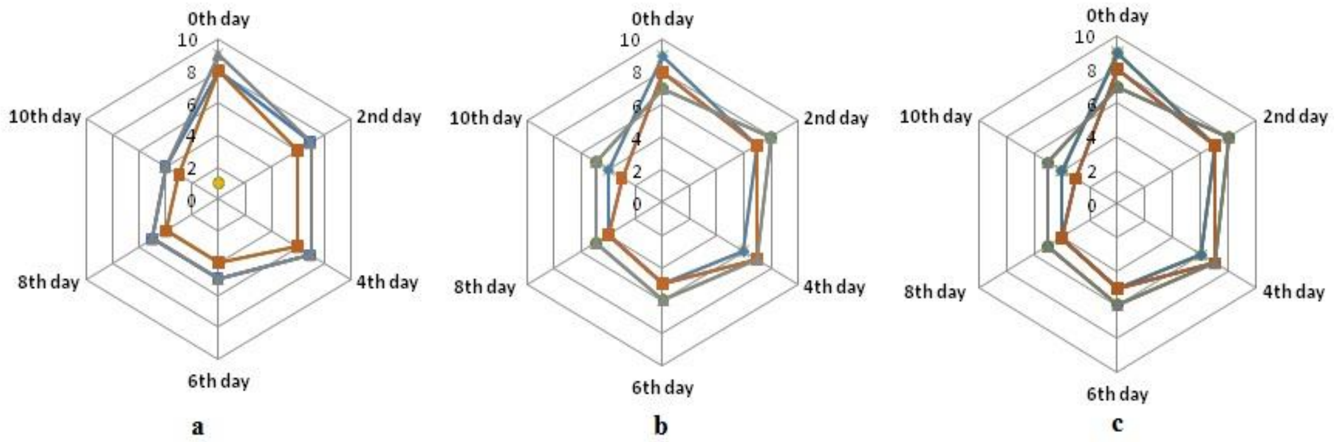

Figure 7 Sensory analysis of overall acceptance of (a) apple, (b) carrot and (c) brinjal.

CONCLUSION

We conclude that the Fe_2O_3 nanoparticle-coated paper having the significant activities which play vital role in enhancing the shelf-life of apples up to a great extent. Also, nanoparticle-incorporated sodium alginate film is encouraged as a novel method. This result reveals that the film involves in enhancing the shelf life of fruit and vegetables to a great extent. Hence, this work has been identified as a novel approach to use the nanotechnology field for packaging film development for food preservation.

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