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Nanoemulsion based alginate organic coating for shelf life extension of okra

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ABSTRACT

Okra holds major share of domestic and export vegetable market, but has a short shelf life owing to desiccation and fungal spoilage. Alginate coating containing nanoemulsified basil (*Ocimum basilicum*. L) oil was attempted for maintaining its postharvest quality and preventing spoilage. O/W nanoemulsion was prepared by using basil oil with synthetic surfactant and a naturally sourced surfactant: Tween 20 and aqueous extract of *Sapindus mukorossi* using ultrasonication respectively. Alginate coatings with basil oil nano-emulsified with Tween 20 (ATNE) and *Sapindus* extract (ASNE) were compared for their effect on PLW (Physiological loss in weight), colour, texture and acceptability of okra pods stored at 5 ± 1 °C and 24 ± 2 °C. Coatings were able to retard loss of moisture, colour and firmness during storage. Compared to 10.05% weight loss in uncoated pods (control), PLW in ASNE and ATNE was recorded as 7.38% and 8.32% respectively after 4 days of cold storage. Increase in L^* value during storage was 26.39% for control pods compared to 14.98% in coated pods. a^* value and browning index revealed better effectiveness of ASNE coating for colour retention during storage. Developed formulations were found effective against spoilage fungi *Penicillium chrysogenum* and *Aspergillus flavus*. Effective concentration for 50% inhibition of pathogens was determined using probit analysis. EC_{50} values were lower for *sapindus* emulsified basil oil over Tween 20 based nano-emulsion. *In vivo* trials on okra inoculated with *Penicillium chrysogenum* and *Aspergillus flavus* yielded promising results. Thus alginate coating with basil oil nano-emulsified with *Sapindus* extract can emerge as a promising non-chemical approach towards extending post-harvest quality and shelf life of okra.

1. Introduction

Vegetables play a significant role in Indian horticulture contributing to 59.64% of total horticulture production with estimated production of about 168.51 million tonnes from an area of 9.46 million hectares. India is the second largest producer of vegetables in the world next to China. Amongst vegetables, Okra (*Abelmoschus esculentus* (L.) Moench) is most popular vegetables throughout the world for its delicious and nutritious edible green pods. Recent findings indicate that its mucilage can help in plasma replacement or blood volume expansion (Liu et al., 2018; Petropoulos, Barros, & Ferreira, 2018). Trade in okra has immense potential as a major foreign exchange earning crop, accounting for about 72.9% of the India's export of fresh vegetables excluding potato, onion, garlic *etc.* (Kodandaram et al., 2017; NHB, 2014). For export purpose, the quality criterion for okra pods should be green, tender, 4–5 ridged and approximately 6–8 cm in length (Singh & Pandey, 1993). Generally, okra is harvested without following any safety measures resulting in mechanical injuries to the ridges of pods which lead to darkening of ridges, shriveled pods, moulds growth and other storage diseases (Singh & Pandey, 1993; Cantwell & Trevor, 2002; Anonymous, 2004). Postharvest loss of okra ranges from 50 to 72% and in majority cases, *Aspergillus flavus, Pencillium chrysogenum* cause major fungal soilage (Dilip, Jain, Rajani, & Sharma, 2013).

Edible coating is an environmental friendly technology for application on many food products as protective coating to minimize the rate of desiccation, gaseous exchange and associated oxidation processes (Nawab, Alam, & Hasnain, 2017). In these edible films and coatings, several active ingredients can also be incorporated into polymer matrix, for the purpose of enhancing safety or improving quality attributes (Acevedo-Fani, Soliva-Fortuny, & Martín-Belloso, 2017). Edible coatings supplemented with nanoemulsion-based solutions may contribute towards extension of shelf-life of horticulture produce by reducing moisture migration, gaseous exchange, oxidative reactions, besides suppressing pathogenic growth and physiological disorders (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2015; Sessa, Ferrari, & Donsì, 2015).

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Lately, essential oils are being explored for their potential to serve as secondary preservatives for safe and eco-friendly ways for extension of shelf life of perishable horticulture products, due to their antimicrobial activity (Solgi & Ghorbanpour, 2014). Essential oils are complex, volatile compounds naturally present in aromatic plants and these oils contribute to their defense mechanisms. Several researchers have attempted the application of essential oil in formation of nanoemulsions or the purpose of use as drugs, nutraceuticals, flavors, antimicrobial agents and antioxidants (Acevedo-Fani et al., 2017; Bhargava, Conti, da Rocha, & Zhang, 2015; Donsì, Cuomo, Marchese, & Ferrari, 2014).

Edible coatings containing nanoemulsified essential oil have been employed by several researchers for reducing postharvest decay of fruits (Otoni, Pontes, Medeiros, & Soares, 2014; Salvia-Trujillo et al., 2015). Tzortzakis (2007) was successfully able to reduce fruit decay and improve postharvest quality of tomato and strawberries through use of cinnamon and eucalyptus oil. Similarly Abd-Alla, El-Gamal, El-Mougy, and Abdel-Kader (2014) have reported that treatment with basil oil aids in controlling the crown rot and anthracnose in bananas and thus prolongs its storage life. However not many reports exist on shelf life enhancement of vegetables like okra. Use of surfactant to decrease surface tension and enable formation of nanoemulsified droplets of essential oil during the process of nanoemulsification is a common practice (Homayoonfal, Khodaiyan, & Mousavi, 2014; McClements & Rao, 2011). Most studies employ synthetic surfactants. However demands for organic and green technologies for food preservation, challenge us to look for alternative and effective surfactants. Among different classes of biosurfactants, one of the important is saponin, a non-volatile surface active glycoside, available from plants belong to the family sapindaceae (*Sapindus* sp.). Among these plants, *Sapindus mukorossi* (Soap Nut) is widely available as a wild forest tree in Indo-Gangetic plains and sub-Himalayans regions of India (George & Shanmugam, 2014; Ghagi, Satpute, Chopade, & Banpurkar, 2011). The pericarp of these fruits, a good source of saponin, is commonly used as detergent and its extract is commercially used as foam stabilizing and emulsifying agent in cleansers, shampoos and cosmetics (Ghagi et al., 2011).

In view of these facts, the present investigation was conducted with following objectives (i) Development of basil oil based nanoemulsions using natural and synthetic emulsifiers and (ii) Validating use of antimicrobial nanoemulsion coating for extension of shelf life and preventing spoilage of okra.

2. Materials and methods

2.1. Materials

Okra (*Abelmoschus esculentus* L. Moench) harvested at commercial maturity stage were procured from Division of Vegetable Science, IARI, New Delhi. Food-grade Sodium alginate was procured from Molychem, Mumbai, India, was used to form edible coatings. Calcium chloride (Qualikems Fine Chemicals Pvt. Ltd., New Delhi, India) was used for initiating crosslinking reaction with sodium alginate to form the continuous edible coating on the fruit surface. Basil essential oil (*Ocimum basilicum*) provided by Mentha and Allied Products Private Limited, New Delhi, India, was introduced in coating for the purpose of antimicrobial action. The main components in basil oil were methyl chavicol (60.19%) and Linalool (17.08%) certified by the company and verified by us using GCMS. Non-ionic surfactant, Tween 20 (Polyoxyethylene sorbitan monolaurate) was purchased from Central Drug House (P) Ltd, New Delhi, India as a emulsifier and *Sapindus mukorossi* (Reetha) pericarp powder was procured from Khadi products Pvt Ltd, India for use as a natural surfactant. Reagents, culture media for pathological assay were procured from Himedia, Mumbai, India. The strains of fungal species *Penicillium chrysogenum* and *Aspergillus flavus* were obtained from Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi. Ultra-pure water obtained from a Milli-Q filtration system was used for preparing solutions.

2.2. Preparation of basil oil nanoemulsion

The emulsions were prepared according to the Salvia-Trujillo et al. (2015), Ghosh, Mukherjee, and Chandrasekaran (2013) with slight modifications. Briefly, the coarse emulsion was prepared by mixing the basil oil (0.5% v/v) in milli-q water with surfactant Tween 20 (0.2% v/v) and sapindus extract (0.2-0.4% w/v) separately. This concentration of basil oil was selected based on the previous reports on MIC against fungal strains (Pandey, Singh, & Tripathi, 2014). The prepared solution was blended using a magnetic stirrer (IKA C-MAG HS7) for about 5–7 min followed by mixing in an electric blender (3000 rpm/ 2 min). These prepared coarse emulsions were subjected to Ultrasonication treatment (Misonix, Ultrasonic Liquid Processors). Ultrasonication (US) treatment was given to the coarse emulsion through sonotrode having maximum probe diameter of 13 mm containing a piezoelectric crystal to obtain nanoemulsions. The temperature increase during ultrasonication was minimized through pre-cooling and dipping in ice bath during the course of ultrasonication. The variables in this investigation included amplitude (15–45 μm), duration of treatment (0.5–3.0 min.), and type of surfactant (Tween 20 or Sapindus extract).

2.3. Characterization of Nanoemulsion

2.3.1. Particle size and z-potential

The droplet size of the developed nanoemulsion solutions was determined by using dynamic light scattering (DLS) with a zetasizer laser diffractometer (Model: SZ-100 Nano particle analyzer) at 633 nm at room temperature (25 °C) (Acevedo-Fani et al., 2017). Nano particle analyzer (SZ-100) is based on Dynamic Light Scattering (DLS), which determines the scattering intensity which naturally fluctuates due to the Brownian motion of macromolecules or particles in suspension. The system had dual solid state laser diodes at 633 nm (near-infrared) wavelength as optical light sources with refractive index of 1.487. To avoid multiple scattering effects, samples were diluted with Milli-Q water (1:10) prior to analysis.

2.3.2. Stability of emulsion

Stability of nanoemulsion was determined by centrifuging at 3500 rpm for 30 min and examining for phase separation (Robledo et al., 2018). The stability tests were also conducted for samples stored in refrigerator (4.0 ± 0.5 °C) using the protocol as per Karthikeyan, Jeeva, Jerobin, Mukherjee, and Chandrasekaran (2012) and Ghosh et al. (2013).

2.3.3. Transmission Electron Microscopy (TEM)

Transmission electron microscopy used to examine the morphology of prepared nanoemulsions. The liquid nano formulation was coated on the carbon grid with the help of forceps and kept for 1–2 min. After washing with 10–15 drops of distilled water and the grid was stained with 2% Uranyl acetate (Negative stain). After drying the grid (200 mesh size) was viewed under TEM (Model No: JEM 1011, 100 kV microscope, JEOL-Japan) with an ultra-high resolution pole piece (Robledo et al., 2018).

2.3.4. Coating of okra fruits for storage study

2% (w/v) Sodium alginate (SA) (Molychem, Mumbai, India) solution was prepared according to Poverenov et al. (2014) with slight modifications. The SA powder was suspended in milli-Q water and kept on hot plate magnetically stirred at 70 °C for 2 h to get completely uniform solution without any lumps. This solution was then cooled to room temperature. Okra fruits were selected based on fruit length, firmness, and freedom from mechanical damage. Prior to coating, the fruits were washed, rinsed, and surface dried by keeping under fan at