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Effect of chitosan and alginate based coatings enriched with pomegranate peel extract to extend the postharvest quality of guava (*Psidium guajava* L.)

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ABSTRACT

The influence of chitosan (1% w/v) and alginate (2% w/v) coatings in combination with pomegranate peel extract (PPE; 1% w/v) on quality of guavas (cv Allahabad *safeda*) were studied. Restricted changes were recorded in respiration rate, ripening index, and instrumental colour values in case of the coated samples as compared to the control for 20 days at 10 °C. Samples coated with chitosan enriched with PPE (CHE) proved to be the most effective treatment in maintaining the overall fruit quality. Ascorbic acid, total phenolics, total flavonoids contents and antioxidant activity were recorded with restricted losses of 29%, 8%, 12%, 12% (DPPH) and 9% (FRAP), respectively for CHE samples at the end of storage. A higher degree of correlation ($r > 0.918$) was established between various phytochemicals and AOA. PPE enriched coatings was proved efficient in maintaining the quality of guavas during 20 days of low temperature storage.

1. Introduction

Guava (*Psidium guajava* L.) is a major fruit crop of India, which belongs to the family *Myrtaceae*. The edible portion in guava is approximately 93% (Haque, Saha, Karim, & Bhuiyan, 2009). It is a crop of important nutritional significance because of its high nutritional value due to polyphenols, carotenoids and ascorbic acid (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006). However, being a climacteric fruit, ripening process continues even after harvesting. The fruit exhibits a high respiration rate due to high metabolic activities, thus contributing to its rapid perishability (Singh & Pal, 2008). Usually guava fruit becomes mealy upon storage beyond 6–8 days under ambient temperature (Tandon, Singh, & Kalra, 1989). It is susceptible to chilling injury when stored below 10 °C (Reyes & Paull, 1995). Thus, the spoilage and losses of this horticultural produce requires attention during transportation and marketing.

Healthy eating and healthy lifestyle is on high demand currently at the global level. This concept has raised the need for better preservation of the perishables such as fruits and vegetables, as they are an abundant source of bioactive compounds, vitamins and minerals (Diaz-Mula, Serrano, & Valero, 2012). Edible coatings have been used to enhance the shelf life of whole as well as fresh-cut fruits and vegetables. They could be good alternative to chemically synthesized preservatives, besides being cost effective. They have been proven to retard physiological processes such as respiration, degradation of cell wall, transpiration and also restrict microbial action; thereby preserving the quality of the fruit and vegetable (Ali, Muhammad, Sijam, & Siddiqui, 2011). In recent years, a lot of research work has been carried out on application of various kinds of edible coatings. Generally, they are based on polysaccharides, proteins and lipids for extending the shelf life of various horticultural commodities (Chen et al., 2016; Gardesh et al., 2016; Khaliq, Mohamed, Ali, Ding, & Ghazali, 2015; Suseno, Savitri, Sapei, & Padmawijaya, 2014; Synowiec et al., 2014). Out of these coating materials, polysaccharide based edible coatings such as chitosan and alginate are gaining popularity in recent times.

Chitosan is a cationic polysaccharide, which is produced by the deacetylation of chitin, obtained from crustacean shells. It is biodegradable, non-toxic, antimicrobial, capable of entrapping bioactive compounds and essential oils (Aloui et al., 2014) It forms a semi-permeable membrane and creates a modified internal atmosphere, thereby, reducing respiration and transpiration in fruits and vegetables. Action of chitosan varies depending upon the degree of deacetylation as higher the degree of deacetylation, higher the antimicrobial function (Pagliarulo et al., 2016). However, its usage is limited due to its insolubility in water at neutral pH. Chitosan coatings have demonstrated positive effects on fruit quality under cold storage; as reported in case of carambola (Gol, Chaudhari, & Rao, 2015) and papaya (Ali et al., 2011).

Alginate is obtained from the brown seaweed belonging to the family *Phaeophyceae*. It has been proven to be a potential coating material for causing delay in the ripening process and its effectiveness enhances

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on incorporation of any antioxidant or antimicrobial agents. Alginate comprises of colloidal properties such as thickening, stabilizing etc and therefore, it could be effectively used in coating and film preparation (Benavides, Villalobos-Carvajal, & Reyes, 2012). Studies on the application of alginate coating on whole fruits have demonstrated the enhancement of shelf-life of strawberry (Peretto et al., 2017), mandarin (Chen et al., 2016), grapes (Aloui et al., 2014), carambola (Gol et al., 2015), sweet cherry fruit (Diaz-Mula et al., 2012).

Edible coatings could also be enriched with certain additives such as antibrowning and antimicrobial agents, which in turn enhance the performance of the native edible coating material (Dhall, 2013). However, enriching the edible coatings with natural plant extracts and its application on various fruits and vegetables is gaining popularity over the use of chemical preservatives. Use of chitosan coating enriched with moringa leaf extract on avocado (Tesfay & Magwaza, 2017) and alginate coating enriched with grape seed extract on grapes (Aloui et al., 2014), have demonstrated improvement in their overall quality.

Pomegranate (*Punica granatum* L.) is considered as a highly nutritious fruit that belongs to the *Punicaceae family*. A considerable amount of bioactive compounds is also present in its non-edible portion such as peel (Ismail, Sestili, & Akhtar, 2012). Pomegranate peel is an agricultural waste and its non-edible part accounts for about 50% of the total fruit weight. It is a rich source of phenolics and flavonoids, which deliver antimicrobial, antidiabetic, antimutagenic and antioxidant properties (Kazemi, Karim, Mirhosseini, & Hamid, 2016; Xi, He, & Yan, 2017). Therefore, enrichment of pomegranate peel extract (PPE) into edible coatings could possibly enhance the phytochemical status of guava.

Previous studies have showed that guava coated with chitosan and stored under low temperature conditions suppressed the ripening process and maintained the overall quality (Hong, Xie, Zhang, Sun, & Gong, 2012). However, to the best of our knowledge, there is no report on the effect of chitosan and alginate coatings enriched with pomegranate peel extract on post-harvest quality of any horticultural produce during storage. Therefore, the objective of this work was to assess the effects of chitosan and alginate coatings on maintenance of the postharvest quality with emphasis on the phytochemical status of guava fruit during low temperature storage.

2. Materials and methods

2.1. Raw material, coating preparation and treatments

Guavas (cv Allahabad *safeda*) having uniform size, colour, maturity state without defects were procured from the farms of Indian Agricultural Research Institute (IARI), Pusa, New Delhi, India and stored at 4 °C until processing. The fruits were phytosanitized by washing in chlorinated water (15 ppm) thoroughly for 5 min and allowed to dry naturally.

Chitosan (low molecular weight, 75% deacetylated), gallic acid, glacial acetic acid and sodium alginate from E-Merck Ltd (Mumbai, India), Folin–Ciocalteu reagent, L-ascorbic acid, and DCPIP (2,6-dichlorophenolindophenol) from Qualigens (Mumbai, India), DPPH (2, 2-Diphenyl-1-picrylhydrazyl) and TPTZ (2,4,6-Tris(2-pyridyl)-*s*-triazine) were procured from Sigma-Aldrich, Germany whilst the other chemicals and solvents used in the study were from S.D. Fine Chemicals Ltd. (Mumbai, India).

Chitosan solution (1% w/v) was prepared by dissolving it in glacial acetic acid (1% v/v) with homogenizer (IKA T25 digital ULTRA-TURRAX, Bengaluru, India) at a speed of 800 rpm for 2 min at room temperature with a final pH of 5.2. Glycerol (0.75%) was added as plasticizer. Alginate solution was prepared by dissolving sodium alginate powder (2% w/v) in distilled water using a magnetic stirrer (Spinot, Tarsons, New Delhi, India) for 1 h at a controlled temperature of 70 °C, until the mixture became clear. 10% glycerol as a plasticizer was added after cooling the alginate solution. Calcium chloride solution (2% w/v) was also used as a firming agent in alginate solution.

Peels from pomegranates (cv *bhagwa*) obtained from IARI, New Delhi were utilized to prepare pomegranate peel extract (PPE). The peels were dried in a dehydrator (MAC, New Delhi, India) at 60 °C for 72 h. 100 g of dried peels were finely ground (40-mesh sieve) in a blender (Philips, India) and kept overnight in 1 L 80% (v/v) ethanol at 25 °C for the purpose of extraction. This solution was further centrifuged at 10,000 rpm at a temperature of 4 °C for 20 min for maximum extraction of total phenolics. The solution was then filtered using Whatman filter paper No. 1. The filtrate was concentrated using rotary evaporator (Hahnvapor, Hahnshin Scientific, Korea) at 40 °C. The obtained extract was stored under refrigerated temperature until further use. This PPE was incorporated at 1% level in chitosan and alginate solutions.

A total No. of 90 fruits were divided into five groups and were dipped in the following solutions for 1 min (1) 1% chitosan + PPE (CHE), (2) 1% chitosan (CH), (3) 2% alginate + PPE (ALE), (4) 2% alginate (AL) and (5) distilled water as control. The treated samples were manually dried and stored in plastic mesh trays at 10 °C with 90–95% relative humidity. Three fruits were taken from each treatment and sampled at an interval of 4 days till 20 days of storage.

2.2. Respiration rate

Respiration rate of the fruits was determined using three fruits per treatment at regular intervals of 4 days during the storage period. The respiration rate was measured at 25 °C and the guavas were equilibrated at this temperature before the measurements were taken. Guavas with known weight were placed in 1000 mL airtight plastic container fitted with a rubber septum on lid. A headspace analyzer (Dansensor, Checkmate 9900, Denmark) consisting of a syringe was inserted into the container through the rubber septum, to measure the respiration rate in terms of mg CO_2 released per kg per h.

2.3. Ripening index

Ripening Index (RI) was determined as a ratio of total soluble solids (TSS) and titratable acidity (TA). 10 g of fruit pulp was homogenized using a blender (Philips, India) with 40 mL of distilled water; followed by filtration with muslin cloth. The TA was determined by titrating 10 mL juice with 0.1 N NaOH using phenolphthalein till the solution turned light pink in colour (pH = 8.1) (AOAC, 1990a). The results were expressed as% of citric acid. TSS of the sample was determined using a digital refractometer (Atago, Japan).

2.4. Colour

Surface colour of the samples was determined using Labscan XE colorimeter (HunterLab, Inc., Reston, VA, USA) to assess the *L*-(lightness), *a*-(greenness) and *b*-(yellowness) values at 400–700 nm range spectral resolution of 10 nm and a wavelength accuracy of 1 nm under CIE Illumination D65 and d/10° illumination. Standardization was done using a black tile followed by a white tile. Total colour difference (TCD) was calculated using *L*, *a*, *b*-values as per Eq. (1):

$$TCD = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

where ΔL, Δa *and* Δb represents the difference in *L*, *a*, *b*-values at a particular interval from the respective initial values.

2.5. Total phenolics and total flavonoids

Total phenolics content in the fresh sample was determined according to the Folin-Ciocalteau procedure (Singleton, Orthofer, & Lamuela-Ranventos, 1999). Briefly, 5 g of sample was extracted with 80% ethanol. To 0.1 mL aliquot of the sample, 2.9 mL